1   **The evolution of vocal duets and migration in New World warblers (Parulidae)**

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14 **ABSTRACT**

15 Vocal duets occur when two individuals vocalize in temporal coordination. In birds, duet

16 participation functions to cooperatively defend shared resources, localize mates, and in some

17 species, guard the mate. Previous work indicates that duetting tends to co-evolve with a non-

18 migratory lifestyle, probably because the absence of migration facilitates greater cooperation

19 between mates. We examined the evolution of duetting and migration in New World warblers

20 (Parulidae), a group that has been largely ignored by duetting research. Of the 95 species in our

21 analysis, we found evidence of duetting in 19 (20%) species, and evidence of migration in 45

22 (47.4%) species. Ancestral character reconstruction indicated that the last common ancestor of

23 the New World warblers did not duet. Duetting evolved multiple times in this group, including

24   two early origins and several more recent origins. Migration was present in the last common

25   ancestor, and was lost several times. Both duetting and migration exhibit phylogenetic signal. A

26   phylogenetically explicit correlation analysis revealed a significant negative relationship between

27   duetting and migration, in keeping with findings from other avian taxa. This study, the first

28   description of the evolution of duetting in a large avian family with a temperate-zone origin,

29   supports the hypothesis that duetting co-evolves with a sedentary natural history in birds.

30

31   **Keywords:** ancestral character reconstruction, duet, migration, threshold model, warbler

32

33   **<u>INTRODUCTION</u>**

34   Vocal duets are acoustic signals that occur when two individuals vocalize in temporal

35   coordination (Hall 2009, Logue and Krupp 2016). Duetting is relatively common in birds,

36   occurring in 4-18% of species (Hall 2009, Tobias et al. 2016), including an estimated 1102

37   species of songbirds (Tobias et al. 2016). Duets are often composed of coordinated male and

38   female song, but may also include vocalization types other than song (Hall 2009, Benedict

39   2010). Cooperative territory defense is the primary function of duet participation in birds,

40   however duets are also used for contact maintenance and mate guarding in some species (Hall

41   2009).

42

43   Across diverse avian taxa, duetting evolves in concert with long-term pair bonds (Hall 2004,

44   Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). Pair-bond stability is thought to

45   promote cooperation, which in turn favors the evolution of signals that facilitate cooperation, like

46   duetting (Hall 2004, Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). Migration can

47   limit pair bond duration, which may explain why duetting is negatively associated with migration

48   in songbirds (Ens et al. 1996, Jeschke and Kokko 2008, Benedict 2008, Logue and Hall 2014,

49   Tobias et al. 2016). This evolutionary association between sedentary life-histories and duetting

50   may drive global geographic patterns in duetting: most duetting species breed outside of north-

51   temperate latitudes, where latitudinal migration is most common (Hall 2009, Logue and Hall

52   2014).

53

54   Previous studies on the evolution of avian duetting are based on taxonomically diverse samples

55   (Hall 2004, Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). Evolutionary studies that

56   focus on specific taxa can complement taxonomically diverse studies by asking whether general

57   patterns apply to the focal taxon (Odom et al. 2015). Consistent results in multiple clades would

58   lend support to the general conclusion. Conversely, divergent results would demand explanation.

59   Taxonomically restricted samples are also useful for the reconstruction of ancestral traits,

60   because they permit detailed exploration of evolutionary history and set the stage for future

61   taxon-specific investigations (Price et al. 2009, Price 2009, Odom et al. 2015).

62

63   Many New World warblers sing duets, but in contrast to well-studied duetting groups like wrens

64   (Troglodytidae), antbirds (Thamnophilidae), and blackbirds (Icteridae), there is virtually no peer-

65   reviewed research on duetting in warblers (Hall 2009). Females of many Parulidae species sing,

66   offering the potential for male-female song duets to evolve (Najar and Benedict 2015, Matthews

67   et al. 2017). Female warblers also produce call notes that are used in some duets (Staicer 1992).

68   All members of this clade are socially monogamous and territorial while breeding, as is typical

69   of species that duet (Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). Atypically for

70   duetting groups, however, this clade has a migratory ancestor that bred in the North-temperate

71   zone (Winger et al. 2012).

72

73   Several studies have demonstrated links between duetting and non-migratory life histories

74   among songbirds generally (Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). A

75   previous study found no evolutionary association between female song and migration in New

76   World warblers (Najar and Benedict 2015), but duet evolution does not always parallel female

77   song evolution (Odom et al. 2015), leaving open questions about what forces promote duetting

78   behavior in this clade. New World warblers' diversity (107 species, Lovette et al. 2010) and

79   proclivity to duet (≥ 19 duetting species, this paper) suggest they would be a useful taxon for

80   duetting research. For the present study, we analyzed the evolution of duetting and migration in

81   the New World warblers (family: Parulidae). Our goals were to describe the diversity of duetting

82   and migration in extant taxa, characterize the evolutionary histories of these two traits, and test

83   for their correlated evolution.

