The evolution of wood warbler flight calls: Species with similar migrations produce acoustically similar calls

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Diverse animal species engage in long-distance migrations. Many migrants travel in groups, and communication within these groups may be important to survival and successful migration. We examined migration and communication in 36 species of wood warblers (Parulidae), songbirds that breed in North America and migrate in mixed-species flocks to their wintering grounds. During migration, wood warblers produce short vocalizations called "flight calls." The function of flight calls and the patterns of acoustic similarity between species are poorly understood. We investigated whether acoustic similarity of flight calls of different species of warbler reflects the similarity in their migratory journeys or their phylogenetic relatedness. We found that phylogeny, similarity in breeding latitude, and overlap in the timing of migration predict acoustic flight call similarity across warbler species. Further, we found that phylogeny, similarity in migration distance, and overlap in wintering range predict acoustic flight call similarity in a subset of 12 species with highly similar calls, although this analysis has a small sample size. We conclude that migratory similarity may be an important force driving the evolution of acoustically similar calls in wood warblers, in addition to phylogenetic relatedness. Acoustic convergence in these species may facilitate communication between individuals with similar migrations.

KEY WORDS: Acoustic convergence, flight calls, migration, wood warblers.

Although most animal communication occurs between members of the same species (Marler 1957; Jarvis 2004; Tobias and Seddon 2009), communication does occur across species boundaries (Tobias and Seddon 2009; May-Collado 2010). Interspecific communication has been documented in mixed-species foraging flocks of songbirds (Grava et al. 2012), different species of tamarin monkeys (Windfelder 2001), mixed-species foraging aggregations of dolphins (May-Collado 2010), brood-parasitic African birds (Edwards et al. 2012), and the well-known example of African honeyguides and mongooses (Isack and Reyer 1989). Although most examples of interspecific communication are from sedentary or resident species, interspecific communication may also occur among mobile, migratory species.

Many animals migrate in mixed-species groups (Laursen 1978; Boland 1990), wherein interspecific communication might help animals to avoid predators, or to navigate over dangerous or unfamiliar terrain (Somveille et al. 2020). In spite of these potential fitness benefits, few studies have explored interspecific communication between animals during migration. The evolution of mutually intelligible signals, recognizable by more than one migratory species, may be important for mixed-species flocks of migrants. For example, if heterospecific animals migrate between similar wintering and breeding grounds, they may benefit by traveling in mixed-species flocks during migration (Winger et al. 2019), and selection may favor the evolution of similar acoustic signals across species. If animals exchange acoustically similar calls with other animals participating in similar migratory journeys, vocalizations may serve as acoustic signals used in collective decision making (Winger et al. 2019), which may be especially important when animals travel with individuals sharing similar migratory destinations.

Communication during migration may be important for songbirds, such as wood warblers (Parulidae). Wood warblers are
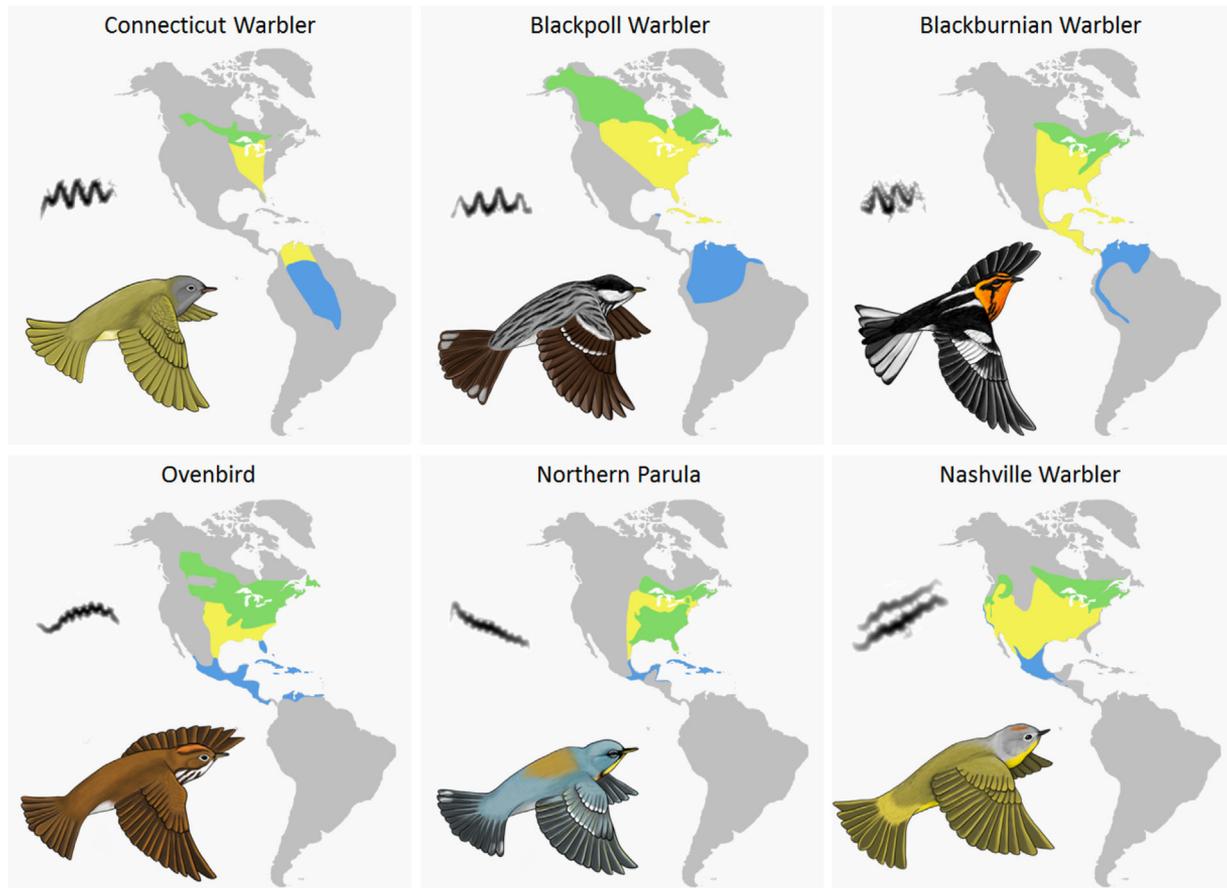
Figure 1. Range maps and flight call spectrograms for six species of migratory wood warblers. The top three species have similar long-distance migrations and acoustically similar flight calls, whereas the bottom three species have more varied migrations and dissimilar flight calls.

