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RESEARCH PAPERS

Strong differences in migratory connectivity patterns among species of Neotropical-Nearctic migratory birds revealed by combining stable isotopes and abundance in a Bayesian assignment analysis

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Abstract

Aim: Conservation planning for migratory species requires knowing how populations are connected throughout the annual cycle. We measured migratory connectivity for five migratory songbird species by combining stable-hydrogen isotopes in feathers (δ^2H_F) with citizen science data on breeding range abundance.

Location: Caribbean Basin and North America.

Taxa: Neotropical migratory birds: *Mniotilta varia*, *Setophaga ruticilla*, *Seiurus aurocapilla*, *Setophaga americana*, and *Setophaga discolor*.

Methods: We analyzed δ^2H_F in feathers grown on North American breeding grounds and sampled at 20 Caribbean Basin sites across most of the non-breeding range of each species. We made Bayesian assignments to breeding origin by combining δ^2H_F values and North American Breeding Bird Survey abundance data. For each species, we used cluster analysis to group Caribbean sites into non-breeding regions that shared birds with similar breeding ground assignment probabilities. We then mapped assignment probabilities for the non-breeding regions of each species onto breeding ground δ^2H_F surfaces and estimated the strength of migratory connectivity.

Results: Apart from some general similarities, the five species demonstrated different patterns of migratory connectivity. Species with larger non-breeding ranges exhibited stronger migratory connectivity compared to those with smaller non-breeding ranges. Despite geographical overlap, birds from each non-breeding region had mostly unique breeding ground origins.

Main conclusions: The contrasting patterns of migratory connectivity shown by the five species suggest that geographic distribution during the non-breeding period is a poor indicator of breeding ground origin. Our results indicate that full annual cycle conservation planning, which requires migratory connectivity information, should proceed on a species-specific basis.

KEYWORDS
Bayesian assignments, Caribbean Basin, conservation, migratory connectivity, Neotropical-Nearctic migratory birds, species distribution, stable isotopes

1 | INTRODUCTION

Migratory organisms travel between distant breeding and non-breeding areas, resulting in annual ranges that can span oceans and continents. Knowledge about the spatial linkages among these locations, known as migratory connectivity, is advancing rapidly (Flockhart et al., 2013; Ruegg et al., 2014), but remains unavailable for most species. Migrants experience diverse threats that vary in severity across the annual cycle, including habitat loss (Stanley et al., 2015), climate change (Tøttrup et al., 2012), harvest pressure (Holdo et al., 2010), infrastructure collisions (Baerwald et al., 2008), and predation (Loss et al., 2013). These threats can be compounded through seasonal interactions, which occur when conditions in one part of the annual cycle affect survival or reproduction later on in the year (Legagneux et al., 2012; Marra et al., 1998). With over 100 migratory taxa facing extinction (CMS, 2020) and many others showing steep population declines (Wilcove & Wikelski, 2008), quantifying migratory connectivity is essential for integrating the complex ecology of these species into a framework that aids conservation and management (Marra et al., 2019).

Stable isotope analysis is a powerful tool for inferring geographic origins of migratory species and has been pivotal for understanding migratory connectivity in birds (Sullins et al., 2016), bats (Sullivan et al., 2012), and insects (Hallworth et al., 2018; Hobson et al., 2018). In North America, stable-hydrogen isotope ratios in growing season precipitation (δ^2H_P) show a strong north-south gradient and correlate strongly with the δ^2H of locally grown tissues, particularly in the eastern region of the continent (Hobson & Wassenaar, 1997). In many species, adult birds complete breeding and then molt their flight feathers locally, incorporating δ^2H_P values from the supporting food web. Because stable-hydrogen isotope values of feathers (δ^2H_F) are mostly inert once incorporated into tissues, feathers sampled at distant non-breeding sites reached after autumn migration can estimate the location of growth at the end of the breeding season.

Neotropical-Nearctic migratory songbirds represent an ideal system to estimate migratory connectivity by using stable isotopes. These small-sized species spend about 3 months of the year on their North American breeding grounds, 6–8 months on their tropical wintering quarters, and 2 months on migration. Collectively, their population dynamics are complex, with steep declines in some regions and modest increases in others (Sauer et al., 2014). Mark–recapture approaches, including continental-scale banding efforts, yield extremely low return rates despite years of effort (Webster et al., 2002). Genetic markers, by themselves, are typically uninformative because there is little range-wide population structure (Clegg et al., 2003; Lovette et al., 2004), although recent applications of next-generation sequencing show promise (Ruegg et al., 2014). Miniaturization of satellite transmitters and light-level geolocators has enabled year-round tracking of a growing number of species, but most Neotropical-Nearctic migrants are too small to carry these devices and sample sizes are often small. Recent work indicates that δ^2H_F values can generate different assignments to breeding origin compared to potentially more accurate geolocators, but Bayesian methods that incorporate additional information can remedy this bias (Rushing et al., 2017). Such approaches are increasingly recognized as essential for developing more accurate estimates of migratory connectivity (Rundel et al., 2013).

In this study, we assessed migratory connectivity between tropical non-breeding areas and temperate breeding grounds for five species of Neotropical-Nearctic migratory bird: Black-and-white warbler (*Mniotilta varia*), American redstart (*Setophaga ruticilla*), Ovenbird (*Seiurus aurocapilla*), Northern parula (*Setophaga americana*), and Prairie warbler (*Setophaga discolor*). We collected over 800 tail feathers from 10 countries throughout the Caribbean Basin and analyzed their stable-hydrogen isotope values (δ^2H_F). We then performed Bayesian assignment of δ^2H_F values to North American breeding grounds by incorporating information on breeding season abundance from the North American Breeding Bird Survey (BBS) and estimated the strength of migratory connectivity. This approach allowed our analyses to reveal range-wide patterns of migratory connectivity that reflected regions where these species had a high probability of occurrence.

2 | MATERIALS AND METHODS

Feather samples were collected from 1995 to 2010 at 20 sites in 10 countries throughout the Caribbean Basin, encompassing the majority of the non-breeding range for each of the five species. Dominant vegetation varied across sites, but included mangrove forest, thornscrub, mixed broadleaf forest, limestone forest, and coffee farms that ranged in elevation from 5 to 200 m. Birds were captured in mist nets, aged and sexed using criteria from Pyle (1997), banded with a United States Geological Survey aluminum leg band, and released. The third tail feather was removed from each bird before its release and stored in a paper envelope for later isotope analysis. The total sample size was 826 feathers but differed among species owing to variation in non-breeding season distribution and abundance (Black-and-white warbler: n = 140, American redstart: n = 213, Ovenbird: n = 214, Northern parula: n = 121, and Prairie warbler: n = 138).