84

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86

87   **METHODS**

88

89   Our analyses used a recent phylogeny that includes 107 New World warbler species (Lovette et

90   al. 2010). The phylogeny was based on mitochondrial DNA (4695-4699 continuous base pairs)

91   and nuclear DNA (six introns, totaling 4602 aligned nucleotides), and was built using maximum

92   likelihood. Outgroups were pruned from the tree prior to analysis.

93

94 We scored duetting from *The Handbook of the Birds of the World* (hereafter, HBW: del Hoyo et

95 al. 2011), *New World Warblers* (Curson 1994), peer-reviewed papers, and queries of recording

96 archives from the Macaulay Library (date: Nov. 3, 2016; query terms: "Parulidae" and "duet"),

97 and Xeno-Canto.org (date: Nov. 9, 2016, query terms: "duet" or "antiphonal" in "Type" or

98 "Remark" fields, and all current and recent Parulidae genera in "Genus" field).

99

100 Birds were scored as duetting (coded 1) if one published source (HBW, New World warblers, or

101 a peer-reviewed paper) or two different recordists (from the MacCaulay or Xeno-Canto archives)

102 indicated that the species duets. If a published source contained detailed descriptions of the

103 species' male song (e.g., note number, note or song structure, pitch, context) with no mention of

104 duetting, the species was scored as non-duetting (coded 0). Species that could not be

105 unambiguously scored as duetting or non-duetting were labeled 'unknown' (12 of the 107

106 (11.2%) New World Warbler species). Species who were scored 'unknown' were not included in

107 the analyses. Migration was scored from the HBW. Species with subspecies that were

108 heterogeneous for migration were assigned the average of their subspecies scores and then

109 rounded to the nearest whole value (e.g., 1.0 if three of five subspecies migrate). Species that

110 only migrated altitudinally were scored as non-migratory (following Winger et al. 2012, Logue

111 and Hall 2014), based on the assumption that altitudinal migration is less likely to break up pair

112 bonds than is latitudinal migration. Scores and sources of scoring information are in the

113 electronic supplementary materials (ESM Tables S3 & S4).

114

115 We used the "threshold model" to reconstruct ancestral character states (Revell 2013). The

116 threshold model assumes discrete characters can be represented over evolutionary time by an

117 underlying continuous variable called "liability". Each of our models allowed two character

118 states: duetting / not duetting and migratory / non-migratory. Liabilities below a fixed threshold

119 correspond to one character state, and those above the threshold correspond to the other.

120 Character states of extant species were treated as prior probabilities, and liability evolved by

121 Brownian motion. We used a Bayesian Markov Chain Monte Carlo (MCMC) to sample liability

122 values at all nodes. The result is the proportion of MCMC generations in which the liability lies

123 above the threshold for each node. This proportion can be interpreted as the likelihood that a

124 given node exhibited the character.

125

126 We created a long MCMC (50 million generations) because we were running many variables

127 simultaneously ($N = 189$ variables, corresponding to the number of tips and internal nodes).

128 Relative to short MCMCs, long MCMCs offer more generational time for variables to stabilize.

129 Burn-in periods vary depending on the size of the chain, and the rate of stabilization among the

130 variables. Some of our variables were slow to stabilize, so we programmed a 20% burn-in time,

131 rather than the conventional 10% (Gordon et al. 2015, Lerp et al. 2016). Subsampling

132 generations eases the computational burden of running large MCMCs. We took one sample

133 every 100 generations.

134

135 We used effective sample size (ESS) and Geweke's diagnostic to assess the MCMCs. Sequential

136 samples from an MCMC are not independent, so the number of samples is not an accurate

137 measure of the independent sample size. ESS accounts for this non-independence by penalizing

138   samples according to their degree of intercorrelation. It is generally desirable to maximize the

139   ESS for all variables. Geweke's diagnostic tests the stability of the MCMC by comparing the

140   distribution of scores from the beginning of the post-burn-in period to the distribution of scores

141   from the end of the MCMC. If the MCMC has stabilized sufficiently by the end of the burn-in

142   period, the beginning of the post-burn-in and the end of the chain should appear to be from the

143   same distribution. We set alpha to 0.05. It is generally desirable to have nonsignificant results of

144   Geweke's diagnostic. In addition to these metrics, we visually inspected all MCMC traces. By

145   definition, MCMC's are never truly stable, but those with sufficient long-term stability for

146   accurate inference produce traces that look like rectangular "hairy caterpillars."