a biodiverse group of 119 species (Lovette et al. 2010; Gill et al. 2019), at least 47 of which migrate between temperate breeding grounds in North America and south-temperate or tropical wintering grounds in southern North America, Central America, or South America (Evans and O'Brien 2002; Birdlife International 2019). The 47 migratory species routinely produce short, structurally simple calls known as "flight calls" (Evans and Mellinger 1999; Farnsworth 2005). Wood warblers migrate nocturnally (Hamilton 1962; Alerstam et al. 2009; Winger et al. 2019) and produce flight calls when they travel in mixed species flocks composed of many migrants (Farnsworth 2005). Flight calls show a high degree of acoustic similarity across species (Evans and O'Brien 2002; Landsborough et al. 2019) but patterns of acoustic similarity and the forces responsible for this similarity are largely unknown. One prior study found that species with higher frequency calls were associated with taller forests containing open canopies but found no relationship between acoustic similarity and other ecological traits (Farnsworth and Lovette 2008).

Although flight calls are well described, their function is poorly understood. In wood warblers, flight calls are thought to stimulate migratory restlessness (Ball 1952; Hamilton 1962) and to facilitate contact between flock-mates (Evans and O'Brien 2002; Farnsworth 2005; Morris et al. 2016; Winger et al. 2019), but there is limited direct behavioral evidence for these functions across multiple species. If wood warbler flight calls facilitate contact, acoustically similar flight calls may be advantageous for warbler species that share migratory routes. The particular flight calls of 12 warbler species in the genera *Setophaga, Oporornis, Parkesia*, and *Helmitheros* are so similar that they are routinely lumped together in a common bioacoustic category termed the "Zeep complex" (Smith et al. 2014; Landsborough et al. 2019). One unifying feature between the species within the "Zeep complex" is that many species share similar migration patterns: long-distance journeys, fast migration transit times, and South American wintering grounds (e.g., Fig. 1, Rodewald 2015). Although there is a range of migratory behaviors and migration routes within the Zeep complex, many species have

very similar migrations. Similarly, a different group of wood warbler species (the "Upsweep complex"), appears to share superficially similar calls and potentially similar migration patterns (Evans and O'Brien 2002; Smith et al. 2014). Other migratory species in wood warblers appear to have dissimilar calls and dissimilar migration patterns (e.g., Fig. 1). Whether similarity in migrations is connected to similarity in flight calls across species has been investigated in only one prior study which found no significant relationship (Farnsworth and Lovette 2008). However, newly available data on bird distributions make investigating these patterns a worthwhile endeavor.

Under what situations would we expect birds to evolve acoustically similar flight calls? If multiple species migrating at the same time experience higher survival rates by remaining within flocks that follow similar migratory routes, acoustically similar flight calls may be under selection to signal similarity in migratory routes and thereby promote group cohesion. Under this coevolutionary scenario, flight calls may have initially been similar within single-species flocks as they traveled between the same breeding and wintering grounds. Selection may have favored individuals who produced calls more similar to other species if they tended to gather in larger mixed species flocks. With shared migratory patterns, species with similar flight calls may have benefitted from reduced disorientation during migration or increased the chance of finding high quality stop-over sites or avoiding predators. Thus, individuals of different species with similar calls may have experienced higher survival during migration than individuals with dissimilar calls.

In this study, we examined relationships between the acoustic similarity of wood warbler flight calls and the similarity of their migratory journeys. We investigated the hypothesis that similarity in migration—including the overlap of breeding ranges, migration ranges, wintering ranges, distances between breeding ranges, distance between wintering ranges, lengths of migratory journeys, and timing of migration—is associated with acoustic similarity in warbler flight calls. We refer to this idea as the "Migration Similarity Hypothesis." Our primary prediction is that warblers with geographically similar breeding ranges, migration ranges, wintering ranges, breeding latitudes, wintering latitudes, lengths of migratory journeys, and timing of migration will produce flight calls that are acoustically similar. We also test the hypothesis that patterns of acoustic flight call similarity reflect species phylogenetic relatedness, and that associations between call similarity and migration similarity are best explained by common ancestry. We used this phylogenetic hypothesis as a null hypothesis, and we anticipated that patterns of acoustic flight call similarity across species would not be explained by phylogeny alone.

Methods

STUDY SPECIES

We studied 36 species of migratory wood warbler (listed in Table S1). We used this set of 36 species to examine the acoustic properties of flight calls and migration overlap because the flight calls of these 36 species are well known, and these 36 species represent migratory wood warbler species with a wide variety of migration routes. We chose calls for study that were available in the most well studied flight call database (Evans and O'Brien 2002). We did not include the 11 additional migratory North American wood warblers because their calls are poorly known and there were limited flight call recordings available for these species. Many of these species are western wood warblers; eastern wood warbler flight calls have been more extensively described (Table S1).

MIGRATION SIMILARITY ANALYSES: GEOGRAPHIC OVERLAP

We calculated similarity in geographic overlap of the breeding, migratory, and wintering ranges of the 36 species of migratory wood warblers, using data from the NatureServe repository (Birdlife International 2019). We defined migration range as the range a species can be found in only during migration, but excluding breeding and wintering ranges (although it is noteworthy that many species migrate throughout areas where some individuals breed or winter). We calculated geographic overlap (in km^2) for each pair of species of warblers, using a custom R script modified from prior research (Simpson et al. 2021), which utilizes the RGEOS and RGDAL packages (Bivand et al. 2015; Bivand and Rundel 2016). We compared the percent overlap of each pair of species in three different comparisons: (1) pairwise breeding range overlap, (2) pairwise migration range overlap, and (3) pairwise wintering range overlap (see Supplemental Figure 1). Pairwise overlap was calculated by measuring the number of square kilometers that the ranges of each pair of species overlapped, and then dividing by the area (in km^2) of the total range of both species in the pair (overlap was calculated as a percentage, to facilitate comparisons between species with broad ranges and species with restricted ranges). Our overlap data were organized as three matrices of pairwise overlaps between the breeding ranges, migration ranges, and wintering ranges of all 36 species.

