Avian Behavior, Ecology, and Evolution

Disentangling the mechanisms of signal evolution in Tyrannidae flycatchers, part II: plumage elaboration evolved with migration behavior, but is also affected by diet, climate, and drift

Desenredando los mecanismos de la evolución de las señales en atrapamoscas de la familia Tyrannidae, parte II: la elaboración en el plumaje evolucionó con el comportamiento migratorio, pero también está afectada por la dieta, el clima y la deriva

Sydney M. Miller [1], Jacqueline Schoen [1], Matthew W. Reudink [1] (iD) and Sean M. Mahoney [1,2] (iD)

ABSTRACT. Animal coloration is an important communication signal that varies among taxa and affects survival and reproduction. Species-specific color is influenced by a variety of factors including phylogeny, predation, sexual selection, light and resource availability, ecological context, and/or species recognition. Tyrant flycatchers are useful to study color evolution because they are the largest family of birds, occur across broad environmental gradients, and although many genera are monomorphic with drab plumage, some are very colorful. Given the complexity of factors involved, the mechanisms influencing plumage color evolution in Tyrant flycatchers likely involve multiple drivers. In the second of our two-part paper on signal evolution in this avian family, we harnessed a large plumage color database of female and male Tyrannidae species (n = 399) to test the relative importance of geography (climate, latitude), ecology (migration behavior, forest cover, diet), and heterospecific proximity on plumage color evolution. From phylogenetically controlled analyses, we found that female color was largely driven by climate and male color was more exaggerated in migratory species. Sexual dichromatism was also affected by climate and diet, and was more pronounced in migratory species, possibly as a result of color loss in females. Pairwise comparisons between heterospecific color differences and geographic distance were generally weak and consistent with expectations under drift, which contrasted our song results (presented in Part I), but analyses at finer taxonomic scales revealed color may be used for conspecific recognition in some genera. Together with our paper on Tyrannidae song evolution, the color results reflect the importance of testing multiple drivers of signal evolution at different taxonomic scales because flycatcher color variation may have arisen through genetic drift, from a loss of female color in migratory species, and for species recognition in some genera. Future work could formally test the correlation between song and color variation, and use stochastic character mapping to confirm if Tyrannidae dichromatism resulted from female color loss in migratory species.

RESUMEN. La coloración en los animales es una señal importante en la comunicación que varía entre taxones y afecta la supervivencia y la reproducción. El color específico a la especie esta influenciado por una variedad de factores incluyendo la filogenia, depredación, selección sexual, luz y disponibilidad de recursos, contexto ecológico y/o reconocimiento de especies. Los atrapamoscas son útiles para estudiar la evolución del color, porque son la familia más grande de aves, ocurren a lo largo de amplios gradientes ambientales y, a pesar que muchos géneros son monomórficos con plumajes poco vistosos, algunos son muy coloridos. Dada la complejidad en los factores involucrados, los mecanismos que influencian la evolución en la coloración del plumaje de atrapamoscas probablemente involucran múltiples determinantes. En la segunda parte de nuestro estudio enfocado en la evolución de las señales en esta familia de aves, utilizamos una gran base de datos de coloración del plumaje de machos y hembras de la familia Tyrannidae (n=399), con el fin de poner a prueba la importancia relativa de la geografía (clima, latitud), la ecología (comportamiento migratorio, cobertura boscosa, dieta) y proximidad hetero específica sobre la evolución del color del plumaje. De los análisis controlados por filogenia, encontramos que el color de las hembras es mayoritariamente determinado por el clima y el color en los machos es más exagerado en especies migratorias. El dicromatismo sexual también estuvo influenciado por el clima y la dieta y fue más pronunciado en especies migratorias, probablemente como producto de la pérdida del color en las hembras. Comparaciones pareadas entre las diferencias en el color hetero-específico y la distancia geográfica fueron generalmente débiles y consistentes con las predicciones de la deriva, lo cual es contrastante con los resultados en el canto (presentado en la Parte I), pero los análisis a una escala taxonómica más fina revelaron que el color puede ser usado en el reconocimiento conespecífico en algunos géneros. En conjunto con nuestro estudio en la evolución del canto en Tyrannidae, los resultados del color reflejan la importancia de poner a prueba múltiples determinantes de la evolución de señales a escalas taxonómicas diferentes, pues la variación en el color en atrapamoscas pudo haberse originado por deriva genética por medio de una pérdida del color de las hembras en especies migratorias y para reconocimiento específico en algunos géneros. Futuros estudios pueden formalmente poner a prueba la correlación entre el canto y la variación en el color, y utilizar el mapeo estocástico de caracteres para confirmar si el dicromatismo en la familia Tyrannidae resultó de la perdida del color de las hembras en especies migratorias.

Key Words: *drift; evolution; flycatcher; plumage coloration; species recognition; Tyrannidae*

[1]Department of Biological Sciences, Thompson Rivers University, Kamloops, BC, Canada, [2]School of Natural Resources and the Environment, The University of Arizona, Tucson, AZ, USA

INTRODUCTION

In birds, feather colors and patterns are important visual signals that vary within and among species (Andersson 1994). Plumage evolution can be driven by both adaptive (natural and sexual selection) and neutral processes (Bradbury and Vehrencamp 1998). Natural selection can play an important role in shaping plumage coloration because plumage characteristics of birds may be under selection to match the environment and aid in crypsis or to help with thermoregulation (Wolf and Walsberg 2000). For example, Gloger's rule (1833), which has received empirical support among bird studies (reviewed by Zink and Remsen 1986, Delhey 2019, but see Marcondes et al. 2021), predicts that more heavily pigmented animals should be found closer to the equator where it is more humid, and less pigmented animals should be found at higher latitudes where it is more arid. Humid areas are hypothesized to have higher bacteria loads and the heavily pigmented feathers found in these areas may be less susceptible to bacterial degradation (Burtt and Ichida 2004). Lighter colored feathers may also aid in crypsis in arid environments; however, a study testing Gloger's rule with species exhibiting carotenoid-based pigments found the opposite pattern, in which upper plumage parts became lighter in more mesic habitats (Chui and Doucet 2009).

