Title: Evolution of breeding plumages in birds: A multiple-step pathway to seasonal dichromatism in New World Warblers (Aves: Parulidae)

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Abstract

Bird feathers serve multiple functions through their physical structure and coloration, but the evolution of functional novelty in bird feathers remains poorly understood. We investigated how selective pressures gave rise to seasonal coloration change in the feathers of the New World Warblers (Aves: Parulidae), a family with a remarkable diversity of plumage, molt, and life history strategies. Seasonal color changes in the plumages of migratory warblers are hypothesized to reflect a tradeoff between natural and sexual selection on the breeding and non-breeding distributions. We used comparative methods including phylogenetic path analysis to examine nested hypotheses relating to the evolution of seasonal dichromatism (i.e. breeding and nonbreeding plumages) and the molts that produce these plumages. We found that biannual molts likely evolved in response to increased feather wear and that changes in feather coloration evolved after the biannual molt itself. These results demonstrate that structural needs, not seasonal selection on coloration, drive the evolution of molt strategies in Parulidae. Importantly, once a biannual molt evolves, it served as a preadaptation for seasonal changes in plumage color. These results reveal how life history strategies act upon multiple and separate feather functions to drive the evolution of feather replacement patterns and bird coloration.

Evolution of breeding plumage in birds: the relative influence of life history, color change, and environment on the evolution of biannual molt in the New World warblers (Aves: Parulidae).

Abstract: Many species of birds show distinctive seasonal breeding and nonbreeding plumages. A number of hypotheses have been proposed for the evolution of this seasonal dichromatism, specifically related to the idea that birds may experience variable levels of sexual selection relative to natural selection throughout the year. However, these hypotheses have not addressed the selective forces that have shaped molt, the underlying mechanism of plumage change. Here, we examined relationships between life-history variation, the evolution of a seasonal molt, and seasonal plumage dichromatism in the new world warblers (Aves: Parulidae), a family with a remarkable diversity of plumage, molt, and life history strategies. We used phylogenetic comparative methods and path analysis to understand how and why distinctive breeding and nonbreeding plumages evolve in this family. We found that color change alone poorly explains the evolution of patterns of biannual molt evolution in warblers. Instead, molt evolution is better explained by a combination of other life-history factors, especially migration distance and foraging stratum. We found that the evolution of biannual molt and seasonal dichromatism are decoupled, with a biannual molt appearing earlier on the tree, more dispersed across taxa and body regions, and correlating with separate life-history factors than seasonal dichromatism. This result helps explain the apparent paradox of birds that molt biannually but show breeding plumages that are identical to the nonbreeding plumage. We find support for a two-step process for the evolution of

distinctive breeding and non-breeding plumages: that prealternate molt evolves primarily under selection for feather renewal, with seasonal color change sometimes following later. These results reveal how life history strategies and a birds' environment act upon multiple and separate feather functions to drive the evolution of feather replacement patterns and bird coloration.

Introduction

When subject to dissimilar selective forces, traits that arose for one function often diversify to serve another (Barve and Wagner 2013). Bird feathers are as diverse in purpose as they are in form, reflecting repeated evolution of novel functions since their origin in early Archosauria (Dimond et al. 2011, Seebacher 2003). The array of feather functions in birds is the product of separate, and potentially competing, selective forces that have influenced the evolution of feather structure and color over time (Dunn et al. 2015). Broadly, feather diversity is shaped by natural selection imposed by environmental conditions and by social selection (Dale et al. 2015, Lyon and Montgomerie 2012). Selection often produces bright or gaudy plumages in response to social competition (Rubenstein and Lovette 2009, Karubian 2002, Sætre et al.1994, West-Eberhard 1979), while other selective forces on feathers may enhance structural integrity for functions such as flight and thermoregulation; or produce cryptic plumages to help birds hide from their predators and prey. Selective forces vary throughout a birds' annual cycle, and this variability has been hypothesized to lead to the distinctive breeding and non-breeding plumages shown by many species, *i.e.* seasonal dichromatism (Mulder et al. 1994). Plumage color change in birds has long interested researchers (Holmgren and Hedenström 1995, Tökölyi et al. 2008, Simpson et al. 2015, Beltran et al. 2018, McQueen et al. 2019), but much remains to be discovered about the selective forces that shaped seasonal changes in avian plumage coloration.

Feathers are lightweight and in order to maintain feather function, all birds replace their feathers at least once per year through molt. Without well-timed molts, birds can quickly lose functions of feathers such as thermoregulation and flight. Seasonal dichromatism is commonly acquired through biannual molts that produce plumages with disparate phenotypes. While much study has focused on evolution of structure and color in feathers (Prum 2005, Dale et al. 2015), our understanding of the selective forces and evolutionary pathways which gave rise to disparate molt patterns and strategies remains poor. The annual, complete molt all birds undergo is termed the *prebasic* molt, and generates the *basic* plumage. In addition to the prebasic molt, many species of birds undergo a second molt within their annual cycle, termed the *prealternate* molt, which generates the *alternate* plumage and typically corresponds to what is colloquially known as the breeding plumage (Wolfe et al. 2014). The prealternate molt varies broadly in presence and extent among taxa, as well as the amount of phenotypic change it produces. Many species of birds have alternate plumages that are identical to their basic plumages, while others exhibit markedly different alternate and basic plumages. Some are so different that basic and alternate plumaged birds of the same species were originally described as separate species,*e.g.* Black-bellied Plover (*Pluvialis squatarola* ; Poole et al. 2016). Different species of birds exhibit diverse molt strategies across the globe (Stresemann and Stresemann 1966). What factors have influenced the evolution of divergent molt strategies? When feathers are replaced more than once a year, is this in response to needs to replace worn feathers, or to grow feathers with a new phenotype?