We compiled NatureServe data for wood warbler total migration distance, centroid of breeding range, and centroid of wintering range (Simpson et al. 2015). We manipulated these data using the Geographic Distance Matrix Generator (version 1.2.3) to convert decimal degrees to total distances, in kilometers, between the centroids for each pair of species (Simpson et al. 2015). We generated a matrix of pairwise migration distance comparisons by subtracting the distance migrated by each species pair. We used the centroids of each species' breeding and wintering

ranges to examine the differences in breeding and wintering latitudes between each species (Fig. S1). We did this by subtracting the centroid of pairs of species' breeding and wintering range, and then generating a matrix for all pairwise combinations. To eliminate negative similarity values, we calculated the absolute value of the difference in breeding latitudes and wintering latitudes.

## MIGRATION SIMILARITY ANALYSES: TIMING OF MIGRATION

In addition to the aforementioned six features of the migration route of 36 species, we also conducted an analysis of migration timing (including both geographic and temporal data), to examine whether temporal overlap in migration might predict acoustic flight call similarity. Although many species overlap geographically in mid-May, there are differences in the mean peak abundance of species. Abundance data show that many species with simultaneous migration have slightly different mean peak abundances (Binford 2006) that influences the composition of warbler flocks. To measure variation in the timing of migration, we used the ebirdst package (Auer et al. 2020) in R to download geographic raster files of weekly relative abundance for 35 species of wood warblers across the Americas. We were unable to include the 36th species in our analysis, the Connecticut Warbler, due to problematic raster files and excluded this species from the analysis. We subsetted the geographic raster files for all 35 species to only the weeks in April and May; these months correspond with the bulk of warbler migration in the Americas (Auer et al. 2020). We used these data to evaluate geographic overlap for each species pair during each week of migration in April and May. We summarized these weekly overlap values as one mean monthly overlap value for each species pair per month. This produced a value—which we refer to as "mean migration overlap"—that reflected the mean overlap of each species pair across the month of April and across the month of May. We did not focus on fall migration in this paper because many species have longer migratory periods in the fall where more species overlap for longer periods of time; we chose to focus on spring migration, because each species' shorter migratory period in the spring allowed us to estimate a migratory period during a narrower time interval. We used mean monthly overlap as an indicator of the similarity in migration timing for pairs of species in both April and May. We calculated pairwise timing overlap for each month as a separate matrix showing all possible species pairwise overlaps.

Altogether, our analyses of migration relied on eight pairwise species measurements: (1) overlap in breeding range; (2) overlap in migration range; (3) overlap in wintering range; (4) similarity in breeding latitude, (5) similarity in wintering latitude, (6) similarity in total migration distance, (7) mean April migration timing overlap, and (8) mean May migration timing overlap.

ACOUSTIC SIMILARITY ANALYSES

We examined acoustic properties of warbler flight calls using flight call libraries from Evans and O'Brien (2002) and recordings from previous studies (i.e., recordings from Sanders and Mennill 2014b; Landsborough et al. 2019). In total, we measured the spectrographic properties of 864 warbler flight calls (mean: 5.0 ± 0.6 per species; range: 3–7). We measured calls in AviSoft SAS-Lab Pro (R. Specht 2002). We used Avisoft's automatic measurement tool, thereby minimizing human subjectivity in measurements, with an amplitude threshold of -20 dB relative to peak amplitude. We measured the following ten features for each call: call duration (ms), time delay from the start of the call to the point of peak amplitude (ms), maximum frequency (Hz), peak frequency (Hz), minimum frequency (Hz), mean frequency bandwidth (Hz), mean peak frequency (Hz), time delay from the start of the call to the point of peak frequency (msec), mean slope (Hz; see description below), and total number of harmonics (Fig. 2). To calculate the mean frequency bandwidth and mean peak frequency, we measured those features across the duration of the call at 0.01 second intervals; given that most calls are 60–200 ms long, this resulted in 6–20 measurements for each call, from which we calculated the average. To calculate mean slope, we sampled the call at 0.01 s intervals and measured peak frequency at each point; we calculated the frequency difference between each subsequent pair of points, and then calculated the average as a measure of change in slope across the call. To calculate total number of harmonics in an unbiased way, we used the pulse wave analysis function in AviSoft to select and measure frequency peaks of each flight call; we set the hysteresis parameter detection for frequency peaks at 10 dB less than the maximum and measured the detected peaks at this hysteresis level.