Isotope analyses were done at the Stable Isotope Mass Spectrometry Facility of the Smithsonian Institution in Suitland, MD. Feathers were washed in a 2:1 chloroform:methanol solution to remove surface oils and air dried under a fume hood for 48 h. After transport to the laboratory, feathers were allowed to equilibrate with the local atmosphere for at least 72 h. A small sample

of each feather (0.30–0.35 mg) was packed into a silver capsule, combusted at 1350°C in an elemental analyzer (Thermo TC/EA), and introduced online to an isotope ratio mass spectrometer (Thermo Delta V Advantage) via a Conflo IV interface. Four in-house keratin standards were run for every 10 unknowns to account for exchangeable and non-exchangeable H in feather samples (IAEA-CH-7: δ^2H = −100.3‰ Vienna standard mean ocean water [VSMOW]; Caribou Hoof Standard: δ2H = −197‰ VSMOW; Kudu Horn Standard: δ2H = −54.1‰ VSMOW; and Spectrum Keratin Fine Powder: δ2H = −121.6‰ VSMOW). Non-exchangeable H was determined by linear regression with the CHS and KHS keratin standards. We report non-exchangeable H in per mil units (‰) relative to the VSMOW-Standard Light Antarctic Precipitation scale. Replicate samples of the Spectrum Keratin Fine Powder standard and duplicate samples run for one in five to eight feathers indicated that analytical error (±1 SD) was 2‰.

For each species, we developed a spatially explicit probability surface of breeding origins by combining geospatial models of expected δ^2H in growing season precipitation (δ^2H_P; Bowen et al., 2005) and North American BBS abundance (Sauer et al., 2014). A global raster file of δ^2H_P with 5 arc-minute resolution was acquired from Water Isotopes.org (http://wateriso.utah.edu/waterisotopes/pages/data_access/ArcGrids.html). Metabolic tissues such as feathers preferentially store the lighter fraction of H, resulting in δ^2H_F values that are more negative relative to expected δ^2H_P values where feathers were grown. To account for this variation, we applied existing linear regression equations for Neotropical migratory birds based on δ^2H_P values and δ^2H_F values throughout North America (Hobson et al., 2012). We chose the equation for non-ground foragers to adjust American redstart, Black-and-white warbler, Northern parula, and Prairie warbler δ^2H_F values and the equation for ground foragers to calibrate Ovenbird δ^2H_F values. Using the Spatial Analyst extension in ArcGIS 10.1 (ESRI), we applied these equations to the δ^2H_P surface, yielding a new surface of expected δ^2H_F values for each species.

Our modeling goal was to define probable North American breeding ground origins of feathers collected at non-breeding sites throughout the Caribbean Basin. We used Bayes rule to calculate the probability that each raster cell in the expected δ^2H_F surface was the true origin of each δ^2H_F value collected during the nonbreeding season:

$$f(b|a) = \frac{f(a|b)f(b)}{\sum_{b=1}^{B} f(a|b)f(b)},$$

where $f(a|b)$ is the likelihood that a δ^2H_F value originated from a given breeding origin and $f(b)$ is the probability of BBS abundance at a given origin. We modeled $f(a|b)$ as a normal probability density function, a standard approach for assessing tissue origin (Royle & Rubenstein, 2004). Here, the likelihood that each feather originated from a given cell in the δ^2H_F surface was calculated as:

$$f(y^*|\mu_b, \sigma_b) = \frac{1}{\sqrt{2\pi\sigma_b}} \exp\left[-\frac{1}{2\sigma_b}(y^* - \mu_b)^2\right],$$

where y^* is each δ^2H_F value, μ_b is the value of each cell in the expected δ^2H_F surface and σ_b is the standard deviation of the residuals from the linear regression of δ^2H_P on δ^2H_F values. For each species, we calculated likelihoods for all feathers simultaneously and then summed them for each cell, yielding a single value for each cell of the expected δ^2H_F surface. We normalized these likelihoods to probabilities by dividing the likelihood for each cell by the sum of the likelihoods.

Tissue assignments that rely only on such likelihoods implicitly treat bird abundance as a uniform variable. Because birds are usually patchily distributed across space, this assumption can lead to erroneous conclusions about tissue origins. We therefore modeled $f(b)$ as the spatial variation in BBS abundance of each species. Spatially explicit maps of BBS abundance with 400 km^2 cell size were acquired for each species as shapefiles from the Patuxent Wildlife Research Center (www.mbr-pwrc.usgs.gov/bbs). Using the raster conversion tool in Spatial Analyst, shapefiles were converted into rasters of the same cell size, projection, and spatial extent as expected δ^2H_F surfaces. Abundance data were converted into probabilities by dividing individual cell values by the sum of all cells.

Using multiple data sources in a Bayesian analysis without first exploring how they should be weighted could cause one variable to bias assignment probabilities (Rundel et al., 2013). For Neotropical-Nearctic migratory birds, assignments done with isotopes and BBS abundance can be heavily dominated by abundance because many species have local centers of high abundance interspersed with large areas of low abundance, leading to posterior probabilities that merely depict breeding distributions (Hobson et al., 2014; Rushing et al., 2017). Using known origin feathers for the five study species, we previously determined the optimal weighting of δ^2H_F values and abundance that maximized assignment precision and minimized assignment error (Rushing et al., 2017). We used this information to weight δ^2H_F values and abundance differently for each species to make Bayesian assignments to breeding origin (Table S1).

Before assigning δ^2H_F values to breeding range origins in North America, we first evaluated their spatial variation among Caribbean Basin countries using cluster analysis. This approach allowed us to make the most parsimonious assignments possible. For each species, we made Bayesian assignments separately for feathers sampled at each non-breeding site and then performed a hierarchical cluster analysis on the resulting probabilities with the hclust function in program R (R Core Team, 2018). We calculated a dissimilarity matrix of Euclidean distances among feather assignment probabilities and developed cluster solutions with the Ward's *D* method. Finally, we grouped sites that clustered together into non-breeding regions and made final assignments for all feathers within each region.

