Behavioral, morphological, and ecological trait evolution in two clades of New World Sparrows (*Aimophila* and *Peucaea*, Passerellidae)

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The New World sparrows (Passerellidae) are a large and diverse group of songbirds that have been the subject of many studies on ecology, behavior, and evolutionary relationships. Here, we studied two clades of sparrows (*Aimophila, Peucaea*) to examine the evolution of behavioral, morphological, and ecological traits in a phylogenetic framework. Specifically, we inferred phylogenetic relationships in these clades, conducted ancestral state reconstructions, and asked whether patterns of trait evolution extend more broadly to New World Sparrows. Our phylogenetic analyses recovered relationships that support recent taxonomic revisions and improve our understanding of relationships among species within the genera *Aimophila* and *Peucaea*. Analyses of trait evolution revealed that behavioral traits exhibit stronger phylogenetic signal than morphological traits within these genera and more broadly across New World Sparrows. The most highly conserved derived trait was the presence and structure of song duets. Song structure also tended to be maintained within clades once evolved, and there appears to be a trade-off between song complexity and plumage patterning. Habitat covaries with some traits in our focal clades but is not the sole evolutionary driver, because even within lineages that share the same habitat type, species exhibit variation in nesting, plumage patterning, song complexity, and duetting behavior. Our study uncovers interesting patterns of phenotypic evolution in New World Sparrows and highlights the strong phylogenetic signal of behavior in this group. More broadly, our study reinforces the value of examining behavioral, morphological, and ecological traits in combination with phylogenies to reveal patterns of evolution.

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Abstract

The New World sparrows (Passerellidae) are a large and diverse group of songbirds that have been the subject of many studies on ecology, behavior, and evolutionary relationships. Here, we studied two clades of sparrows (*Aimophila, Peucaea*) to examine the evolution of behavioral, morphological, and ecological traits in a phylogenetic framework. Specifically, we inferred phylogenetic relationships in these clades, conducted ancestral state reconstructions, and asked whether patterns of trait evolution extend more broadly to New World Sparrows. ~~Our phylogenetic analyses recovered relationships~~ ~~that~~ support recent taxonomic revisions and improve our understanding of relationships among species within the genera *Aimophila* and *Peucaea*. ~~Analyses of trait evolution revealed that~~ behavioral traits ~~exhibit~~ stronger phylogenetic signal than morphological traits within these genera and ~~more broadly across~~ New World Sparrows. The most highly conserved derived trait was the presence and structure of song duets. Song structure also tended to be maintained within clades ~~once evolved~~, and there appears to be a trade-off between song complexity and plumage patterning. Habitat covaries with some traits in our focal clades but ~~is not the sole evolutionary driver,~~ ~~because~~ even within lineages that share the same habitat type, species ~~exhibit variation~~ in nesting, plumage pattern~~ing~~, song complexity, and duetting ~~behavior~~. Our study uncovers interesting patterns of phenotypic evolution in New World Sparrows and highlights the strong phylogenetic signal of behavior in this group. More broadly, our study reinforces the value of examining behavioral, morphological, and ecological traits in combination with phylogenies to reveal patterns of evolution.

Introduction

Behavioral, morphological, and ecological traits have been used historically to reconstruct evolutionary relationships, and many taxonomic groups were originally designated on the basis of shared, homologous characters (e.g., Hamilton; 1962; Storer, 1955; Wolf, 1977). For example, Lanyon (1984; 1985; 1986; 1988a; 1988b) used similarities in syringeal and cranial morphology, plumage, nesting behavior, and foraging mode to establish generic limits and hypothesize relationships in tyrannid flycatchers, one of the world's largest and most diverse avian radiations. Likewise, Hamilton (1962) inferred species relationships and the origin of sympatry in the avian genus *Vireo* by comparing species-specific characteristics of distribution, habitat preference, foraging ecology, and external morphology. Although recent studies of evolutionary relationships have largely depended on genetic data, studies of trait evolution in a phylogenetic framework can shed light on patterns of phenotypic evolution and diversification.

Phenotypic traits may exhibit differences in phylogenetic signal, or the propensity to recapitulate phylogenetic relationships (Blomberg, 2003), due to variation in trait lability, selective pressures, or random processes (Grant & Grant, 2002). Differences in the rate of phenotypic evolution, as well as multiple gains or losses of a trait within large groups, can lead to patterns of character variation that differ across taxa (Dodd et al. 1999; Mooers et al., 1999). Although morphological traits are thought to change relatively slowly through time and thus have higher phylogenetic signal compared to traits such as behavior (Blomberg et al., 2003), studies have shown that both morphological and behavioral traits can be phylogenetically informative (deQueiroz & Wimberger, 1993; Kamilar & Cooper, 2013) and show conservative (e.g., Cicero & Johnson, 2002; Brumfield et al., 2007; Anderson & Wiens, 2017) as well as labile patterns of evolution (e.g., Cicero & Johnson, 1998; Price & Lanyon, 2004; Price et al., 2007; Barve & Mason, 2015; Fang et al., 2018).

Molecular phylogenies facilitate tests of how ~~different types of~~ traits evolve within clades. For example, Cicero and Johnson (2002) constructed a mitochondrial DNA (mtDNA) phylogeny of the *Empidonax* group of tyrant flycatchers to test Lanyon's (1986) hypothesis of relationships based on morphologic, behavioral, and allozymic characters. In that study, they found strong congruence between mtDNA sequences and other characters used to hypothesize relationships, and reported that some behaviors (e.g., nesting, migratory tendency) are more phylogenetically informative than others (e.g., foraging mode). In comparing song and plumage evolution in *Icterus* orioles, Price et al. (2007) found that both trait classes exhibit high lability between taxa within the clade, yet were conserved when considered across the genus as a whole. Furthermore, they found that songs in *Icterus* were more labile than those in oropendolas (*Psarocolius, Ocyalus*), a closely related group of icterids with highly conserved song features that have proven useful in assessing taxonomic relationships.

The New World sparrows (Passerellidae, formerly Emberizidae) are an excellent group for studying trait evolution in a phyogenetic framework because of their behavioral, morphological, and ecological diversity. These sparrows form a large and diverse lineage of songbirds that have been the subject of numerous molecular and non-molecular studies aimed at resolving their evolutionary relationships (e.g., Wolf, 1977; Patten & Fugate, 1998; Carson & Spicer, 2003; DaCosta et al., 2009; Klicka & Spellman, 2007; Klicka et al., 2014; Bryson et al., 2016; Sandoval et al., 2017). Evolutionary studies of New World sparrows include analyses based on morphology, plumage, soft-part colors, behavior, egg coloration, allozymes, mitochondrial and nuclear gene sequences, and phylogenomic data from ultraconserved elements. Although comparisons among these studies are compounded by differences in taxon and character sampling, together they provide a valuable framework for studying sparrow evolution.

One especially interesting group is the historical genus *Aimophila*, which has been plagued by uncertainty in classifications due to extensive morphological variation. Members of this group were originally classified together based on characteristics of the bill, wings, tail, and feet (Swainson, 1837; Baird, 1858), but ornithologists have long thought that they represent species from unrelated lineages (Ridgway, 1901; Dickey & Van Rossem, 1938; Storer, 1955; Wolf, 1977). Within the past decade, molecular data (e.g., DaCosta et al., 2009; Klicka et al., 2014; Sandoval et al., 2017) have clarified relationships and demonstrated polyphyly of the "*Aimophila*" group. This necessitated a taxonomic revision (Chesser et al., 2010; Remsen et al., 2010) that placed species formerly classified as *Aimophila* into one of three genera (*Aimophila, Peucaea, Rhynchospiza*), recognized the alliance of "*Aimophila*" *quinquestriata* with an unrelated genus *Amphispiza*, and moved some taxa from the related genus *Pipilo* to *Melozone*. Furthermore, species of *Aimophila, Melozone*, and *Pipilo* form a clade separate from *Peucaea* and *Rhynchospiza*.

Prior to molecular phylogenetic studies, species relationships in *Aimophila* sensu lato were hypothesized based on detailed study (Wolf, 1977) of behavioral, morphological, and ecological differences that grouped taxa into one of three ecological "complexes": (1) *Haeomophila* complex (currently *Peucaea*: species *ruficauda, sumichrhasti, humeralis, mystacalis, carpalis*), which radiated in lowland thorn scrub forests of western Mexico and the Pacific lowlands of Central America, and are characterized by simple songs, chatter duets derived mostly from primary songs, prenuptial molt, raised nests, heavy bills, patterned adult plumages, juvenile plumages more similar to adults than in other groups, and mostly delayed skull ossification; (2) *botterii* complex (also *Peucaea*: species *aestivalis, botterii, cassinii*), which occupy weedy, open country in Central and North America, have dull plumages (often with yellow at the bend of the wing), are migratory with more pointed wings, sometimes have spotted first-year plumages, and sing complex songs with chitter duet; and (3) *ruficeps* complex (currently *Aimophila*: species *ruficeps, rufescens, notosticta*), which radiated in pine-oak woodlands of Mexico and Central America and have similar primary songs, chatter duets not derived from primary song, and similar plumage patterns with rusty head stripes or caps. Wolf (1977) also suspected a close relationship between the *ruficeps* complex and towhees in the genus "*Pipilo*" (now *Melozone*) based on behavioral similarities – a hypothesis since supported by molecular data (Spicer & Carson, 2003; DaCosta et al., 2009; Klicka et al., 2014). He was uncertain about the placement of another species *quinquestriata* because of its unique plumage and song traits, and did not include two South American species (currently *Rhynchospiza*: species *stolzmani* and *strigiceps*) in his study.

The detailed phenotypic analysis by Wolf (1977) provides an opportunity to revisit questions about trait evolution (Maddison, 1994) within the two clades of *Aimophila* (plus *Melozone* and *Pipilo*) and *Peucaea* (plus *Rhynchospiza*) in a modern phylogenetic comparative framework. In this study, we focus on whether behavior, morphology, and/or ecology exhibit phylogenetic signal in these two clades, and extend these ideas to a larger group of New World sparrows.

Specifically, we ask which traits identified as informative by Wolf (1977) are phylogenetically conservative or labile. We also use these data to assess the extent to which behavioral and morphological traits are associated with open (grassland) versus closed (arid scrub or pine oak) habitat types. If we find a strong association, then species in the same habitat type (i.e., same ecological group) may have been subjected to either phylogenetic niche conservatism (Pyron et al. 2015) or similar selective pressures that drive local adaptation to the environment (Lenormand 2012). ~~.~~ In either case, we would predict that traits are more conserved among species in similar habitats than among those in different habitats. Whether behavioral traits are more labile (~~e.g.,~~ Blomberg et al., 2003) or conserved (~~e.g.,~~ Brumfield et al., 2007) than morphological traits remains an open question; therefore, we do not have a priori expectations about which traits most closely track phylogenetic relationships in these sparrows.

Material & Methods

DNA sequencing

~~Because of differences among prior studies in taxon and character sampling,~~ we constructed an independent phylogeny of sparrows with a focus on all species formerly in the genus *Aimophila* but that are now divided into three genera: *Peucaea* (22 samples), *Aimophila* (8), and *Rhynchospiza* (4). We also included samples of *Pipilo* (9) and *Melozone* (4) because of their closeness to the *ruficeps complex* based on both behavioral (Wolf, 1977) and molecular (DaCosta et al., 2009) data. In addition, we sequenced two individuals of *Amphispiza quinquestriatia*, which has been classified either in *Aimophila* (Dickinson, 2003) or *Amphispiza* (Chesser et al., 2010), along with *Amphispiza bilineata* (3) and a former congener that is now placed in a monotypic genus (~~*Artemisiospiza belli*~~, 2; Klicka & Spellman, 2007; Chesser et al., 2010; Klicka & Banks, 2011). Using other phylogenetic studies of "*Aimophila*" and passerellid sparrows (Carson & Spicer, 2003; DaCosta et al., 2009; Klicka et al., 2014; Bryson et al., 2016) as a framework for comparison, we added species in 10 other sparrow genera: *Arremon* (4), *Chondestes* (2), *Oriturus* (2), *Spizella* (4), *Pooecetes* (2), *Ammodrammus* (4), *Ammospiza* (2), *Passerculus* (2), *Arremonops* (2), and *Zonotrichia* (2). Our total taxon set included 80 samples from 43 species in the Passerellidae and 4 non-sparrow outgroups (2 Parulidae, 2 Icteridae; Table S1).