Two hypotheses exist to explain the evolution of seasonal dichromatism in birds. The first hypothesis, which we term the *variable needs hypothesis* , concentrates on feather color and states that prealternate molt evolved in response to differential relative levels of social and natural selection throughout the year (Tökölyi et al. 2008, Simpson et al. 2015, McQueen et al. 2019). This hypothesis is based on the observation that social selection for bright plumage is stronger during the breeding season (Hill 1991, Karubian 2002, Butcher and Rohwer 1989), and may be weaker outside the breeding season such that natural selection would favor a more cryptic plumage in order to evade detection by predators and prey (Götmark et al. 1997, Slagsvold et al. 1995). Long-distance migrant birds experience a brief period of intense sexual selection during the breeding season, which is likely reduced on the non-breeding grounds; though male-male competition may play a strong role in winter plumages in at least some species (Reudink et al. 2009). There is evidence that this has likely led to a latitudinal gradient in sexual dichromatism in the New World warblers and orioles (Friedman et al. 2009, Hamilton 1995, Simpson et al 2015). On the other hand, resident species may form pair

bonds all year, and experience more stable relative levels of sexual and nonsexual selection on feather color throughout the year. Under this hypothesis, the prealternate molt evolved similarly to sexual dichromatism – for plumage color. This hypothesis states that prealternate molt evolves in response to variable needs for feather colors induced by changes in the relative strength of sexual and natural selection on feathers throughout a birds' annual cycle.

The second hypothesis, which we term the *feather wear hypothesis* , is focused on feather structure. It is based on an observation that prealternate molts appear to be more common in long-distance migrants than in non-migratory species and does not always produce plumage color change (Fig 1). Pyle and Kayhart (2010) and Wolfe (2011) observed that a prealternate molt that produces feathers with the same coloration as prebasic molt is a widespread phenomenon in birds, and proposed that prealternate molt mat not evolve for breeding plumage necessarily. Instead, they proposed that prealternate molt evolves to replace worn feathers, and then can be co-opted by pressures for seasonal dichromatism. The idea that the realization of selection on plumage color is limited by pre-existing molts is not entirely novel. Rowher and Butcher (1988) investigated delayed plumage maturation in birds, and found that molt limitations explained patterns of plumage color better than explanations based on social selection alone. The *feather wear hypothesis* similarly views feather color development through the lens of molt limitations, and proposes that the relationship between long-distance migration and prealternate molt may be driven by the need to replace feathers worn by ultraviolet radiation, where migration degrades feathers through extended photoperiods experienced throughout the year (Lennox and Rowlands 1969, Surmacki 2008). This idea is supported by theoretical models demonstrating that biannual molt should evolve when poor feather quality has elevated impacts on survival rates (Holmgren and Hedenström 1995). Migrant breeders experience longer days and increased feather wear through bleaching during their summer breeding seasons at temperate latitudes relative to resident tropical species (Fig. 1c.). Thus, the *feather wear hypothesis* is that prealternate molt evolved to replace worn feathers associated with a migratory lifestyle and increased solar exposure during longer days, and then functioned as preadaptation for the evolution of seasonal dichromatism following the *variable needs hypothesis* . The *feather wear hypothesis* does not rule out variable needs for feather colors, but instead proposes a different mechanism for the origin of prealternate molt. The feather wear hypothesis is a multiple-step evolutionary process for the evolution of seasonal dichromatism: prealternate molt evolved to replace feathers, and was subsequently co-opted for seasonal dichromatism in response to differential selective forces at different times of year.

We examined these two hypotheses using the ecologically diverse New World Warbler (Parulidae) family, which exhibit remarkable variation in plumage characteristics and migratory behaviors. Variation in molt strategies in this family are accompanied by gains and losses in migratory behavior (Winger et al. 2011) as well as considerable variation in life history characteristics, making them a suitable system to assess how interactions between separate selective forces influenced the evolution of seasonal dichromatism. To test these hypotheses, we quantified the extent of prealternate molt and seasonal dichromatism in the New World warblers, as well as 31 life history and environmental characteristics that may affect the evolution of prealternate molts and plumage coloration through natural selection.

Methods

Molt and dichromatism scoring

We scored the extent of prealternate molt and plumage dichromatism using a combination of specimen examinations and literature review. All specimens were examined at the LSU Museum of Natural Science. We used a combination of collection date, data from specimen labels, and known molt patterns (Pyle 2007) to classify individuals by age, sex, and molt stage. Species or life stages not available at the LSUMNS were scored from the literature (Pyle 2007) or visual examination of published photographs of plumages (Dunn and Garrett 1997, Stephenson and Whittle 2013). We defined a dichromatic region as a region with visible color or pattern differences between basic and alternate plumage. We scored dichromatism in feather regions as follows: 1 = region completely dichromatic, 0 = no dichromatism in region, and 0.5 = and partial dichromatism or intraspecific variation. In some species, extent of molt and dichromatism differs between the first prealternate molt and definitive prealternate molts. In these cases, we considered only the definitive

prealternate molts (Wolfe et al. 2014). We scored molt extent using the same museum and literature resources, through examination of molt limits (Pyle 1997a). For each body region (Fig 2f), we scored molt as follows: 1= complete replacement of the feathers in the region, 0 = molt absent from the region, and 0.5 = either partial replacement of the feathers in that region, or intraspecific variation in extent of molt.

Life history parameters

A birds' lifestyle and environment likely affect needs for feathers, and so we attempted to quantify many life history and environmental parameters for each species. All calculations of life history and environmental parameters were conducted in R version 3.3.1 (R Core team 2016). Spatial data were extracted using shapefiles of species distributions provided by Birdlife International and NatureServ (Birdlife International 2016). We worked with the shapefiles of spatial distributions of Parulidae using the packages GISTools (Brunsdon and Chen 2014), maptools (Bivand and Lewis-Koh 2016), raster (Hijmans 2016), and geosphere (Hijmans et al. 2005) in R. We chose to quantify life history and environmental parameters that reflect factors that may result in feather wear from solar exposure due to distribution and migratory behavior, as well habitat use and foraging stratum. These parameters are migratory distance, breeding latitude, wintering latitude, day length experienced throughout the year, breeding and winter habitat, breeding and winter foraging stratum, nest type, intensity of solar radiation experienced over the year, intensity of solar radiation experienced in the breeding and wintering range (separately), and precipitation, minimum maximum and mean temperature, and elevation, on the breeding and wintering ranges, separately. We also calculated body mass. Below, we detail how we measured or scored these parameters.

