

Magnolia Warbler flight calls demonstrate individuality and variation by season and recording location

Elliott M. Ress,¹ Andrew Farnsworth,^{1,2} Sara R. Morris,^{3,4,5} Michael Lanzone,⁶ and Benjamin M. Van Doren^{1,7,*}

¹Cornell Lab of Ornithology, Cornell University, Ithaca, New York, USA

²Actions@EBMF, New York, NY, USA

³Department of Biology, Canisius University, Buffalo, New York, USA

⁴Appledore Island Migration Station and Shoals Marine Laboratory (Joint Program of University of New Hampshire and Cornell University), Durham, New Hampshire, USA

⁵Braddock Bay Bird Observatory, Hilton, New York, USA

⁶Cellular Tracking Technologies, Rio Grande, New Jersey, USA

⁷Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

*Corresponding author: vandoren@cornell.edu

Abstract

Flight calls are short vocalizations frequently associated with migratory behavior that may maintain group structure, signal individual identity, and facilitate intra- and interspecific communication. In this study, Magnolia Warbler (*Setophaga magnolia*) flight call characteristics varied significantly by season and recording location, but not age or sex, and an individual's flight calls were significantly more similar to one another than to calls of other individuals. To determine if flight calls encode traits of the signaling individual during migration, we analyzed acoustic characteristics of the calls from the nocturnally migrating Magnolia Warbler. Specifically, we analyzed calls recorded from temporarily captured birds across the northeastern United States, including Appledore Island in Maine, Braddock Bay Bird Observatory in New York, and Powdermill Avian Research Center in Pennsylvania to quantify variation attributable to individual identity, sex, age, seasonality, and recording location. Overall, our findings suggest that Magnolia Warbler flight calls may show meaningful individual variation and exhibit previously undescribed spatiotemporal variation, providing a basis for future research.

Keywords: acoustic signals, flight calls, individual identity, Magnolia Warbler, migration, recording location, seasonality

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LAY SUMMARY

- Flight calls are short vocalizations frequently associated with migratory behavior and may contribute to maintaining flock structure, signaling individual identity, and facilitating intra- and interspecific communication.
- We analyzed acoustic characteristics of Magnolia Warbler (*Setophaga magnolia*) flight calls, examining variation to determine if calls differed by individual, age, sex, season, and recording location.
- Calls from the same individual were significantly more similar to one another than to calls of other individuals. Seasonality and recording location were significantly associated with call characteristics, while calls did not vary significantly by age and sex.
- These findings suggest that it may eventually be possible to identify individual Magnolia Warblers within a small group by their vocalizations. This research helps us better understand Magnolia Warblers' migratory behavior, flight call differences due to geographical location, and the role of seasonality in flight call morphology.

Las llamadas de vuelo de *Setophaga magnolia* demuestran individualidad y variación por estación y ubicación de grabación

RESUMEN

Las llamadas de vuelo son vocalizaciones cortas frecuentemente asociadas con el comportamiento migratorio que pueden mantener la estructura del grupo, señalar la identidad individual y facilitar la comunicación intra- e inter-específica. En este estudio, las características de las llamadas de vuelo de *Setophaga magnolia* variaron significativamente según la estación y la ubicación de la grabación, pero no según la edad

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o el sexo, y las llamadas de vuelo de un individuo fueron significativamente más similares entre sí que las llamadas de otros individuos. Para determinar si las llamadas de vuelo codifican rasgos del individuo que emite las señales durante la migración, analizamos las características acústicas de las llamadas de individuos de *S. magnolia* que migran durante la noche. Específicamente, analizamos las llamadas grabadas de aves capturadas temporalmente en el noreste de los Estados Unidos, incluyendo la Isla Appledore en Maine, el Observatorio de Aves de Braddock Bay en Nueva York y el Centro de Investigación de Aves Powdermill en Pensilvania, para cuantificar la variación atribuible a la identidad individual, el sexo, la edad, la estacionalidad y la ubicación de la grabación. En general, nuestros hallazgos sugieren que las llamadas de vuelo de *S. magnolia* pueden mostrar una variación individual significativa y exhibir una variación espaciotemporal previamente no descrita, lo que proporciona una base para investigaciones futuras.

Palabras clave: estacionalidad, identidad individual, llamadas de vuelo, migración, señales acústicas, *Setophaga magnolia*, ubicación de grabación

INTRODUCTION

Bioacoustic communication consists of a complex and diverse array of sound signals used to convey messages to and elicit responsive behavior from conspecific and/or heterospecific individuals (Penar *et al.* 2020, Green and Marler 1979). The content of and responsive behavior to such communication is determined by the species; social system; behavioral context; function; interests of the involved individuals; and the ability for them to produce, receive, and process a given message (Naguib 2006). Although the precise and accurate meaning of non-human animal signals may be unknown, bioacoustic research in these organisms may allow for the classification, description, and analysis of signals and gives insight into how vocalizations affect behavior in the recipient (Garcia and Favaro 2017). As such, many bioacoustic signals have been described and classified, but our understanding of behavioral contexts associated with flight calls is incomplete.