We extracted genomic DNA from tissue using a modified salt extraction procedure (Miller et al., 1988), and then PCR-amplified and sequenced four protein-coding mitochondrial genes (cyt-*b*, ND2, ATPase 8, COI) and three nuclear gene regions (intron 5 of transforming growth factor beta 2 [TGFb2] and beta-fibrinogen [Fib5], recombination activating gene RAG-1) using various combinations of primers (Table S2). We focused on the core ingroup taxa and putative allies for all loci (total of 5344 bp: 3495 mtDNA, 1849 nDNA), and added mtDNA sequences from GenBank to fill out the taxon sampling (Table S1). PCR-amplification and sequencing were generally successful except for a few samples at some loci. We amplified DNA in 25 µL reactions with a mixture of 2 µL dNTPs (2mM), 2.5 µl BSA (10 mM), 1.5 µL of each primer pair (10 mM), 2.5 µL of buffer (10x) pre-mixed with MgCl2, 0.1 µL of *Taq* polymerase, 1 µL of DNA, and double-distilled water. Amplification steps included an initial denaturation at 93°C for 4 min followed by 30-35 cycles of denaturation (93°C for 30 s), annealing (42-50°C for 30 s), and extension (72°C for 45 s), and a final extension at 72°C for 5 min. Reactions had at least one negative and often a positive control, and we visualized PCR products on agarose gels stained with ethidium bromide. Following amplification, we cleaned the PCR products with Exonuclease

I and Shrimp Alkaline Phosphatase (ExoSAP-IT, US Biochemical Corp.), and sequenced the purified products in both directions using Big Dye terminator chemistry v. 3.1and an AB PRISM 3730 DNA Analyzer (Applied Biosystems). We checked and aligned all sequences using CodonCode Aligner v. 4.0.3 (CodonCode Corporation).

Phylogenetic analyses

We constructed phylogenetic trees using all 80 ingroup and 4 outgroup samples (Table S1) with mitochondrial and nuclear loci by performing Bayesian and maximum-likelihood concatenated analyses alongside species-tree inference. For the concatenated analyses, we first identified the best-performing model of sequence evolution for each locus and codon position for gene regions via Akaike Information Criterion with MrModeltest v. 2.3 (Nylander, 2004). We then constructed Maximum Likelihood (ML) phylogenies using RAxML v7.0.4 (Stamatakis, 2006; Stamatakis et al., 2008), in which we performed 100 iterations of rapid bootstrapping while simultaneously finding the best tree in a single run with a GTR + I + G model of nucleotide substitution for each locus or gene region. We used BEAST v2.5.1 (Drummond & Rambaut, 2007; Drummond et al., 2012; Bouckaert et al., 2014) on the CIPRES Science Gateway (Miller et al., 2010) to conduct concatenated analyses in a Bayesian framework, in which we linked an uncalibrated clock model across loci but applied a separate HKY + I + G model of nucleotide substitution to each locus. We linked the tree prior for all loci and implemented a Yule model of speciation. We selected the Yule model because it is the simplest model of speciation, where each lineage is assumed to have the same constant speciation rate, and is also appropriate for inferring phylogenies among species rather than among populations within species (https://www.beast2.org). We ran the BEAST analysis for 1 x 10^8 generations while sampling every 1000 generations. We discarded the first 10% of sampled generations as burn-in, and assessed convergence and mixing by ensuring ESS scores for each parameter exceeded 300 in Tracer v1.7.5.

We conducted a species-tree analysis using the *BEAST package within BEAST v2.5.1 (Bouckaert et al., 2014). For this analysis, we implemented a Yule speciation model and a constant population model with estimated population sizes for each gene tree and the resultant species tree. We ran the species-tree analysis for 1 x 10^9 generations and removed the first 10% as burn-in. For both the BEAST and *BEAST analyses, we subsequently generated maximum clade credibility trees from a thinned set of 5000 trees that was sampled every 20,000 or 200,000 generations, respectively.

Trait reconstructions

We scored 12 trait variables (9 binary and 3 multi-state) for each species (Table S3). Of these, 11 traits were described in detail by Wolf (1977) and we followed his scheme in assigning values as closely as possible. These included range size, typical habitat, plumage “brightness” (hereafter referred to as patterning), completeness of the postjuvenal molt, presence of a prenuptial molt, nest position, timing of skull ossification, group breeding, song structure, duetting, and duet type. We added geographic distribution as an additional trait in order to reconstruct its history in our focal clades. We assigned trait values based primarily on published information, which we took directly from Wolf (1977) for the species that he included, but we had to interpret and standardize definitions for some traits (e.g., range size, plumage patterning) and for species not studied by Wolf. We used available audio recordings (Wolf, 1977 LP of audio recordings; Macaulay Library) to characterize song structure.

Binary traits used in trait reconstructions and tests of phylogenetic signal are described as follows:

(1) Plumage patterning: Unpatterned species are generally black or tan, but may show small patches of color or clearly delineated markings, such as the facial patterns on *Aimophila sumichrasti*. Patterned species have large patches of color that differ from the rest of the body.

(2) Postjuvenal molt: This molt is complete in species where individuals molt the entire plumage at this life stage, and incomplete in species where individuals molt only part of the plumage.

(3) Prenuptial molt (also known as prealternate molt): This molt is present in some species and absent in others.

(4) Skull ossification: Normal species have fully ossified skulls by the end of the first year. Skull timing is delayed in species where this process takes longer than one year.

(5) Nest position: Ground nesters typically build their nests on the ground. All species that build nests off the ground, regardless of height, are considered to have raised nests.

(6) Group breeding: Species where more than a pair of adults occur together during the breeding season are considered to have groups. For example, Wolf (1977) characterized *Aimophila ruficauda* as having groups because he observed one female, one adult male, and additional first year males in the same breeding flock. This differs from other species where a single pair occurs on a territory. We scored group breeding as present only if the species frequently or regularly breeds in groups.

(7) Song structure: Simple songs consist of a relatively small number of note types, although the notes may be repeated many times. They include songs with consistent syntax, including those that begin with a few introductory notes, followed by a trill. Complex songs include a highly variable array of note types and syntactical constructions. Song structure determinations were based on Wolf (1977), other published reports (e.g., Rodewald, 2015), and examination of sound files (Table S3).

(8) Duetting: Duet is ~~present~~ when two individuals ~~regularly~~ time their vocalizations to ~~coincide~~ in a predictable manner.

(9) Geographic distribution: Northern Temperate species have breeding ranges in North America~~, sometimes extending south into Mexico~~. Middle American species breed between Mexico and Panama.

Multiple-state traits used in trait reconstructions are described as follows:

(10) Range size: We followed Wolf (1977)'s characterization of species as having small, medium, or large breeding ranges, which we measured from his published distribution maps as ca. 240 km long at the longest diameter, 240-800 km long, and over 800 km long, respectively. This trait reflects the restricted distribution of some species (e.g., *A. notosticta*, confined to the mountains of central and northern Oaxaca) compared to more widespread taxa.

(11) Habitat: Arid scrub species coincide with Wolf's (1977) "thorn-scrub" category and live mostly in dry environments characterized by low, bushy vegetation. Pine-oak species live in woodlands that may be dominated by pine and/or oak trees. Grassland species live in open environments with predominantly grassy, herbaceous vegetation. Although this character has three states, we converted it to binary for trait correlation analyses. We designated grassland as

"open" habitat, and both thorn-scrub and pine-oak as "closed" habitat (Boncoraglio and Saino 2007).

(12) Duet type: We used Wolf's (1977) named duet types to indicate duet structure, and only included character states for species that he coded because these designations are somewhat subjective. Squeal duets have broadband elements that sound like squeals. Chitter and chatter duets have similar brief broadband ticking elements. *Aimophila carpalis* gives a unique "warbled" (Wolf 1977) duet.

~~The~~ ML and BEAST trees with the concatenated dataset (mtDNA and nDNA) ~~showed~~ the same topology, so we used the BEAST maximum clade credibility tree to estimate character transition rates and reconstruct ancestral character states. We reconstructed character states on our tree with all samples as well as in the two clades of *Peucaea* (colored blue in Fig. 1) and *Aimophila* (plus the closely related genera *Melozone* and *Pipilo*; colored pink in Fig. 1). We performed ancestral state reconstructions of our categorical traits using a model-fitting approach that allowed for polymorphic character states within the package corHMM (Beaulieu et al., 2017). Polymorphic character states were assigned likelihoods following the methods of Felsenstein (2004), whereby each possible character state was assigned an equal probability. This allowed us to estimate the phenotype of ancestral nodes while incorporating uncertainty in species' phenotypes that were based on missing or incomplete data. We implemented an 'equal rates' model, in which transition rates between any character state were assumed to be equal with an upper bound of 100, while the character state of the root for each group was estimated following differential equations put forth by Maddison et al. (2007) and FitzJohn et al. (2009). After estimating the transition rate matrix, we subsequently calculated the marginal likelihood states at each node.

We used Pagel's (1994) correlation method in Mesquite to test Wolf's hypothesis that individual traits vary in association with habitat for *Peucaea*. In this group, we tested for associations of prenuptial molt, nest location, song structure, and plumage patterning with open and closed habitat. We did not test other traits such as molt or skull ossification because we had no a priori predictions about their relationships, and we lacked the required information for all taxa. Because this test requires binary character states, we did not test non-binary traits. All members of the *Aimophila* clade live in closed habitat, so within-clade tests for effects of habitat are uninformative; however, we tested for a relationship between song complexity and plumage patterning in that group. We ran tests with 10 extra iterations over 10,000 simulations. Extra iterations implement additional searches within the maximum likelihood framework, and the simulation number is used to estimate statistical significance, with higher numbers above 100 returning better p-value estimates based on simulation output (Maddison & Maddison, 2018). Because the tests of Wolf’s specific hypotheses were done on small samples, we followed up on some of the associations they revealed by using the same correlation method to relate song with plumage and habitat use for all species in the tree. We were unable to evaluate additional traits in this way because of missing data across the full tree.

We examined trait lability among all species in the full tree for a subset of behavioral and morphological traits by calculating the *D* statistic, which is suitable for binary, categorical traits (Fritz & Purvis, 2010), using the function phylo.d within the caper package in R (Orme, 2018). Binary traits included in these analyses included plumage patterning, postjuvenal molt, prenuptial molt, skull ossification, nest position, group breeding, song structure, and duetting.