To quantify the migratory connectivity of each species, we used Pearson correlation coefficients to test the relationship between two matrices: the pairwise distance among non-breeding sites and the correlation among breeding origin assignment probabilities at these sites. For this analysis, correlation coefficients closer to −1 indicate that birds wintering nearby had similar probabilities of breeding origin, whereas coefficients closer to 0 indicate that birds wintering nearby had no shared probability of breeding close together. We

calculated Pearson correlation coefficients and confidence intervals from 1000 replicate bootstraps using the *wBoot* R package (Weiss, 2016). To assess significance of Pearson correlation coefficients, we converted matrices into Euclidean distance matrices and performed a Mantel matrix randomization test with 1000 permutations using the R package *ade4* (Chessel et al., 2004; Appendix S1).

3 | RESULTS

Cluster analyses resulted in delineation of two to four non-breeding regions, each supporting birds that had a high probability of a common breeding origin (Figure S1). In most cases, individual birds that spent the tropical non-breeding period nearby one another were more likely to settle in proximity to one another on temperate breeding areas compared to those that overwintered further away.

Cluster analysis separated the Black-and-white warbler (*Mniotilta varia*) non-breeding range into three regions (Figure S1a): (A) Mexico, Belize, and Nicaragua (−95‰ ± −99‰, −91‰; mean ± 95% CI; Figure 1 inset); (B) Jamaica, Cuba, Hispaniola, Bahamas, and Florida (−77‰ ± −81‰, −74‰); and (C) Puerto Rico and St. Martin (−68‰ ± −72‰, −63‰). Warblers overwintering in region A originated primarily from the north central part of the breeding range in Manitoba and Minnesota, but also from further east in Ontario, Quebec, and Newfoundland (Figure 1a). Similar to those from region A, region B captures bred in the north

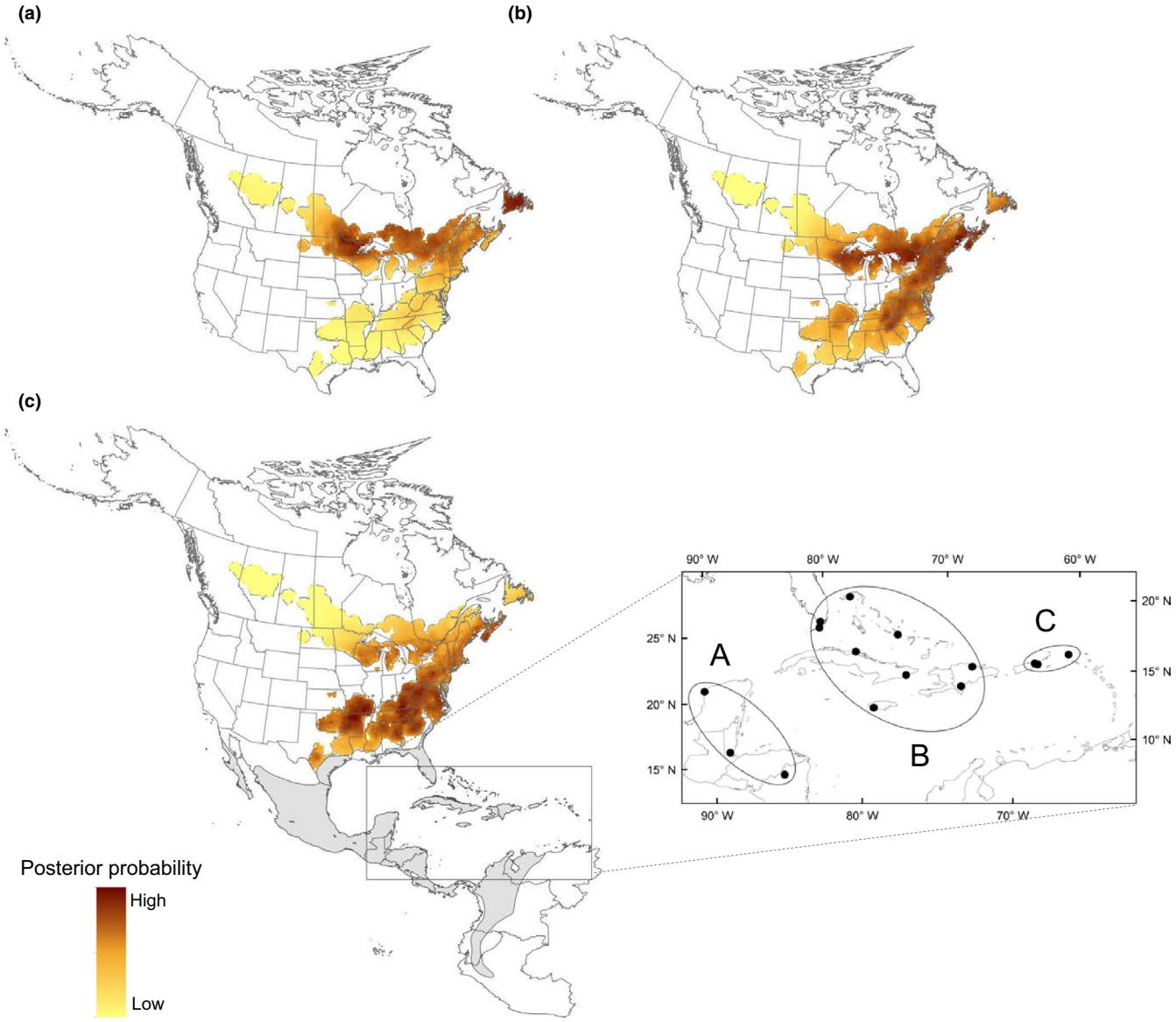

FIGURE 1 Bayesian posterior probability assignments to breeding range origin for Black-and-white warbler calculated by combining stable-hydrogen isotope values in feathers (n = 140) from three Caribbean Basin non-breeding regions (A–C) and breeding ground abundance from the North American Breeding Bird Survey. The non-breeding range is shaded in gray. The inset shows the location of feather collection sites and the non-breeding regions identified with cluster analysis. Maps are displayed in Albers Equal Area projection [Colour figure can be viewed at wileyonlinelibrary.com]

central part of the range, but had high assignment probabilities further east to New England and the Maritime Provinces of Canada (Figure 1b). Region B birds also came from localized areas of the Appalachians, a region extending from Pennsylvania in the north to North Carolina in the south. Region C birds had origins primarily from the southern edge of the breeding range in an area that spanned from North and South Carolina in the east to Missouri and Arkansas in the west (Figure 1c). Models done only with isotopes are shown in Figure S2 for comparison.