Bioacoustic communication may play an important role in the migration of birds. Migration is fundamental to the survival of many birds, providing access to seasonally varying resources such as territory, food sources, and breeding grounds (Winger *et al.* 2012, 2014). Birds use diverse strategies to migrate, and many birds communicate during migration through flight calls. Flight calls consist of short, species-specific, often monosyllabic vocalizations made during flight. These vocalizations are often associated with nocturnal migratory behaviors (Farnsworth 2005; but see Farnsworth 2007). Flight calls may be important in maintaining group structure, coordinating movement, stimulating migratory restlessness, and intra- and interspecific communication (Ball 1952, Hamilton 1962, Evans and O'Brien 2002, Farnsworth 2005, Morris *et al.* 2016, Winger *et al.* 2019).

Passerine birds are primarily active during the day, but enormous numbers engage in nocturnal flights during migration (Dokter *et al.* 2018; Horton *et al.* 2019). The potential advantages of nocturnal migration include decreased predation risks from typically visual diurnal predators (Alerstam 2009, Lank 1989, Mukhin *et al.* 2009; but see DeCandido and Allen 2006); more stable flight conditions at night, with less turbulence and solar-induced thermal disturbances (Alerstam 2009, Kerlinger and Moore 1989); and lower nocturnal temperatures, which decrease the hydration requirements of flight activities (Alerstam 2009, Kerlinger and Moore 1989). Nocturnal migration also allows birds to spend time foraging during the day so that they may fly uninterrupted at night (Alerstam 2009). However, nocturnal migration also presents challenges for birds, including limited visibility—and thus the risk of collisions with human-made or natural structures—and the confounding presence of sensory pollution, especially artificial light (Van Doren *et al.* 2017, Winger *et al.* 2019). Although the proposed functions of flight calls are still largely speculative as the potential benefit to the calling individual is still unclear, communication via flight calls might convey

information to nearby individuals during low visibility flight and to inexperienced birds during migration (Farnsworth 2005). Furthermore, we do not completely understand the ontogeny and origins of flight calls (Farnsworth and Lovette 2005). Moreover, few studies have examined individual variation in flight calling behavior (Griffiths *et al.* 2016, Morris *et al.* 2016, Keen *et al.* 2013, Farnsworth and Lanzone, personal communication).

A more thorough understanding of flight calls could aid our understanding of interactions within and among migratory bird species (Lanzone *et al.* 2009). Some data suggest that flight calls may play a role in signaling traits such as sex, age, or identity, as demonstrated in American Redstarts (*Setophaga ruticilla*) (Griffiths *et al.* 2016) and other Parulid species (Farnsworth and Lanzone, personal communication). The abilities of birds to communicate information about these traits may be useful for facilitating the maintenance of social structure and group cohesion. Deciphering how flight calls vary is also important for the development of acoustic monitoring protocols for counting individual birds during migration (Lanzone *et al.* 2009). The ability to identify individual birds and discern sex and age classes with acoustic data would facilitate novel methods of enumerating passing individuals and providing more comprehensive understanding of interactions with other individuals during migration.

Here, we examine variation in the flight calls of Magnolia Warblers (*Setophaga magnolia*) relating to age, sexual dimorphism, seasonality, recording location, and individual identity. Previous work has documented individual variation, sexual dimorphism, and age-related differences in nocturnal flight calls of Parulid warblers (Griffiths *et al.* 2016, Farnsworth and Lanzone, personal communication). We therefore predicted that Magnolia Warbler flight calls would exhibit individual variation, sexual dimorphism, and age differences.

METHODS

Study Organism

Magnolia Warbler is a small (11–13 cm) Neotropical migrant and a member of a diverse radiation of American passerines, the Parulidae (Lovette and Bermingham 2002). During the breeding season, they occur throughout the boreal forests of Canada, the northern Great Lakes region, New England, and the northern Appalachians. In fall, they migrate south through the eastern half of the US, en route to southern Mexico and Panama (Dunn and Hall 2020). Magnolia Warbler migration patterns differ by season, with birds taking a more easterly route in the fall than in the spring (Crawford and Stevenson 1984). Magnolia Warblers migrate primarily at night and give nocturnal flight calls. Magnolia Warbler flight calls are monosyllabic and described as a “buzzy ‘szzip’ or ‘zeet’ note” (Evans and O'Brien 2002, Farnsworth and Russell 2007,

respectively). The calls average 59.4 ms in duration and a frequency range of 6.5–8.4 kHz (Evans and O'Brien, 2002).