The bounds of the *D* statistic depend on the number of tips in the phylogenetic comparative analysis, but in general, more negative values imply stronger phylogenetic signal (Fritz & Purvis, 2010). The *D* statistic is calculated by comparing the sum of observed sister-clade differences in the evolutionary history of the binary trait ($\sum d_{obs}$) to simulated data sets of sister-clade differences generated by randomly shuffling the tip values of the phylogeny ($\sum d_r$) and another simulated data set generated by Brownian motion ($\sum d_b$). Thus, *D* is comparable across data sets; when *D* is equal to 1, the binary trait in question has a phylogenetically random distribution across the tips of the phylogeny. In contrast, when *D* is equal to 0, the distribution of binary values across the tips is equal to that expected under Brownian motion (Fritz & Purvis, 2010). Values of *D* can fall outside of the range of 0 to 1, such that negative values indicate phylogenetic conservatism beyond that expected by Brownian motion, while values greater than 1 indicate phylogenetic dispersion beyond that expected by random shuffling of tip values (Fritz & Purvis, 2010). This method also allows one to calculate the probabilities that the observed *D* statistic is greater than 0 and less than 1. For each trait, we omitted taxa with unknown or ambiguous character states.

Results

Sequence variation

The complete data set of 84 individuals from 47 species and up to 5344 bp of sequence contained 1740 variable (32.6%) and 1546 (28.9%) potentially parsimony-informative sites. The two clades for which we reconstructed character states had 1324 (24.8%) variable and 1188 (22.2%) parsimony-informative sites. ~~As expected, mtDNA sequences had more variable and parsimony-informative sites than nuclear gene regions.~~ Average nucleotide composition for the mitochondrial genes cyt-*b* and ND2 were similar to values reported in previous studies of this group and related taxa (Klicka & Spellman, 2007; DaCosta et al., 2009), with an excess of cytosine (36%) and a deficiency of guanine (10-13%). Average uncorrected sequence distances among core taxa for the mitochondrial gene regions ~~was~~ 11% in *Peucaea* (6.6-14.8%) and 4.9% in *Aimophila* (3.7%-6.1%). The mean distance between *Aimophila* and the closely related genera *Melozone* and *Pipilo* was 9.1% (range of 7.7% to 11.5%).

Phylogeny

Maximum likelihood (Fig. S1) and Bayesian methods (Fig. 1) of phylogenetic reconstruction produced similar phylogenetic hypotheses, with the strongest support obtained for the concatenated analysis of mtDNA and nuclear sequence data (Fig. 1). With the exception of three genes (ATPase 8, Fib 5, TGFb2), the best model was GTR + I + G for the data partitioned by loci, mtDNA partitioned by codon position, and combined mtDNA and nDNA sequences. For all samples combined, taxa fell into two lineages that received high to moderate support in the phylogenetic analyses. The first included *Peucaea*, *Rhynchospiza, Arremonops*, and *Ammodramus*. Within that lineage, the eight species of *Peucaea* formed a monophyletic group that was strongly supported and distinct from *Rhynchospiza* and the other genera. The second lineage included species in multiple genera, with a strongly supported clade that united species retained in *Aimophila* with species of *Melozone* and *Pipilo.* The species *quinquestriata* was sister to *Amphispiza bilineata* in a lineage that included *Chondestes* and *Spizella*, and those taxa were distant to the clade containing *Aimophila.* The species tree analyses generated a phylogeny that was concordant with the concatenated approaches and many of the same relationships were recovered (Fig. S2). However, the resultant species tree did not have strong posterior probability values for the large majority of nodes, which likely reflects the relatively small number of loci and the small number of individuals per species used in the coalescent-based species tree analysis (Camargo et al., 2012; Fig. S2). A species tree constructed with many more loci also was not able to resolve all relationships within the family (Bryson et al., 2016).

Trait reconstructions: *Peucaea* and *Aimophila* clades

Ancestral state reconstructions (Fig. 2 through Fig. 5) show that both the *Peucaea* and *Aimophila* clades originated in Middle America (Fig. 2), with some members of each clade shifting their ranges northward into the Northern Temperate zone. *Aimophila* species descended from a common ancestor that is predicted to have a large geographic range and a preference for pine-oak (closed) habitat (Figs. 2 and 3). We were unable to reconstruct the geographic range and habitat preference of ancestral *Peucaea* species unequivocally.

Molt patterns, plumage patterning, and timing of skull ossification showed different histories in the two clades. The ancestral species in both clades had partial post-juvenal molts, but they differed in the presence (*Peucaea*) or absence (*Aimophila*) of a prenuptial molt (Fig. 4). Prenuptial molt has been lost once in *Peucaea*, and gained twice within the broader *Aimophila* clade. Evolutionary patterns of plumage coloration likewise differed between clades (Fig. 4). The ancestral *Peucaea* had unpatterned plumage, and there has been a single transition to patterned coloration in one descendant lineage. In contrast, the *Aimophila* clade shows more uncertainty, with multiple probable transitions between unpatterned and patterned plumage. While the ancestral *Aimophila* species had normal skull ossification timing, the skull timing of the *Peucaea* ancestor is uncertain and there is diversity in this trait among modern lineages (Table S3). Three

Peucaea species form a clade with normal skull timing, three species form a clade with delayed skull timing, and a third clade is split with one species in each category.

Ancestral state reconstructions of behavioral traits also showed different patterns. Most species in the two clades live in pairs and do not form larger social groups (Table S3). The only exceptions are *P. ruficauda* and *P. humeralis*. Because their close relative *P. mystacalis* does not form groups, the presence of groups in *P. ruficauda* and *P. humeralis* may represent separate gains of the trait or a single gain with a subsequent loss of the trait in *P. mystacalis.* The ancestral nest type for *Peucaea* is a raised nest (Fig. 3), while the ancestral nest type for *Aimophila* is equivocal. However, members of both clades use both nest locations. Simple songs are the ancestral condition in both clades, with complex songs evolving once among the *Peucaea* group and twice among the *Aimophila* group (Fig. 5). Many members of both clades produce vocal duets (Fig. 5). Duetting clearly represents an ancestral condition among *Peucaea* species that is highly conserved, while duets have been lost at least twice within the *Aimophila* clade (*A. notosticta*, *Pipilo*). Furthermore, duet type shows phylogenetic conservatism in acoustic structure (Fig. 5). *Peucaea* species all sing rapidly modulated “chitter,” “chatter,” or “warble” duets, while all members of the *Aimophila* group with well-described duets produce broadband “squeal” duets.

Trait correlations

Pagel’s (1994) correlation tests showed that preference for closed habitat is correlated with patterned plumage (p = 0.011) and simple songs (p = 0.010) in the *Peucaea* clade. In contrast, open habitat preference is correlated with unpatterned plumage (p = 0.0069) and complex songs (p = 0.011), as well as with ground nesting (p = 0.010), in this clade. Open habitat use is not correlated with prenuptial molt (p = 0.11). All species in the *Aimophila* clade occur in closed habitat, where they exhibit a negative association between vocal and visual signals such that simple song is correlated with patterned plumage (p = 0.021).

Across our full tree (Fig. 1), unpatterned coloration is correlated with transitions into open habitats (p = 0.026), mirroring the results within our two focal clades. Song structure did not correlate with transitions to or from open (p = 0.746) habitat. Plumage patterning correlated with song complexity such that patterned birds tended to have simpler songs (p = 0.045) across all species in our tree.

Measures of trait lability

To examine the lability of behavioral and morphological traits among sparrows through time, we estimated character state changes for eight traits using our full tree that included a broader sampling of our two focal clades and related taxa without missing data (Table 1). We found a range of estimated *D* values, indicating variation in phylogenetic signal among behavioral and morphological traits. For the behavioral traits we examined, presence or absence of duetting behavior exhibited the strongest phylogenetic signal (*D* = -1.72), while group breeding behavior exhibited the weakest phylogenetic signal (*D* = 1.17). Among the morphological traits, skull ossification exhibited the strongest phylogenetic signal (*D* = -1.21), while plumage patterning exhibited the weakest phylogenetic signal (*D* = 0.56). On average, phylogenetic signal was stronger among the four behavioral traits (mean *D* = -0.71) compared to the four morphological traits (mean *D* = -0.31).

Discussion

Phylogenetic relationships of the *Peucaea* and *Aimophila* clades

Several previous phylogenetic analyses have included some of the species in our study: DaCosta et al. (2009) presented a systematic revision of "*Aimophila*" and "*Pipilo*" using mtDNA sequences for these genera plus numerous other taxa (63 species in 28 genera total); Klicka et al. (2014) used both mtDNA and nuclear gene sequences to resolve a phylogeny for all New World sparrows; Bryson et al. (2016) investigated early relationships in the Passerellidae with limited sampling (28 individuals from 8 major clades) using thousands of ultraconserved elements (UCEs); and Sandoval et al. (2017) sampled 81 *Melozone* and two *Aimophila* taxa using both mtDNA and nuclear genes to recover their evolutionary and biogeographic history. Our study corroborates prior results from these studies in (1) supporting the assignment of species to *Peucaea* (*aestivalis, cassinii, botteri, humeralis, mystacalis, ruficauda, carpalis, sumichrasti*), *Rhynchospiza* (*stolzmani, strigiceps*), and *Aimophila* (*notosticta, ruficeps, rufescens*), (2) showing a close relationship between *Aimophila, Melozone,* and *Pipilo,* and (3) resolving *quinquestriata* as the sister to *Amphispiza bilineata,* both of which are distant to all taxa formerly placed in "*Aimophila*". Within *Melozone*, Sandoval et al. (2017) uncovered four lineages and found that some species in that genus were more closely related to *Aimophila* than to other ~~congeneric species~~.

~~We also noted some differences between our study and prior work~~. For one, we found *Peucaea carpalis* and *P. sumichrasti* to be sister to the remaining *Peucaea* with over 95% posterior probability (PP) support in the concatenated analysis (Fig. 1), while DaCosta et al. (2009) could not resolve this relationship; however, support was lower in our species tree (Fig. S2) and in the maximum likelihood tree of Klicka et al. (2014). Another difference was in the clade containing *Aimophila rufescens*, *A. ruficeps*, and *A. notosticta*. While DaCosta et al. (2009) and Klicka et al. (2014) found strong support for a sister relationship between *A. notosticta* and A. *ruficeps* based on mtDNA when all three taxa were included, we recovered a sister relationship between *A. ruficeps* and *A. rufescens* using both mtDNA and nuclear markers in our concatenated analysis (PP > 0.95). Our species tree analysis, on the other hand, was unable to resolve the relationships between these three taxa. The different studies all supported a sister relationship between *Aimophila* and at least some species of *Melozone,* although Sandoval et al. (2017) did not recover monophyly among *Melozone* with more intensive sampling of that genus. Finally, Bryson et al. (2016) found the *Amphispiza* lineage to be sister to all other sparrow taxa sampled including *Peucaea* based on UCE sequence data with thousands of loci, whereas our results showed *Peucaea* to be outside of that lineage. Together, these studies offer a compelling overview of species relationships among *Aimophila*, *Peucaea*, and related sparrow taxa, although additional work is needed to resolve some relationships. Furthermore, they clarify relationships in the three ecological complexes that Wolf (1977) defined, including support for a close affinity between the *ruficeps* complex and species in the genus *Melozone*.

Trait evolution within the *Aimophila* and *Peucaea* clades

~~We found that all species in~~ the *Aimophila* and *Peucaea* clades have Middle American ancestors. Large range size was resolved for the ancestor of the *Aimophila* clade, but range size was equivocal in the *Peucaea* clade and reflected high variability among those species. Four of the eight *Peucaea* species have expanded (3) or moved (1) their ranges from ancestral Middle

America to Northern Temperate locations. Six of the twelve *Aimophila/Melozone/Pipilo* species also have expanded (4) or moved (2) their ranges into Northern Temperate regions. Interestingly, none of the species that showed range shifts are long-distance migrants (Howell and Webb 1995). These results fit with recent work showing that the common ancestor of all species in Passerellidae was likely a tropical endemic (Winger et al., 2014).