147

148   Phylogenetic signal (or phylogenetic inertia) is the tendency for related taxa to be more similar to

149   each other than they are to more distantly related taxa. We used Pagel's lambda to test for

150   phylogenetic signal among species with known characters. This test uses a likelihood ratio to

151   compare the hypothesized tree to a null tree with no phylogenetic signal (Lovette et al. 2010).

152   The null hypothesis is that there is no phylogenetic signal.

153

154   We tested for correlated evolution of duetting and migration using only species with 'known'

155   character scores. To include unknown species would have risked pseudoreplication, because

156   their scores were based on the scores of their congeners. Scores consisted of binary values, so we

157   used χ^2 tests to test for correlations between duetting and migration in extant species. We also

158   tested for a correlation between phylogenetically independent contrasts (Felsenstein 1985).

159   Contrast scores included numbers between one and zero, but did not meet the assumptions of

160   parametric tests, so we used Spearman's rank correlation.

161

162   We used the HBW to score the northern-most and southern-most breeding latitudes of all 95

163   New World warbler species used in the analyses. These points were then averaged to get the

164   central breeding latitude for each species (ESM Table S5).

165

166   All analyses were run in R (R Core Team 2013). Ancestral trait reconstructions used the

167   packages *ape* (Paradis et al. 2004) and *phytools* (Revell 2012). The test for phylogenetic signal

168   also used *phytools*. MCMC diagnostic tools were from the *coda* package (Plummer et al. 2006).

169   The correlation and phylogenetically independent correlation tests were performed using

170   functions from the *psych* (Revelle 2018) and *phytools* packages. The tree diagram was created

171   using the *ggtree* package (Yu et al. 2017). The latitude diagram was created using the *ggplot2*

172   package (Wickham 2016). Data and R code are available in the online supplementary materials.

173

174   **RESULTS**

175

176   Of the 107 New World warbler species, we were able to find sufficient data to score 95 (88.8%)

177   species with confidence. The remaining species were scored 'unknown' and dropped from the

178   analyses. We scored 19 of 95 (20%) New World warbler species as duetting and 76 (80%)

179   species as non-duetting (Table 1). We estimated duetting frequencies of 0% in the genera

180   *Cardellina, Helmitheros*, *Limnothlypis*, *Mniotilta, Oreothlypis, Parkesia, Protonotaria, Seiurus,*

181   *Vermivora*; 8% in *Geothlypis*; 9% in *Setophaga; 20% in Basileuterus; 57% in Myiothlypis; and*

182   *75% in Myioborus.* We scored 47 species (49.5%) as migratory and 48 species (50.5%) as non-

183   migratory. Genus-specific migration frequencies were 0% in *Basileuterus*, *Myioborus. and*

184 *Myiothlypis;* 33% in *Geothlypis*; *68% in Setophaga;* 75% in *Cardellina* and *Oreothlypis; and*