Ornamental colors can evolve through sexual selection when individuals prefer particular colors in mates, leading to higher relative fitness of colorful individuals and, ultimately, an elaboration of those traits (Hill 2015). In birds, females are typically the choosy sex and numerous experimental and observational studies have demonstrated that females prefer males with more elaborate plumage (Hill 2006a). Plumage can act as a direct indicator of male condition or quality, but elaborate plumage can also evolve simply through female preference for specific signals (e.g., through sensory bias, Prum 1990, 2010, Tobias and Hill 1998). Males with elaborate colors may be perceived as higher quality for example, because the acquisition of pigments (e.g., carotenoids) or producing feather nanostructure for structural coloration can be costly, meaning only individuals in good physiological condition during moult can produce the most colorful displays (Hill 2006a, Hill 2015, White 2020). Regardless of the mechanism, if colorful males are more successful at obtaining mates and females prefer more colorful males, elaborate plumage can quickly evolve (Hill 2015). Additionally, sexual versus natural selection can lead to plumage divergence within species, resulting in sexual dichromatism (Badyaev and Hill 2003). Dichromatism arises when there is stronger mate choice pressure on one sex within the species, which results in more elaborate ornamentation in that sex (Bailey 1978, Simpson et al. 2015). Simultaneously, dichromatism can arise through the loss of elaborate ornaments in one sex due to relaxation of sexual selection and/or increased selection for crypsis from predation risk during migration (Simpson et al 2015). At a different level of analysis, sexual selection can drive species radiations by driving the diversification of phenotypic traits associated with mate choice (Cooney et al. 2017). In the presence of strong intra-sex competition, and a change in inter-sex mate preference, the plumage signals that increase mating success can diverge, leading to reproductive isolation (Lande 1981).

Other ecological aspects of a species' environment may influence color evolution. The sensory drive hypothesis predicts signal transmission and reception will optimize under specific environmental contexts (Boughman 2002). In the case of plumage coloration, environmental factors like light availability may affect signal transmission (Endler 1992, 1993). For example, birds that display carotenoid-based colors absorb short and medium wavelengths of light, resulting in transmission of longer wavelengths (red, orange, and yellow; Badyaev and Hill 2003, Hill 2006a). Another way plumage color can be produced is through the interaction of light and nanostructures in the feathers. Composed of a combination of melanin, keratin, collagen, and other biomolecules, feather nanostructures interact with light and transmit blue, green, and iridescent colors (Prum 2006). Regardless of the plumage color, the light availability of an environment (e.g., open grassland vs. forest understory) will affect the conspicuousness of ornate plumage in the habitat (Endler 1992, Endler and Thery 1996).

When many species are living in sympatry and the need for species recognition is heightened, plumage signal divergence is magnified and can result in radiation events (Seddon et al. 2013). Birds that are closely related often have similar plumage color and patterns because of shared ancestry; however, overlapping ranges can cause signals to diverge (Sætre et al. 1997, Seddon 2005). This phenomenon could be explained by the species recognition hypothesis (Sætre et al. 1997, Simpson et al. 2021). In their study of Wood Warblers (*Phylloscopus sibilatrix)*), Simpson et al. (2021) found North American Wood Warblers with overlapping ranges exhibited greater plumage divergence. Similarly, dichromatism may evolve from a need for species recognition (Luro and Hauber 2022). Not all genera support the hypothesis because some congeners exhibit conserved plumage coloration even in sympatry, suggesting species recognition may be multi-modal (McNaught and Owens 2002).

Tyrant flycatchers are a unique group to study the evolution of visual signals because they occur across broad environmental gradients in North, South, and Central America and although there are many monomorphic species with drab plumage, some species are quite colorful (Fig. 1). The mix of conserved, drab plumage traits among many species and high elaboration in others, makes the group a promising subject to help unravel patterns of signal divergence. In our second paper of a two-part study on Tyrannidae signal evolution, we used phylogenetically controlled analyses to investigate how ecology (habitat, diet, migration behavior) and climate (precipitation and temperature) affected plumage color in Tyrant flycatchers to gain insight on how variation arose and is maintained. Additionally, we examined whether species living in closer proximity displayed greater color divergence as a test of the species recognition hypothesis. We predicted (1) plumage of both sexes would be largely driven by migration behavior, diet, climate, and habitat because these aspects have been found to influence coloration in other groups (e.g., Reudink et al. 2015, Simpson et al. 2015, Ward et al. 2021), and (2) we would find less support for the species recognition hypothesis because many Tyrant flycatchers exhibit similar plumage within genera but display pronounced vocal differences (investigated in Part I of this study). We further aimed to broaden the understanding of avian ecology and evolution in historically underrepresented regions because many of the species in our study occur exclusively in Central and South America (Nuñez et al. 2021). Our study also contributes to the growing body of literature on female signaling modalities by assessing female plumage evolution in addition to males (Riebel et al. 2019).