~~Behaviorally, the ancestors of both the *Aimophila* and *Peucaea* clades sang simple songs and did not tend to have large social groups.~~ Two species (*Peucaea humeralis* and *P. ruficauda*) have evolved group-living, but overall this trait appears to be relatively rare and infrequently derived. Complex songs that include many variable elements have evolved three times and are now present in seven of our modern focal species (Fig. 5). Wolf (1977) used song and duet similarity as a justification for grouping species together, and our phylogeny supports those groupings while confirming that shifts in song form occur between but not within groups. Likewise, Marshall (1964) concluded that voice is a good predictor of relationships within the "brown towhee" complex (*Melozone fusca, M. crissalis*, *M. aberti*, *M. albicollis*), especially when used with other attributes. Song structure is known to vary widely across avian species, and other work has shown that song traits may be both conserved and divergent within and among groups (Price & Lanyon, 2002; Price et al., 2007; Snyder & Creanza, 2019). Importantly, Wolf's (and hence our) divisions of songs into "simple" and "complex" reflect only two potential measures of complexity – syllable type diversity and syntax. More detailed song form analysis would be a valuable follow-up to this work and might show that different elements of song complexity are differentially conserved or labile through evolutionary time (Benedict & Najar 2019).

Ancestral habitat use and nesting behavior varied between clades, as did skull ossification timing, molt patterns, and plumage. The *Aimophila* common ancestor might have had patterned plumage, while the *Peucaea* ancestor was likely unpatterned. Modern species in both groups show a range of plumage patterns, which appear to be relatively labile suggesting that color patterning can both appear and disappear. Similar trends have been found in other avian species and across birds more generally (Price et al., 2007; Hoffman et al., 2008; Dunn et al., 2015; Maia et al., 2016; Shultz & Burns, 2017; Marcondes & Brumfield, 2019), showing that evolution may favor elaborate plumage or drabness depending on selective pressures. In addition, there appears to be a negative association between plumage patterning and song complexity, both within our focal clades and across our full phylogeny. Two lineages that contain species with complex songs (*Peucaea cassinii-P. aestivalis-P. botteri* and *Aimophila rufescens-A. ruficeps-A. notosticta*) are characterized by unpatterned plumage, while species in other lineages with simple songs (e.g., *Peucaea mystacalis, P. humeralis, P. ruficauda*) have patterned plumage. Other studies on the evolution of plumage and song complexity in birds have shown that some groups (e.g., cardueline finches, Badyaev et al., 2002) exhibit a similar trade-off whereas other groups (e.g., tanagers, Mason et al., 2014) do not show a correlation between song and plumage elaboration. Such mixed results suggest that the relationship between song and plumage likely depends on a variety of factors, which may include physiological processes (Shutler, 2010) or ecological interactions.

A number of researchers (Morton, 1975; Wolf, 1977; Wiley, 1991; Derryberry, 2009; Mason & Burns, 2015; Derryberry et al., 2018; Crouch & Mason-Gamer, 2019) have suggested that song complexity may be greater in open versus densely vegetated habitats because of the acoustic properties of those habitats (but see Karin et al., 2018 and Hill et al., 2017). Within the *Aimophila* and *Peucaea* clades, we found that complex songs are significantly associated with open grassland habitat, and simple songs are associated with closed (arid scrub or pine-oak) habitat. Such a relationship may result from habitat structure, but might also arise because more grassland species (*Peucaea botteri, P. cassinii, P. aestivalis*) occur in Northern Temperate latitudes where they experience higher environmental variability, which is known to influence bird song complexity (Medina & Francis, 2012; but see Najar & Benedict, 2019). We did not, however, recover the same relationship when all species were included. Therefore, we have tentative support for Wolf's (1977) hypothesis that habitat drives song features within the focal clades, but his observed trend is not universal. It is possible that the observed correlations between habitat and song within the *Aimophila* and *Peucaea* clades results from small samples sizes, because a small number of trait transitions drive these correlations (Maddison & FitzJohn, 2015).

Researchers also have argued that habitat type can drive the evolution of color toward either patterned or unpatterned plumage (Dunn et al., 2015; Shultz & Burns, 2013; Marcondes & Brumfield, 2019). Unpatterned coloration can be advantageous for crypsis in open grassland habitats (Hill & McGraw; 2006; but see Somveille et al., 2016). We found that unpatterned plumage correlated with open grassland habitat among members of the *Aimophila* and *Peucaea* clades, as well as when trait correlation analyses were run using the full tree. Thus, unlike his ideas about habitat and song, Wolf's hypotheses regarding the influence of habitat on plumage evolution may apply broadly across the Passerellidae.

Lability versus stability of behavioral and morphological traits

Although behavioral traits are expected to be more labile than morphological traits (Blomberg et al., 2003), we found that the behavioral traits identified by Wolf (1977) exhibited stronger phylogenetic signal across our full tree than the morphological traits (Revell et al., 2008). In particular, prenuptial molt and plumage patterning showed low phylogenetic signal and high lability. This result is counterintuitive for the prenuptial molt, because molt strategies in birds are integral to their life history (e.g., Terrill, 2017, 2018) and are not predicted to be highly labile. In contrast, concordant with our findings, studies on diverse taxa have shown that plumage patterning is generally quite labile across avian clades (Omland & Lanyon, 2000). Lability in this trait is associated with a variety of biotic and abiotic attributes, such as variation in mating systems (Møller & Birkhead, 1994; Price & Whalen, 2009) and light environments (Shultz & Burns, 2013; Marcondes & Brumfield, 2019). The species studied here all have similar monogamous mating systems, but patterning was correlated with habitat across Passerellidae, providing a potential selective factor shaping patterning. Future work studying this variability would be informative.

Traits with high phylogenetic signal included song structure, duetting, nest location, group breeding, skull ossification, and postjuvenal molt. The most highly conserved trait was ~~the presence of~~ duetting, which was frequent across the tree but ~~had a small number of~~ evolutionary origins. Both song structure and duet type tended to be conserved ~~within lineages~~, such that close relatives used similar sounds. Complex song is often attributed to sexual selection (Andersson, 1994), while duetting is ~~considered under social selection for~~ pair bond maintenance and territory defense (Logue & Hall, 2014). For song structure, the phylogenetic signal in our focal clades came primarily from the derivation and maintenance of complex song in two lineages (Fig. 5). ~~Some other groups have shown similar conservation of complex song~~ (Price & Lanyon, 2002; Tietze et al., 2015), ~~but this pattern is not universal~~ (Price et al., 2007). For this study, we followed Wolf (1977) in defining song complexity based on the number and variety of note types in the species-typical song. Although debate exists about what metrics of song best describe “complexity” (Pearse et al, 2018; Najar & Benedict, 2019; Benedict & Najar, 2019), increased complexity reflects higher syllable diversity in the species we studied and is conserved in related lineages. This result might suggest that closely related species are under similar selective pressures for maintenance of song structure, potentially relating to visual signaling or habitat as discussed above (Panhuis et al., 2001; Boncoraglio & Saino, 2007).

Duet vocalizations are derived and maintained in many of the focal species our study. Avian duets have been shown to perform a range of functions, including joint resource defense, mate defense, and pair coordination (Hall, 2009; Dahlin & Benedict, 2014). Work on the genera *Melozone* and *Peucaea* has demonstrated that duets of different species have similar functions in resource defense, providing a possible selective pressure maintaining this trait (Benedict, 2010; Illes, 2015; Sandoval et al., 2018). Similarly, studies of other New World avian clades have shown that vocal duet presence and form are often evolutionarily conserved (Mann et al., 2009; Mitchell et al., 2019). This pattern is likely driven by life-history traits such as monogamy, territoriality, and sedentariness, which are shown by many of the species included in our analysis (Benedict, 2008; Logue & Hall, 2014). Most strikingly, duet type (Fig. 5) in addition to duet presence is conserved, as noted by Wolf (1977). Our focal species therefore provide a valuable system for future analyses examining how territorial behavior throughout the year and the length of pair bonds might promote evolutionary stability in behavioral traits. Overall, the strong phylogenetic signal found for vocal traits and other behaviors, including nest location and group breeding, counters a general assumption that behavioral traits are more labile than morphological traits (Blomberg et al. 2003).

Conclusions

This study extends our current understanding of the evolution of New World sparrows. In addition to providing additional data on species relationships, we combined genetic and phenotypic data to assess behavioral, morphological, and ecological traits in a phylogenetic context. Habitat appears to be an important driver of trait evolution within our two focal clades, but does not reliably predict song evolution across all sparrows in the phylogeny. Thus, the influence of habitat is not consistent across all traits of birds within the Passerellidae. On the other hand, the correlations of unpatterned plumage with open habitats and complex songs do hold across *Aimophila*, *Peucaea*, and more broadly in New World sparrows, suggesting that habitat may influence the evolution of some traits. Our analyses found varying levels of phylogenetic signal among different traits, and showed that behavioral traits exhibit stronger signal than morphological traits, contrary to general expectations.

Acknowledgements

Sakina Palida assisted with the molecular lab work. Rauri Bowie helped with analyses and comments, and Michael Patten and five anonymous reviewers provided comments that improved earlier drafts of this paper. The Macaulay Library provided recordings on a CD, and the University of California Berkeley Language Center digitized Wolf's (1977) recordings from LP.

We thank the curators and collections staff at the following museums for loans of tissue samples used in this study and for providing information on voucher numbers (Supplemental Table S1): Biodiversity Institute and Natural History Museum, University of Kansas (Town Peterson, Mark Robbins); Burke Museum of Natural History and Culture, University of Washington (Sharon Birks, John Klicka); Colección Nacional de Aves, Instituto de Biología, Universidad Nacional Autónoma de México (Patricia Escalante); Field Museum of Natural History (John Bates, Shannon Hackett, David Willard); Museo de Zoología de la Facultad de Ciencias, Universidad Nacional Autónoma de México (Blanca Hernández, Adolfo Navarro); Museum of Natural Science, Louisiana State University (Donna Dittman, Fred Sheldon); and Royal Ontario Museum (Brad Millen).

References

American Ornithologists' Union. 1983. *Check-list of North American Birds, 6th edition.* American Ornithologists' Union, Lawrence, KS.

Anderson SR, Wiens JJ. 2017. Out of the dark: 350 million years of conservatism and evolution in diel activity patterns in vertebrates. *Evolution* **71**:1944–1959

Andersson MB. 1994. *Sexual Selection*. Princeton: Princeton University Press.

Badyaev A, Hill G, Weckworth B. 2002. Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. *Evolution* **56**:412–419

Baird SF. 1858. Birds. In: Baird SF, CassinJ, Lawrence GN, eds. *Pacific Railroad Reports*. U.S. Government, Washington.

Baltz ME, Latta SC. 1998. Cape May Warbler (*Setophaga tigrina*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Barve S, Mason NA. 2015. Interspecific competition affects evolutionary links between cavity nesting, migration and clutch size in Old World flycatchers (Muscicapidae). *Ibis* **157**:299-311

Beaulieu JM, Oliver JC, O'Meara B. 2017. corHMM: Analysis of Binary Character Evolution. R package version 1.22. https://CRAN.R-project.org/package=corHMM.