Flight Call Recordings

Lanzone *et al.* (2009) pioneered an approach to record the flight calls of captive migratory birds by placing birds in recording chambers and eliciting vocalizations with playback of conspecifics and heterospecifics. We obtained audio recordings of temporarily captured wild migrant Magnolia Warblers from Lanzone *et al.* (2009) and Morris *et al.* (2016), which adapted the original protocol. Recordings were made at 3 locations across the northeastern United States: Appledore Island Migration Station (AIMS) on Appledore Island off the coast of Maine; Braddock Bay Bird Observatory (BBBO) in western New York state; and Powdermill Avian Research Center (PARC) in southwestern Pennsylvania. Magnolia Warblers do not breed in any of these locations; thus, all individuals were migrants. At all locations, researchers captured wild birds in mist nets, measured and banded them, placed them in a recording chamber, and released them after recording. Each facility used the protocols originally developed by Lanzone *et al.* (2009). Flight calls recorded using these methods are similar to calls recorded diurnally and nocturnally from birds in free flight (Lanzone *et al.* 2009). Researchers used playback of conspecifics and heterospecifics to elicit vocal responses. At PARC, multiple recording chambers were in the same room so that multiple birds could be recorded at the same time. At AIMS and BBBO, only one bird was present at a time for recording. In addition, the conspecific playback stimuli at AIMS and BBBO included 2 adult and 3 young birds to simulate age proportions in migrant populations, whereas age proportions were not as closely controlled at PARC. AIMS data were collected in spring only, whereas both BBBO and PARC include recordings from both spring and fall. Our measured audio recordings used a sampling frequency of 22 kHz, a bit depth of 16 bits per sample, and a .wav file type. A full description, methods, and protocols are available in the studies by Morris *et al.* (2016) and Lanzone *et al.* (2009).

Individual Metadata

We compiled data on captured individuals from the original data sources, including bird age, sex, and band number. To simplify analyses, we assigned birds to 2 age classes: young and adult. We classified birds less than one year old as “young” and birds greater than one year old as “adults” based on reported USGS age classes (Celis-Murillo *et al.* 2022). We chose this binary age classification to simplify the analysis and because chronological age (<1 year vs > 1 year old) is well suited for the ontogenetic questions addressed here. Birds assigned the USGS age class “after hatch year” (AHY) during spring were classified as unknown age in this analysis because these birds could be either greater than or less than 1 year old.

We compiled a total of 3,091 calls from 351 individuals. We removed 74 calls that were either (1) inaccurately attributed to Magnolia Warblers ($n = 6$) or (2) had a low signal-to-noise ratio, preventing accurate measurement ($n = 68$). The resulting final dataset included 3,017 calls from 277 individuals: 1,843 from BBBO, 627 from AIMS, and 547 from PARC. There was an average of 10.89 calls bird⁻¹ (SD = 14.98, range: 1–143). Of these calls, 1,187 are from males, 569 are from females, and 1,261 are from birds of unknown sex. Six hundred and fifty-nine of the calls are from adult birds, 2,213 are

from young birds, and 145 are from birds of unknown age; 2,159 of these calls were recorded in the fall and 858 calls in the spring.

Acoustic Measurements

We used Raven Pro 1.6 sound analysis software (K. Lisa Yang Center 2023) to make spectrographic measurements of individual flight calls. We used a 256-point Fast Fourier Transform with 230-sample Hann windows and a hop size of 40 samples. We drew bounding boxes around each vocalization, using both spectrogram and waveform views to aid in defining call start, end, and high- and low-frequency boundaries. We drew spectrographic bounding boxes blind to location, season, or individual identity.

A single person drew all the bounding boxes, and all measurements were calculated either by Raven Pro or by our analysis code (details below); no measurements were taken by hand. To ensure the repeatability of our measurements, we took a subset of 30 calls and drew new bounding boxes 3 separate times for each call in Raven Pro. We determined that the measurement error was negligible and our measurements were repeatable.

We measured 39 different acoustic characteristics. These included attributes of frequency, amplitude, bandwidth, duration, and slope measured in Raven Pro 1.6. From the peak frequency contour generated with Raven Pro (Figure 1), we derived additional metrics designed specifically to capture variation in Magnolia Warbler flight calls. These included the number of modulations in the call (each modulation comprising a “peak” and a “valley”), the mean slope between adjacent peaks and valleys, and other related measurements. We used the *extrema()* function in the R package *Rlibeemd* to identify local maxima and minima (i.e., “peaks” and “valleys”) (Helske and Luukko 2021). In the case of flat regions (plateaus) in the data, this function takes the center point of the flat region as the extremal point. A full list of measurements can be found in Supplementary Material Table S1; Raven measurements are illustrated in Figure 1.

Statistical Analysis

Principal component analysis

To summarize the 39 call measurement variables into a smaller set of meaningful predictors, we performed principal component analysis (PCA) using the R package *FactoMineR* (Lê *et al.* 2008). We retained 3 orthogonal principal components that together explained 64.8% of variation in call measurements. We limited our analysis to the first 3 principal components because they explained the majority of variation while also being easily interpretable. Specifically, PC1 explained 32% of total variation in acoustic measurements and correlated strongly with frequency variables; higher values of PC1 indicated higher-pitched calls (Supplementary Material Table 1). PC2 explained 20% of total variation and correlated strongly with variables associated with the duration of a call; higher values of PC2 indicated longer-duration calls (Supplementary Material Table 1). PC3 explained 13% of total variation and correlated with variation in call frequency bandwidths; higher values of PC3 indicated narrower-bandwidth calls with shallower modulations (Supplementary Material Table 1).