185 100% in *Helmitheros*, *Limnothlypis*, *Mniotilta*, *Parkesia*, *Protonotaria*, *Seiurus*, and *Vermivora.*
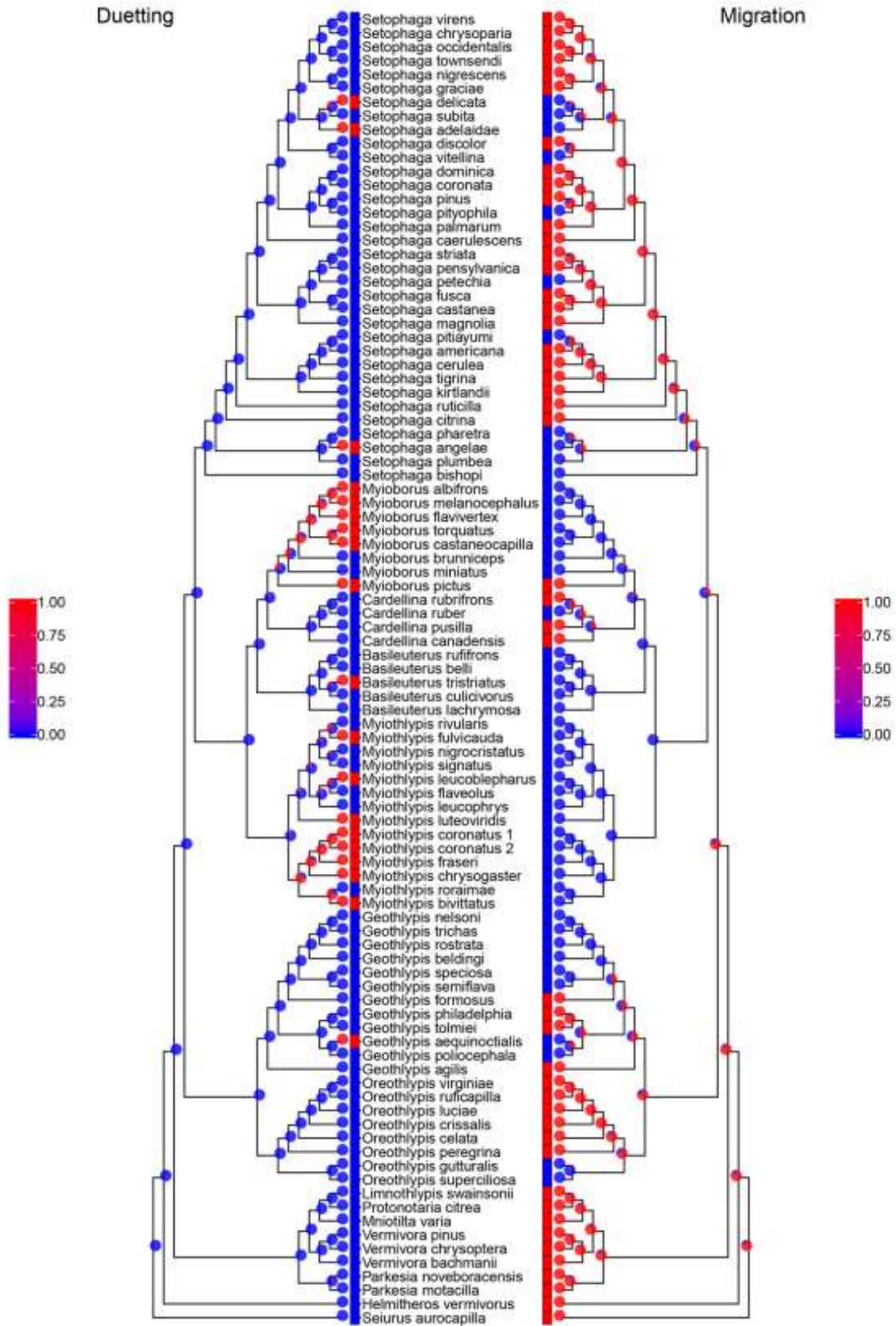
186

	Duetting	Non-duetting
Migratory	46	30
Non-migratory	18	1

187

188 **Table 1.**

189

190 Reconstructions of ancestral traits are shown in Figure 1. The ancestral trait reconstruction for

191 duetting was robust with 187 of 189 (99%) estimates based on ESS's >200 and 42 of 189

192 (22.2%) Geweke's diagnostic indicating stable MCMCs. The migration reconstruction was

193 similarly robust: 187 of 189 (99%) variables achieving an effective sample size >200 and 72 of

194 the 189 (38.1%) variables indicating stable MCMC's. None of the variables had both significant

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

Duetting

Setophaga virens
Setophaga chrysoparia
Setophaga occidentalis
Setophaga townsendi
Setophaga nigrescens
Setophaga graciae
Setophaga delicata
Setophaga subita
Setophaga adelaidae
Setophaga discolor
Setophaga vitellina
Setophaga dominica
Setophaga coronata
Setophaga pinus
Setophaga pityophila
Setophaga palmarum
Setophaga caerulescens
Setophaga striata
Setophaga pensylvanica
Setophaga petechia
Setophaga fusca
Setophaga castanea
Setophaga magnolia
Setophaga pitiayumi
Setophaga americana
Setophaga cerulea
Setophaga tigrina
Setophaga kirtlandii
Setophaga ruticilla
Setophaga citrina
Setophaga pharetra
Setophaga angelae
Setophaga plumbea
Setophaga bishopi
Myioborus albifrons
Myioborus melanocephalus
Myioborus flavivertex
Myioborus torquatus
Myioborus castaneocapilla
Myioborus brunniceps
Myioborus miniatus
Myioborus pictus
Cardellina rubrifrons
Cardellina ruber
Cardellina pusilla
Cardellina canadensis
Basileuterus rufifrons
Basileuterus belli
Basileuterus tristriatus
Basileuterus culicivorus
Basileuterus lachrymosa
Myiothlypis rivularis
Myiothlypis fulvicauda
Myiothlypis nigrocristatus
Myiothlypis signatus
Myiothlypis leucoblepharus
Myiothlypis flaveolus
Myiothlypis leucophrys
Myiothlypis luteoviridis
Myiothlypis coronatus 1
Myiothlypis coronatus 2
Myiothlypis fraseri
Myiothlypis chrysogaster
Myiothlypis roraimae
Myiothlypis bivittatus
Geothlypis nelsoni
Geothlypis trichas
Geothlypis rostrata
Geothlypis beldingi
Geothlypis speciosa
Geothlypis semiflava
Geothlypis formosus
Geothlypis philadelphia
Geothlypis tolmiei
Geothlypis aequinoctialis
Geothlypis poliocephala
Geothlypis agilis
Oreothlypis virginiae
Oreothlypis ruficapilla
Oreothlypis luciae
Oreothlypis crissalis
Oreothlypis celata
Oreothlypis peregrina
Oreothlypis gutturalis
Oreothlypis superciliosa
Limnothlypis swainsonii
Protonotaria citrea
Mniotilta varia
Vermivora pinus
Vermivora chrysoptera
Vermivora bachmanii
Parkesia noveboracensis
Parkesia motacilla
Helmitheros vermivorus
Seiurus aurocapilla