Benedict L. 2008. Occurrence and life history correlates of vocal duetting in North American passerines. *Journal of Avian Biology* **39**:57-65

Benedict L. 2010. California towhee vocal duets are multi-functional signals for multiple receivers. *Behaviour* **147**:953-978

Benedict L, Najar NA. 2019. Are commonly used metrics of bird song complexity concordant? *The Auk: Ornithological Advances* **136**:uky008

Blomberg SP, Garland T Jr, Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**:717-745

Boncoraglio G, Saino N. 2007. Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Functional Ecology* **21**:134-142

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C.-H, Xie D, Suchard M, Rambaut A, Drummond A. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. – *PLoS Computational Biology* **10**: e1003537 doi:10.1371/journal.pcbi.1003537

Brumfield RT, Tello JG. Cheviron ZA, Carling MD, Crochet N., Rosenberg KV. 2007. Phylogenetic conservatism and antiquity of a tropical specialization: Army-ant-following in the typical antbirds (Thamnophilidae). *Molecular Phylogenetics and Evolution* **45**:1–13

Brush T. 1998. Olive Sparrow (*Arremonops rufivirgatus*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Bryson RW Jr, Faircloth BC, Tsai WLE, McCormack JE., Klicka J. 2016. Target enrichment of thousands of ultraconserved elements sheds new light on early relationships within New World sparrows (Aves: Passerellidae). *The Auk* **133**:451-458

Byers C, Curson J, Olsson U. 1995. *Sparrows and Buntings: A Guide to the Sparrows and Buntings of North America and the World*. Boston: Houghton Mifflin Company.

Camargo A, Avila LJ, Morando M, Sites JW Jr. 2012. Accuracy and precision of species trees: Effects of locus, individual, and base pair sampling on inference of species trees in lizards of the *Liolaemus darwinii* group (Squamata, Liolaemidae). *Systematic Biology* **61**:272–288

Carson RJ, Spicer GS. 2003. A phylogenetic analysis of the emberizid sparrows based on three mitochondrial genes. *Molecular Phylogenetics and Evolution* **29**:43-57

Chesser RT, Banks RC, Barker FK, Cicero C, Dunn JL, Kratter AW, Lovette IJ, Rasmussen PC, **Remsen JV Jr, Rising JD, Stotz DF, Winker K. 2010**. Fifty-first supplement to the American Ornithologists' Union Check-list of North American Birds. *The Auk* **127**:726-744

Cicero C, Johnson NK. 1998. Molecular phylogeny and ecological diversification in a clade of New World songbirds (genus *Vireo*). *Molecular Ecology* **7**:1359-1370

Cicero C, Johnson NK. 2001. Higher level phylogeny of New World vireos (Aves: Vireonidae) based on sequences of multiple mitochondrial DNA genes. *Molecular Phylogenetics and Evolution* **20**:27-40

Cicero C, Johnson NK. 2002. Phylogeny and character evolution in the *Empidonax* group of tyrant flycatchers (Aves: Tyrannidae): a test of W. E. Lanyon's hypothesis using mtDNA sequences. *Molecular Phylogenetics and Evolution* **22**:289-302

Crouch NMA, Mason-Gamer RJ. 2019. Identifying ecological drivers of interspecific variation in song complexity in songbirds (Passeriformes, Passeri). *Journal of Avian Biology* **2019**:e02020

DaCosta JM, Spellman GM, Escalante P, Klicka J. 2009. A molecular systematic revision of two historically problematic songbird clades: *Aimophila* and *Pipilo*. *Journal of Avian Biology* **40**:206-216

Dahlin CR, Benedict L. 2014. Angry birds need not apply: a perspective on the flexible form and multifunctionality of avian vocal duets. *Ethology* **120**:1-10

De Queiroz A, Wimberger PH. 1993. The usefulness of behavior for phylogeny estimation: levels of homoplasy in behavioral and morphological characters. *Evolution* **47**:46-60

Derryberry EP. 2009. Ecology shapes bird song evolution: variation in morphology and habitat explains variation in White-crowned Sparrow song. *American Naturalist* **174**:24-33

Derryberry EP, Seddon N, Derryberry GE, Claramunt S, Seeholzer GF, Brumfield RT, Tobias JA. 2018. Ecological drivers of song evolution in birds: disentangling the effects of habitat and morphology. *Ecology and Evolution* **8**:1890–1905

Desjardins P, Morais R 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. *Journal of Molecular Biology* **212**:599-634

Dickey DR, van Rossem AJ. 1938. *The Birds of El Salvador.* Chicago: Field Museum of Natural History Zoological Series 23.

Dickinson EC, ed. 2003. *The Howard and Moore Complete Checklist of the Birds of the World, 3rd Edition*. United Kingdom: Christopher Helm.

Dobbs RC, Martin PR, Martin TE. 1998. Green-tailed Towhee (*Pipilo chlorurus*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Dodd ME, Silvertown J, Chase MW. 1999. Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. *Evolution* **53**:732-744

Drummond AJ, Rambaut,A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **214**:1–8

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**:1969–1973

Dunn PO, Armenta JK, Whittingham LA. 2015. Natural and sexual selection act on different axes of variation in plumage color. *Science Advances* **1**:e1400155

Emlen ST. 1997. Predicting family dynamics in social vertebrates. In: KrebsJR, Davies NB, eds. *Behavioural Ecology: An Evolutionary Approach*. 4th edition. Oxford: Blackwell Publishing. 228-252.

Falls JB, Kopachena JG. 1994. White-throated Sparrow (*Zonotrichia albicollis*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Fang Y, Mao-Ning T, Chih-Ming H. 2018. Asynchronous evolution of interdependent nest characters across the avian phylogeny. *Nature Communications* **9**:1863

Felsenstein J. 2004. *Inferring phylogenies*. Sunderland: Sinauer Associates.

FitzJohn RG, Maddison WP, Otto SP. 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. *Systematic Biology* **58**:595–611

Fleischer RC, Olson SL, James HF., Cooper AC. 2000. Identification of the extinct Hawaiian Eagle (*Haliaeetus*) by mtDNA sequence analysis. *The Auk* **117**:1051-1056

Fritz SA, Purvis A. 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conservation Biology* **24**:1042-1051

Grant PR, Grant BR. 2002. Unpredictable evolution in a 30-year study Darwin's Finches. *Science* **296**:707-711

Greenlaw JS. 1996a. Eastern Towhee (*Pipilo erythrophthalmus*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Greenlaw JS. 1996b. Spotted Towhee (*Pipilo maculatus*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Greenlaw JS, Rising JD. 1994. Saltmarsh Sharp-tailed Sparrow (*Ammodramus caudacutus*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Hall ML. 2009. A review of vocal duetting in birds. *Advances in the Study of Behavior* **40**:67-121

Hamilton TH. 1962. Species relationships and adaptations for sympatry in the avian genus *Vireo. The Condor* **64**:40-68

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of birds through DNA barcodes. *PLoS Biology* **2**:1657-1663

Hill GE, McGraw KJ, eds. 2006. *Bird Coloration: Function and Evolution*. Volume 2. Cambridge: Harvard University Press.

Hill SD, Pawley MDM, Ji W. 2017. Local habitat complexity correlates with song complexity in a vocally elaborate honeyeater. *Austral Ecology* **42**:590-596

Hilty S. 2003. *Birds of Venezuela*. 2nd edition. Princeton: Princeton University Press.

Hofmann, CM, Cronin TW, Omland KE. 2008. Evolution of sexual dichromatism. 1. Convergent losses of elaborate female coloration in New World orioles (*Icterus* spp.). *The Auk* **125**:778-789

Howell SNG, Webb S. 1995. *A Guide to the Birds of Mexico and Northern Central America*. Oxford: Oxford University Press.

Illes AE. 2015. Context of female bias in song repertoire size, singing effort, and singing independence in a cooperatively breeding songbird. *Behavioral Ecology and Sociobiology* **69**:139-150

Irestedt M, Johansson US, Parsons TJ, Ericson PGP. 2001. Phylogeny of major lineages of suboscines (Passeriformes) analysed by nuclear DNA sequence data. *Journal of Avian Biology* **32**:15-25

Johnson RR, Haight LT. 1996. Canyon Towhee (*Pipilo fuscus*). – In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Johnson MJ, Van Riper C. III, Pearson KM, 2002. Black-throated Sparrow (*Amphispiza bilineata*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Jones SL, Cornely JE. 2002. Vesper Sparrow (*Pooecetes gramineus*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Kamilar JM, Cooper N. 2013. Phylogenetic signal in primate behaviour, ecology and life history. *Philosophical Transactions of the Royal Society B* **368**:20120341

Karin,BR, Cicero C, Koo MS, Bowie RCK. 2018. The role of history and ecology as drivers of song divergence in Bell's and Sagebrush sparrows (*Artemisiospiza*, Aves: Passerellidae). *Biological Journal of the Linnean Society* **125**:421-440

Klicka J, Banks RC. 2011. A new generic name for some sparrows (Aves: Emberizidae). *Zootaxa* **2793**:67-68

Klicka J, Barker KF, Burns KJ, Lanyon SM, Lovette IJ, Chaves JA, Bryson RW Jr. 2014. A comprehensive multilocus assessment of sparrow (Aves: Passerellidae) relationships. *Molecular Phylogenetics and Evolution* **77**:177-182

Klicka J, Spellman G. 2007. A molecular evaluation of the North American "Grassland" sparrow clade. *The Auk* **124**:537-551

Knapton RW. 1994. Clay-colored Sparrow (*Spizella pallida*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Kunzmann MR, Ellison K, Purcell KL, Johnson RR, Haight LT. 2002. California Towhee (*Pipilo crissalis*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Lanyon WE. 1984. A phylogeny of the kingbirds and their allies. *American Museum Novitates* **2797**:1-28

Lanyon WE. 1985. A phylogeny of the myiarchine flycatchers. In Buckley PA, Foster MS, Morton ES, eds. Neotropical Ornithology. *Ornithological Monographs* 36:361-380

Lanyon WE. 1986. A phylogeny of the thirty-three genera in the *Empidonax* assemblage of tyrant flycatchers. *American Museum Novitates* **2846**:1-64

Lanyon WE. 1988a. A phylogeny of the thirty-two genera in the *Elaenia* assemblage of tyrant flycatchers. *American Museum Novitates* **2914**:1-57

Lanyon WE. 1988b. A phylogeny of the flatbill and tody-tyrant assemblage of tyrant flycatchers. *American Museum Novitates* **2923**:1-41

Lenormand T. 2012. From local adaptation to speciation: Specialization and reinforcement. *International Journal of Ecology* **2012**, Article ID 508458, 11 pages.

Logue DM, Hall ML. 2014. Migration and the evolution of duetting in songbirds. *Proceedings of the Royal Society B: Biological Sciences* **281**:20140103

Lowther PE. 1993. Brown-headed Cowbird (*Molothrus ater*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Lowther PE. 2005. Le Conte's Sparrow (*Ammodramus leconteii*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Maddison DR. 1994. Phylogenetic methods for inferring the evolutionary history and processes of change in discretely valued characters. *Annual Review of Entomology* **39**:267-292

Maddison WP, FitzJohn RG. 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. *Systematic Biology* **64**:127-136.