After PCA, we constructed linear mixed models to test for associations among call characteristics and age, sex, season

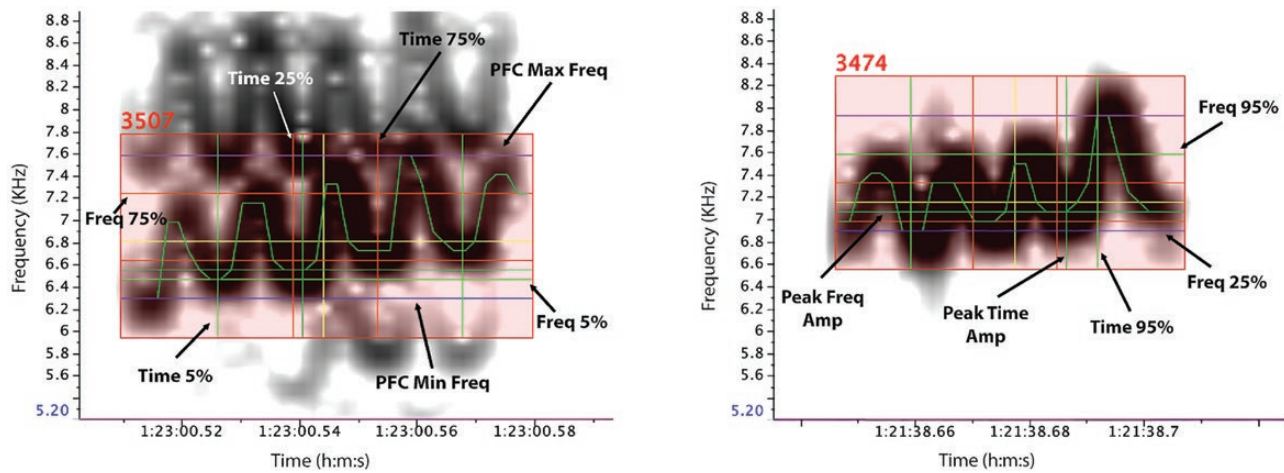


FIGURE 1. Flight calls of Magnolia Warbler (*Setophaga magnolia*) with measurements made by Raven Pro. Graphs show spectrograms of calls from different individuals (Hann Window, FFT window size 256 samples, hop size of 40 samples, 22,050 Hz sample rate, y-axis limit of 5.58–10.3 kHz, x-axis limit of 0.256 s per line). Colored vertical and horizontal lines illustrate a subset of spectrographic measurements. The peak frequency contour, as denoted by the curvilinear dark green line, is the peak frequency measurement for each spectrogram slice. Call 3,507 (left) shows a greater number of modulations than call 3,474 (right). More noise is present in call 3,507 (left).

(spring vs. fall), and location (AIMS, BBBO, or PARC) using the R package *lme4* (Bates et al. 2015). Response variables were each of the principal components (PC1, PC2, or PC3). We included a random effect of individual identity to account for correlation among calls given by the same bird. Fixed effects were age, sex, season, and location. We also tested two biologically plausible interactions of age*sex and age*season, as we hypothesized that birds of different ages might demonstrate different sex- and season-related effects. We evaluated statistical significance of each term using the *drop1()* function with a chi-squared test in *lme4*. If the interaction terms were not statistically significant, we removed the interactions to simplify the model. We checked model residuals for linearity and confirmed that residuals were distributed acceptably under an assumption of normality.

Individual variation

Next, we examined the degree of consistency among an individual's flight calls. We analyzed multiple flight calls from each bird to determine if an individual's calls were more similar to each other than to calls of other individuals. We quantified individual variation in calls using 2 analyses. In the first analysis, we performed repeatability analyses using the R package *rptR* (Stoffel et al. 2017) on the 3 principal components representing call characteristics. Our repeatability models included only one random effect of individual identity. We did not control for any fixed effects in the repeatability models to avoid overstating individual variation. This analysis quantified the proportion of variation in each call characteristic that is attributable to individual identity. We calculated confidence intervals using bootstrap resampling with 1,000 iterations (Gaussian data type; nboot = 1,000; npermut = 0). In the second analysis, we followed the approach of Griffiths et al. (2016) and calculated similarity metrics among individual calls with an unsupervised random forest model (ntree = 4,999, proximity = T, x = Y.raw, all other settings default) (Liaw and Wiener 2002) using the proximity scores, or closeness, between each pair of calls ($n = 2,898$). In contrast to the repeatability analysis, an unsupervised random forest considers information from all principal components

together instead of calculating scores separately for each principal component. The resulting proximity scores can be interpreted as measures of overall similarity between two calls based collectively on the underlying measurements. For each individual bird, we calculated (1) the average similarity score among calls made by that individual and (2) the average similarity score between calls made by the focal bird and those of other birds. We then performed a paired *t*-test to determine whether an individual's calls were statistically more similar to each other than to calls made by other birds.

Finally, we performed 2 additional analyses. In the first, we separately calculated repeatabilities for adults and young birds, fall and spring, and the 3 recording locations. We compared confidence intervals to determine if the level of individual consistency in flight call characteristics differed by age, seasonality, or recording location. Previous work on vocal behavior has shown that younger birds can show higher variation (and therefore lower repeatability) in vocal signals (de Kort et al. 2009, Vehrencamp et al. 2013). In the second additional analysis, we performed the *Random Forest* analysis on a balanced subset of the data. The full dataset has imbalanced sample sizes among individuals (range: 1–143 calls per individual). Unlike the repeatability analysis, the *Random Forest* analysis does not explicitly account for unbalanced sample sizes among individuals, so our results could be biased towards individuals with more measured calls. We therefore conducted this additional analysis to ensure that our conclusions were not affected by sample size imbalances among individuals. Specifically, of birds that produced 5 or more calls, we randomly selected 5 calls per individual, such that the subsampled dataset contained exactly 5 calls from each of 153 birds (765 calls total). We then repeated the *Random Forest* analysis with this balanced, subsampled dataset.