Migration

1.00
0.75
0.50
0.25
0.00

1.00
0.75
0.50
0.25
0.00

196

197   **Figure 1.**

198

199   Our duetting reconstruction indicates that the last common ancestor of the New World warblers

200   did not duet (likelihood(duetting) = 0.016). We found strong evidence that duetting evolved

201   relatively early in *Myioborus* (likelihood (duetting) = 0.771), and *Myiothlipis* (likelihood

202   (duetting) = 0.765). In both groups, duetting appeared after the lineages lost migratory behavior.

203   It is unlikely that the most recent common ancestors of *Cardellina* (likelihood (duetting) =

204   0.041), *Setophaga* (likelihood (duetting) = 0.005), or *Myiothlypis* (likelihood (duetting) = 0.113)

205   duetted. The basal nodes of *Basileuterus*, *Geothlypis*, *Oreothlypis*, and the basal clade that

206   includes *Limnothlypis* and *Parkesia* show little or no evidence of duetting. The lineages leading

207   to extant duetters outside of *Myiothlipis* and *Myioborus* (e.g., in *Setophaga*) appear to have

208   evolved duetting recently.

209

210   Our migration reconstruction suggests the last common ancestor of all New World warblers

211   probably migrated (likelihood(migration) = 0.824). The last common ancestors of the genera

212   *Myioborus* (likelihood(migration) = 0.133), *Basileuterus* (likelihood(migration) = 0.050)*,* and

213   *Myiothlypis* (likelihood(migration) = 0.0.015) probably did not migrate. Reconstructions are

214   equivocal for the last common ancestors of C*ardellina* (likelihood(migration) = 0.406)*,* and

215   *Setophaga* (likelihood(migration) = 0.436). There is stronger evidence of migration in the last

216   common ancestors of the clade comprising *Oreothlypis* and *Geothlypis* (likelihood(migration) =

217   0.773), and the basal clade that includes *Limnothlypis* and *Parkesia* (likelihood(migration) =

218   0.904)*.* There is also some evidence of migration at the first split of *Setophaga*

219   (likelihood(migration) = 0.571) and the last common ancestor of *Geothlypis*

220   (likelihood(migration) = 0.615). Our reconstructions suggest that transitions to non-migratory

221 natural histories began early in the diversification of the New World warblers, but duetting

222 evolved more recently.

223

224 The tests for phylogenetic signal were statistically significant for both duetting ($\lambda = 0.99$, p <

225 0.0001) and migration ($\lambda = 0.87$, p < 0.0001). Without controlling for phylogeny we found a

226 significant, negative correlation between duetting and migration among known, extant species

227 (Chi square: x-squared = 18.57, p < 0.0001, df = 1). After controlling for phylogeny with the

228 method of independent contrasts, the correlation remained significant (Spearman's rank

229 correlation: rho = -0.25, p = 0.015, df = 94). Duetting was associated with lower breeding

230 latitudes and migration was associated with higher breeding latitudes in our sample (Fig. 2).