Fig. 1. A. Tyrannidae species occur across broad latitudinal, climatic, and ecological gradients (range centroids are shown by black dots). Tyrant flycatchers are a useful group to investigate plumage color evolution because although heterospecific coloration can be variable with species expressing carotenoid- and melanin-based pigments (B-D), coloration among some congeners is highly conserved (E-G). To assess color variation, we used published color scores from Dale et al. (2015) in which Tyrannidae ranged 35.1-72.1. Photos B and D represent higher scores (*Pyrocephalus rubinus*, 72.1, *Pitangus sulphuratus*, 59.0). Photos E-G exhibit low color scores (range 38-42.5). Species with primarily black coloration have higher color scores (H. *Knipolegus aterrimus*, 64.7; I. *Arundinicola leucocephala*, 63.4), and species with a combination of whites, blacks, and grays are intermediate (C. *Sayornis nigricans*, 55.8, J. *Tyrannus forficatus*, 50.4). Photo credits: B. *P. rubinus* (iNaturalist user miscbr), C. *S. nigricans* (Isaac Rath), D. *P. sulphuratus* (Dario Sanches), E. *Empidonax oberholseri* (Frank Fogarty), F. *E. traillii* (Michael Friedman), G. *E. minimus* (iNaturalist user milkocj), H. *K. aterrimus* (iNaturalist user avocat), I. *A. leucocephala* (Ricardo Gagliardi), J. *T. forficatus* (Luis Trinchan). Photo numbers can be found in acknowledgments.
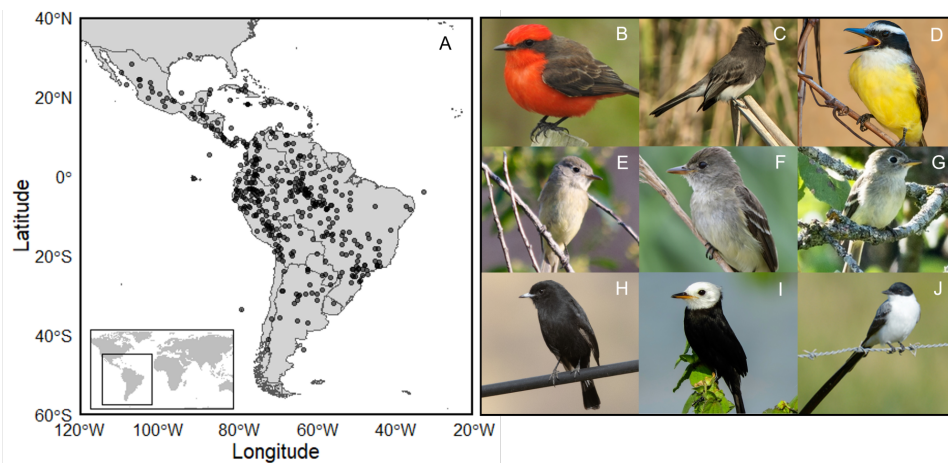
METHODS

Data collection

We obtained Tyrannidae plumage color data for 399 species (Appendix 1) from Dale et al. (2015), who digitally scanned images from the *Handbook of the Birds of the World* to score plumage color on males and females of each species from six patches (crown, forehead, nape, throat, upper breast, and lower breast), found to be consistently included in the illustrations and important for avian communication (Dale et al. 2015). Color patch values were measured from 400 randomly selected pixels to obtain red-green-blue (RGB) values (Dale et al. 2015). The RGB values were then corrected using species with similar coloration (see Dale et al. 2015) and averaged to provide single plumage color scores for males and females of each Tyrannidae species. To estimate dichromatism, we used the absolute values of the difference between the female color score from the male color score (Thibault et al. 2022). We previously used Dale et al. (2015)'s color scores to test different hypotheses about the evolutionary mechanisms of passeriform plumage coloration (Thibault et al. 2022).

Color scores provide a measurement for the general degree of color elaboration in each species and sex, where higher values indicate more colorful species, and are ideally suited for our large-scale comparative study of Tyrannidae (Dale et al. 2015). Human-biased color estimates provide useful proxies of color variation, particularly for interspecific comparative studies and for groups that are not UV sensitive (Seddon et al. 2010). Visual sensitivities for all Tyrant flycatchers are not known, however Brown-crested Flycatchers (*Myiarchus tyrannulus*), Eastern Phoebes (*Sayornis*

phoebe), and Eastern Kingbirds (*Tyrannus tyrannus*) are violet sensitive (Ödeen and Håstad 2003, Aidala et al. 2012), suggesting UV signals are less important in Tyrannidae. This is consistent with the notion that UVS sensitive species typically communicate visually using structural blue plumage (Ödeen et al. 2012), a color rare in Tyrannidae (Gray 1996). Majority of Tyrannidae exhibit black, gray, and rufous plumage color, which typically exhibits modest amounts of UV reflectance (Eaton and Lanyon 2003). As a further test of the utility of their approach, Dale et al. (2015) verified color scores were highly correlated with UV-visual spectrometry, as measured on museum specimens ($R^2 = 0.67$, P < 0.0001; Dale et al. 2015, see their Extended Data Fig. 1 and Extended Data "Plumage scores validation analysis" section). Therefore, although our color score approach certainly underestimates color variation, we do not anticipate this has a large effect on assessing general interspecific color differences, and about the ecology and evolution of visual signals among Tyrannidae. We also endeavored to estimate carotenoid-based color in Tyrannidae by calculating chromaticity for each species (Methods and Results presented in Appendix 2, Dey et al. 2014).