The American redstart (*Setophaga ruticilla*) non-breeding range clustered into four discrete regions (Figure S1b): (A) Mexico (−126‰ ± −139‰, −114‰; Figure 2 inset); (B) Cuba, Bahamas, and Florida (−99‰ ± −102‰, −96‰); (C) Jamaica, Hispaniola, and Puerto Rico (−84‰ ± −87‰, −80‰); and (D) St. Martin and Dominica (−71‰ ± −75‰, −68‰). Redstarts sampled in non-breeding region A bred in the northwestern reaches of the range in British Columbia and Alberta (Figure 2a). Region B individuals originated along the United States–Canada border from Saskatchewan and Minnesota in

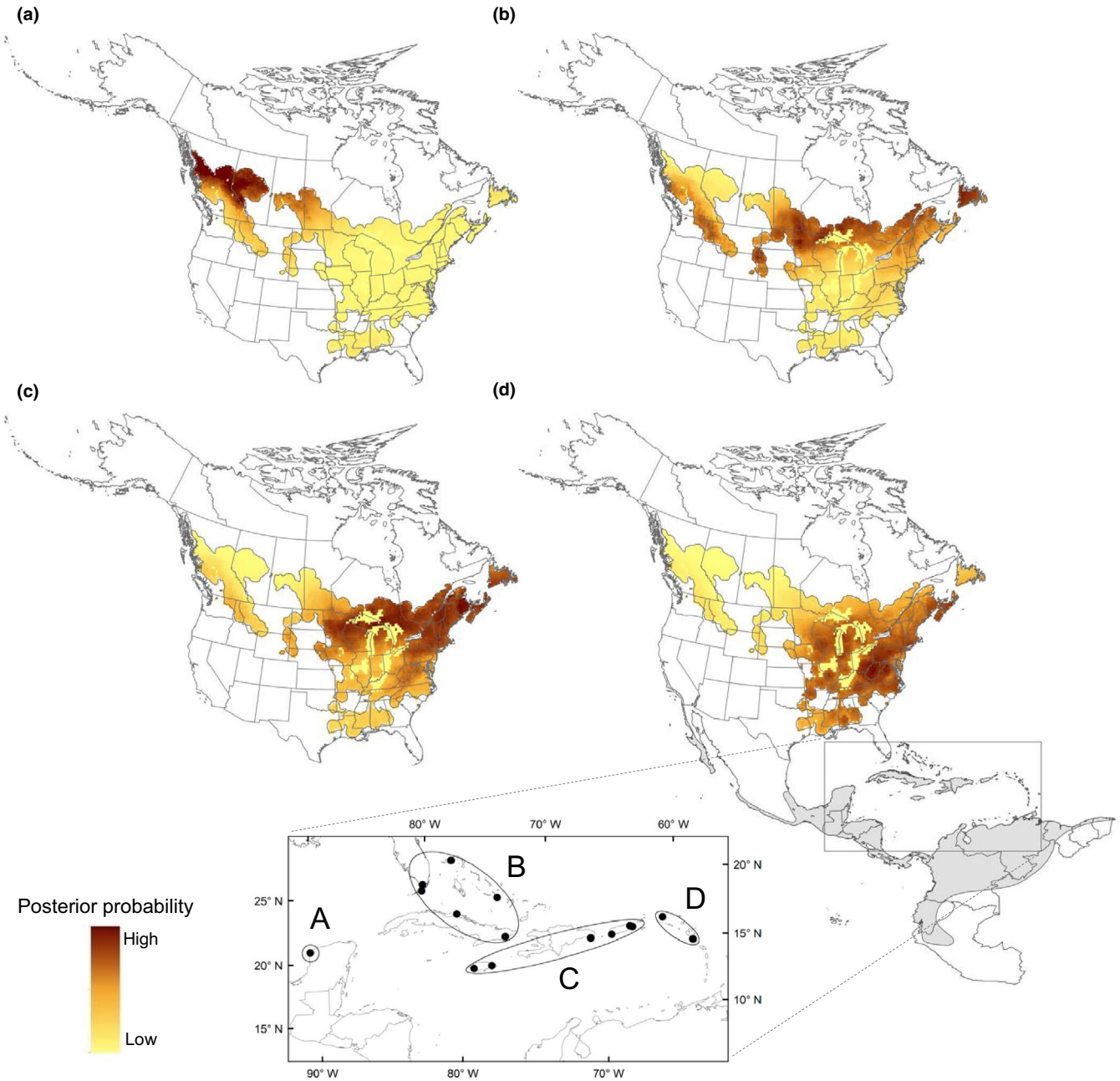

FIGURE 2 Bayesian posterior probability assignments to breeding range origin for American redstart calculated by combining stable-hydrogen isotope values in feathers (n = 213) from four Caribbean Basin non-breeding regions (A–D) and breeding ground abundance from the North American Breeding Bird Survey. The non-breeding range is shaded in gray. The inset shows the location of feather collection sites and the non-breeding regions identified with cluster analysis. Maps are displayed in Albers Equal Area projection [Colour figure can be viewed at wileyonlinelibrary.com]

the west to Newfoundland in the east (Figure 2b). Assignments of regions B and C birds overlapped considerably, but the latter showed a more eastern and southern breeding distribution (Figure 2c). Region D captures came primarily from the eastern extent of the range, an area encompassing Maine in the north to North Carolina in the south (Figure 2d). For comparison, models done only with isotopes appear in Figure S3.

Cluster analysis divided the Ovenbird (*Seiurus aurocappila*) non-breeding range into three regions (Figure S1c): (A) Nicaragua and Belize (−94‰ ± −104‰, −86‰; Figure 3 inset); (B) Mexico, central Cuba, Jamaica, northern Bahamas, and Florida (−69‰ ± −65‰, −62‰); and (C) eastern Cuba, southern Bahamas, Hispaniola, Puerto Rico, and St. Martin (−79‰ ± −82‰, −77‰). Region A Ovenbirds originated in the north central portion of the breeding range in Minnesota, Wisconsin, Manitoba, and Ontario (Figure 3a). Individuals captured in region B had origins extending across the southern half of the breeding range, with the majority in an area extending from Pennsylvania in the north to a broad area of the south from South Carolina to Arkansas (Figure 3b). Similar to those overwintering in region A, region C birds originated from the Great Lakes Region, but also has high assignment probabilities to northeastern part of the range, mainly in Ontario, Quebec, and northern New England and New York (Figure 3c). Isotope-only models are given in Figure S4 for comparison.