Migratory distance and Latitude

To estimate migratory distance, we divided species into three categories: Migrants, which have no spatial overlap between their breeding and nonbreeding distributions; non-migrants, which have complete overlap between breeding and nonbreeding distributions, and partial migrants, which have some overlap between breeding and nonbreeding distributions. Non-migrants were always set to zero migratory distance. Using shapefiles of breeding and non-breeding distribution (Birdlife 2016) we calculated six separate estimates of migratory distance: 1: distance between the mid latitudes of each distribution, 2 & 3: distance between the maximum and minimum latitudes of each distribution respectively, 4: distance between maximum latitude of breeding distribution and minimum latitude of nonbreeding distributions, 5: distance between minimum latitude of the breeding distribution and maximum latitude of the nonbreeding distribution, and 6: the great circle distance between the centroids of the points. We used linear models to examine the autocorrelation between these variables and chose the first measure of migratory (distance between mid-latitudes) distance to use in further analysis, because it best predicted the other measurements of migration. We calculated the latitude of the breeding and winter ranges of each species as the mean latitude value of each shapefile.

Solar Radiation, Day Length, and Climate Variables

We calculated solar radiation, day length, temperature, precipitation, and elevation values for each species by extracting spatial data from the distribution shape files used to calculate migratory distance. Data were extracted separately for breeding (May-July) and non-breeding (Nov-Feb) seasons. Although nonbreeding and partial migrant birds may reside in the same location for 12 months, we extracted values from the same periods for all species for consistency. The solar radiation and day length datasets were acquired from the NASA Langley Research Center Atmospheric Science Data Center Surface meteorological and Solar Energy (SSE) web portal supported by the NASA LaRC POWER Project (Nasa 2008). We estimated radiation as the average insolation incident on a horizontal surface per month (hereafter solar radiation) over the course of a year in units of kWh/m^2/month. We estimated daylight hours as the average daylight hours a species experiences per month (hrs/month). We separated solar radiation into radiation experienced in the breeding and winter ranges separately and combined for an overall average. We also created a new variable to estimate total solar exposure by multiplying solar radiation by day length. We extracted ten climatic variables from WorldClim 1.4 (Hijmans et al. 2005) at 2.5 minutes resolution. We extracted breeding and nonbreeding range values from maximum, minimum, and mean temperature, precipitation, and altitude data

sets. Temperature is provided and degrees Celsius x 10. To extract solar radiation, day length, and climate variables, we generated 10,000 points randomly within each distribution map polygon. We extracted data from each variable layer at each of the 10,000 points for the breeding and winter months, in the appropriate polygon for each species. We then calculated the mean value for each variable in the breeding and winter distributions.

Habitat and Stratum

We created a scoring system for habitat and stratum that roughly estimated solar radiation exposure by species. We scored habitats using the following codes: 0 = tall deciduous forest, 1 = coniferous/montane forest, 2 = riparian/secondary/gallery forest, or broad forest type use, 3 = stunted/young forest, 4 = forest edge, 5 = scrub/marshes, 6 = open habitat. We rated stratum by relative stratum within a habitat using the following codes: 0= ground or near ground. 1= understory/undergrowth, 2 = midstory, 3= subcanopy, 4 =canopy/edge/open. Using data from Dunn and Garrett (1997), Curson (2010), Stevenson and Whittle (2013), Rodewald (2015) and Schulenberg (2019) we scored the habitat and foraging stratum during the breeding and wintering periods for each species. We also scored the stratum of nest placement and the nest type from these sources. We coded nest types as the following: 0 = cavity, 1 = dome/closed, 2 = open cup.

Analysis

We conducted all phylogenetic analyses using a recent, multilocus phylogeny of the Parulidae (Lovette et al. 2010).

Model Selection and Phylogenetic Signal

We fit models of evolution to molt and dichromatism to understand how phylogenetic history and selection may interact with these traits, as well as to inform phylogenetic comparative analyses involving these two traits. To select models of evolution for molts and dichromatism, we fit various models of evolution to the data and phylogeny. We fit models of character evolution using Brownian motion (BM), Orstein-Uhlenbeck (OU), and Early-burst (EB) (Butler 2004) models in the package geiger in R (Harmon et al. 2008). We fit models of continuous traits for feather regions and extent of molts and dichromatism, and models of discrete traits for presence of molts and dichromatism. We extracted the sample size-corrected AIC (AICc) values and parameters from the BM, OU, and EB models for cross-model comparisons and converted these values to AIC weights to compare models (Revell 2012). We compared the AICc weights for these three models by calculating AICc weights for each feather tract and for presence and extent of prealternate molt, and seasonal dichromatism. To assess the best model across body regions, we calculated AICc weighted parameter values across feather regions by weighting rate parameters by AICc weights and summed these weighted parameters for molts and dichromatism. We calculated phylogenetic signal as Pagel's Lambda in phytools (Revell 2012) for each molt and sexual and seasonal dichromatism for each body region, as well as presence and extent of molts and dichromatism.

The difference between gains and losses of traits can be important to understand how traits change and interact over evolutionary time. We were interested in knowing when and how often seasonal dichromatism and prealternate molt were gained and lost, and whether these transitions provided insight into the relationship between prealternate molt and seasonal dichromatism. We evaluated the number of transitions and the probability that rates of gains and losses were significantly different for presence of molts and dichromatism by reconstructing ancestral states under equal rates (ER) and all rates different (ARD) models; we compared the log-likelihoods of each model using a likelihood ratio test to obtain a p-value for rejection of the ER model in favor of the more complex ARD model. This method allowed us to ask if rates of gains and losses of molts and dichromatism were significantly different from equal. We used a similar test, based on Pagel (1994) to test if the evolution of prealternate molt is dependent on long-distance migration, through comparison of likelihood ratios of dependent and independent models of evolution (Figure 1g).