231
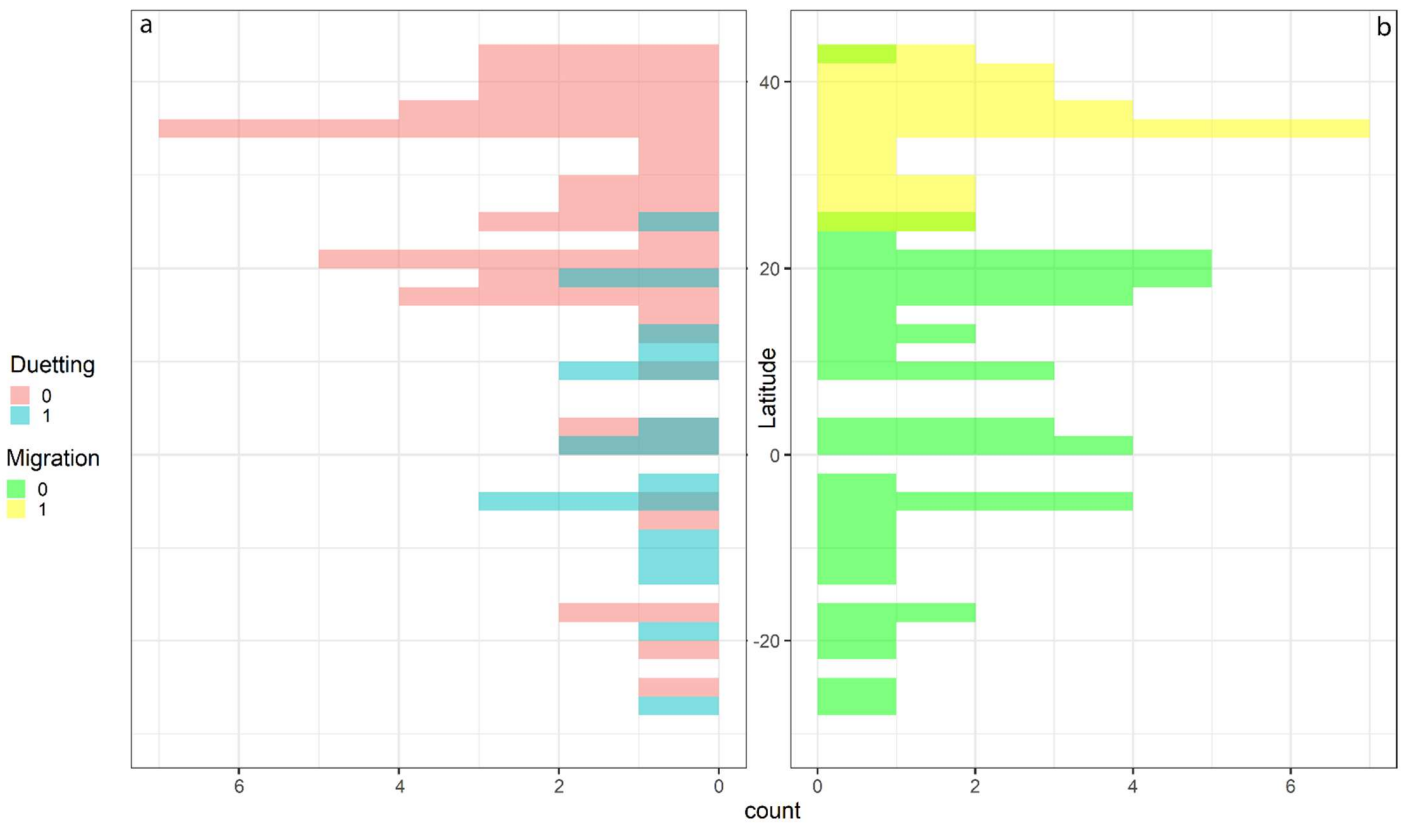
232

233 **Figure 2.**

234

DISCUSSION

235 **DISCUSSION**

236

237 Our analysis of the prevalence of vocal duetting in extant New World warblers indicated that

238 duetting occurs in 20% of New World warbler species we were able to score. This estimate is

239 similar to the estimates for New World blackbirds (18%, Odom et al. 2015), songbirds (15.6%,

240 Logue and Hall 2014), and all birds (~16%, Tobias et al. 2016). Duetting is non-randomly

241 distributed among New World warblers, with the highest concentration in the genera *Myioborous*

242 and *Myiothlypis.*

243

244 There is strong evidence that the last common ancestor of the extant Parulidae did not duet, and

245 that duetting originated multiple times in the family. The ancestral trait reconstruction suggests

246 duetting evolved twice during the early diversification of the family: at or near the common

247 ancestor of *Myioborous* and after the first split of *Myiothlypis*. There have also been several

248 recent gains of duetting, especially in *Setophaga* and *Myiothlypis*. Duetting appears to have been

249 lost in the lineages leading to *Myioborous brunniceps* and *Myiothlypis rorimae*. Both of those

250 species, however, are poorly known to science, so further investigation may reveal that they duet.