Based on the 10 acoustic measurements for all calls, we performed a Principal Components Analysis (PCA) in JMP (version 14; SAS Institute 2019) to produce a smaller number of multivariate measures that summarize the measured acoustic features of calls. The PCA gave rise to four unrotated principal components with Eigenvalues greater than 1.0. The first principal component (PC1) explained 31.5% of the variance and received strong loadings (i.e., loadings greater than a threshold of 0.4) from call duration, time to peak amplitude, mean bandwidth, time to peak frequency, mean slope, and number of harmonics. The second principal component (PC2) explained 16.1% of the variance and received strong loadings from min frequency and mean peak frequency. The third principal component (PC3) explained 12.4% of the variance in the data and received strong loadings from call duration, max frequency, peak frequency, and number of harmonics. The fourth principal component (PC4) explained 9.5% of the variance in the data and received strong loadings from call duration, and mean bandwidth (Table S2).
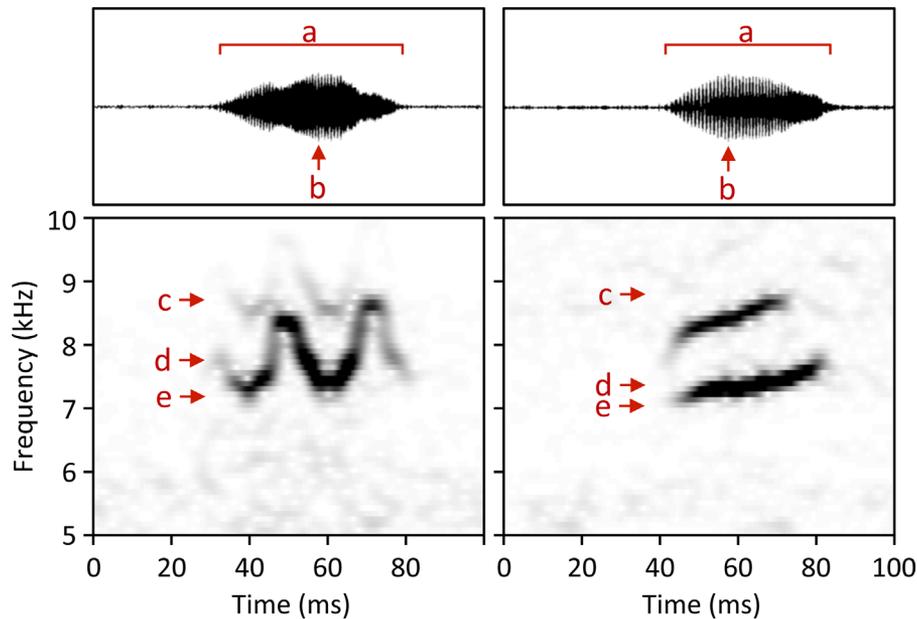
Figure 2. Recordings of flight calls of two warbler species shown as a waveform (top) and sound spectrogram (bottom), showing spectrotemporal features measured in this study. For each call we measured (A) call duration, (B) time to peak amplitude, (C) maximum frequency, (D) peak frequency, and (E) minimum frequency, as well as five additional measurements that are not shown: mean frequency bandwidth, mean peak frequency, time to point of peak frequency, mean slope, and number of harmonics.

EUCLIDEAN DISTANCE CALCULATIONS

We performed a hierarchical cluster analysis in JMP (version 14; SAS Institute, Cary, NC) on the four principal components summarizing acoustic features, with the centroid of each warbler species as an object identity. This analysis clustered the centroids of each warbler species position in *n*-dimensional space using a maximum-likelihood algorithm.

We constructed an index of call similarity for each species by plotting the Euclidean distance between each species' flight call in n-dimensional space (Sosa-Lopez et al. 2016; Fig. 3). We used the same approach as for migration similarity to export Euclidean distances as a pairwise matrix with the same species order. Cluster analysis of the acoustic measurements of the flight calls of 36 species of wood warblers revealed six clusters of species with acoustically similar calls (Fig. 3). Nine of 12 members of the "Zeep complex" fell into one cluster, while the remaining three "Zeep complex" species (Hooded Warbler, Kentucky Warbler, Northern Waterthrush) clustered with a second group of warblers that included Common Yellowthroat and Chestnut-sided Warbler; members of the "Upsweep complex" (Black-throated Blue Warbler, Black-throated Green Warbler, Blue-winged Warbler, Golden-winged Warbler, Mourning Warbler, Orange-crowned Warbler, Nashville Warbler, Prothonotary Warbler, Swainson's Warbler, Tennessee Warbler) fell within two clusters (Fig. 3).

PHYLOGENETIC DISTANCE ANALYSES

We calculated phylogenetic distance from a published time calibrated ultrametric wood warbler phylogeny comprising 107 migratory and non-migratory species (Lovette et al. 2010). The wood warbler tree file was pruned in *R* using the *Ape* package (Paradis et al. 2019) to remove all but the 36 migratory species under investigation (Fig. 4). We extracted the branch lengths of the pruned phylogeny and converted this to a matrix of pairwise patristic distances between branch lengths for each species in the analysis.

We restricted analyses to 36 species of wood warblers because these were the species with extensive call libraries suitable for acoustic analyses. When further species are studied, it will be useful to expand analyses to more species. Even though we used a subset of the total warbler phylogeny, our analyses are robust for detecting the influence of phylogeny on acoustic flight call structure, given that we followed the methods employed by multiple prior studies using incomplete phylogenies to test for effects of phylogenetic relatedness (Farnsworth and Lovette 2007; Farnsworth and Lovette 2008; Miller et al. 2019).
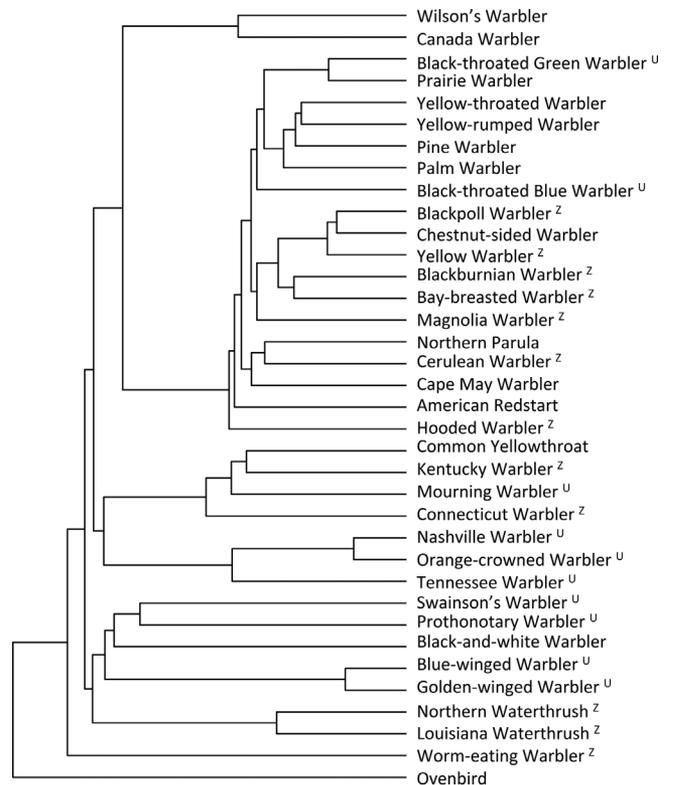
STATISTICAL ANALYSIS AND MATRIX MODELING