Tyrannidae plumage color may be under selection to optimize transmission given the light conditions in the environment (Endler 1993). To test this hypothesis, in our models, we included estimates of forest cover from Mikula et al. (2020), a study that assessed acoustic adaptation of peak song frequency in all Passeriformes birds. Briefly, Mikula et al. (2020) estimated tree cover using data from the Copernicus Global Land Cover project (Buchhorn et al. 2020). For each geographic location of bird song recordings in their study, they extracted the percentage of tree cover in a 100

m² quadrant using the "exactextractr" package in R (Baston 2020). They then calculated species-specific tree cover percentages as the mean of all conspecific recordings in their dataset. In our analyses, we used the mean forest percentage cover for each Tyrannidae species from Mikula et al. (2020). The geographic extent of Mikula et al. (2020) broadly overlapped with that in our study, so the forest cover estimates are well suited to use here. Some species (n = 18) were missing forest cover data, so we imputed data using the mean value from congeners (n = 13) or the overall mean (n = 5).

Diet may also influence plumage color expression due to differences in resource availability (McGraw and Hill 2001), thus we included diet breadth in our models. We characterized diet breadth for each species using Wilman et al. (2014), who estimated the frequency of consumption of 10 major food types in which the frequency of use for each food type is recorded between 0 (never used) and 1 (approximately used exclusively). Data were unavailable for n = 4 species (*Calyptura cristata*, *Piprites chloris*, *P. griseiceps*, and *P. pileata*), so we gathered diet data from Birds of the World (https://birdsoftheworld.org). We then built a similarity matrix of nutritional content for each food type from Sayol et al. (2018) and calculated a breadth index using Rao's quadratic entropy in the R package "indicspecies" (De Cáceres and Legendre 2009), in which larger diet breadth values represent broader diets (Appendix 1). The breadth values estimate the species' diets but also account for differences in the resources, making them more accurate diet estimates (De Cáceres et al. 2011). Previous research testing diet differences among species calculated breadth using similar methodology (e.g., Sol et al. 2014).

Plumage color may be under thermoregulatory and/or feather degradation resistance pressure (Gloger 1833, Burtt and Ichida 2004; and see Marcondes et al. 2021), so we sampled maximum temperature of the warmest month (BIO5) and annual precipitation (BIO12) climate variables from WorldClim 2 (Fick and Hijmans 2017), based on the species' range latitude and longitude centroids. We gathered range centroids and maximums from the Birdlife database (http://datazone.birdlife.org/home). Some species were lacking climate data (n = 18), so we used the mean values from congeners. In one case, a species was the sole member of the genus (*Nesotriccus ridgwayi*), therefore the climate variables reflect the family mean. We also used the species range centroids to assess the species recognition hypothesis (Simpson et al. 2021).

Finally, because there may be a trade-off between plumage coloration and migratory behavior due to the predation pressure, increased sexual selection, or relaxed female social selection (Badyaev and Hill 2003, Friedman et al. 2009, Simpson et al. 2015), we included in our models migration behavior from Tobias et al. (2022). We included migration as a binary variable (non-migratory and migratory) based on the classifications from Tobias et al. (2022). One species (*Calyptura cristata*) was missing migratory behavior, so we consulted Birds of the World and classified it as non-migratory. Our resulting dataset included 399 species for each sex.

Phylogenetic methods and statistical analysis
To control for non-independence of related species, we downloaded 1000 potential phylogenies from https://birdtree.org (Jetz et al. 2012, 2014; Hackett All Species, Hackett et al. 2008)

for all Tyrannidae species. We then used "TreeAnnotator" in BEAST v.1.10.1 (Suchard et al. 2018) to construct a maximum clade credibility tree using 1% burn in and mean node heights (Pageau et al. 2020, Thibault et al. 2022).

All analyses were performed in R 3.5.3 (R Core Team 2017). To assess the evolutionary mechanism of Tyrannidae plumage color, we built Pagel's lambda (λ) character evolution models with phylogenetic generalized least square (PGLS) statistical models, using the "nlme" package (Pinheiro et al. 2018). To determine which variables, or combination of variables, best explained color variation, we used stepwise model reduction in the "AICcmodavg" package (Mazerolle 2020) based on Akaike information criterion (AIC) and Bayesian information criterion (BIC). First, we constructed a full model, which included either female color score, male color score, or dichromatism as the response variable, and the latitude of the range centroid (absolute value), diet breadth, forest cover, annual precipitation, the maximum temperature of the warmest month, and migration as the predictor variables. Distributions of predictors varied so we scaled each term to a mean of 0 prior to analyses. Separate models were constructed for each sex and dichromatism. Next, we carried out model reduction for all possible models using the "StepAIC" function in the MASS package (Venables and Ripley 2002) and compared candidate models based on AIC and BIC (Burnham and Anderson 2003).

We also used phylogenetic path analyses using the R package "phylopath" (van der Bijl 2018) to assess the direct and indirect effects of the variables from the top PGLS models. We first built candidate path analyses informed by the PGLS models and then ranked models using an information theory approach based on C-statistics (Shipley 2009). Information theory evaluates the conditional independencies of each model and assigns a C statistic. The models are ranked based on the change in C statistic (ΔCICc) between models, where lower C scores are optimized and models within 2 ΔCICc of the top model are competitive and thus also supported. The top phylogenetic path analysis model was then selected as the model with lowest C statistic and models within 2 ΔCICc were subsequently averaged in the final model (for more details please see von Hardenberg and Gonzalez-Voyer 2013). We did not conduct a phylogenetic path analysis in cases where a single predictor variable was identified in our PGLS model selection.