251 We conclude that duetting has probably been gained more than it has been lost in the New World

252 warblers. The recent gains (and possible losses) of duetting are evidence that duetting can evolve

253 rapidly, while the finding of strong phylogenetic signal indicates that duetting has been

254 conserved over evolutionary time in some lineages. Similar patterns were found in the New

255 World blackbirds: the common ancestor did not duet, gains of duetting exceeded losses, and

256 duetting evolved both recently and earlier in the clade's evolutionary history (Icteridae; Odom et

257 al. 2015). The occurrence of both ancient and recent evolutionary transitions from non-duetting

258 to duetting make Parulidae and Icteridae model families for research on duet evolution. For

259 example, we might use these groups to test whether duets tend to increase in structural and

260 temporal coordination over evolutionary time.

261

262 We scored 49.4% of the New World warblers in our sample as migrating latitudinally. This is

263 similar to the results of two previous efforts to score migration in Parulidae, which arrived at

264 estimates of 45.4% and 46.7%, respectively (Winger et al. 2012, Najar and Benedict 2015). The

265 difference in estimates is largely attributable to the fact that, unlike the present study, those

266 studies scored partial migrants (as opposed to rounding them). Additionally, in the case of the

267 2015 study, altitudinal migrants were also scored as migratory. New World warblers are more

268 prone to migration than are birds in general, of which ~ 18.5% are estimated to migrate (Rolland

269 Jonathan et al. 2014). Like duetting, migration was heterogeneously distributed throughout the

270 family, and exhibited phylogenetic signal. The last common ancestor of Parulidae probably

271 migrated. This finding is reflected in the high migration rates among the basal taxa and matches

272 the conclusions of two previous studies (Winger et al. 2014, Najar and Benedict 2015). Ten

273 genera are mostly migratory, and three large genera do not migrate at all (including both of the

274 genera with the highest duetting rates). Early losses of migration occurred in the last common

275 ancestor of *Geothlypis*, and the last common ancestor of the clades *Myioborus* through

276 *Setophaga*. There was an early secondary evolution of migration in *Setophaga*. There is also

277 evidence of recent losses (*Setophaga delicata* through *S. adelaidae*, *S. vitellina, S. pityophila, S.*

278 *pitayumi, Geothlypis semperi*, and *Oreothlypis peregrina* through *O. superciliosa*) and gains of

279 migration (*C. rubrifons* through *C. canadensis*, *G. formosus* through *G. tolmiei*, and *G. agilis*).

280

281   Migration is generally a labile trait in birds (Pulido 2007). Our findings largely support the idea

282   that migration is evolutionarily labile in New World warblers. We also see evidence that

283   migration status is conserved in some lineages, a finding that is supported by the significant

284   result from our test for phylogenetic signal. Our results replicate Winger et al.'s (2012) finding

285   of phylogenetic signal for migration in Parulidae. That study used different scoring and analytic

286   methods, strengthening the value of this replication. In this study we found that Pagel's λ for

287   migration and duetting were similar, but the value for duetting was slightly higher (0.95 versus

288   0.89 for migration), suggesting a stronger phylogenetic signal for duetting than for migration.

289   Some vocal traits change too rapidly to produce phylogenetic signal (Rheindt et al. 2004). Others

290   can be conserved over time, both revealing and reflecting evolutionary history (Price and Lanyon

291   2002). The results of this study suggest that duetting in New World warblers is a vocal trait that

292   evolves slowly enough to produce phylogenetic signal, but is labile enough to have evolved

293   recently in the phylogeny.

294

295   We believe our trait reconstructions for both duetting and migration are valid despite the

296   occurrence of some low ESS's and significant Geweke's diagnostics. The low ESS's occurred on

297   short branches with homogenous local environments. Those conditions generate strong

298   correlations between subsequent generations (poor mixing). We used a large number of

299   generations to increase the ESSs, but that decision also increased the number of significant

300   Geweke's diagnostics. Importantly, the significant Geweke's diagnostics were attributable to

301   high power, rather than large effects (ESM text, Figs. S1 & S2). The similarity in diagnostic

302   metrics between our two reconstructions, coupled with confirmation of the migration results

303 from other studies (Winger et al. 2012, Najar and Benedict 2015), gives us confidence in our

304 duet evolution results.