Ancestral State Reconstruction and Rates of Evolution

To understand the evolutionary history of prealternate molt and seasonal dichromatism, among separate

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species and feather regions, we constructed ancestral state estimates of molts and dichromatism as discrete variables by feather region (Figure 4). We conducted ancestral state reconstruction of presence of molts and dichromatism on the whole body, and by feather region. To convert continuous characters to presence, we converted any nonzero integer to a 1, to indicate that the molt or dichromatism is present in the region of interest. We then evaluated the probability of presence and absence of molts and dichromatism for the entire body and by feather region at each node using a likelihood framework in the package APE (Paradis et al. 2004) in R. We conducted model testing by reconstructing ancestral states under both equal rates (ER) are all-rates-different (ARD) models and used likelihood ratio tests to choose the best model with which to reconstruct ancestral states to help us understand whether we were correctly evaluating the rates of gains and losses over time. We also evaluated molts and dichromatism as continuous characters, scored as the number of feather regions involved, and reconstructed their ancestral states to evaluate their ancestral states and rates of evolution as continuous characters across the bodies of these birds.

Phylogenetic mixed models for molt and dichromatism extents

We built phylogenetic mixed models to predict the presence and extent of prealternate molt and seasonal dichromatism to understand the relative influences of life history and environmental variables on these traits. To build mixed models of exogenous correlates of molt extents, we first conducted pairwise phylogenetic generalized least squares analysis over extents of molt, dichromatism, and exogenous correlates (extended data table 1) using the package caper (Orme et al. 2013) in R. We examined pairwise PGLS results for strength and significance of interactions and used these interactions to build sets of mixed models to test for the effects of exogenous drivers on extents of molts and dichromatism by examining pairwise interaction between molts, dichromatism, and ecological data, as well as covariation between life history and ecological correlates. We evaluated these mixed models using caper, MuMIn (Barton 2016) and Nlme (Pinheiro et al. 2016) in R, and organized the models using information theory by ranking models by their AICc score (Table 1).

Phylogenetic ANOVA of drivers of molt and dichromatism in feather regions

Identifying those feather regions being replaced by the prealternate molt can provide clues as to why this molt evolves To investigate how migratory distance interact with molts and dichromatism within individual feather regions, we conducted a phylogenetic-controlled analysis of variance (ANOVA), for each feather region using the package phytools (Revell 2012) in R. We investigated the influence of migratory distance on prealternate molt within feather regions by comparing these continuous characters to presence and absence of prealternate molt (Fig. 2g). We then conducted Holm's sequential Bonferroni post-hoc tests on the phylogenetic ANOVA results to correct for simultaneous test runs.

Phylogenetic path analysis

Because the *feather wear hypothesis* is a multiple-step hypothesis, it is important to be able to parse direct and indirect relationships between variables. We investigated these direct and indirect relationships using a phylogenetic path analysis, following the method outlined by and von Hardenbeg and Gonzalez-Voyer (2016) as explained in Garamszegi, Ed. (2014). Phylogenetic path analysis has several advantages when assessing multivariate relationships, especially in its ability to discriminate between direct and indirect effects between variables, and in its consideration of multiple interactions at once. To evaluate the multivariate interactions in this system, we used results from PGLS analyses to inform 12 separate hypotheses of direct and indirect effects within prealternate molt, seasonal dichromatism, migration distance and foraging stratum (supplemental fig. 1). We used a d-sep based path analysis to build sets of phylogenetic-controlled model equations, which we evaluated using the package caper (Orme et al. 2013) in R. We then used an information theory approach based on a C statistic (Sjipley 2016) to rank candidate models. The C-statistic evaluates ranks the conditional independencies within the models and produces CIC_c score for each model. We used P-values and CICc (Von Hardenberd and Gonzales-Voyer 2013) scores to evaluate the probability and information content of the C-statistic, respectively. We used P-values of the C statistic to identify a subset of models that we were not able to reject, and then ranked models by their CICc score to evaluate the likelihood of each candidate model.

RESULTS

Ancestral State Reconstruction

We found support for an Ornstein-Uhlenbeck (OU) model (AICc weight = .96), for presence of prealternate molt, but support for Brownian motion (BM) evolution (AICc weight = .60) for extent of prealternate molt. We found support for a BM model for both presence (AICc weight = .56) and extent (AICc weight = .57) of seasonal dichromatism.

The feather regions more involved in prealternate molt, namely the head, breast, belly, and back, showed higher rates of evolution relative to other feather regions (Fig. 4). We reconstructed a partial prealternate molt at the root of the tree, only on the head, with no associated seasonal dichromatism, and several gains and losses of both seasonal dichromatism and prealternate molt (Fig. 4), which agrees with our transition analysis (Fig. 1) that prealternate molt can be gained and lost over time, over separate lineages (Fig. 1e).

Phylogenetic generalized linear models assessing exogenous correlates among extents of molts and dichromatism

For individual pairwise comparisons between variables, we found that extent of seasonal dichromatism was best predicted by extent of prealternate molt (adjusted R^2= .312, p<.001), and day length (adjusted R^2= .065, p=.046), and migration distance (adjusted R^2= .072, p =.039) which were correlated with prealternate molt. Seasonal dichromatism was also significantly correlated to foraging stratum (adjusted R^2= .078, p=.032), which was not correlated with prealternate molt. The extent of prealternate molt was significantly correlate with extent of seasonal dichromatism (adjusted R^2= .312, p<.001), day length (adjusted R^2= .16, p = .001), migration distance (adjusted R^2= .188, p = .013), and breeding latitude (adjusted R^2= .109, p = .013); figure 1).

16 mixed models significantly predicted the extent of prealternate molt with significance of p<.05, and we ranked these models using the sample size-adjusted information theory criterion AIC_c(Table 1). The top model for extent of prealternate molt outperformed all other models by a sizable margin, and the top two models combined accounted for the majority of the AIC weight. Top models that predicted the extent of prealternate molt generally included day length, solar radiation both in the breeding and nonbreeding season, and migratory distance as predictor variables. In all, we found fifteen models that predicted the extent of seasonal dichromatism with significance of p<.05; and these models included the extent of prealternate molt, foraging stratum both in the breeding and nonbreeding season, and migratory distance (Table 1). Top models were evaluating seasonal dichromatism more evenly weighted than models for prealternate molt, with the top two models produced similar AIC_cvalues, and the third and fourth models produced similar AIC_c values. All four of these top models, which accounted for the majority of the AIC_c weight, included extent of prealternate molt and foraging stratum. Foraging stratum, both in the breeding and nonbreeding season, was the main predictor variable in the top models for the extent of seasonal dichromatism that was not associated with top models of prealternate molt.