To assess the relationship between color differences and geographic distance, we constructed pairwise distance matrices between all comparisons of species' range centroids and their color scores. To calculate color distances, we used the "vegdist" function in the Vegan package for R, using Euclidean distances (1 dimension; Oksanen et al. 2013). We then calculated linear geographic distances (km) between all species comparisons from the species' range centroids from above using the "geodist" package (van Etten 2017). We subsequently conducted a Mantel test, under the hypothesis that color distances would be greater if species ranges were closer in proximity (i.e., trait distance is negatively related to geographic distance). Although Mantel tests may suffer from low statistical power (Harmon and Glor 2010), the debate is ongoing (Hardy and Pavoine 2012). However, it is generally agreed upon that Mantel tests used to test comparative hypotheses violate assumptions of independence (Harmon and Glor 2010, Hardy and Pavoine 2012). We therefore addressed this

Table 1. Full and final model selection results assessing the relationships between climatic and ecological variables on Tyrannidae plumage color (female, male, dichromatism). Lambda (λ) indicates the phylogenetic signal of the response variable and was estimated using Pagel's λ model of character evolution in a phylogenetic generalized least square analysis. Note: K: number of model parameters; BIC: Baysian information criterion score; AICc: Akaike's information criterion score corrected for small sample sizes; ΔAIC: change between full and reduced model AICc; AICcWt: AICc weights, indicating the probability a model is the most parsimonious model; Cum.Wt: cumulative model weights; LL: log-likelihood.

Variable	Model	Model terms	K	BIC	AICc	ΔAICc	AICcWt	Cum. Wt	LL	λ
Female	Final	Latitude + Annual precipitation	5	2383.7	2363.91	0	0.96	0.96	-1176.88	0.37
	Full	Latitude + Diet breadth + Forest cover + Annual precipitation + Maximum temperature of the warmest month + Migration	9	2405.77	2370.33	6.42	0.04	1	-1175.93	0.37
Male	Final	Migration	4	2542.76	2526.91	0	0.98	0.98	-1259.4	0.38
	Full	Latitude + Diet breadth + Forest cover + Annual precipitation + Maximum temperature of the warmest month + Migration	9	2570.19	2534.75	7.84	0.02	1	-1258.14	0.38
Dichromatism	Final	Latitude + Diet breadth + Migration	6	2128.27	2104.55	0	0.9	0.9	-1046.17	0.29
	Full	Latitude + Diet breadth + Forest cover + Annual precipitation + Maximum temperature of the warmest month + Migration	9	2144.28	2108.84	4.29	0.1	1	-1045.19	0.29

issue using a phylogenetically controlled Mantel test in the package "evolqg" for R (Melo et al. 2016). We conducted Mantel tests for females, males, and dichromatism at two taxonomic scales: first at the family scale and then at the genus-level as a test assessing the risk of hybridization. For the genus-level analysis, we only included genera with n ≥ 10 species, restricting our analyses to n = 24 genera, and we treated genera as separate hypotheses.

RESULTS

In our dataset, female color score ranged 36.1 to 62.4 (median = 44.9), males ranged 35.1 to 72.1 (median = 45.1), and dichromatism ranged 0 to 30.7 (median = 1.4). Typically, species in our dataset with red, yellow, and black plumage had high plumage scores, gray and rufous species had low scores, and species with a mix of gray, white, and black had intermediate scores (Fig. 1). Structural blue plumage is rare in Tyrannidae species (Gray 1996) and exhibited intermediate scores (e.g., *Tachuris rubrigastra* female color score = 50.9, male = 56.8).

The final model for female Tyrannidae color (Table 1) included range latitude and annual precipitation (latitude: $X^2{}_{1396}$ = 2.93, p = 0.09, ϕ = 0.07 [0-0.18]; annual precipitation: $X^2{}_{1396}$ = 2.50, p = 0.11, ϕ = 0.06 [0-0.17]; Table 2). Although the PGLS results were not statistically significant, effect size confidence intervals did not overlap 0, suggesting colorful females were from lower latitudes and in areas with less annual precipitation (Fig. 2A and 2B). Male color scores (Table 1) were best predicted by migration behavior ($X^2{}_{1397}$=12.96, p=0.0003, ϕ=0.17 [0.07-0.27]; Table 2), where more colorful males were migratory (Fig. 2C). Tyrannidae dichromatism was best predicted by the reduced model (Table 1), and included latitude ($X^2{}_{1395}$ = 3.42, p = 0.06, ϕ = 0.08 [0-0.18]), diet breadth ($X^2{}_{1395}$ = 3.43, p = 0.06, ϕ = 0.08 [0-0.19]), and migration ($X^2{}_{1395}$ = 7.26, p = 0.007, ϕ = 0.13 [0-0.23], Table 2). Migration was statistically significant, but effect size confidence intervals for latitude and diet breadth did not overlap 0 (Table 2). Species with pronounced dichromatism were found at higher latitudes (Fig. 2D) and had narrower diet breadths (Fig. 2E). Similarly, to male coloration, dichromatic species were more likely to be migratory (Fig. 2F).