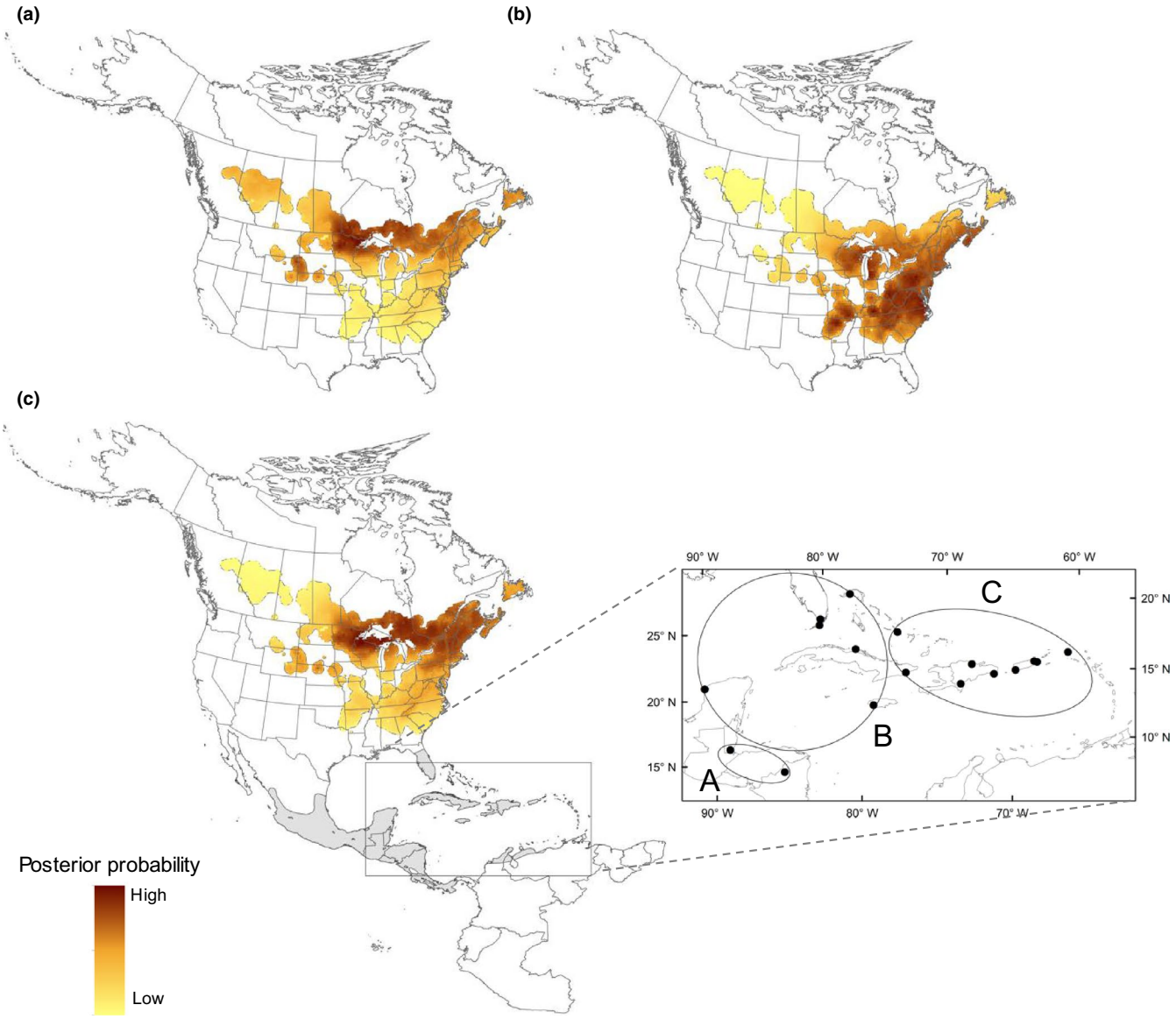

FIGURE 3 Bayesian posterior probability assignments to breeding range origin for Ovenbird calculated by combining stable-hydrogen isotope values in feathers (n = 214) from three Caribbean Basin non-breeding regions (A–C) and breeding ground abundance from the North American Breeding Bird Survey. The non-breeding range is shaded in gray. The inset shows the location of feather collection sites and the non-breeding regions identified with cluster analysis. Maps are displayed in Albers Equal Area projection [Colour figure can be viewed at wileyonlinelibrary.com]

Cluster analysis identified two non-breeding regions for Northern parula (*Setophaga americana*), indicating that birds overwintering closest together and farthest apart were likely to originate from similar breeding areas, whereas those at intermediate distances lacked common breeding distributions (Figure S1d): (A) Mexico, Cuba, Hispaniola, Puerto Rico, and St. Martin (−96‰ ± −100‰, −93‰; Figure 4 inset); and (B) Jamaica and Bahamas (−78‰ ± −87‰, −69‰). Parulas in region A had high assignment probabilities exclusively to northern breeding areas (Figure 4a). Birds wintering in Mexico had breeding distributions along the United States–Canada border west of the Great Lakes, while those in the eastern Caribbean originated primarily from northern New England. As with region A birds, those from region B had assignment probabilities to northern breeding areas, but also had moderately high assignments to the southern part of the breeding range (Figure 4b). For comparison, models done with isotopes only are presented in Figure S5.

The Prairie warbler (*Setophaga discolor*) non-breeding range clustered into three regions (Figure S1e): (A) Mexico, Jamaica, Puerto Rico, and St. Martin (−54‰ ± −55‰, −52‰; Figure 5 inset); (B) Cuba, Bahamas, and Hispaniola (−60‰ ± −57‰, −53‰); and (C) Florida (−44‰ ± −49‰, −38‰). Region A Prairie warblers had high assignment probabilities to the central and southern parts of the breeding range, including northern Alabama, South Carolina, and Virginia (Figure 5a). Birds overwintering in region B had assignment probabilities to central and northern portions of the range (Figure 5b). Assignment probabilities for this region overlapped with those from region A in the center of the breeding range, but were also further north into Pennsylvania, New Jersey, and southern New England. Region C warblers had high assignment probabilities to extreme southern end of the breeding distribution, primarily in Arkansas, Mississippi, and southern Alabama (Figure 5c). Isotope-only models are in Figure S6 for comparison.