Phylogenetic ANOVA of feather regions

We found a that the positive correlation between migratory distance and prealternate molt was repeated across feather regions. In general, migratory distance predicted whether a feather region was replaced during prealternate molt (Fig. 2). This relationship was significant in the head (F = 13, p=.002), breast (F=15.5,p=.001), back (F=12.47,p=.033), belly (F=14.8,p=.013), and tertials (F=11.1,p=.015).

Phylogenetic path analysis

Two path models were strongly favored by information theory analyses, with roughly equivalent CICc values. These were models 2 and 3 (fig. 4), both of which proposed that prealternate molt and foraging stratum are direct parent variables of seasonal dichromatism and that migration distance is a direct parent of variable of day length, and only differed in whether migration distance or day length was a direct parent of prealternate molt. The best model that proposed a conditional independency for prealternate molt was model 5, the next

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best model after models 2 and 3, though this model showed a marked jump in its CICc value compared to models 2 and 3. (fig. S2).

Discussion

The feather wear vs. variables needs hypotheses

The *variable needs hypothesis* , which proposes that prealternate molt evolves in response to birds' needs to change colors, predicts coevolution of prealternate molt and seasonal dichromatism. We found discrepancies in the best fit models of evolution, timing, pattern and external correlates of evolution of between the prealternate molt and seasonal dichromatism. The character that we studied with the strongest evidence for selection, as interpreted by the ratio of likelihood for an Ornstein-Uhlenbeck model to a Brownian Motion model, was presence of prealternate molt. This may imply that prealternate molt itself is under stronger selection (Butler and King 2004) than the color change it produces, which fit slightly better to a model of Brownian motion. We interpret this as support for the *feather wear hypothesis* , because this hypothesis predicts stronger selection on molt patterns than on seasonal dichromatism. The life history characteristics that best predicted prealternate molt were migration distance, day length, and solar radiation experienced on the breeding grounds (Fig 1, table 1). Top models for seasonal dichromatism all included prealternate molt and foraging stratum on the breeding and nonbreeding ranges. Combined with the results of the path analysis, we interpret these results as evidence for prealternate molt and seasonal dichromatism evolving along separate pathways.

The *feather wear* hypothesis invokes preadaptation in the relationship between prealternate molt and seasonal dichromatism. The*variable needs hypothesis* may predict synchronous evolution of prealternate molt and seasonal dichromatism, whereas the *feather wear hypothesis* predicts that prealternate molt should precede seasonal dichromatism and correlate with separate external parameters When we investigated the evolutionary timing of these characters, prealternate molt appeared to arise before seasonal dichromatism, and in more species and feather regions (Fig. 4). The idea that a character can evolve in response to selection for one function, and then be co-opted to serve another, has been well-explored in evolutionary biology (Bock 1959). Preadaptation has been implicated in the evolution of a wide array of evolutionary novelties (Cheney and Seyfarth 2005, Ketola et al. 2013, Schiestl and Cozzolino 2008, Quiñones and Pen 2017), and is an important phenomenon to understand when investigating how traits evolve. Our ancestral state reconstruction suggests that prealternate molt is a preadaptation, rather than an adaptation for seasonal dichromatism. We do not present these results as a rebuttal to variable needs for feather color. Clearly, needs for feather colors vary with life-history and latitudinal gradients in social behavior (Friedman et al. 2009), needs for crypsis on migration (Simpson et al. 2015) and nest stratum (Martin and Badyaev 1996), though we found little support for a relationship between nest stratum or nest type and prealternate molt or seasonal dichromatism. Our findings suggest that latitudinal gradients likely do play a role in the evolution of color change in feathers once prealternate molt is present. From these results, we propose a two-step pathway for the evolution of disparate breeding and nonbreeding plumages in warblers: a biannual molt evolves in response to structural needs for feathers, and then serves as a preadapted mechanism for seasonal dichromatism.

Immediacy of needs helps explain why structure may influence molt more than needs for color change. Structural functions provided by feathers are more immediately necessary for survival of birds than colors that function for social signaling. Without feathers, chicks are poikilothermic (Whittow and Tazawa 1991) and reliant on their parents for warmth. In adult birds, worn feathers directly influence survival through decrease of important functions such as flight (Swaddle et al. 1996). Timing of molt appears to be so important that experimentally malnourished birds will undergo a molt in spite of losing up to 40% body mass in the process, instead of delaying molt (Murphy et al. 1988). Because of more immediate implications on survival, it may make sense that selection on feather structure is stronger than on color change, and that selection on feather structure may be more likely to influence the evolution of molt strategies.

Life History and Environmental correlates of molts and color change

Phenotypic evolution is the result of repeated interactions between selective pressures and pre-existing struc-

tures available for selection to act upon, in addition to neutral drift. While Gould and Lewontin (1979) has been widely criticized for oversimplifying adaptation hypotheses (Pigliucci and Kaplan 2000), a general point that it explains well is that selection can only work upon biological features that exist, and that contemporary uses for a biological structure may not fully explain why that structure originally evolved. While it may make intuitive sense that prealternate molt is "for" a breeding plumage, and indeed some naming conventions (*e.g.* prenuptial molt, prebreeding molt) imply this causative relationship; it is important to disentangle direct and indirect causation when attempting to understand how selection interacts with phenotypic evolution over time (Hardenberg and Gonzalez-Voyer 2013). Phylogenetic path analysis produced two top models, both of which found that the extent of prealternate molt was driven directly by migratory distance and cumulative annual day length.. This suggests that seasonal dichromatism is connected indirectly to migratory distance through prealternate molt (Fig 3b). The models suggested that seasonal dichromatism was determined by the presence of the prealternate molt and foraging stratum, with birds foraging in more open strata experiencing more extensive prealternate molts and seasonal dichromatism. This generally agrees with previous findings that sexual selection operates more strongly in canopy birds, which tend to be more visually oriented (Gomez and Théry 2004, Shutler and Weatherhead 1990), resulting in brighter plumages (Shultz and Burns 2013). From a structural standpoint, canopy birds may also experience greater solar exposure. Indeed, one of the few tropical groups of birds with a known prealternate molt are the becards (*Pachyramphus* ; Johnson and Wolfe 2018) which show identical alternate and basic plumages, and inhabit canopy and forest edge habitats. Importantly, breeding season foraging stratum, when combined with extent of prealternate molt, strongly predicted extent of seasonal dichromatism, but did not by itself predict extent of prealternate molt (table 1). This suggests that selective pressure on plumage color acts on seasonal dichromatism only after prealternate molt has evolved for other reasons and then provides a structural canvass for sexual selection to paint upon.