305

306 Our results support previous findings that latitudinal migration and duetting are negatively

307 correlated in birds (Benedict 2008, Logue and Hall 2014, Tobias et al. 2016). We scored all

308 duetting New World warblers as non-migratory, and a phylogenetically controlled correlation

309 showed a significant relationship between these two traits. Because the ancestral New World

310 warbler was migratory and non-duetting, this pattern necessarily results from gains of duetting in

311 lineages that also lost migration. Estimated trait values at nodes within the phylogeny (Fig. 1)

312 suggest that losses of migration typically occurred first, and then duets evolved in sedentary

313 species. A previous study linking these two traits found that the relationship between them was

314 largely driven by losses of duetting in migratory species (Logue and Hall 2014). Results

315 presented here indicate that both gains and losses of these traits may be integral in larger

316 patterns, and that changes to migration behavior may drive changes in signaling. As in previous

317 studies, we suspect pair bond duration mediates the relationship between migration and duetting,

318 and we encourage further study of the social behavior of the many species of New World

319 warblers for which pair bond duration is unknown. As in previous studies, duetting and

320 sedentariness were associated with tropical and southern hemisphere breeding (Fig. 2, Hall 2004,

321 2009; Logue and Hall 2014).

322

323 Past research suggests that there is little, if any, phylogenetic signal for female song in New

324 World warblers, and no correlation between female song and migration (Najar and Benedict

325 2015). Duetting in Parulidae does not rely on the presence of female song because some New

326   World warblers that duet lack female song (e.g., *S. adelaidae*, which duets with a *pip* call

327   (Staicer 1992) and some with female song do not duet (e.g., *S. ruticilla*). The evolutionary

328   dissociation between female song and duetting contrasts with New World blackbirds, in which

329   female song is a pre-requisite for vocal duetting (Odom et al. 2015). Although female song and

330   duetting are not perfectly correlated in New World warblers, these two traits exhibit similar

331   patterns within the group. An early New World warbler ancestor with female song occurred

332   within the genus *Myiothlypis,* and this genus showed high rates of both female song and duetting

333   (Najar and Benedict 2015). Duets are also prevalent in *Myioborus*, but it was impossible to

334   accurately score female song in most of that group because the songs of these species are poorly

335   described (Najar and Benedict 2015). In both Najar & Benedict (2015) and the present study, the

336   accuracy of analyses is limited by the available data. It is likely that we have incorrectly scored

337   some tropical duetting species as non-duetters because the vocal behavior of many tropical

338   warbler species is poorly known. Our published dataset will permit updated analyses as more is

339   learned about duetting behavior in tropical warblers.

340

341   Patterns of vocal evolution in New World warblers differ from patterns of vocal evolution in the

342   closely related and well-studied New World blackbirds (Odom et al. 2015). In that family,

343   female song is negatively correlated with non-migratory behavior, but after controlling for the

344   presence of female song, the association between sedentariness and duetting is not significant

345   (Odom et al. 2015). Odom et al. (2015) argue that, in blackbirds, female song is a prerequisite for

346   the evolution of duetting, but that different selective pressures may promote the two signal types

347   (Odom et al. 2015). An alternative hypothesis is that similar selective forces underlie female

348   song and duetting but duetting takes longer to evolve. In warblers, duets are not restricted to

349 lineages with female song, so the evolution of duetting may be less constrained than it is in

350 blackbirds. New World warblers are not unique in this regard: many duetting species do not

351 produce learned songs at all (Hall 2004, 2009). Comparisons between the Icteridae and the

352 Parulidae highlight the multiple avenues through which duets may evolve and covary with

353 migratory behavior.

354

355 To summarize, our results describe a hypothesized evolutionary history of duetting and

356 migration in New World warblers. There were sufficient character transitions in both duetting

357 and migration to robustly establish the evolutionary relationship between these two traits. We

358 found support for the inverse evolutionary link between duetting and migration, adding to the

359 evidence for the generality of this pattern across multiple groups with varied traits. These

360 findings provide a point of comparison with related taxa, and a foundation for future research on

361 the evolution of duetting and migration in New World warblers.

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368

369 **Author Contributions**

370   D.L. conceived of the idea and design; L.M., N.N., and J.C. collected data; L.M., D.L., L.B., and

371   N.N wrote or substantially edited the paper; L.M. and D.L. designed and developed methods;

372   L.M. and J.C. analyzed the data; and L.M. and D.L. contributed resources and funding.

373

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459

460 **APPENDIX**

461 **Figure captions:**

462 **Table 1.** Table depicting the co-occurrence of duetting and migratory character for 95 New

463 World warblers. Numbers indicate number of species that exhibit the characters orthogonal to

464 them.

465

466 **Figure 1.** Threshold reconstruction of duetting (left tree) and migration (right) for 95 New World

467 warblers. Circles at branch tips represent posterior estimates, and squares represent priors.

468

469 **Figure 2.** The distribution of breeding latitudes as a function of (a) duetting and (b) migration in

470 a sample of 95 New World warbler species. Positive latitudes are in degrees north, and negative

471 latitudes are in degrees south.

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