We used Multiple Mantel Tests to test whether species pairwise breeding range overlap, migration range overlap, wintering range overlap, distance between breeding latitudes, distance between
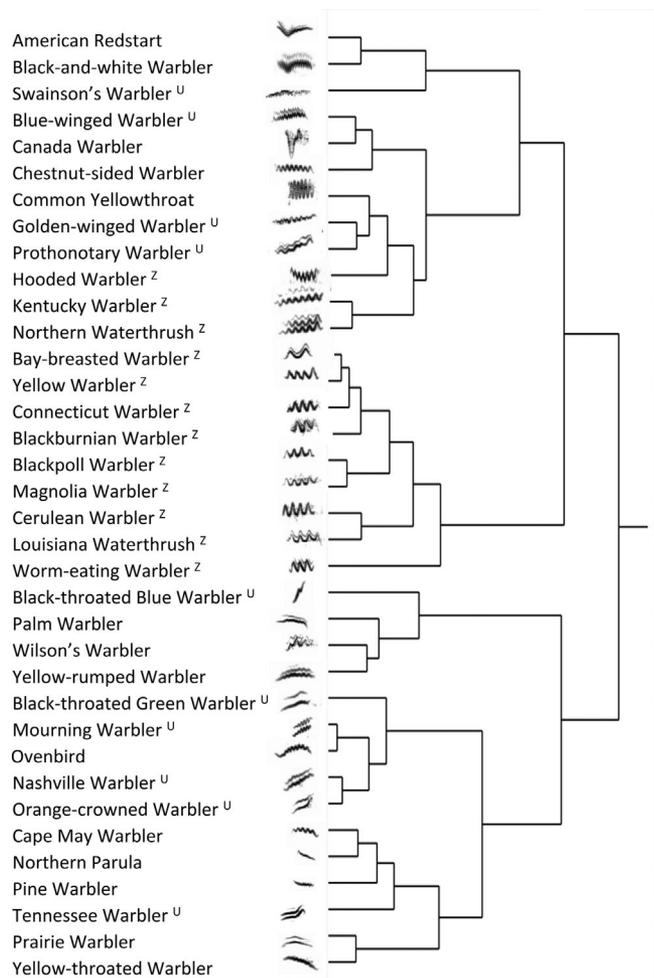
Figure 3. Dendrogram clustering wood warbler (*Parulidae*) flight calls based on a Hierarchical Cluster Analysis using acoustic features of their flight calls. Warbler species were clustered based on the Euclidean distances between centroid positions of 36 wood warbler flight calls. Species with "Z" superscript denote members of the "Zeep Complex" and species with "U" superscript denote members of the 'Upsweep Complex.

Figure 4. Phylogeny of 36 species of wood warblers (Parulidae) used in study, based on patristic distances in Lovette et al. 2010. Species with "Z" superscript denote members of the "Zeep Complex" and species with "U" superscript denote members of the "Upsweep Complex.

wintering latitudes, total migration distance, and phylogeny predict flight call similarity (Mantel 1967; Miller et al. 2019). Mantel tests are commonly used in ecology to assess whether matrices of ecological or genetic data are correlated with response variables (Miller et al. 2019). Multiple Mantel tests were modified from Mantel's original matrix regression technique (Mantel 1967) to simultaneously control for the contribution of multiple predictor variables (Revell 2012). We used multiple Mantel tests to assess whether phylogenetic relatedness and similarity in all six measures of geographic overlap in pairs of wood warbler species were correlated with acoustic flight call similarity. We chose to use Mantel tests as opposed to as an alternative approach, such as Phylogenetic least squares regression (PGLS), because Mantel tests were most appropriate for the similarity

matrices that governed the structure of our data. For example, PGLS would not have been appropriate for our similarity value data which represented a comparison between two species rather than a value for each species independently. All Mantel tests were conducted within the Phytools (Revell 2012) R package. Multiple Mantel tests used to compare multiple predictors were equivalent to partial Mantel correlations (Mantel 1967). For each test we ran 1000 permutations to generate *P*-values from the raw matrix data.

We performed the same analysis techniques to see if breeding range overlap, migration range overlap, wintering range overlap, breeding latitude, wintering latitude, and total migration distance predict acoustic flight call similarity in two subsets of the 36 warbler species (Table S1). Subsets included separate analyses of 12 acoustically similar species commonly referred to as the "Zeep complex" (Table S1) and 10 species of acoustically similar species commonly referred to as the "Upsweep complex" (Smith et al. 2014). The justification for sub-phylogeny analyses was that these groups of flight calls are so acoustically similar that they are often grouped together into *a priori* bioacoustic categories below the phylogeny level (e.g., Smith et al. 2014; Landsborough et al. 2019). We wanted to see if the traits used in the broader study

Table 1. Results from Multiple Mantel Matrix Regression with phylogeny and spatial migration overlap as predictors of acoustic flight call similarity in 36 species of migratory wood warbler (*Parulidae*).

Mantel test			
Predictor	Estimate	*t*	*P*-value
(intercept)	4.2	4.08	0.99
Phylogeny	0.05	5.6	0.008
Breeding Overlap	1.9	2.8	0.23
Migration Overlap	2.8	3.7	0.28
Winter Overlap	0.6	0.9	0.72
Breeding Latitude	0.1	2.9	0.04
Winter Latitude	0.05	1.4	0.53
Migration Distance	0.0002	2.08	0.34
Multiple R^2	0.079		
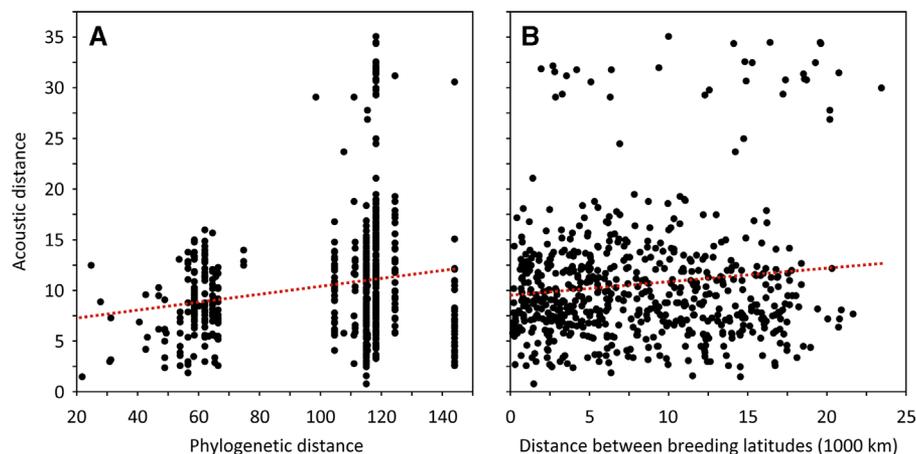
Figure 5. Scatterplots showing (A) acoustic distance of wood warbler flight calls for 36 species of wood warblers and phylogenetic distance, and (B) relationship between acoustic distance of wood warbler flight calls and distance between breeding latitude (degrees). Acoustic distances represent the Euclidean distances between the mean position of pairs of warbler flight calls. Phylogenetic distances represent the patristic distance between branch lengths of species pairs in the comprehensive wood warbler phylogeny. Distance between breeding latitudes represents the degrees latitude between pairs of warbler breeding, measured at the centroid position of each species range. Red line shows line-of-best-fit.