The five species varied in the degree of correlation between the distance among non-breeding sites and the Bayesian posterior assignment probabilities among individuals at these sites (Table 1). Black-and-white warbler and American redstart had strong negative Pearson correlation coefficients, suggesting relatively strong migratory connectivity (Figure 6a,b). The correlation coefficient for Ovenbird was larger in comparison, consistent with moderate connectivity (Figure 6c). In contrast, Northern parula and Prairie warbler had Pearson coefficients closer to 0, indicating that birds from nearby non-breeding sites likely mix on the breeding grounds (Figure 6d,e).

4 | DISCUSSION

The proliferation of migratory connectivity research has been motivated by the growing recognition that a spatially explicit view of the full annual cycle is needed to extend knowledge about population ecology (Marra et al., 2019; Webster et al., 2002) and to optimize conservation decisions (Martin et al., 2007; Small-Lorenz et al., 2013). This trend has been spurred by advances in stable isotope assignment methods and by innovations in on-board tracking of animals (Stanley

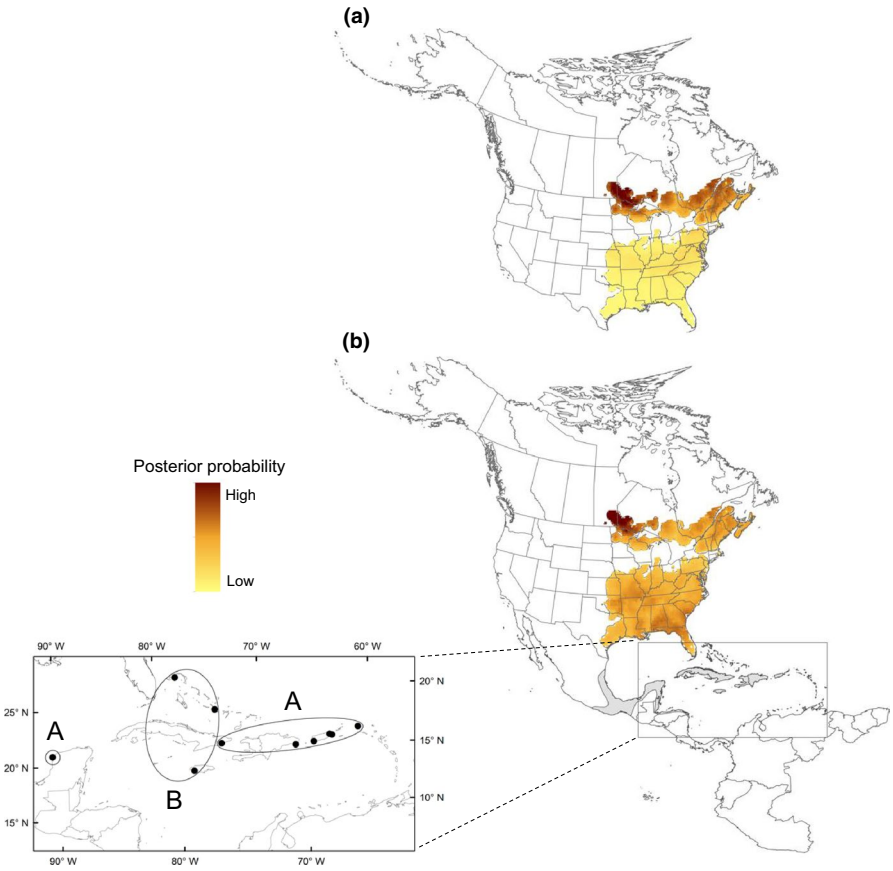

FIGURE 4 Bayesian posterior probability assignments to breeding range origin for Northern parula calculated by combining stable-hydrogen isotope values in feathers (n = 121) from two Caribbean Basin non-breeding regions (A,B) and breeding ground abundance from the North American Breeding Bird Survey. The non-breeding range is shaded in gray. The inset shows the location of feather collection sites and the non-breeding regions identified with cluster analysis. Maps are displayed in Albers Equal Area projection [Colour figure can be viewed at wileyonlinelibrary.com]