Past studies have found that sexual dichromatism can evolve through the loss of a gaudy plumage among female migratory birds (Simpson et al 2015). Similarly, we find that at least in some cases, year-round monochromatism evolved through loss of the prealternate molt. It is important to consider phylogenetic context in the evolution of different types of dimorphism because trait gains and losses may mean different things over evolutionary time. For example, Simpson (2015) found sexual dichromatism in warblers stems from loss of bright coloration in females, and Hoffmann et al (2009) found a similar pattern in oriole plumage. From the perpsective of migratory distance, Winger and Lovette (2011) showed that resident warblers were more likely to be examples of lineages that had lost long-distance migration. We found gains and losses of both seasonal dichromatism and prealternate molt, and, importantly, we found that losses of long-distance migration were associated with loss of prealternate molt. Froelich et al. (2005) and Tökölyi et al. (2008) proposed that the relationship between migratory distance and seasonal dichromatism is caused by earlier breeding in resident species which limited their ability to molt; however, resident species do not appear to be limited in their molts when compared to migrant birds, as they show increased molt-breeding overlap (Johnson et al. 2012) and protracted molts (Kiat et al. 2019, Terrill 2018). Furthermore, it is likely that migrant birds are limited in their molt timing, as they generally complete prealternate molt before beginning spring migration (Pyle 1997b). Without a prealternate molt, nonmigratory warblers are often the same color throughout the year, and resident warblers fall into two categories, those that are either gaudy all year, or cryptic all year. These findings suggest that variable needs for feather color alone are not strong enough to maintain a biannual molt in these birds, without an external force acting on the structural integrity of their feathers, and long-distance migration directly impacts structural integrity. Furthermore, pressures that affect the latitudinal gradients in sexual dichromatism and seasonal dichromatism likely differ because each is derived from a different mechanism. While sexual dichromatism can be associated with the *prebasic* molt and result in a year-long plumage aspect, seasonal dichromatism results in discrepancies between the *prebasic* and *prealternate* molt, and results in seasonally variable plumage aspects.

Molt and coloration across feather regions

Selective pressures on plumage may vary across birds' bodies (Marcondes and Brumfield 2018, Dale et al 2015). We know that molt in different species of birds varies in which feathers are molted and when (Stresemann and Stresemann 1966), but, despite some hypotheses being put forward (Howell 2010), little work has

investigated the interplay between feather function and molt patterns across feather regions. Among species of warblers, certain feather regions were repeatedly more or less likely to be involved in prealternate molt. Despite variation in prealternate molt extent, ancestral state reconstruction suggested that the prealternate molt evolved in a stereotyped manner (Fig. 2g; Fig. 4). The head is involved in prealternate molt in all species, amnd then most frequently followed by the back, breast, belly feathers and wing coverts. Prealternate molt rarely replaces other parts of the body, including wing and tail feathers, which are often shaded from the sun by covert feathers and each other. The feather regions more involved in prealternate molt appear to be those more exposed to the sun on a perched bird (Fig 2f). Although the wing and tails are prominent features on birds, when folded, each individual remex is almost entirely shaded by coverts and other remiges. (Fig. 2). It may also be the case that the larger and stronger remiges are costlier to replace than body feathers, but we interpret this evidence as at least suggestive that feathers that are more exposed to the sun are more likely to be replaced in prealternate molt. This pattern was confirmed by both ANOVA analysis and ancestral state reconstruction, where feather regions most strongly associated with prealternate molt were also correlated with long-distance migration (Fig 2g, Fig. 4), and showed increased rates of evolution in seasonal dichromatism and prealternate molt (Fig. 4). In each feather group, we recovered the same positive relationship between migratory distance and likelihood of replacement in prealternate molt (Fig. 2g). We found gains and losses of long-distance migration and prealternate molt; and, importantly, found no gains of prealternate molt in birds without long-distance migration, but a high transition rate to prealternate molt in lineages with long-distance migration (Fig. 1g). The predictable evolution of prealternate molt in regions of the body more exposed to the sun, coupled with a lack of seasonal dichromatism in lineages which recently evolved prealternate molt, lends support to the feather wear hypothesis for the evolution of prealternate molt.

Feather wear and structural needs

Photodegradation is a primary source of feather structure atrophy in feathers (Ito et al. 2017, Pearlstein et al. 2014). The main variables that predict extent of prealternate molt are migration distance, day length, and foraging stratum. Migration distance likely affects feather degradation through increased overall day length (Fig. 1a). Long-distance migrants experience longer days overall because they experience long summer days in the temperate zone, but escape short winter days. For example, the longest-distance migrant in our dataset, and one of the most seasonally dichromatic species with one of the most extensive prealternate molts, the Blackpoll Warbler (*Setophaga striata*) experiences an average of 1.7 more hours of daylight each day, or 621 more hours of ultraviolet exposure each year, when compared to the species exposed to the least amount of ultraviolet radiation, the Masked Yellowthroat (*Geothlypis aequinoctialis*), which also shows no seasonal dichromatism and no prealternate molt. Additionally, many warbler species exhibit prealternate molts that do not result in seasonal dichromatism, this phenomenon may seem paradoxical from the standpoint of hypotheses focused on coloration as the evolutionary catalyst for the prealternate molt, but makes sense within the context of the *feather wear hypothesis.*

Evidence from other taxa outside the New World Warblers provides additional context for the relationship between prealternate molt and seasonal dichromatism. The most extensive prealternate molts in birds occur in three species of long-distance migrants that breed, winter, and migrate in open, solar-exposed environments: Franklin's Gull (*Leucophaeus pipixcan* ; Howell 2010) , Bobolink (*Dolichonyx oryzivorus* ; Renfew et al. 2011) and Willow Warbler (*Phylloscopus trochilus;* Underhill et al. 1992) . Bobolink shows seasonal change in feather color, but Willow Warbler does not, and Franklin's Gull only shows a partial plumage color change. In these species, anecdotally, migration distance and habitat better predict prealternate molt than color change. The Willow Warbler is an extreme example: this species completely replaces all feathers twice a year, but the basic and alternate plumages are indistinguishable. Further research into this phenomenon should expand beyond the new world warblers to other groups of birds, as well as attempt to measure relative feather degradation rates in association with life history, habitat, and environment in birds, and study groups with more variable social systems. Other resident species of birds with strong variable selection on feather color, such as Ptarmigans (Beltran et al. 2018) may indeed have molts that evolve solely for variables needs of feather color. Overall, our results demonstrate the importance of molt strategies in the functional

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diversification of feathers and illuminates the value of considering interactions between different functional requirements for birds in the evolution of feather function.