predicted acoustically similar calls within these call groups below the full phylogeny.

Finally, we conducted a third set of analyses testing whether pairwise species temporal overlap in migration predicts flight call similarity. We used the same Mantel tests to see if pairwise species mean migration overlap for April and May predicts flight call acoustic similarity in 35 species of wood warblers (see above for why there is a difference in sample size). We also included phylogeny as a predictor in this model.

Results
MIGRATION SIMILARITY VERSUS ACOUSTIC SIMILARITY OF FLIGHT CALLS
Across 36 species of migratory wood warblers, phylogeny and breeding latitude were correlated with acoustic similarity in flight calls (Multiple Mantel test; Table 1). Phylogeny predicted acoustic similarity in flight calls: species that were closely related produced more similar flight calls (Multiple Mantel test; $t = 5.6$; $P = 0.008$ $n = 36$; Table 1; Fig. 5A). Similarity in breeding latitude also predicted acoustic similarity in flight calls, even after accounting for the effect of phylogeny: species with similar breeding latitudes produced more similar flight calls (Multiple Mantel test; $t = 2.9$, $P = 0.04$; Fig. 5B). Migration distance, similarity in winter latitude, and three measures of migratory overlap (breeding range overlap, migration overlap, and winter range overlap) did not show a relationship with acoustic similarity in flight calls across 36 species of wood warblers (Multiple Mantel test; Table 1).

Within the "Zeep complex," a subset of 12 species with highly similar flight calls, phylogeny, and two migratory predictors were correlated with acoustic similarity (Multiple

Table 2. Results from Multiple Mantel Matrix Regression with phylogeny and spatial migration overlap as predictors of acoustic flight call similarity in 12 species of migratory wood warbler (*Parulidae*) referred to as the "Zeep complex".

Mantel Test			
Predictor	Estimate	*t*	*P*-value
(intercept)	0.03	0.07	0.99
Phylogeny	0.01	2.9	0.04
Breeding Overlap	0.009	0.03	0.92
Migration Overlap	0.5	1.1	0.46
Winter Overlap	0.9	2.2	0.04
Breeding Latitude	0.03	1.6	0.10
Winter Latitude	2.5	0.3	0.23
Migration Distance	0.0001	2.1	0.02
Multiple R^2	0.11		

Table 3. Results from Multiple Mantel Matrix Regression with phylogeny and spatial migration overlap as predictors of acoustic flight call similarity in 10 species of migratory wood warbler (*Parulidae*) termed the "Upsweep complex".

Mantel Test			
Predictor	Estimate	*t*	*P*-value
Intercept	0.4	0.4	0.99
Phylogeny	0.01	1.7	0.04
Breeding Overlap	0.8	1.2	0.40
Migration Overlap	0.6	1.1	0.53
Winter Overlap	0.3	0.4	0.71
Breeding Latitude	0.03	0.9	0.45
Winter Latitude	0.02	0.8	0.46
Migration Distance	0.00005	0.7	0.65
Multiple R^2	0.27		

Mantel test; Table 2). Phylogeny predicted acoustic flight call similarity: species that were closely related produced more similar flight calls ($t = 2.9$; $P = 0.04$; Table 2). Similarity in total distance migrated between breeding and wintering grounds predicted acoustic flight call similarity: species with similar lengths of migration had more similar flight calls (Multiple Mantel test; $t = 2.1$; $P = 0.02$, $n = 36$; Table 2). Wintering range overlap also predicted acoustic flight call similarity: species with overlapping wintering ranges produced more similar flight calls (Multiple Mantel test; $t = 2.3$; $P = 0.04$; Table 2).

Within the "Upsweep complex," a second subset of 10 warbler species with highly similar calls, only phylogeny predicted acoustic flight call similarity in this group: species in the "Upsweep complex" that were closely related produced more similar flight calls (Multiple Mantel test; $t = 1.7$, $P = 0.04$, $n = 36$; Table 3). Migration overlap, breeding overlap, winter overlap, breeding latitude, winter latitude, and migration distance were not predictors of flight call similarity (Multiple Mantel test; Table 3).
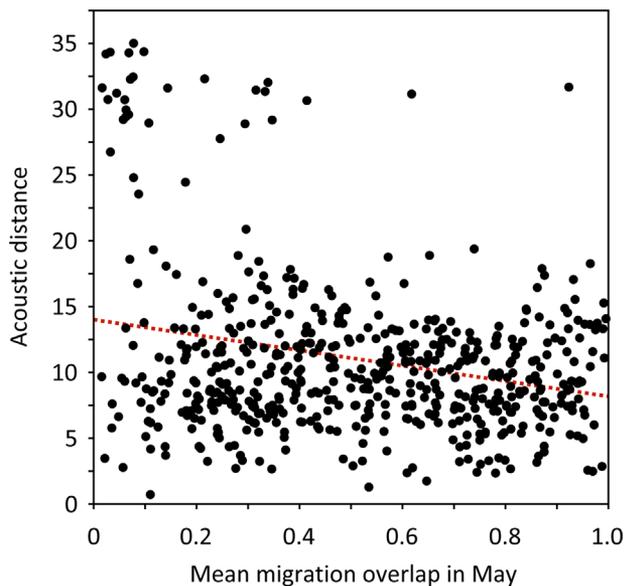
Across 35 species of wood warblers with migration timing data, mean migration overlap in May, the period of highest species overlap, predicted acoustic flight call similarity: species that had similar peak timings of migration produced acoustically similar flight calls (Multiple Mantel Test; $t = -5.3$, $P = 0.03$, $n = 35$; Table 4; Fig. 6). Specifically, species that had similar peak timings of migration through the Americas summed over each week in May had similar flight calls (Fig. 6). Phylogeny also predicted acoustic flight call similarity: species that were closely related produced more acoustically similar calls. We did not find a similar relationship for mean migration time overlap in April (Multiple Mantel Test; $t = -0.22$, $P = 0.92$, $n = 35$; Table 4).

