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## ABSTRACT

Vocal duets occur when 2 individuals vocalize in temporal coordination. In birds, duet participation functions to cooperatively defend shared resources, localize mates, and in some species, guard the mate. Previous work indicates that duetting tends to co-evolve with a non-migratory lifestyle, probably because the absence of migration facilitates greater cooperation between mates. We examined the evolution of duetting and migration in New World warblers (Parulidae), a group that has been largely ignored by duetting research. Of the 95 species in our analysis, we found evidence of duetting in 19 (20%) species, and evidence of migration in 45 (47.4%) species. Ancestral character reconstruction indicated that the last common ancestor of the New World warblers did not duet. Duetting evolved multiple times in this group, including 2 early origins and several more recent origins. Migration was present in the last common ancestor and was lost several times. Both duetting and migration exhibit phylogenetic signal. A phylogenetically explicit correlation analysis revealed a significant negative relationship between duetting and migration, in keeping with findings from other avian taxa. This study, the first description of the evolution of duetting in a large avian family with a temperate-zone origin, supports the hypothesis that duetting co-evolves with a sedentary natural history in birds.

**Key words:** ancestral character reconstruction, duet, migration, threshold model, warbler

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## RESUMEN

Los duetos vocales suceden cuando dos individuos vocalizan en coordinación temporal. En las aves, la participación en un dueto funciona para defender cooperativamente recursos compartidos, localizar parejas y, en algunas especies, para proteger a la pareja. Trabajos previos indican que el dueto tiende a co-evolucionar con un estilo de vida no migratorio,

Across diverse avian taxa, duetting evolves in concert with long-term pair bonds (Hall 2004, Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). Pair-bond stability is thought to promote cooperation, which in turn favors the evolution of signals that facilitate cooperation, like duetting (Hall 2004, Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). Migration can limit pair-bond duration, which may explain why duetting is negatively associated with migration in songbirds (Ens et al. 1996, Benedict 2008, Jeschke and Kokko 2008, Logue and Hall 2014, Tobias et al. 2016). This evolutionary association between sedentary life histories and duetting may drive global geographic patterns in duetting: most duetting species breed outside of north-temperate latitudes, where latitudinal migration is most common (Hall 2009, Logue and Hall 2014).

Previous studies on the evolution of avian duetting are based on taxonomically diverse samples (Hall 2004, Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). Evolutionary studies that focus on specific taxa can complement taxonomically diverse studies by asking whether general patterns apply to the focal taxon (Odom et al. 2015). Consistent results in multiple clades would lend support to the general conclusion. Conversely, divergent results would demand explanation. Taxonomically restricted samples are also useful for the reconstruction of ancestral traits, because they permit detailed exploration of evolutionary history and set the stage for future taxon-specific investigations (Price 2009, Price et al. 2009, Odom et al. 2015).

Many New World warblers sing duets, but in contrast to well-studied duetting groups like wrens (Troglodytidae), antbirds (Thamnophilidae), and blackbirds (Icteridae), there is virtually no peer-reviewed research on duetting in warblers (Hall 2009). Females of many Parulidae species sing, offering the potential for male-female song duets to evolve (Najar and Benedict 2015, Matthews et al. 2017). Female warblers also produce call notes that are used in some duets (Staicer 1992). All members of this clade are socially monogamous and territorial while breeding, as is typical of species that duet (Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). Atypically for duetting groups, however, this clade has a migratory ancestor that bred in the North-temperate zone (Winger et al. 2012).

Several studies have demonstra

evolutionary time by an underlying continuous variable called “liability.” Each of our models allowed 2 character states: duetting/not duetting and migratory/non-migratory. Liabilities below a fixed threshold correspond to 1 character state, and those above the threshold correspond to the other. Character states of extant species were treated as prior probabilities, and liability evolved by Brownian motion. We used a Bayesian Markov Chain Monte Carlo (MCMC) to sample liability values at all nodes. The result is the proportion of MCMC generations in which the liability lies above the threshold for each node. This proportion can be interpreted as the likelihood that a given node exhibited the character.

We created a long MCMC (50 million generations) because we were running many variables simultaneously ( $K = 189$  variables, corresponding to the number of tips and internal nodes). Relative to short MCMCs, long MCMCs offer more generational time for variables to stabilize. Burn-in periods vary depending on the size of the chain, and the rate of stabilization among the variables. Some of our variables were slow to stabilize, so we programmed a 20% burn-in time, rather than the conventional 10% (Gordon et al. 2015, Lerp et al. 2016). Subsampling generations eases the computational burden of running large MCMCs. We took 1 sample every 100 generations.