Conclusions:

Based on the observation that prealternate molt often involves feathers that appear to be identical between basic and alternate plumage, Pyle and Kayhart (2010) proposed that prealternate molt may evolve to replace sun-exposed feathers, then later be co-opted for seasonal dichromatism. We examined this hypothesis across warblers and found that it better explains patterns of evolution of prealternate molt than color change alone. Here, we present evidence that selection on coloration and structure interact in complex ways to influence the evolution of molts and plumages in warblers. Namely, we find that color change poorly explains the evolution of the molts that produce these changes. This suggests that biannual molt acts as a preadapted platform for color change, instead of evolving in direct response to needs for color change. These results provide a more nuanced understanding of plumage evolution in birds by incorporating the mechanism for plumage generation. Rohwer and Butcher (1988) made a novel contribution to our understanding of delayed plumage maturation in birds by arguing that molt must be understood first in order to understand plumage maturation in birds. They found that the breeding-season driven hypotheses lose support when molt is studied, and that the limitations of preexisting molts explain delayed plumage maturation in birds better that social selection on the breeding grounds. Our results largely agree with this study, in that we find needs for plumage change do not appear to be able to influence the evolution of molt strategies, instead they only influence the phenotypes of feathers produced within molt strategies that have evolved for other reasons. Similarly, needs for seasonal color change may be present in species, but the translation of that need into phenotype may be limited by the extent of prealternate molt. Following our results and those of Rohwer and Butcher (1988) that hypotheses about the role of social selection on feather color may look different when viewed through the lens of molt, we encourage other authors studying the evolution of plumage to consider molt strategies when attempting to understand mechanisms of feather evolution.
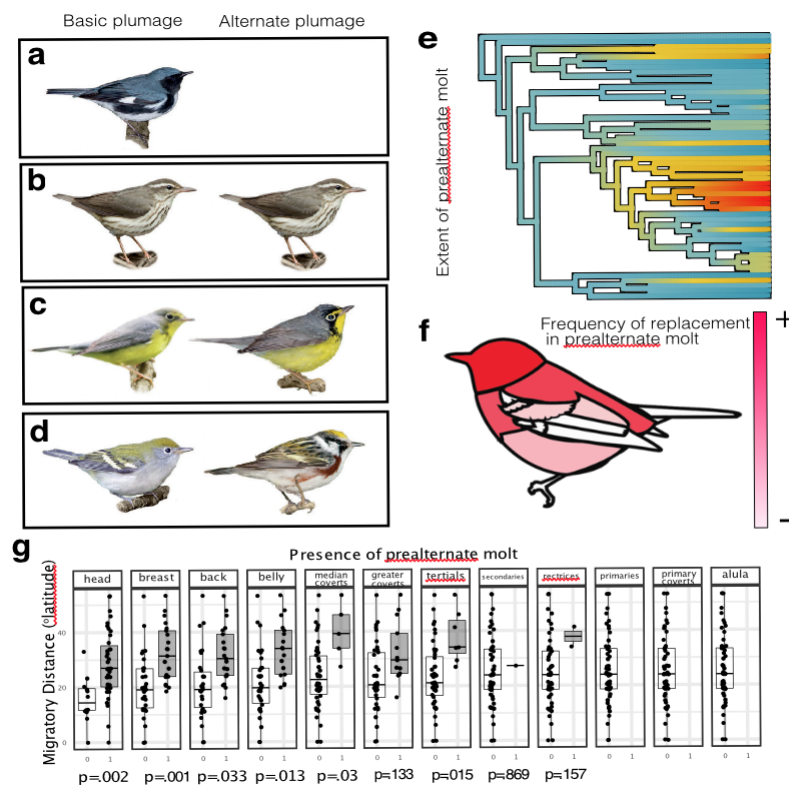
Feather color has attracted much attention, especially into selective processes that may have produced the diversity of coloration present in birds' feathers (Darwin 1871, Wallace 1891, Chaine and Lyon 2008, Li et al. 2010, Payne 1984). Both natural and sexual selection play roles in the colors of feathers as well as diversification of birds (Barraclough et al. 1995, Møller and Cuervo 1998, Stoddard and Prum 2007, Marcondes and Brumfield 2019). However, the molts that produce these feathers have been largely ignored in these studies. Birds show a diverse array of molt strategies (Stresemann and Stresemann 1966), but how and why different species of birds have different strategies for the timing and patterns with which they replace their feathers remains poorly known. How selection interacts with molt, and not just feather phenotype, is an essential question because molt is the underlying mechanism of feather production. How molts provide limitations and opportunities for seasonal change may be of widespread importance for understanding evolution of avian color at a broader scale. For example, some juvenile birds are brightly colored, and then lose this bright coloration in the highly conserved preformative molt that occurs shortly after fledging (Pyle 2009). It may be that the preformative molt provides an opportunity for these chicks to respond to selection from parental choice in the nest (Lyon et al. 1994) without being "stuck" in a bright plumage for their entire first year of life.
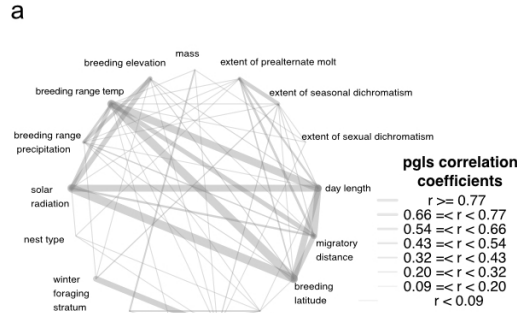
The needs for seasonal dichromatism likely vary by latitude and social system in birds (Friedman et al. 2009, Simpson et al 2015), but these results suggest that they may not be the primary factor influencing the evolution of prealternate molt in the New World warblers. A major study into global variation in seasonal plumage coloration in birds found that seasonal color change is more uncommon than predicted by social systems and predation risk (McQueen et al. 2019). We believe our study sheds some light on this conundrum. From the viewpoint of the *feather wear hypothesis* , the answer to this problem is that needs for seasonal color change alone are not strong enough to influence molt patterns in many species, and so seasonal color change can only evolve within the context of pre-existing molts. This is similar to how pre-existing molts limit the phenotypic realization of plumage maturation (Rohwer and Butcher 1988). A two-step relationship between a birds' needs for feather color change and the response of phenotypic evolution to those needs