RESULTS

Variation by Age, Sex, Seasonality, and Location

In linear mixed models (LMM) of call characteristics, for all 3 principal components, the age*sex and age*season interaction terms did not reach statistical significance

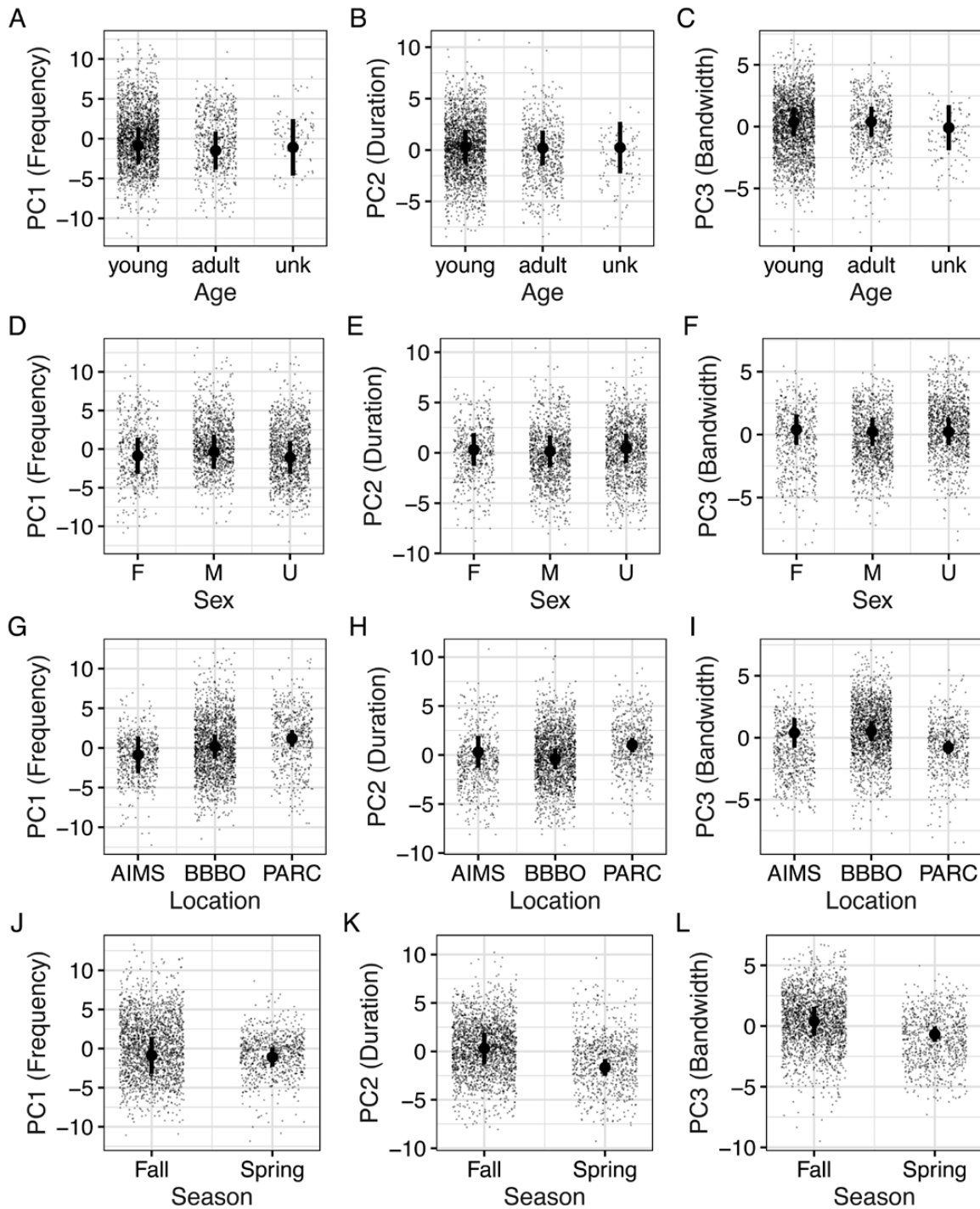


FIGURE 2. Mean predictions and 95% confidence intervals from linear mixed models of PC1 (frequency), PC2 (duration), and PC3 (bandwidth) for each fixed effect of age, sex, location, and season. Jittered raw data are also plotted.

(age*sex: PC1: $P = 0.42$, PC2: $P = 0.78$, PC3: $P = 0.85$; age*season: PC1: $P = 0.45$, PC2: $P = 0.75$, PC3: $P = 0.81$) (Figure 2). Therefore, after removing all interaction terms, the 3 final models each contained only 4 fixed effects: age, sex, season, and location.