We used effective sample size (ESS) and Geweke’s diagnostic to assess the MCMCs. Sequential samples from an MCMC are not independent, so the number of samples is not an accurate measure of the independent

*L* and *M*

Reconstructions of ancestral traits are shown in [Figure 1](#). The ancestral trait reconstruction for duetting was robust with 187 of 189 (99%) estimates based on an ESS

>200 and 42 of 189 (22.2%) Geweke's diagnostic indicating stable MCMCs. The migration reconstruction was similarly robust: 187 of 189 (99%) variables achieving an ESS >200 and 72 of the 189 (38.1%) variables indicating stable MCMCs. None of the variables had both significant



**FIGURE 1.** Threshold reconstruction of duetting (left tree) and migration (right tree) for 95 New World warblers. Circles at branch tips represent posterior estimates, and squares represent priors.

Geweke's and an ESS <200. All MCMC traces indicated relative stability over generational time.

Our duetting reconstruction indicates that the last common ancestor of the New World warblers did not duet (likelihood [duetting] = 0.016). We found strong evidence that duetting evolved relatively early in *M. ...* (likelihood [duetting] = 0.771), and *M. ...* (likelihood [duetting] = 0.765). In both groups, duetting appeared after the lineages lost migratory behavior. It is unlikely that the most recent common ancestors of *C. ...* (likelihood [duetting] = 0.041), *M. ...* (likelihood [duetting] = 0.005), or *M. ...* (likelihood [duetting] = 0.113), duetted. The basal nodes of



that both gains and losses of these traits may be integral in larger patterns, and that changes to migration behavior may drive changes in signaling. As in previous studies, we suspect pair-bond duration mediates the relationship between migration and duetting, and we encourage further study of the social behavior of the many species of New World warblers for which pair-bond duration is unknown. As in previous studies, duetting and sedentariness were associated with tropical and southern hemisphere breeding (Figure 2; Hall 2004, 2009, Logue and Hall 2014).

Past research suggests that there is little, if any, phylogenetic signal for female song in New World warblers, and no correlation between female song and migration (Najar and Benedict 2015). Duetting in Parulidae does not rely on the presence of female song because some New World warblers that duet lack female song (e.g., *Mniotilta*, which duets with a pip call; Staicer 1992), and some with female song do not duet (e.g., *Geothlypis*). The evolutionary dissociation between female song and duetting contrasts with New World blackbirds, in which female song is a prerequisite for vocal duetting (Odom et al. 2015). Although female song and duetting are not perfectly correlated in New World warblers, these 2 traits exhibit similar patterns within the group. An early New World warbler ancestor with female song occurred within the genus *Mniotilta*, and this genus showed high rates of both female song and duetting (Najar and Benedict 2015). Duets are also prevalent in *Mniotilta*, but it was impossible to accurately score female song in most of that group because the songs of these species are poorly described (Najar and Benedict 2015). In both Najar and Benedict (2015) and the present study, the accuracy of analyses is limited by the available data. It is likely that we have incorrectly scored some tropical duetting species as non-duetters because the vocal behavior of many tropical warbler species is poorly known. Our published dataset will permit updated analyses as more is learned about duetting behavior in tropical warblers.

Patterns of vocal evolution in New World warblers differ from patterns of vocal evolution in the closely related and well-studied New World blackbirds (Odom et al. 2015). In that family, female song is negatively correlated with non-migratory behavior, but after controlling for the presence of female song, the association between sedentariness and duetting is not significant (Odom et al. 2015). Odom et al. (2015) argue that, in blackbirds, female song is a prerequisite for the evolution of duetting, but that different selective pressures may promote the 2 signal types (Odom et al. 2015). An alternative hypothesis is that similar selective forces underlie female song and duetting but duetting takes longer to evolve. In warblers, duets are not restricted to lineages with female song, so the evolution of duetting may be less constrained than it is in blackbirds. New World warblers are not unique in this regard: many

duetting species do not produce learned songs at all (Hall 2004, 2009). Comparisons between the Icteridae and the Parulidae highlight the multiple avenues through which duets may evolve and covary with migratory behavior.

To summarize, our results describe a hypothesized evolutionary history of duetting and migration in New World warblers. There were sufficient character transitions in both duetting and migration to robustly establish the evolutionary relationship between these 2 traits. We found support for the inverse evolutionary link between duetting and migration, adding to the evidence for the generality of this pattern across multiple groups with varied traits. These findings provide a point of comparison with related taxa, and a foundation for future research on the evolution of duetting and migration in New World warblers.

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**Ethics statement:** Animal care is not relevant to this study, because we did not collect data from living birds.

**Author contributions:** D.M.L. conceived of the idea and design; L.R.M., N.N. and J.C. collected data; L.R.M., D.M.L., L.B. and N.N. wrote or substantially edited the paper; L.R.M. and D.M.L. designed and developed methods; L.R.M. and J.C. analyzed the data; and L.R.M. and D.M.L. contributed resources and funding.

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