may not necessarily be unexpected. Selection for color and structure on feathers likely interact in complex ways. For example, sexual selection on feathers may act as a "bridge" between peaks on natural selective landscapes for feather structure (Persons and Currie 2019). Our results provide evidence for similar "bridges" across adaptive landscapes, where naturally selected molts may provide bridges between spaces on a social selection landscape, in this case between year-round monochromatism, and seasonal dichromatism. Further research into the interplay between different types of selection on the evolution of molts and plumages in birds could consider groups with disparate social systems, as well as quantification of feather degradation. We suggest that molt should be considered when attempting to understand the evolution of plumages in birds.
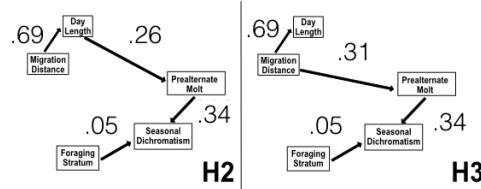
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Top path models

Response Variable	model	Adjusted R²	p	AICc	Δ AIC	AIC weight
Extent of Seasonal Dichromatism	extent of prealternate molt + breeding foraging stratum	0.54	<.001	106.3	0	0.890
Extent of Seasonal Dichromatism	extent of prealternate molt + winter foraging stratum	0.5	<.001	110.7	4.394	0.099
Extent of Seasonal Dichromatism	extent of prealternate molt + winter foraging stratum + day length	0.5	<.001	116.6	10.317	0.005
Extent of Seasonal Dichromatism	extent of prealternate molt + winter foraging stratum + breeding foraging stratum	0.5	<.001	116.72	10.443	0.005
Extent of Seasonal Dichromatism	extent of prealternate molt	0.36	<.001	119.5	13.257	0.001
Extent of Seasonal Dichromatism	migratory distance	0.09	0.008	131	30.093	0
Extent of Seasonal Dichromatism	day length	0.07	0.0119	131.8	31.043	0
Extent of Seasonal Dichromatism	minimum breeding temperature	0.06	0.017	132.7	31.557	0
Extent of Seasonal Dichromatism	breeding foraging stratum	0.06	0.0219	133.1	31.576	0
Extent of Seasonal Dichromatism	average winter temperature	0.06	0.0318	134.1	31.845	0
Extent of Seasonal Dichromatism	winter foraging stratum	0.05	0.0346	134.3	32.217	0
Extent of Seasonal Dichromatism	breeding maximum temperature	0.05	0.0387	134.5	32.614	0
Extent of Seasonal Dichromatism	breeding latitude	0.03	0.0458	134.8	33.270	0
Extent of Seasonal Dichromatism	winter foraging stratum + breeding foraging stratum	0.07	0.0489	134.9	33.595	0
Extent of Seasonal Dichromatism	breeding solar radiation	0.23	0.0594	135.2	33.610	0
Extent of Prealternate Molt	migratory distance + day length + breeding solar radiation	0.28	<.001	173.8	0	0.369
Extent of Prealternate Molt	day length + breeding solar radiation	0.22	0.0014	175.8	2	0.136
Extent of Prealternate Molt	migratory distance	0.19	0.0014	176.7	2.9	0.086
Extent of Prealternate Molt	migratory distance + breeding solar radiation	0.2	0.0025	177	3.2	0.074
Extent of Prealternate Molt	day length + migratory distance	0.19	0.0037	177.8	4	0.05
Extent of Prealternate Molt	day length + breeding solar radiation + solar radiation	0.21	0.0043	178	4.2	0.045
Extent of Prealternate Molt	migratory distance + winter solar radiation	0.16	0.0031	178.3	4.5	0.039
Extent of Prealternate Molt	day length	0.17	0.0057	178.7	4.9	0.032
Extent of Prealternate Molt	migratory distance + winter solar radiation	0.17	0.0062	178.9	5.1	0.029
Extent of Prealternate Molt	day length + solar radiation	0.17	0.0062	178.9	5.1	0.029
Extent of Prealternate Molt	migratory distance + solar radiation	0.17	0.0064	179	5.2	0.027
Extent of Prealternate Molt	day length + breeding precipitation	0.15	0.0104	180	6.2	0.017
Extent of Prealternate Molt	day length + winter solar radiation	0.2	0.0102	180.2	6.4	0.015
Extent of Prealternate Molt	day length + breeding foraging stratum winter foragin stratum + breeding solar radiation	0.15	0.012	180.3	6.5	0.014
Extent of Prealternate Molt	day length + breeding minimum temperature	0.14	0.0132	180.5	6.7	0.013
Extent of Prealternate Molt	day length + breeding foraging stratum	0.14	0.0129	180.5	6.7	0.013
Extent of Prealternate Molt	day length + winter foraging stratum	0.12	0.0352	182.9	9.1	0.004

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Supporting Information

Hosted file

`image6.emf` available at https://authorea.com/users/333884/articles/459975-title-evolution-of-breeding-plumages-in-birds-a-multiple-step-pathway-to-seasonal-dichromatism-in-new-world-warblers-aves-parulidae

Supplemental Table 1. molt, plumage, and life history scores for warbler species included in these analyses.

Supplemental Table 2: Presence of prealternate molt and seasonal dichromatism by species. 1= present, 0=absent, 0.5=partial, or intraspecific variation present.