Both PC2 (call duration) and PC3 (call bandwidth) were significantly associated with season and capture location (Figure 2H, I, K, L). Birds assayed in spring showed shorter duration calls (PC2) than in the fall ($P = 0.006$) (Figure 2K). Likewise, calls differed significantly among locations; birds from BBBO

had shorter duration calls than those from PARC ($P = 0.01$) (Figure 2H). Considering call bandwidth (PC3), calls recorded in fall showed significantly shallower bandwidths than did those in spring ($P = 0.04$) (Figure 2L). Also, birds from BBBO gave shallower-bandwidth calls than those from PARC ($P = 0.002$) (Figure 2I). For both duration (PC2) and frequency (PC3), calls recorded at AIMS showed broader variation that was not obviously distinct from either BBBO or PARC.

Call frequency (PC1) was not significantly associated with any of the fixed effects ($P(\text{age}) = 0.53$, $P(\text{sex}) = 0.48$,

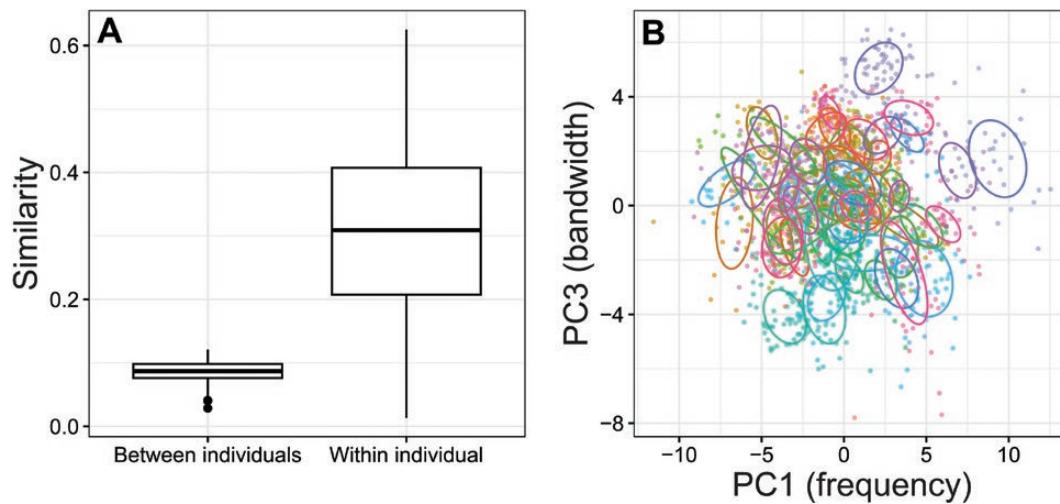


FIGURE 3. Individual consistency in flight calls. **(A)** Boxplot depicting similarity scores for calls made by different individuals (left) and calls made by the same individual (right). **(B)** Scatterplot showing individual variation in 2 principal components (PC1 and PC3). Each colored ellipse and corresponding points represent the calls of one individual; ellipses are drawn to contain 50% of an individual's data points. For clarity, only individuals with at $n \geq 15$ measured calls are included.

$P(\text{season}) = 0.83$, $P(\text{location}) = 0.17$ (Figure 2A, D, G, J). Duration (PC2) and bandwidth (PC3) were not significantly associated with bird age and sex (PC2: $P(\text{age}) = 0.95$, $P(\text{sex}) = 0.80$; PC3: $P(\text{age}) = 0.67$, $P(\text{sex}) = 0.83$) (Figure 2B, C, E, F).

Overall effects of each variable, including additional test statistics, p -values, and other effects are presented in Supplementary Material Tables 2–7.

Individual Variation

Repeatability analyses quantified the proportion of variation in call characteristics attributable to individual identity and demonstrated strong consistency within an individual bird's calls for all 3 principal components. The mean call similarity score (derived from an unsupervised random forest) for calls made by the same individual was 0.3071 (range: 0.0162–0.6052, $SD = 0.1232$). The mean similarity score for calls made by different individuals was a substantially lower 0.0858 (range: 0.0276–0.1221, $SD = 0.0170$). Calls given by an individual were therefore significantly more similar to each other than to those given by other individuals (t -test, $t = 29.041$, $df = 226$, $P < 2.2 \times 10^{-16}$) (Figure 3). This finding was unchanged when using a balanced subset of the data ($n = 5$ per individual; $t = 25.545$, $df = 152$, $P < 2.2 \times 10^{-16}$).

Repeatabilities did not differ meaningfully across seasons or age classes, but the flight calls recorded at PARC showed lower repeatability than those recorded at BBBO and AIMS for PC1 and PC2 (Figure 4).

DISCUSSION

Magnolia Warbler flight calls varied significantly by season and location in call duration and bandwidth, but not frequency. Moreover, calls made by an individual were distinctly repeatable in frequency, duration, and bandwidth characteristics. These findings suggest that Magnolia Warbler flight calls show meaningful individual variation and exhibit meaningful spatiotemporal variation. We did not find any consistent differences based on age or sex for

any call characteristics. To our knowledge, this is one of few studies characterizing these acoustic traits in Magnolia Warblers, highlighting numerous opportunities for future research.