Fig. 2. Tyrannidae plumage color varied by ecology (diet breadth and migration), climate (precipitation), and geography (latitude). Figures show plumage variation for females (A, B), males (C), and sexual dichromatism (D, E, F). From phylogenetic generalized least square (PGLS) models, male color scores (C) were significantly higher and dichromatism (F) was more pronounced in migratory species (indicated by asterisks). White dots on boxplots represent mean values.
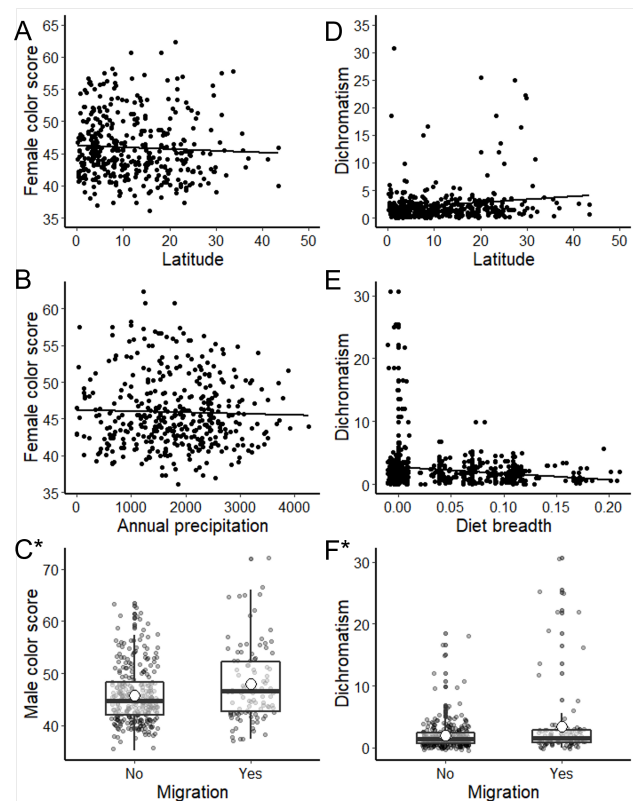
Table 2. Akaike's information criterion score (AIC) and Baysian information criterion score (BIC) selected model results from phylogenetic generalized least square models demonstrating the effects of climate and ecology on Tyrannidae color. Also included are effect sizes (ϕ, [95% CI]) for main effects. Significant results are shown in bold text.

Response	Main Effect	Model df	Residual df	X^2	p	ϕ
Female color	Latitude	1	396	2.93	0.09	0.07 [0, 0.18]
	Annual precipitation	1	396	2.5	0.11	0.06 [0, 0.17]
Male color	**Migration**	**1**	**397**	**12.96**	**0.0003**	**0.17 [0.07, 0.27]**
Dichromatism	Latitude	1	395	3.42	0.06	0.08 [0, 0.18]
	Diet breadth	1	395	3.43	0.06	0.08 [0, 0.19]
	Migration	**1**	**395**	**7.26**	**0.007**	**0.13 [0, 0.23]**

Our phylogenetic path analyses revealed direct and indirect effects of ecology and climate on female coloration and dichromatism (Appendix 3). In the top path model for females, annual precipitation had a direct, albeit weak, negative effect on coloration (coef. = -0.06, SE = 0.04) and latitude had a relatively strong, indirect negative effect on precipitation (coef. = -0.49, SE = 0.05, Fig. 3), suggesting more colorful females were found in dry, low latitude areas. The top model for dichromatism included a direct but weakly negative influence of range latitude (coef. = -0.01, SE = 0.03) and diet breadth (coef. = -0.004, SE = 0.03; Fig. 3). Migration positively influenced dichromatism indirectly through latitude (coef. = 0.05, SE = 0.06) and latitude positively influenced diet breadth (coef. = 0.02, SE = 0.03; Fig. 3). We did not conduct a path analysis for male Tyrannidae because our PGLS model identified migration behavior as the sole predictor.

Fig. 3. Tyrannidae color evolution is directly and indirectly affected by climatic, ecological, and geographic factors. Schematic shows final phylogenetic generalized least square, i.e., PGLS-informed phylogenetic path analysis models testing the relative importance of ecological, climatic, and geographic variables on female plumage color (top panel) and sexual plumage dichromatism (bottom panel) evolution in Tyrannidae. Solid arrows represent positive effects while dashed arrows represent negative effects, and the arrows are scaled by their relative effect. Model coefficients are indicated adjacent to arrows.
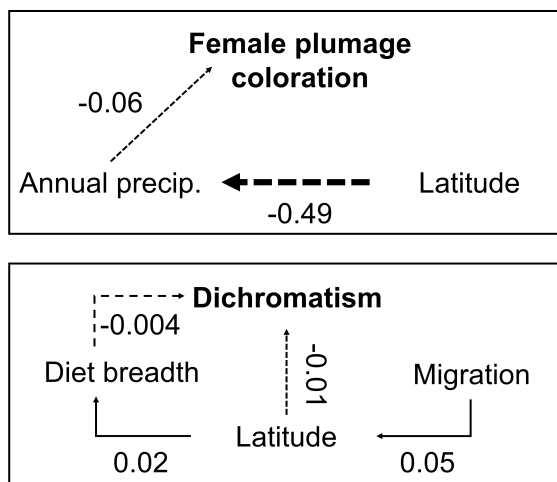
Support for the species recognition hypothesis depended on the taxonomic analysis level (Table 3). From a phylogenetic Mantel test of all Tyrannidae, female color did not show a statistically significant relationship with geographic distance, however confidence intervals did not overlap with 0, suggesting a positive relationship (Table 3; r = 0.02, p = 0.18, CI: 0.01-0.03). Male coloration was positively related to distance (Table 3; r = 0.06, p = 0.02, CI: 0.05-0.07), but dichromatism did not vary with geographic distance (Table 3; r = 0.009, p = 0.39, CI: 0.003-0.02). From genus-level phylogenetic Mantel tests, we found *Poecilotriccus* female coloration was positively related to geographic distance (Table 3; r = 0.51, p = 0.002, CI: 0.30-0.67). Female *Myiarchus* coloration was negatively related to distance based on non-zero overlapping confidence intervals, but the result was not statistically significant (Table 3; r = -0.13, p = 0.93, CI: -0.26 - -0.002). Male coloration and geographic distance in *Phylloscartes* were negatively related (Table 3; r = 0.11, p = 0.04, CI: -0.01-0.23), and positively related in *Poecilotriccus* (Table 3; r = 0.38, p = 0.01, CI: 0.15-0.57). Male *Empidonax* coloration showed a positive correlation to geographic distance among congeners from non-zero overlapping CIs, however the result was not statistically significant (Table 3; r = 0.27, p = 0.07, CI: 0.09-0.44). Dichromatism distances were negatively related to geographic proximity in *Elaenia* because CIs did not overlap 0, but p was > 0.05 (Table 3; r = -0.17, p = 0.92, CI: -0.32 - -0.02). All other relationships between color and geographic distances among congeners were not statistically significant (Table 3).