Maddison WP, Maddison DR. 2003. Mesquite: A Modular System for Evolutionary Analysis. Version 1.0. http://www.mesquiteproject.org

Maddison DR, Maddison WP. 2018. Pagel's Correlation Method. Mesquite Version 3.6. http://www.mesquiteproject.org/Pagel%20Correlation%20Method.html

Maddison WP, Midford PE, Otto SP. 2007. Estimating a Binary Character's Effect on Speciation and Extinction. *Systematic Biology* **56**:701–710

Maia R, Rubenstein DR, Shawkey MD. 2016. Selection, constraint, and the evolution of coloration in African starlings. *Evolution* **70**:1064–1079

Mann NI, Dingess KA, Barker FK, Graves JA, Slater PJB. 2009. A comparative study of song form and duetting in neotropical *Thryothorus* wrens. *Behaviour* **146**:1-43

Marcondes RS, Brumfield RT. 2019. Fifty shades of brown: macroevolution of plumage brightness in the Furnariida, a large clade of drab Neotropical passerines. *Evolution* **73**: 704–719

Marini MA, Hackett SJ. 2002. A multifaceted approach to the characterization of an intergeneric hybrid manakin (Pipridae) from Brazil. *The Auk* **119**:1114-1120

Marshall JT. 1964. Voice in communication and relationships among brown towhees. *The Condor* **66**:345-356

Martin JW, Carlson BA. 1998. Sage Sparrow (*Amphispiza belli*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Martin JW, Parrish JR. 2000. Lark Sparrow (*Chondestes grammacus*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Mason NA, Burns KJ. 2015. The effect of habitat and body size on the evolution of vocal displays in Thraupidae (tanagers), the largest family of songbirds. *Biological Journal of the Linnean Society* **114**:538–551

Mason NA, Shultz AJ, Burns KJ. 2014. Elaborate visual and acoustic signals evolve independently in a large, phenotypically diverse radiation of songbirds. *Proceedings of the Royal Society B* **281**:20140967

Medina I, Francis CD. 2012. Environmental variability and acoustic signals: a multi-level approach in songbirds. *Biology Letters* **23**:928-931

Middleton AL. 1998. Chipping Sparrow (*Spizella passerina*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). New York: Institute of Electrical and Electronics Engineers, Inc. 10.1109/GCE.2010.5676129

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**:1215

Mitchell LR, Benedict L, Cavar J, Najar N, Logue DM. 2019. The evolution of vocal duets and migration in New World warblers (Parulidae). *The Auk: Ornithological Advances* **136**:ukz003

Møller AP, Birkhead TR. 1994. The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution* **48**:1089-1100

Mooers A Ø, Vamosi SM, Schluter D. 1999. Using phylogenies to test macroevolutionary hypotheses of trait evolution in Cranes (Gruinae). *American Naturalist* **154**:249-259

Morton ES. 1975. Ecological sources of selection on avian sounds. *American Naturalist* **109**:17-34

Najar N, Benedict L. 2019. The relationship between latitude, migration and the evolution of bird song complexity. *Ibis* **161**:1-12

Nylander JAA. 2004. MrModeltest. Version 2.3. Uppsala: Evolutionary Biology Centre, Uppsala University.

Omland KE, Lanyon SM. 2000. Reconstructing plumage evolution in Orioles (*Icterus*): Repeated convergence and reversal in patterns. *Evolution* **54**:2119–2133

Orme D. 2018. The caper package: Comparative analysis of phylogenetics and evolution in R. https://rdrr.io/cran/caper/man/caper-package.html

Pagel M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London B* **255**:37-45

Panhuis TM, Butlin R, Zuk M, Tregenza T. 2001. Sexual selection and speciation. *Trends in Ecology and Evolution* **16**:364-371

Patten MA, Fugate M. 1998. Systematic relationships among the Emberizid sparrows. *The Auk* **115**:412-424

Pearse WD, Morales-Castilla I, James LS, Farrell M, Boivin F, Davies TJ. 2018. Complexity is complicated and so too is comparing complexity metrics-A response to Mikula et al.(2018). *Evolution* **72**:2836-2838

Petit LJ. 1999. Prothonotary Warbler (*Protonotaria citrea*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Phillips AR, Farfan RP. 1993. Distribution, migration, ecology, and relationships of the Five-striped Sparrow, *Aimophila quinquestriata*. *Western Birds* **24**:65-72

Price JJ, Friedman NR, Omland KE. 2007. Song and plumage evolution in the New World orioles (*Icterus*) show similar lability and convergence in patterns. *Evolution* **61**:850-863

Price JJ, Lanyon SM. 2002. Reconstructing the evolution of complex bird song in the oropendolas. *Evolution* **56**:1514-1529

Price JJ, Lanyon SM. 2004. Patterns of song evolution and sexual selection in the oropendolas and caciques. *Behavioral Ecology* **15**:485-497

Price JJ, Whalen LM. 2009. Plumage evolution in the oropendolas and caciques: Different divergence rates in polygynous and monogamous taxa. *Evolution* **63**:2985-2998

Pyle P. 1997. *Identification Guide to North American Birds, Part I*. Bolinas: Slate Creek Press.

Pyron RA, Costa GC, Patten MA, Burbrink FT. 2015. Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. *Biological Reviews* **90**:1248-1262.

Remsen JV Jr, Areta JI, Cadena CD, Jaramillo A, Nores M, Pacheco J F, Pérez-Emán J, Robbins MB, Stiles FG, Stotz DF, Zimmer K. J. 2010. *A classification of the bird species of South America*. Chicago: American Ornithological Society. http://www.museum.lsu.edu/~Remsen/SACCBaseline.html

Revell LJ, Harmon LJ, Collar DC. 2008. Phylogenetic signal, evolutionary process, and rate. *Systematic Biology* **57**:591–601

Ridgely RS, Tudor G. 1994. *The Birds of South America. Vol. 2: The Suboscine Passerines*. Austin: University of Texas Press.

Ridgway R. 1901. *The Birds of North and Middle America*. Washington, D.C.: Bulletin of the United States National Museum.

Rising JD, Williams PL. 1999. Bullock's Oriole (*Icterus bullockii*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Laboratory of Ornithology.

Rodewald PG (ed). 2015. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology. https://birdsna.org

Sandoval L, Epperly KL, Klicka J, Mennill DJ. 2017. The biogeographic and evolutionary history of an endemic clade of Middle American sparrows: *Melozone* and *Aimophila* (Aves: Passerellidae). *Molecular Phylogenetics and Evolution* **110**:50-59

Sandoval L, Juárez R, Villarreal M. 2018. Different messages are transmitted by individual duet contributions and complete duets in a species with highly overlapped duets. *The Open Ornithology Journal* **11**:56-67

Sandoval L, Méndez C, Mennill DJ. 2013. Different vocal signals, but not prior experience, influence heterospecific from conspecific discrimination. *Animal Behaviour* **85**:907-915

Shultz AJ, Burns KJ. 2013. Plumage evolution in relation to light environment in a novel clade of Neotropical tanagers. *Molecular Phylogenetics and Evolution* **66**:112–125

Shultz AJ, Burns KJ. 2017. The role of sexual and natural selection in shaping patterns of sexual dichromatism in the largest family of songbirds (Aves: Thraupidae). *Evolution* **71**:1061-1074

Shutler D. 2010. Sexual selection: When to expect trade-offs. *Biology Letters* **7**:101-104

Snyder KT, Creanza N. 2019. Polygyny is linked to accelerated birdsong evolution but not to larger song repertoires. *Nature Communications* **10**: 884

Somveille M, Marshall KLA, Gluckman T-L. 2016. A global analysis of bird plumage patterns reveals no association between habitat and camouflage. *PeerJ* **4**:e2658

Sorenson MD, Balakrishnan CN, Payne RB. 2004. Clade-limited colonization in brood parasitic finches (*Vidua* spp.). *Systematic Biology* **53**:140-153

Stamatakis A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**:2688

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* **57**:758–771

Storer RW. 1955. A preliminary survey of the sparrows of the genus *Aimophila*. *The Condor* **57**:193-201

Swainson W. 1837. *On the Natural History and Classification of Birds*. London: Longman, Rees, Orme, Brown, Green, Longman, Taylor.

Tenney CR. 1997. Black-chinned Sparrow (*Spizella atrogularis*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Terrill RS. 2017. Evolutionary interactions of feather molt in birds. Louisiana State University Doctoral Dissertations 4419. https://digitalcommons.lsu.edu/gradschool_dissertations

Terrill R S. 2018. Feather growth rate increases with latitude in four species of widespread resident Neotropical birds. *The Auk: Ornithological Advances* **135**:1055–1063

Thompson CW. 1991. The sequence of molts and plumages in Painted Buntings and implications for theories of delayed plumage maturation. *The Condor* **93**:209-235

Tietze DT, Martens J, Fischer BS, Sun YH, Klussmann-Kolb A, Päckert M. 2015. Evolution of leaf warbler songs (Aves: Phylloscopidae). *Ecology and evolution* **5**:781-798**Vickery PD. 1996**. Grasshopper Sparrow (*Ammodramus savannarum*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Wheelwright NT, Rising JD. 2008. Savannah Sparrow (*Passerculus sandwichensis*). In: Rodewald PG, ed. *The Birds of North America*. Ithaca: Cornell Lab of Ornithology.

Wiley RH. 1991. Associations of song properties with habitats for territorial oscine birds of eastern North America. *American Naturalist* **138**:973-993

Winger BM, Barker FK, Ree RH. 2014. Temperate origins of long-distance seasonal migration in New World songbirds. *Proceedings of the National Academy of Sciences* **111**:12115-12120

Willoughby EJ. 1991. Molt of the genus *Spizella* (Passeriformes, Emberizidae) in relation to ecological factors affecting plumage wear. *Proceedings of the Western Foundation of Vertebrate Zoology* **4**:247-286

Wolf LJ. 1977. ~~Species relationships in the avian genus *Aimophila*~~. *Ornithological Monographs No. 23* (with accompanying LP of audio recordings). Lawrence: American Ornithologists Union.

Figure 1

Concatenated analysis of phylogenetic relationships in the Passerellidae.

Maximum clade credibility tree for concatenated analysis (4 mitochondrial and 3 nuclear genes) and all taxa using BEAST. Asterisks indicate posterior probability values of 0.95 or higher. Taxa in black were originally classified as *Aimophila* prior to recent revision (DaCosta et al. 2009). The two clades outlined by boxes are the focus of detailed analyses of trait evolution. Bird illustrations are provided courtesy of Lynx Edicions.