Several studies have examined flight calls of nocturnally migrating passerine birds, but few have evaluated these calls for individual, age, and sex variation (Griffiths et al. 2016, Morris et al. 2016, Farnsworth and Lanzone, personal communication). Previous work has found both individual variation and sexual dimorphism in the flight calls of American Redstarts (Griffiths et al. 2016) and other Parulids (Farnsworth and Lanzone, personal communication). Because Magnolia Warblers are congeneric (genus *Setophaga*), we hypothesized that Magnolia Warblers would exhibit similar patterns. Indeed, our findings of strong individual variation in flight calls and the absence of age-related variation are consistent with the results of Griffiths et al. (2016), who found evidence for individual consistency but no significant differences between age classes in redstarts. Griffiths et al. (2016) only examined flight calls recorded in fall at one location, so they were not able to test for seasonal or location-based differences.

In this study, Magnolia Warblers did not demonstrate sexual dimorphism in their vocalizations. This contrasts with American Redstarts, which do show sex-based variation in flight calls (Griffiths et al. 2016). Magnolia Warblers and American Redstarts both exhibit protandry, as males arrive at breeding grounds earlier than females. However, both species lack consistent differences in stopover behavior (e.g., refueling index, stopover duration, and rate of mass gain) (Morbey et al. 2018). Magnolia Warblers also show some sex-based differences in responsiveness to flight calls, unlike redstarts, which lack this difference (Tegeler et al. 2018). The cause of the apparent difference between these species in the degree of sexual dimorphism in call characteristics is unclear.

Annual cycle timing and capture location played larger roles in flight call variation than anticipated. The recordings used here were collected in Maine (AIMS), Pennsylvania (PARC), and New York (BBBO), and we observed the most

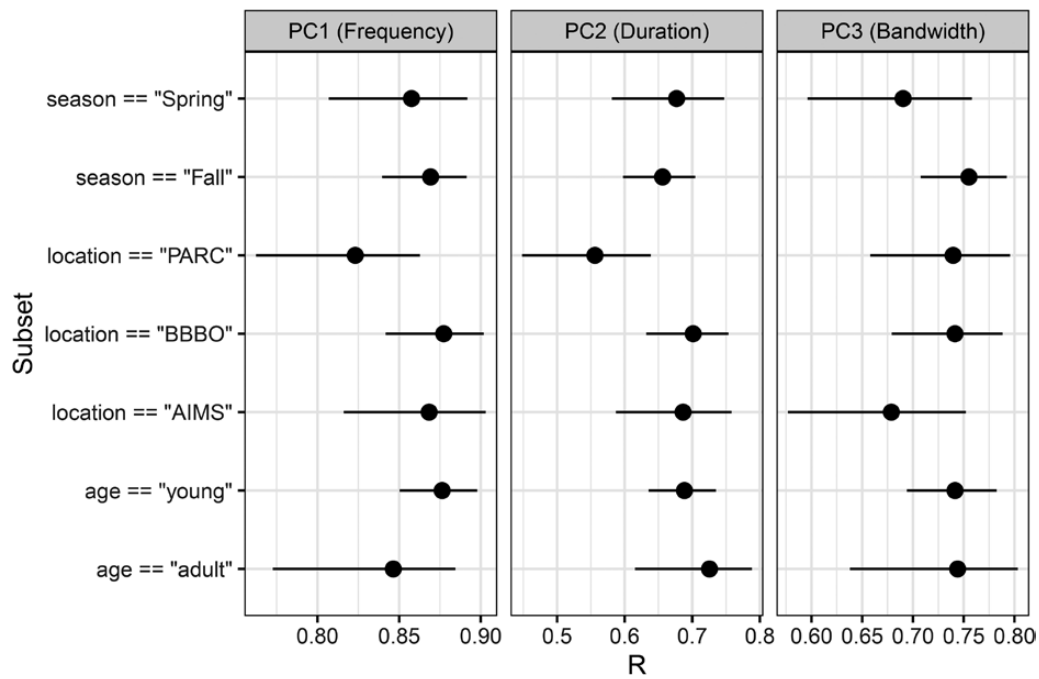


FIGURE 4. Individual repeatability analyses of flight call characteristics for season, recording location, and age. Bars represent 95% confidence intervals. There are no significant differences for age groups or seasonality, since all confidence intervals overlap the mean estimates. There is a significant difference in individual repeatabilities between PARC and BBBO for PC1 and between PARC and BBBO/AIMS for PC2.

consistent differences between Pennsylvania and New York sites; the Maine site generally showed larger variance in call characteristics. Other species of birds exhibit regional variation in their flight calls (Adkisson 1981, Reynolds *et al.* 2010); Magnolia Warblers might exhibit regional variation in their flight calls as well. Migrating birds traverse broad fronts and diverse routes to travel from their breeding grounds in the north to their wintering grounds in the south (Dokter *et al.* 2018, La Sorte *et al.* 2018). It is possible that the birds captured at different stations originated from different breeding populations.

Location-based variation could also be a result of a difference in study design, such as different elicitation stimuli, testing environment, or sampling procedures. However, AIMS, BBBO, and PARC used nearly identical experimental protocols. One difference among studies is that the recording chambers at AIMS and BBBO were housed in trailers, whereas PARC used a small room in a building. Additionally, AIMS and BBBO used balanced calling stimuli in order to simulate age proportions in migrant populations (2 adult, 3 young), whereas the stimuli used at PARC were not as closely age-adjusted. As shown in Figure 4, the flight calls recorded at PARC showed lower repeatability than those recorded at BBBO and AIMS for PC1 and PC2. It is possible this difference in the experimental environment contributed to location-based variation we see in our data. Further, environmental factors such as the lighting (time of day) or atmospheric conditions could contribute to differences in calling behavior (Horton *et al.* 2015). Future studies should consider studying birds across larger spatial distances to investigate additional geographic variation in vocalizations and behavior.