DISCUSSION

Plumage coloration is an important visual signal that aids in mate choice, predator avoidance, thermoregulation, and species recognition (Bradbury and Vehrencamp 1998). In our study, we simultaneously tested geographic and ecological factors of plumage variation in Tyrannidae, a speciose Passeriformes family, using a large-scale dataset of the Americas. Our phylogenetic comparative analyses revealed variation in plumage coloration among female and male Tyrant flycatchers was associated most strongly with migration behavior and heterospecific differences were consistent with expectations under drift. To a lesser extent, color varied by dietary and climatic factors, both of which may influence how feathers are grown (Hill 2006a). We also incorporated a phylogenetic path analysis to disentangle the direct and indirect effects of this complex and important visual signal.

From our PGLS, latitude and climate correlated with female plumage and dichromatism, but the results were not statistically significant. This contrasts with previous findings of pronounced

Table 3. Tyrannidae coloration showed mixed support for the species recognition hypothesis among genera. Table summarizes phylogenetically controlled Mantel test results comparing Euclidean color distances and geographic proximity (km). Significant relationships based on alpha = 0.05 (p) are in bold and in italics are Mantel correlation estimates (r) whose confidence intervals (CI) did not overlap 0 but p > 0.05. n refers to the number of species in the analysis.

Genus	Female plumage color				Male plumage color				Dichromatism				n
	r	95% LCI	95% UCI	p	r	95% LCI	95% UCI	p	r	95% LCI	95% UCI	p	
All genera	*0.02*	*0.01*	*0.03*	*0.18*	**0.06**	**0.05**	**0.07**	**0.02**	*0.009*	*0.003*	*0.02*	*0.39*	399
Phylloscartes	0.08	-0.04	0.20	0.09	**0.11**	**-0.01**	**0.23**	**0.04**	-0.02	-0.15	0.10	0.60	46
Myiarchus	*-0.13*	*-0.26*	*-0.002*	*0.93*	-0.07	-0.20	0.06	0.75	0.07	-0.06	0.20	0.25	44
Hemitriccus	0.02	-0.12	0.15	0.43	0.03	-0.10	0.17	0.32	-0.03	-0.16	0.11	0.61	42
Elaenia	-0.10	-0.26	0.06	0.71	-0.04	-0.19	0.12	0.57	*-0.17*	*-0.32*	*-0.02*	*0.92*	36
Empidonax	-0.01	-0.21	0.18	0.50	0.27	0.09	0.44	0.07	0.11	-0.09	0.29	0.18	*30*
Contopus	0.07	-0.14	0.27	0.28	0.12	-0.09	0.32	0.19	0.01	-0.20	0.21	0.35	28
Muscisaxicola	-0.07	-0.29	0.16	0.52	-0.02	-0.24	0.20	0.40	0.16	-0.07	0.37	0.18	26
Tyrannus	-0.12	-0.34	0.10	0.88	-0.03	-0.25	0.20	0.52	-0.16	-0.37	0.06	0.99	26
Phyllomyias	0.15	-0.10	0.38	0.09	-0.005	-0.25	0.24	0.46	-0.09	-0.33	0.15	0.83	24
Poecilotriccus	**0.51**	**0.30**	**0.67**	**0.002**	**0.38**	**0.15**	**0.57**	**0.01**	-0.18	-0.40	0.06	0.79	24
Knipolegus	0.11	-0.19	0.39	0.20	0.04	-0.25	0.33	0.38	0.03	-0.27	0.32	0.35	20
Ochthoeca	-0.05	-0.34	0.25	0.58	0.12	-0.18	0.40	0.27	-0.001	-0.29	0.29	0.42	20