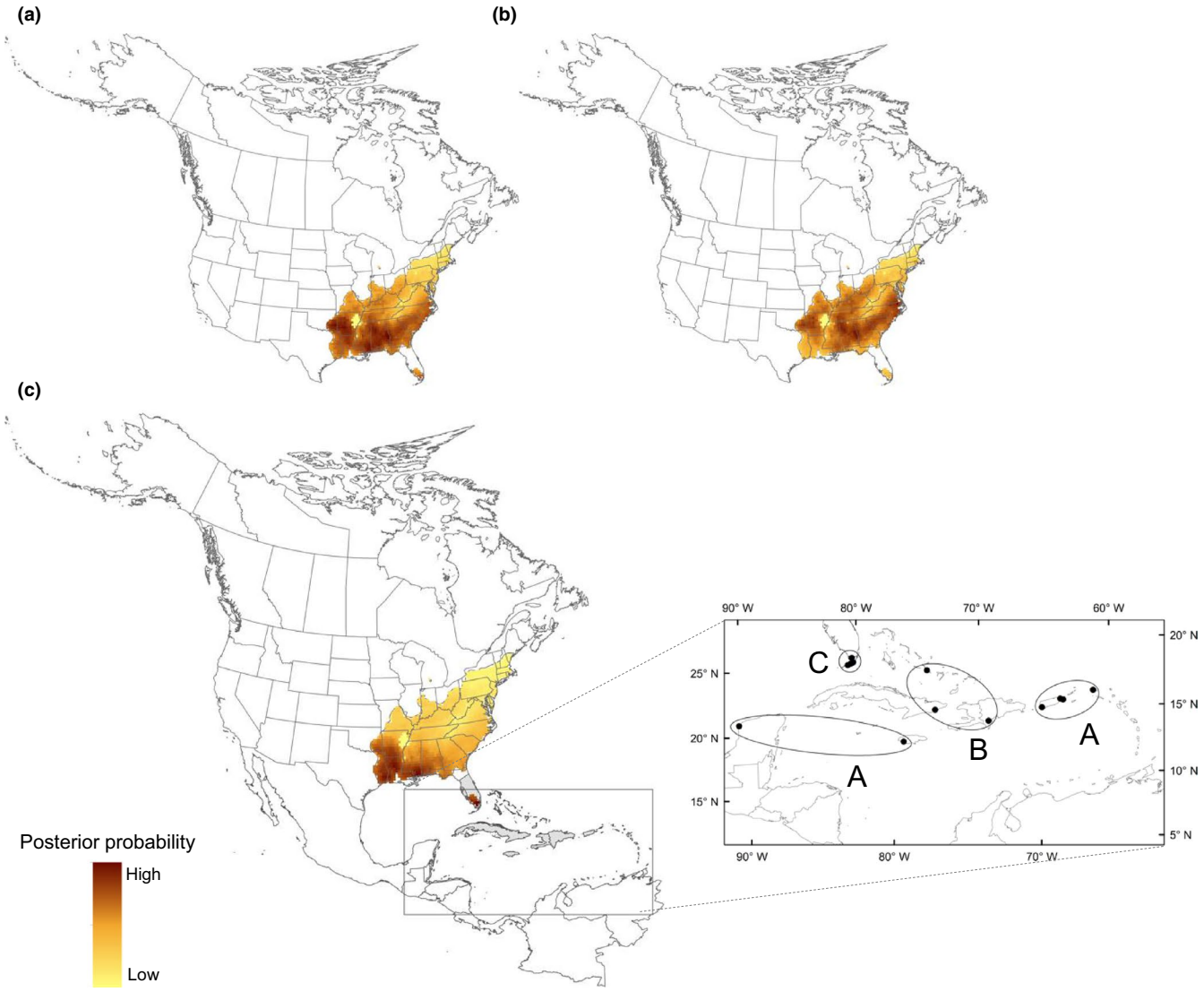

FIGURE 5 Bayesian posterior probability assignments to breeding range origin for Prairie warbler calculated by combining stable-hydrogen isotope values in feathers (n = 138) from three Caribbean Basin non-breeding regions (A-C) and breeding ground abundance from the North American Breeding Bird Survey. The non-breeding range is shaded in gray. The inset shows the location of feather collection sites and the non-breeding regions identified with cluster analysis. Maps are displayed in Albers Equal Area projection [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2015; Stutchbury et al., 2009). Despite these developments, range-wide assessment of migratory connectivity remains uncommon (Block et al., 2011; Flockhart et al., 2013; Norris et al., 2006). By combining stable-hydrogen isotope values sampled in the non-breeding period with patterns of breeding season abundance generated by citizen scientists through the North American BBS, we provide among the most thorough connectivity assessment to date for Neotropical-Nearctic migratory birds. Our estimates of geographic origin indicate few generalities among species, suggesting that shared annual range geography alone does not predict migratory connectivity.

Combining citizen science-derived information on breeding abundance from the BBS with stable-hydrogen isotopes enabled us to better localize assignments to specific parts of the breeding distribution. Because we weighted isotopes and BBS abundance based on previous analyses of known origin birds of the same species, the resulting assignments likely minimized both assignment error and assignment area (Rushing et al., 2017). Despite the potential benefit of incorporating abundance into assignments, there are also limitations. Abundance models may obscure patterns of connectivity by lowering assignment probability when true origins are to areas of low breeding abundance. Future connectivity research that calculates assignments by combining isotopes and abundance should therefore first evaluate abundance models with known origin birds and compare them with isotope-only models (Rushing et al., 2017). Other data sources have shown promise when incorporated with isotopes into assignments, including morphological traits (Rushing et al., 2014), molecular genetics (Kelly et al., 2005; Ruegg et al., 2014), and hunting pressure (Hobson et al., 2009). These approaches

offer similar advantages over assignments that rely on stable isotopes alone, where tissues receive equal assignment probabilities to regions of high and low breeding abundance or other indicators of breeding distribution.

TABLE 1 Pearson correlation coefficients, their 95% CI, and *p-values* from Mantel matrix randomization tests used to assess significance of coefficients for five species of Neotropical migratory birds. Coefficients estimate the strength of migratory connectivity and measure the correlation between pairwise distances among non-breeding sites and pairwise correlations among assignment probabilities at these sites

Species	Coefficient	95% CI	Mantel *p-values*
Black-and-white warbler	−0.73	−0.81, −0.63	<0.001
American redstart	−0.70	−0.79, −0.58	<0.001
Ovenbird	−0.44	−0.57, −0.30	<0.001
Northern parula	−0.02	−0.29, 0.24	0.250
Prairie warbler	0.06	−0.13, 0.23	0.378

The five species varied markedly in the strength of their migratory connectivity. Although sample size prevented a formal analysis, species with larger non-breeding ranges exhibited stronger patterns of connectivity compared to those with smaller ranges. Black-and white warbler and American redstart, which have the most extensive non-breeding range, showed strong migratory connectivity, and Ovenbird, with a comparatively smaller non-breeding distribution, had moderate connectivity. In contrast, Northern parula and Prairie warbler, which had the smallest non-breeding ranges, displayed low or diffuse connectivity. These patterns suggest that higher non-breeding population spread may promote stronger patterns of migratory connectivity (Finch et al., 2017). Unlike migratory connectivity estimates for species directly tracked with geolocators or satellite tags that rely on distance matrices of individual breeding and non-breeding sites (Ambrosini et al., 2009; Sarà et al., 2019), our measure of breeding distance was the correlation among breeding ground assignment probabilities among individuals at non-breeding sites. Thus, our estimates of connectivity strength may be particular to our assignment model, and other methods of assessing connectivity