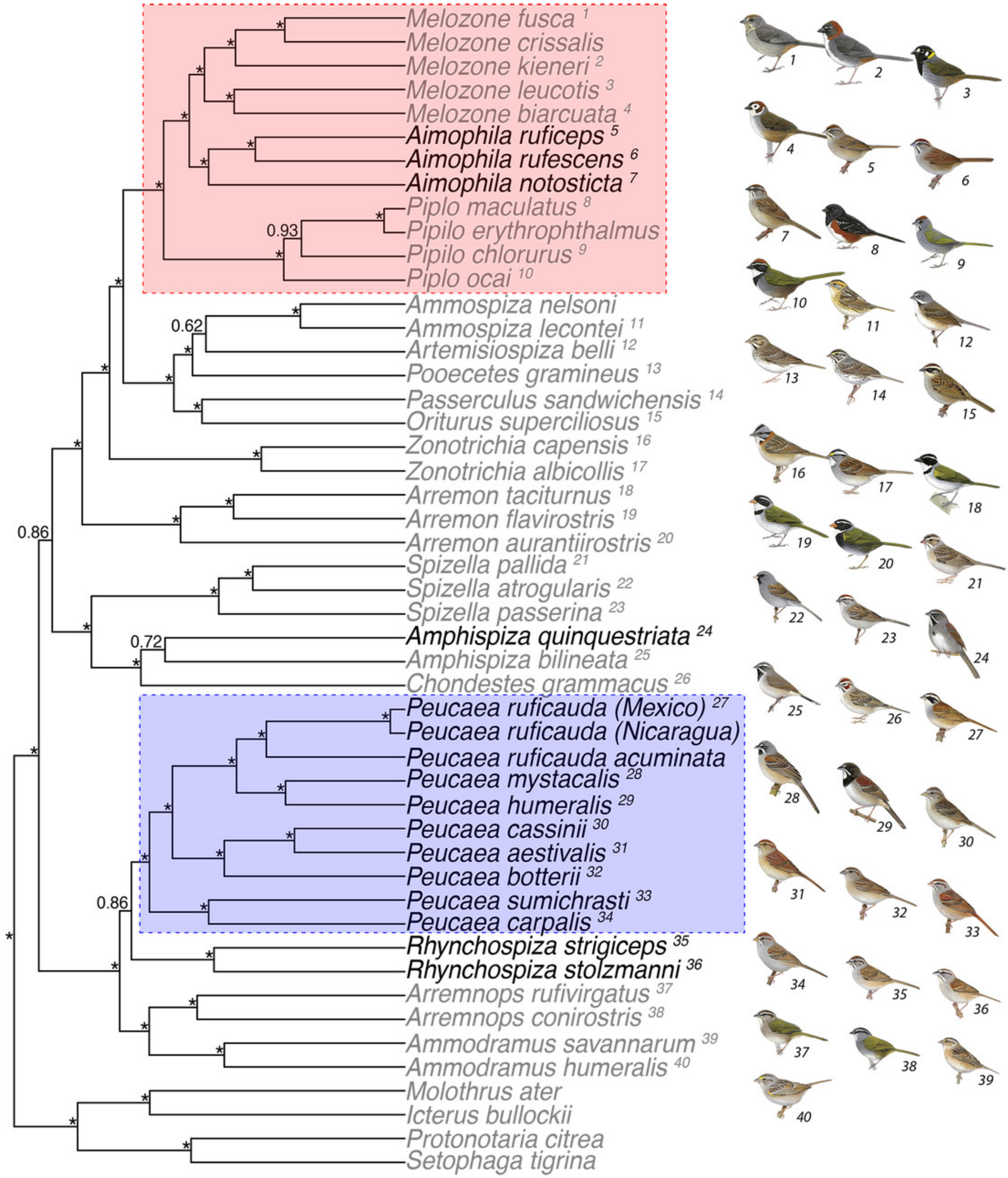
Melozone fusca 1
Melozone crissalis
Melozone kieneri 2
Melozone leucotis 3
Melozone biarcuata 4
Aimophila ruficeps 5
Aimophila rufescens 6
Aimophila notosticta 7
Piplo maculatus 8
Pipilo erythrophthalmus
Pipilo chlorurus 9
Piplo ocai 10
Ammospiza nelsoni
Ammospiza lecontei 11
Artemisiospiza belli 12
Pooecetes gramineus 13
Passerculus sandwichensis 14
Oriturus superciliosus 15
Zonotrichia capensis 16
Zonotrichia albicollis 17
Arremon taciturnus 18
Arremon flavirostris 19
Arremon aurantiirostris 20
Spizella pallida 21
Spizella atrogularis 22
Spizella passerina 23
Amphispiza quinquestriata 24
Amphispiza bilineata 25
Chondestes grammacus 26
Peucaea ruficauda (Mexico) 27
Peucaea ruficauda (Nicaragua)
Peucaea ruficauda acuminata
Peucaea mystacalis 28
Peucaea humeralis 29
Peucaea cassinii 30
Peucaea aestivalis 31
Peucaea botterii 32
Peucaea sumichrasti 33
Peucaea carpalis 34
Rhynchospiza strigiceps 35
Rhynchospiza stolzmanni 36
Arremnops rufivirgatus 37
Arremnops conirostris 38
Ammodramus savannarum 39
Ammodramus humeralis 40
Molothrus ater
Icterus bullockii
Protonotaria citrea
Setophaga tigrina
0.93
0.62
0.86
0.72
0.86
*

Figure 2

Trait reconstructions for geographic distribution and range size in two focal clades.

Maximum-likelihood based trait reconstructions of geographic distribution and range size among the *Peucaea* and *Aimophila* clades. Multiple colors at branch tips indicate the presence of multiple character states. Multiple colors on nodes indicate the probability of each character state.

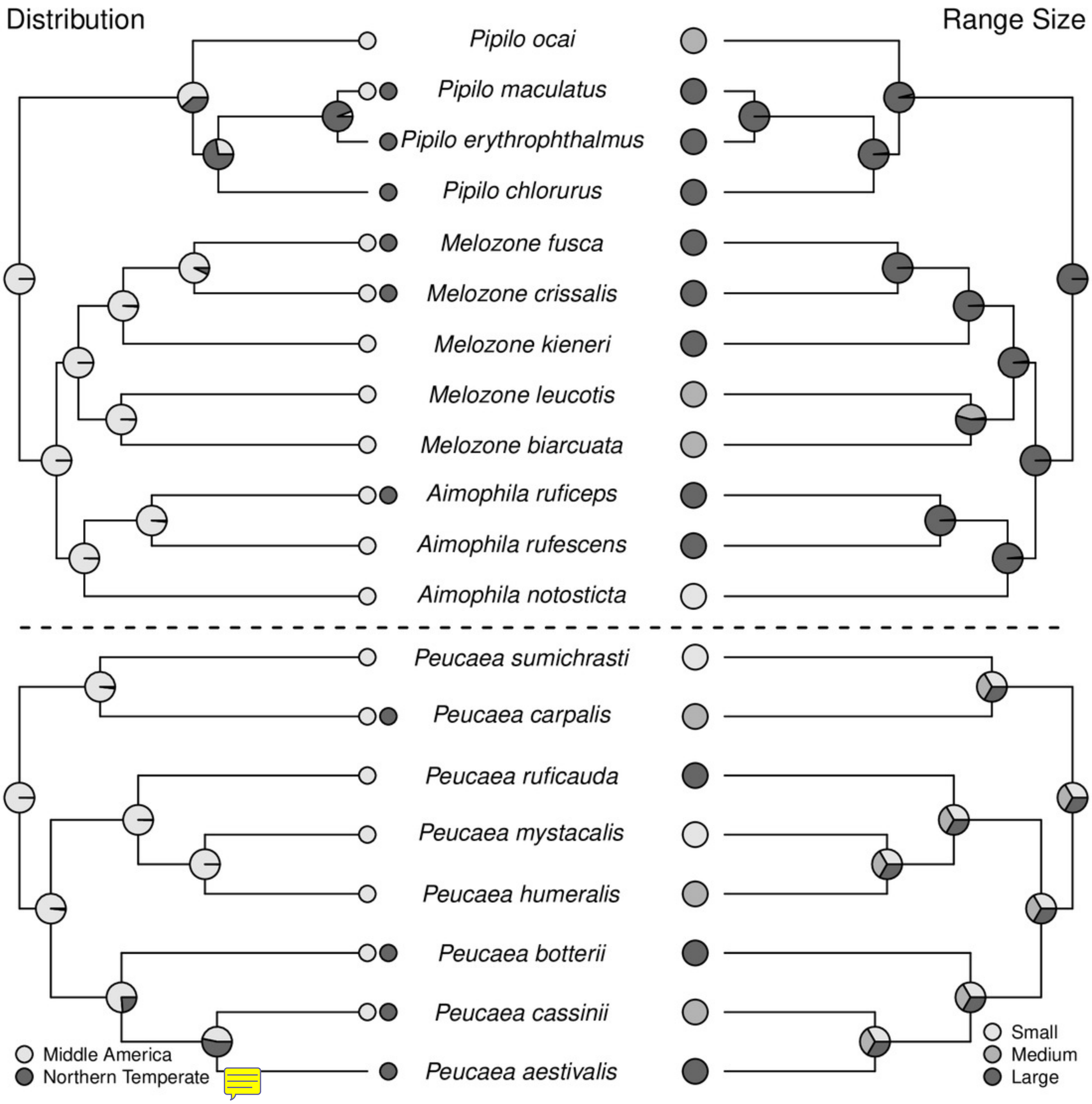
Distribution
Range Size
Pipilo ocai
Pipilo maculatus
Pipilo erythrophthalmus
Pipilo chlorurus
Melozone fusca
Melozone crissalis
Melozone kieneri
Melozone leucotis
Melozone biarcuata
Aimophila ruficeps
Aimophila rufescens
Aimophila notosticta
Peucaea sumichrasti
Peucaea carpalis
Peucaea ruficauda
Peucaea mystacalis
Peucaea humeralis
Peucaea botterii
Peucaea cassinii
Peucaea aestivalis
Middle America
Northern Temperate
Small
Medium
Large

Figure 3

Trait reconstructions for habitat type and nest placement in two focal clades.

Maximum-likelihood based trait reconstructions of habitat type and nest placement among the *Peucaea* and *Aimophila* clades. Multiple colors at branch tips indicate the presence of multiple character states. Multiple colors on nodes indicate the probability of each character state.

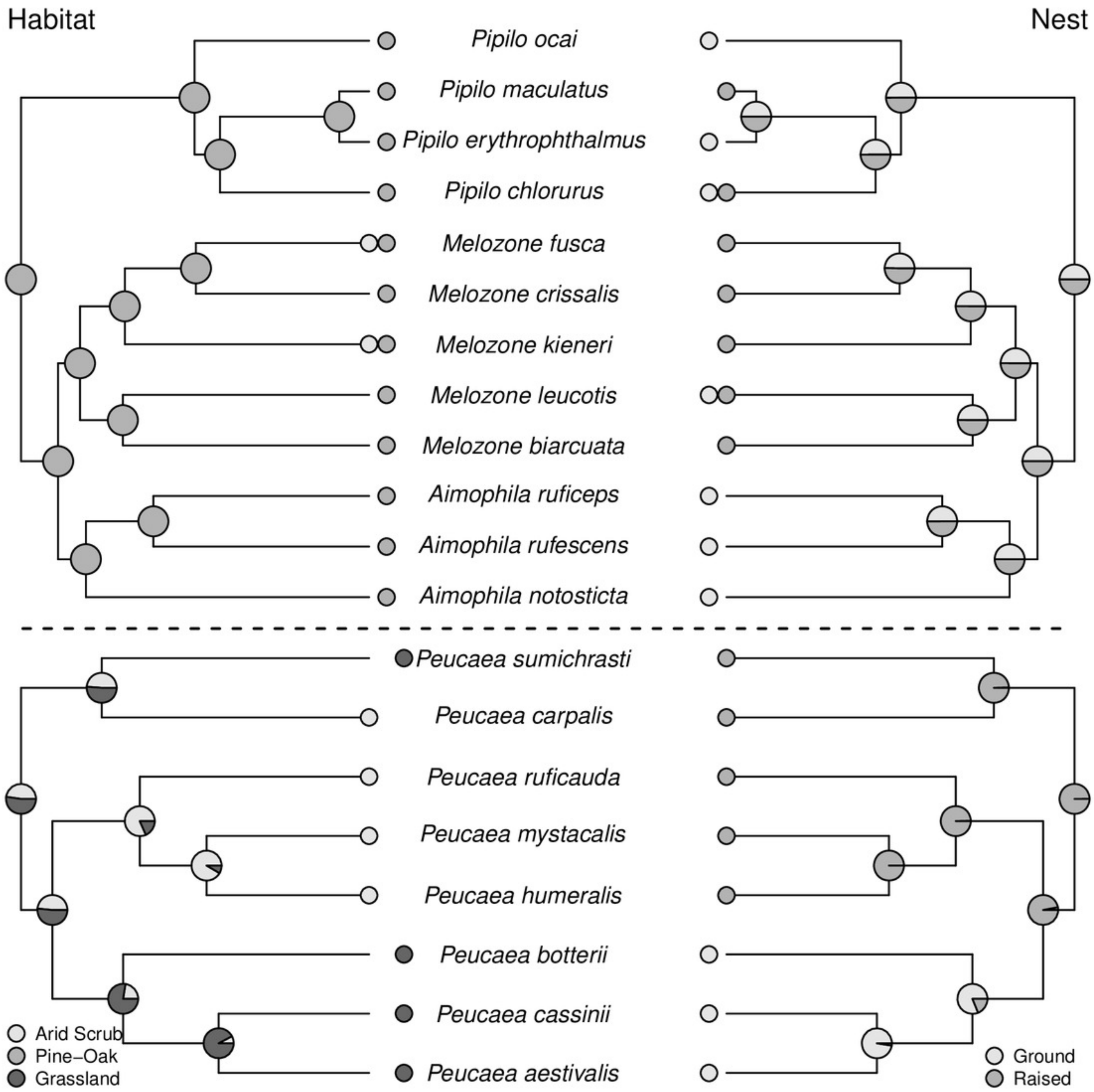
Habitat
Nest
Pipilo ocai
Pipilo maculatus
Pipilo erythrophthalmus
Pipilo chlorurus
Melozone fusca
Melozone crissalis
Melozone kieneri
Melozone leucotis
Melozone biarcuata
Aimophila ruficeps
Aimophila rufescens
Aimophila notosticta
Peucaea sumichrasti
Peucaea carpalis
Peucaea ruficauda
Peucaea mystacalis
Peucaea humeralis
Peucaea botterii
Peucaea cassinii
Peucaea aestivalis
Arid Scrub
Pine–Oak
Grassland
Ground
Raised

Figure 4

Trait reconstructions for plumage patterning and prenuptial molt in two focal clades.