Surprisingly, seasonality played a significant role in flight call variation. Flight calls recorded in spring showed shorter durations and deeper bandwidths than those recorded during fall migration. Previous studies have noted a decreased

rate of flight call production between migratory seasons (Farnsworth 2007, Farnsworth and Lovette 2005, Smith *et al.* 2014). However, to our knowledge, this study is the first to evaluate the effect of seasonality on measured acoustic traits. One possible explanation for this seasonal difference in call characteristics are differences in migratory behavior between spring and fall. Spring migration is generally more fast-paced than in fall, as birds attempt to secure prime breeding locations (Nilsson *et al.* 2013). Another explanation may relate to geography; migrating birds frequently take different routes in fall compared to the spring (Mellone *et al.* 2015, Higuchi 2012). This means that individuals from different breeding populations may pass through the same areas in spring versus fall, so underlying geographic variation in call characteristics across the breeding range could potentially explain observed seasonal differences. Body mass and other related physical and physiological characteristics that vary by season could also affect differences in flight call characteristics. However, we are unsure of the magnitude of this effect or if it even exists, as this is beyond the scope of this study.

Other bird species, such as the American Redstart and Superb Starling (*Lamprolornis superbus*), show individual variation in their flight calls (Griffiths *et al.* 2016, Keen *et al.* 2013). Consistent with this finding, we found that Magnolia Warblers show meaningful individual variation in their flight calls. Consistency in an individual's flight call may aid other individuals in discriminating members of a group (Emlen 2010, Godard 1991, Molles and Vehrencamp 2001, Stoddard *et al.* 1991). Signaling individual variation may also be an important way of cooperating in a socially complex animal society (Keen *et al.* 2013) and could allow individual birds to benefit directly and indirectly (Kennedy *et al.* 2009).

With limited visual references during nocturnal flight, individually distinct flight calls might be important in maintaining

associations among migrating birds. If flight call identification methods improve and if Magnolia Warbler flight calls demonstrate repeatability over time, the ability to distinguish individuals by call could also allow scientists to track birds by their acoustic signals, similar to tracking movements of migrating whales to and from their breeding grounds (Stranistreet *et al.* 2013). This would facilitate tracking birds without capture, banding, or physical contact. Our finding of high individual repeatability in call characteristics suggests the possibility that individual Magnolia Warblers could be distinguished by call within small groups, which should be explored in future work.

Flight calls were recorded from temporarily captured birds in elicited response to conspecific playback of recorded flight calls. These artificial conditions could have altered the characteristics of the flight calls or the response itself. Technological advancements that would allow for contactless recording of spontaneous flight calls in a natural environment and documentation of visual characteristics at night without light would greatly increase the ability to obtain recordings while minimizing observer effects.

Another limitation of this study is that we were not able to assess the repeatability of individual calls over an extended period of time, since the birds captured were released immediately after recording, and no birds were recorded a second time. Sharp and Hatchwell (2005) demonstrated repeatability of individual variations in recorded contact calls from free-flying Long-tailed Tits (*Aegithalos caudatus*) over an entire breeding season. Keen *et al.* (2013) also showed sustained individual variation of flight calls of free-flying Superb Starlings over an extended period of time. These results would suggest that it is possible that the individual variation noted in Magnolia Warbler flight calls could be repeatable over time. This is an interesting avenue for future studies.

In conclusion, Magnolia Warblers demonstrated individual variation in their flight calls as well as significant variation by season and capture location. Although this study demonstrates individual variation in flight calls, identifying individual birds from acoustic measurements will require additional research. As technology and methods improve, our ability to create an “acoustic fingerprint” for individual birds within a group may become possible. Through continued research and collection of more flight call data, we will gain a better understanding of migratory behavior, differences in flight calls across space, and the role of seasonality in flight call behavior. Such an understanding would be broadly beneficial to ornithology, migration ecology, and monitoring and applied conservation.

Supplementary material

Supplementary material is available at *Ornithology* online.

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Ethics statement

This research was conducted under and complied with the Cornell University Office of Research Integrity and Assurance (ORIA).

Conflict of interest statement

The authors declare that we have no competing interests.

Author contributions

E.M.R., A.F., S.R.M., M.L., and B.M.V.D. conceived the idea, design, experiment (supervised research, formulated question or hypothesis). E.M.R., A.F., S.R.M., M.L., and B.M.V.D. performed the experiments (collected data, conducted the research). E.M.R., A.F., S.R.M., and B.M.V.D. wrote the paper (or substantially edited the paper). M.L., S.R.M., E.M.R., and B.M.V.D. developed or designed methods. E.M.R. and B.M.V.D. analyzed the data. E.M.R., M.L., A.F., S.R.M., and B.M.V.D. contributed substantial materials, resources, or funding.

Data availability

Analyses reported in this article can be reproduced using the data and code provided by Ress *et al.* (2023).

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