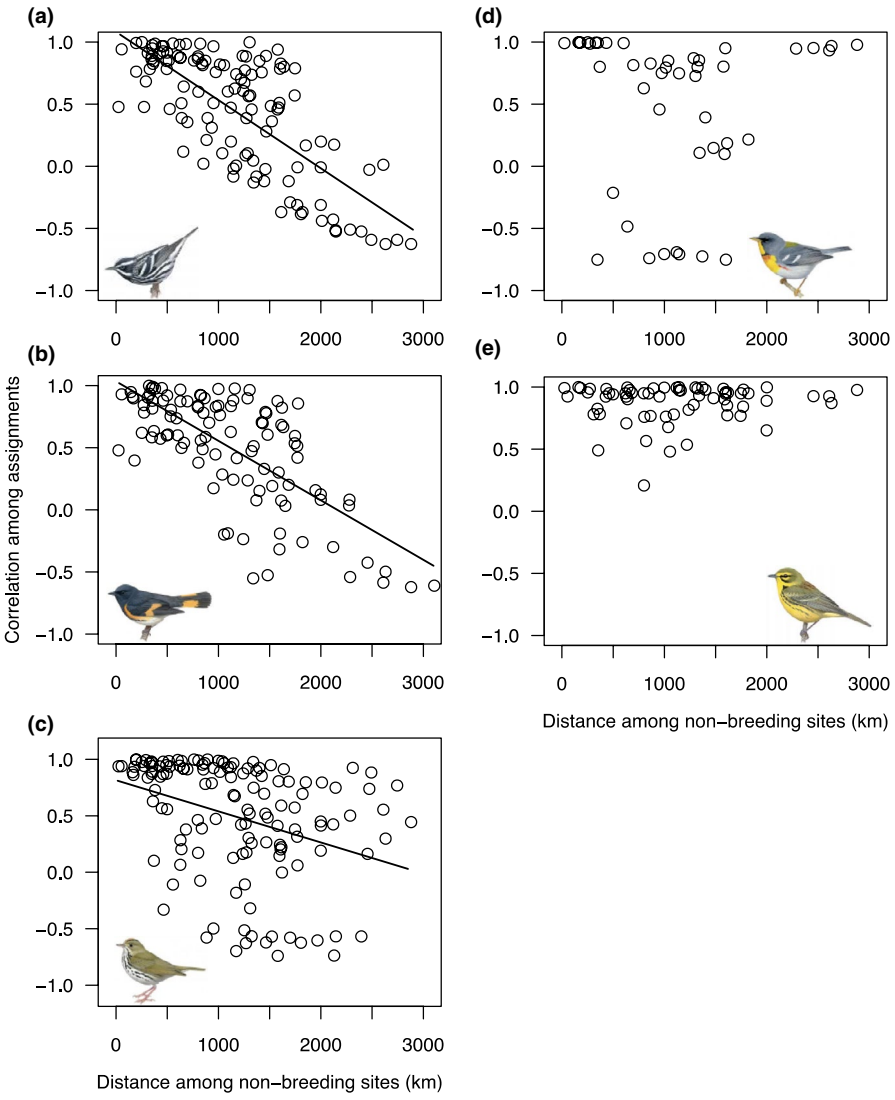

FIGURE 6 Correlations between the pairwise distance among non-breeding sites and pairwise correlations among assignment probabilities at these sites for (a) Black-and-white warbler (n = 140), (b) American redstart (n = 213), (c) Ovenbird (n = 214), (d) Northern parula (n = 121), and (e) Prairie warbler (n = 138) [Colour figure can be viewed at wileyonlinelibrary.com]

of these species may yield different results. Nonetheless, our approach offers future research with stable isotopes a new method for estimating migratory connectivity.

Posterior assignment probabilities based on cluster analysis yielded reasonably well-resolved connectivity patterns, with unique breeding associations that differed among species. The interspecific variation in assignments was especially clear when considering linkages between specific non-breeding and breeding populations. For example, New England had a high probability of receiving breeding birds from across the majority of non-breeding sites that we sampled. Black-and-white warbler that migrated to New England had the highest probability of overwintering in Cuba, Bahamas, and Florida. In contrast, Redstarts with the highest probability of breeding in this region overwintered further south and east from an area extending from Jamaica to Puerto Rico. Finally, Northern parula with a high probability of New England breeding origins spent the non-breeding season as far west as Mexico. These contrasting patterns of migratory connectivity suggest that shared non-breeding geography in the Caribbean does predict common breeding ground origin.

Despite the interspecific variation in assignments, there were two emergent patterns in assignments when considering range extent and size. First, three species had breeding ranges that spanned the United States-Canada continental divide: Black-and-white warbler, American redstart, and Ovenbird. Feathers for these species sampled in the western extent of the non-breeding range in Belize and Nicaragua had high assignment probabilities to northwestern margins of the breeding range. Second, birds that overwintered in St. Martin and Dominica at the eastern edge of the non-breeding range had high assignment probabilities to the southeastern United States. Thus, large difference in non-breeding longitude appears to be the primary geographic factor separating western and eastern breeding populations, a pattern found in other species through analyses of stable isotopes and neutral genetic markers (Clegg et al., 2003; Kelly et al., 2005).

Neotropical-Nearctic migratory birds spend the majority of the year in the tropics, but management decisions usually occur on breeding grounds. Our results have several important implications for the conservation of migratory birds in both the breeding and non-breeding period. Non-breeding sites within approximately 1000 km of one another had the greatest probability of having wintering birds that bred in the same geographic area in North America. This result means that conservation investments targeted for any one species for range wide conservation are best distributed at distances in excess of 1000 km. However, conservation investment aimed at securing non-breeding habitat for species breeding in a particular region cannot simply choose to protect one non-breeding region.

Spatial conservation planning for migratory species is in its infancy relative to other systems, but there is growing interest in closing this gap. Efforts must include biogeographic considerations from throughout the annual cycle and migratory connectivity for populations of conservation interest. Stable isotope estimates of connectivity such as those we present here should be part of this effort, and the growing collections of feather archives and existing collections of specimens in museums will permit connectivity assessments for numerous species without the need for additional fieldwork and the high cost of transmitter technology (Hobson et al., 2014). Our findings should be thought of as a working hypotheses for how populations of these species are connected throughout the annual cycle. An interesting test of this hypothesis would be to deploy geolocators on individuals of these species across a gradient of breeding ground assignment probabilities. For example, geolocators deployed on Ovenbirds in northern New England should have approximately a 75% probability of migrating to the Hispaniola or Puerto Rico and a 25% probability of migrating to the Florida, Bahamas, Cuba, and Jamaica. Such explicit tests would lend predictive rigor to the study of migratory connectivity and are likely to advance our knowledge of these patterns.

Refining our understanding of the migratory connectivity of migratory animals is essential to their conservation. This information, combined with demographic data, will allow for the determination of when and where in the annual cycle species and populations are limited (Studds et al., 2017). Finally, assessing the strength of migratory connectivity for species with similar annual ranges may also reveal insights about fundamental biology such as the evolution of life history strategies (Marra et al., 2019) and responses to global change (Small-Lorenz et al., 2013).

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DATA AVAILABILITY STATEMENT

Isotope data from this research is available on the Smithsonian Migratory Bird Center website data depository: https://nationalzoo.si.edu/migratory-birds/data. R code for assignments is available from https://github.com/studdsc/migratory.connectivity.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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