Maximum-likelihood based trait reconstructions of plumage patterning and presence or absence of a prenuptial molt among the *Peucaea* and *Aimophila* clades. Multiple colors at branch tips indicate the presence of multiple character states. Multiple colors on nodes indicate the probability of each character state.

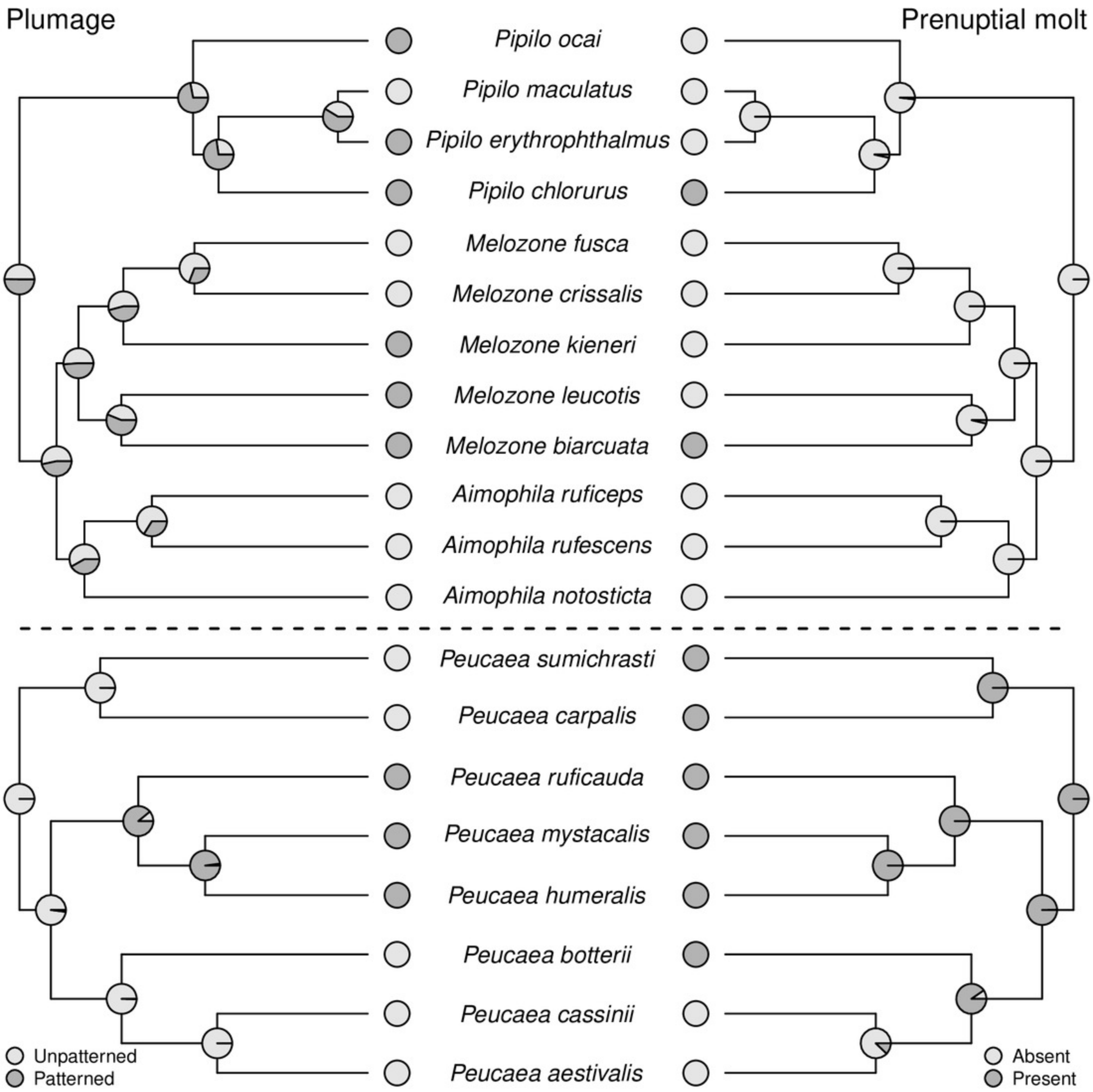
Plumage
Prenuptial molt
Pipilo ocai
Pipilo maculatus
Pipilo erythrophthalmus
Pipilo chlorurus
Melozone fusca
Melozone crissalis
Melozone kieneri
Melozone leucotis
Melozone biarcuata
Aimophila ruficeps
Aimophila rufescens
Aimophila notosticta
Peucaea sumichrasti
Peucaea carpalis
Peucaea ruficauda
Peucaea mystacalis
Peucaea humeralis
Peucaea botterii
Peucaea cassinii
Peucaea aestivalis
Unpatterned
Patterned
Absent
Present

Figure 5

Trait reconstructions for song structure and duet type in two focal clades.

Maximum-likelihood based trait reconstructions of song structure and duet type among the *Peucaea* and *Aimophila* clades. Multiple colors at branch tips indicate the presence of multiple character states. Multiple colors on nodes indicate the probability of each character state.

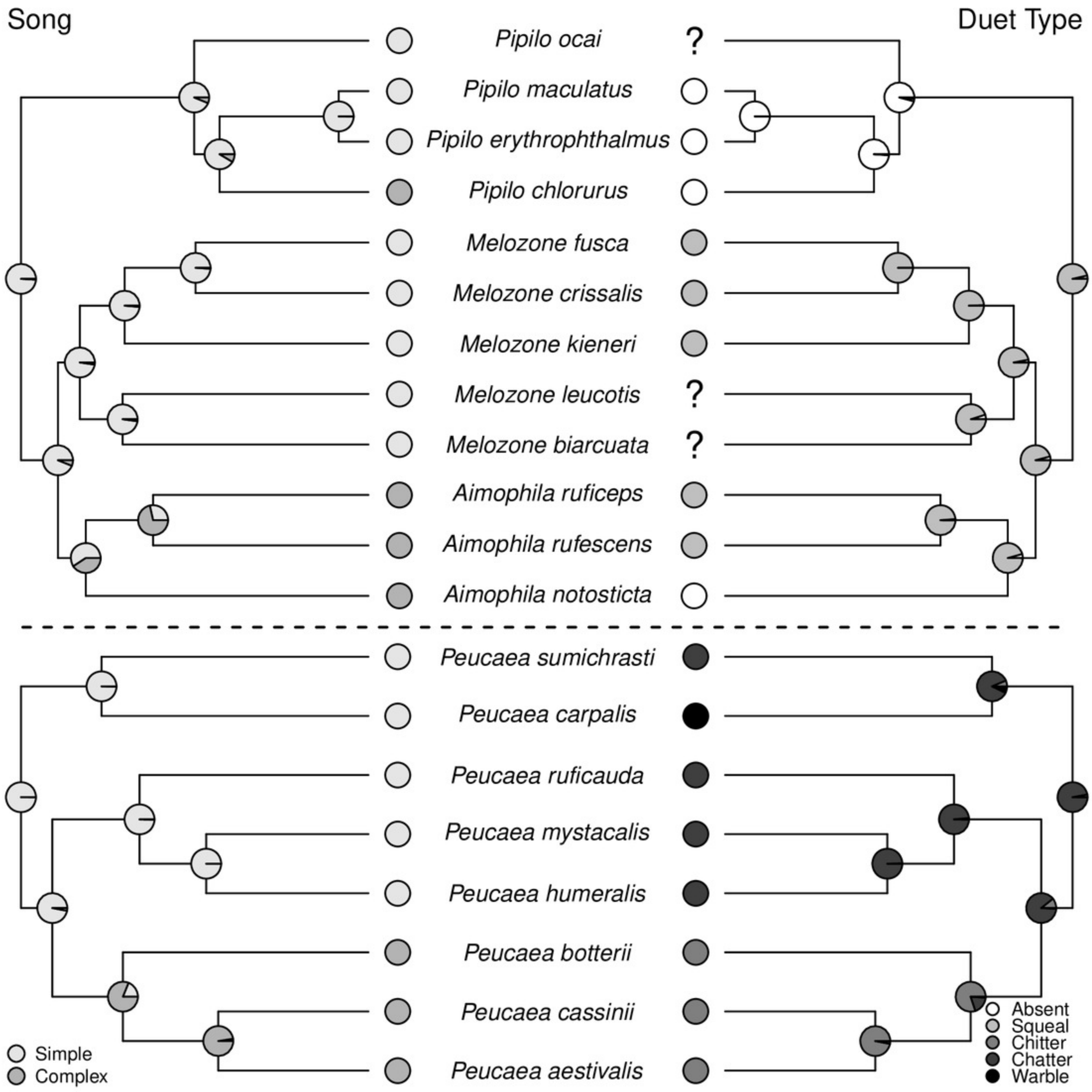
Song
Duet Type
Pipilo ocai
Pipilo maculatus
Pipilo erythrophthalmus
Pipilo chlorurus
Melozone fusca
Melozone crissalis
Melozone kieneri
Melozone leucotis
Melozone biarcuata
Aimophila ruficeps
Aimophila rufescens
Aimophila notosticta
Peucaea sumichrasti
Peucaea carpalis
Peucaea ruficauda
Peucaea mystacalis
Peucaea humeralis
Peucaea botterii
Peucaea cassinii
Peucaea aestivalis
?
?
?
Simple
Complex
Absent
Squeal
Chitter
Chatter
Warble

Table 1(on next page)

Estimates of sister-clade differences and binary trait lability.

Estimates of the sum of sister-clade differences and trait lability (*D* statistic) in binary behavioral and morphological traits across the phylogeny depicted in Figure 1.

Table 1:

Estimates of phylogenetic signal and the sum of sister-clade differences in binary behavioral and morphological traits across the phylogeny depicted in Figure 1.

	# of Taxa	Sum of sister-clade differences	*D* statistic	$P_{D > 0}$	$P_{D < 1}$
Behavioral traits					
Group breeding	45	8.44	1.17	0.16	0.51
Nest position	39	9.69	-1.05	0.93	**0.00**
Song type	46	10.00	-1.25	0.96	**0.00**
Duetting	39	7.28	-1.72	0.99	**0.00**
		Mean = 8.85	Mean = -0.71		
Morphological traits					
Postjuvenal molt	35	6.83	-0.93	0.84	**0.01**
Prenuptial molt	42	15.13	0.34	0.32	**0.09**
Plumage brightness	47	27.54	0.56	**0.00**	**0.00**
Skull ossification	33	5.69	-1.21	0.88	**0.00**
		Mean = 13.80	Mean = -0.31		