Discussion

We found that the acoustic similarity of wood warbler flight calls is correlated with phylogenetic relatedness of species, similarity in breeding latitudes, and mean temporal migration overlap in May. In a smaller subset of 12 acoustically similar species in the

Table 4. Results from Multiple Mantel Matrix Regression with phylogeny and mean temporal migration overlap in April and May as predictors of acoustic flight call similarity in 35 species of migratory wood warblers (*Parulidae*).

Mantel Test			
Predictor	Estimate	*t*	*P*-value
Intercept	9.1	7.5	0.68
Phylogeny	0.05	5.06	0.02
April Mean Timing Overlap	−0.25	−0.22	0.92
May Mean Timing Overlap	−5.5	−5.3	0.03
Multiple R^2	0.12		

Figure 6. Scatterplot showing acoustic distance of wood warbler flight calls for 35 species of wood warblers and mean migration overlap during the month of May. Acoustic distances represent the Euclidean distances between the mean position of pairs of warbler flight calls. Mean migration overlap in May represents the degree of similarity in timing of migration between pairs of warbler migrations during May. Red line shows line-of-best-fit.

"Zeep complex," a collection of species often grouped together because their flight calls are highly acoustically similar (Evans and O'Brien 2002), we found that similarity in total length of migration, overlap in their wintering range, and phylogeny were correlated with acoustic similarity of flight calls. In a subset of 10 other species in the "Upsweep complex," only phylogenetic relatedness corresponded with acoustic similarity. While these results suggest that shared evolutionary history plays a role in shaping flight call acoustic structure, similarity in migratory routes also influences the acoustic similarity of wood warbler flight calls, both within the larger group of 36 species we analyzed, and the subset of 12 species in the "Zeep complex."

We found a strong effect of phylogeny on acoustic similarity in flight calls, a pattern that held true across the entire group of 36 species, as well as two restricted subsets of birds with highly similar flight calls. Previous independent studies have also found a strong effect of phylogeny in warbler flight calls; the acoustic similarity among 47 species of wood warblers showed a strong effect of phylogeny (Farnsworth and Lovette 2008). Therefore, our research adds an independent confirmation that closely related animals share acoustically similar flight calls. This pattern holds true across other types of vocalizations, and across other taxa. For example *Streptopelia* doves show a phylogenetic effect of acoustic similarity in their breeding vocalizations (de Kort and ten Kate 2001), corvids show an effect of phylogeny on their contact calls (Laiolo and Rolando 2003), and non-human primates show an effect of phylogeny in their alarm and distress calls (Hauser 1993).

Beyond the well-known effect of phylogeny, we were interested in whether similarity in migration might explain additional variation in warbler flight calls. We hypothesized that warblers migrating in mixed-species groups would benefit from acoustically similar flight calls, an idea which we called the "Migration Similarity Hypothesis." The rationale for this hypothesis was that among distantly related warbler species that flock together (Winger et al. 2019), similarity in migratory overlap and timing of migration may be an important driver of flight call evolution. Under the "Migration Similarity Hypothesis," warblers would use acoustically similar flight calls as aural signals indicating shared migrations. Across the entire set of 36 warbler species, we found that similarity in breeding latitude and similarity in mean migration overlap in May, averaged over each week of May, corresponded with acoustic similarity of flight calls. We interpret these results as support for the "Migration Similarity Hypothesis": acoustically similar flight calls frequently uttered by these nocturnally migrating species (Smith et al. 2014) may have evolved to increase flock contact with flockmates (Winger et al. 2019) sharing similar migratory routes at the same time. This would imply that there is a direct benefit to using flight calls as acoustic signals for identifying flockmates sharing similar migrations. This also implies that there is a direct benefit for migrating in groups that share geographically similar migratory routes at the same time. Our results support the idea that species that overlap in migration, both geographically and temporally, may

have experienced selection for acoustically similar calls as signals of shared migrations. This could have evolved if selection favored individuals that produce acoustically similar calls to other species with similar migrations, whenever both species migrate together in mixed-species flocks. This may have reduced disorientation during migration or increased the chance of finding high quality stop-over sites and avoiding predators. Thus, individuals of different species with similar calls may have experienced higher survival during migration than those individuals within each species who produced dissimilar calls. Selection for acoustically similar wood warbler flight calls may have been high in the last 50,000 years of glacial flux, when shifting migratory routes may have driven the need for increased communication (Somveille et al. 2020).

In the group of warblers with flight calls so similar they are often grouped together as the "Zeep complex" (Evans and O'Brien 2002; Landsborough et al. 2019), we found that phylogeny is a predictor of acoustic flight call similarity, however, similarity in total length of migration and winter range overlap are also predictors of acoustic call similarity even after accounting for the effects of phylogeny in this group. The "Zeep complex" includes two species in monotypic genera that are phylogenetically distant from other members of the group—the Connecticut Warbler (Genus *Oporornis*) and the Worm-eating Warbler (Genus *Helmitheros*)—that exhibit extremely similar calls to *Setophaga* Zeep members (Evans and O'Brien 2002). We posit that both species may have evolved acoustically similar calls to other Zeep members despite being phylogenetically distant, due to overlapping migrations with other members of the "Zeep complex." For example, Connecticut and Blackpoll Warbler are two species within the "Zeep complex" that in spite of relatively distant phylogenetic relatedness, both make nonstop transoceanic migrations from maritime Canada to the Caribbean coast of South America (DeLuca et al. 2015; McKinnon et al. 2017; DeLuca et al. 2019). If migrating in groups is only beneficial among individuals with similar migratory patterns, this may highlight why flight calls among "Zeep complex" species have evolved acoustic similarity. We note that analyses of the Zeep and Upsweep groups include a small number of species, and therefore these results should be interpreted cautiously.

In the ten species within the "Upsweep complex" (Evans and O'Brien 2002), we did not find a relationship between acoustic similarity of flight calls and similarity of migration. More research is needed to determine if other ecological or environmental factors besides phylogenetic relatedness drive the evolution of these calls. This group is less acoustically similar, however, than the "Zeep complex" and has substantially more variation in wintering and migratory range (Birdlife International 2019). This is supported by the results of our acoustic flight call cluster analysis and prior work (Evans and O'Brien 2002). Individual species

in the "Upsweep complex" category are often easier to identify by their more divergent flight calls and perhaps some do not belong in this category, suggesting a reevaluation of the "Upsweep complex" (Smith et al. 2014).

It is worth noting that we chose to measure geographic overlap in breeding, migration, and winter, similarity in breeding latitude, similarity in wintering latitude, and similarity in length of migration as predictors of migration similarity but we also accounted for temporal occurrence of migrants. We evaluated the mean migration overlap in April and May, averaged over each week per month, separately, because nearly all North American warbler species overlap temporally during migration although each has slightly different mean peak abundances (Birdlife International 2019). Many species overlap for some part of May but fewer migrate in April when many individuals are still on wintering grounds. This may be why temporal migratory overlap of species in May, but not April, predicted acoustic flight call similarity. Temporal migratory overlap is likely an important factor contributing the evolution of acoustic flight call similarity, because temporal and geographic overlap are both needed for warblers to coexist in migration. The data in our analyses were summarized weekly, but it is possible that weekly summarization of timing of migration could mask trends in species departures and arrivals which happened below the weekly level. More fine-scale data on the species composition and calling rates of warbler flocks in active migration would be very useful to examine the function of flight calls, but these data are not available and are difficult to obtain because warblers migrate under cover of darkness. Additional studies directly recording the flight calls of warbler species in active migration would be a welcome approach to learn about the behaviour and species composition of migrating neotropical warblers.

Flight calls are also produced in contexts outside of active migration, including among newly fledged family groups, and among foraging individuals (Farnsworth and Lovette 2008). The function of flight calls in these contexts should be studied in more detail and may further influence the evolution of these vocalizations beyond what we have studied here. Flight calls, however, appear to be uttered more frequently during migration than in non-migratory periods which could indicate they are most important as migratory calls. In addition, the attenuation of high frequency calls such as flight calls (Horton et al. 2015) could play a factor in the reception of these calls by migrant individuals and impose constraints on their signal evolution. Acoustic attenuation of flight calls as it related to signal evolution should be studied in more detail.

Several other families of songbirds (Order: Passeriformes) also use flight calls. These species also flock together during migration and may use flight calls to increase flock contact with flockmates (Winger et al. 2019). Patterns of acoustic similarity

have not been described in species beyond warblers. Descriptive accounts (Evans and O'Brien 2002), however, suggest the calls within some genera of sparrows are highly similar, in particular the calls of species in the Genera *Spizella*, *Zonotrichia*, and *Melospiza*. Calls of buntings in the genus *Passerina* and the family Cardinalidae also have highly similar calls (Evans and O'Brien 2002). Further examples of flight call acoustic similarity should be investigated across songbird species as recordings of species become available and increase in quality. Calls of these species are increasingly available and future analysis may reveal if call evolution has driven acoustic convergence in multiple passerine species that share similar migration routes and migration timings.

Very little information is available about the role of acoustic communication in migratory animals. Migrants that travel in tightly spaced groups, where distances between individuals are small, may not require acoustic signals during migration because the animals are in direct contact or immediate visual range. Migrants that are widely spaced or travel during low visibility conditions may be more likely to use auditory cues. Humpback whales (*Megaptera novaeangliae*) sing during migration and their songs can be used as indicators of their intended migratory route (Clapham and Mattila 1990). The importance of such acoustic signals in other migrants is not known. More information is needed to determine how auditory signals have evolved to communicate information about migration routes in diverse systems. This will be important to elucidate how widespread auditory communication is in migrating animals, or if it is particularly restricted to migrating songbirds where visual contact is not possible during nocturnal migration (Alerstam 2009). It will be important to investigate these ideas while also considering alternative hypotheses for the evolution of acoustic signals.

Conclusions

After examining 36 migratory wood warbler species to understand if warblers with similar migrations have acoustically similar calls, we found correlations between similarity of migration overlap and acoustic similarity of flight calls. After controlling for phylogeny, which was also correlated with acoustic similarity of flight calls, we found that warbler flight calls were acoustically more similar among: (1) species with similar breeding latitudes, (2) species with similar timings of migration during May, (3) species with similar total lengths of migration, and (4) species with similar wintering range overlap. This research provides context for the selective pressures acting on flight call structure.

AUTHOR CONTRIBUTIONS
Z.G.G. and D.J.M. conceived of and designed the study. Z.G.G. collected the data. Z.G.G. and R.K.S. analyzed the data. Z.G.G. and D.J.M. wrote the manuscript. All authors edited the manuscript.

DATA ARCHIVING
All data used in analyses will be publicly available at the University of Windsor Data Repository and Dryad. The data used in this paper are available at the following link: https://datadryad.org/stash/share/f7rWYMjUu14mSwqKhEoeLNKvFKiky51WNZsAtFoItgo

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplemental Table 1. List of 36 wood warbler species used to examine correlations between acoustic similarity of wood warbler flight calls, phylogenetic distance, and similarity in migration.

Supplemental Figure 1. Example of paiwise migratory similarity measurements shown two migratory wood warblers: Connecticut Warbler (left) and Blackpoll Warbler (right).

Supplemental Table 2. Loadings of variables in Principle Components Analysis of the acoustic structure of 36 wood warbler flight calls.