dichromatism at higher latitudes (Friedman et al. 2009, Simpson et al. 2015), suggesting the effect varies among families. Our results could be driven by the broad variation of dichromatism in Tyrannidae (color score range = 0-30.7). For example, the most dichromatic species in our dataset, Vermillion Flycatcher (*Pyrocephalus obscurus*, color score = 30.7), is found at relatively low latitudes in North and Central America. Conversely, Great-shrike Tyrant (*Agriornis lividus*) is more monotypic (color score = 0.7) and occurs at relatively high latitudes in Chile and Argentina. Similarly, neotropical migrants such as *Empidonax*, *Contopus*, *Myiarchus*, and *Tyrannus* flycatchers can occur at higher latitudes and are relatively monotypic. Although studies testing latitudinal effects on morphology typically use range centroids (Simpson et al. 2015, Tobias et al. 2022), maximum range latitude may reveal patterns in Tyrannidae. Indeed, a univariate test of maximum range latitude suggests a negative effect of maximum latitude in females, but no relationship in males (females: estimate = -0.07, X^2_{1397} = 4.89, p = 0.02; males: estimate = 0.003, X^2_{1397} = 0.006, p = 0.94). Female color is consistent with historical evidence of latitudinal gradients (Gloger 1833, Görnitz 1923), suggesting colorful plumage is more prevalent in low latitude tropical areas (i.e., "Simple Gloger's rule," sensu Rensch 1929), however the direct and indirect paths of climate and latitude identified from our path analyses support the hypothesis that latitudinal effects on plumage coloration is more complex than previously thought (i.e., "Complex Gloger's rule," sensu Rensch 1929, Delhey 2019, Marcondes et al. 2021). In our path analysis for females, we identified a direct effect of annual precipitation. Species in wetter climes may be under pressure to express bacteria- or abrasion-resistant melanin-based plumage, which are both more prevalent in mesic climates (Burtt 1986, Burtt and Ichida 2004). Dichromatism was also influenced by latitude in the path analysis, but the coefficient was relatively weak, and the stronger latitudinal influence was indirect through diet. Precipitation may mediate resource availability (Williams 1951, 1961) and can limit foraging efficiency and/or invertebrate activity (Williams 1951, Taylor 1963, Grüebler et al. 2008), thereby reducing nutrient intake required for coloration (Laczi et al. 2020). Although we did not explicitly test the "Complex

Gloger's rule" (sensu Rensch 1929), Tyrannidae exhibit eumelanin and phaeomelanin plumage and species occur across broad climatic gradients, so the group may prove useful in evaluating the generalizability of the so-called "Complex Gloger's rule" (Rensch 1929, Delhey 2019).

Consistent with the hypothesis that migratory behavior influences plumage coloration, we found pronounced Tyrannidae male color and dichromatism in migratory species. In other avian taxa, the association between migration and sexual dichromatism results from a loss of elaboration in females (Friedman et al. 2009, Simpson et al. 2015) and there are several non-mutually exclusive hypotheses to explain this finding. First, pronounced dichromatism may arise from losses of female plumage elaboration for crypsis during migration (Caldwell 1986, Slagsvold et al. 1995, Huhta et al. 2003). Second, relaxed social selection for females at higher latitudes may reduce territoriality (West-Eberhard 1983, Irwin 1994, Tobias et al. 2011). Third, enhanced migratory male coloration may result from increased sexual selection at breeding areas (Badyaev and Hill 2003). And finally, recent evidence suggests dichromatism evolved for conspecific recognition (see Luro and Hauber 2022). Given female Tyrannidae coloration did not significantly vary with migration behavior, our results are consistent with the notion of pronounced dichromatism in migratory species due to the loss of female elaboration. To better understand the evolution of dichromatism in Tyrannidae, future studies could conduct ancestral state reconstructions to confirm whether dichromatism results from a loss or gain of elaboration and whether this pattern is associated with gains of migratory behavior (Badyaev and Hill 2003).

We did not find support for the light environment affecting tyrannid color evolution because no final models included forest cover. Similarly in Part I of our study in which we investigated the evolution of Tyrannidae song, our models failed to find support for acoustic adaptation (using forest cover), and song variation was mostly associated with morphology (body and bill size; Schoen et al. 2023). Other taxa are sensitive to light environments, such as *Phylloscopus* warblers, in which an

individual's plumage contrasts with the light environment (Marchetti 1993). Recent comparative studies indicate an interaction between light environments and climate affects plumage coloration (Gomez and Théry 2004, Marcondes et al. 2021) and indeed our models revealed plumage coloration was influenced by climate. We did however find positive effects of forest cover on carotenoid-based plumage coloration using chromaticity as a proxy (see Appendix 2; Dey et al. 2014), perhaps due to greater variation in carotenoid resources in forests (Grether et al. 2001, Jones et al. 2010), but spectrometry is needed to confirm if carotenoid signals are indeed associated with forest cover.

The species recognition hypothesis predicts differences among species signaling has evolved for conspecific recognition (Sætre et al. 1997, Seddon 2005). We conducted an analysis that included all Tyrannidae and found female color and dichromatism was not significantly related to geographic proximity, however confidence intervals suggested a weakly positive effect. Males were significantly positive, but the effect was also weak. The positive effects of geographic distance may be evidence of drift (Hill 2006b), and given that many flycatcher species are habitat specialists, heterospecific color differences may have arisen from geographic isolation and/or natural selection (e.g., Johnson and Cicero 2002). In contrast, Tyrannidae song (Schoen et al. 2023) at the family scale may have evolved for species recognition or to minimize signal overlap (Liou and Price 1994, Seddon 2005, Simpson et al. 2021). Genus-level tests of color differences offered some evidence for species recognition because female *Myiarchus* color and *Elaenia* dichromatism were negatively related to geographic proximity. Interestingly, *Myiarchus* song from Part I of our study also showed a negative relationship to congener proximity, suggesting correlated evolution of song and plumage and multi-modal signaling in species recognition (Schoen et al. 2023). Other genera showed positive relationships between color and congener proximity including *Empidonax* male, *Phylloscartes* male, and *Poecilotriccus* female and male coloration. *Empidonax* flycatchers are typically thought to be a cryptic group (e.g., Prescott 1987) and congener song differences did not vary with geographic proximity (Schoen et al. 2023), but our color results suggest differences arose from genetic drift (Hill 2006b). *Poecilotriccus* congener plumage differences also varied positively, as did their song difference (Schoen et al. 2023), offering evidence of similar evolutionary trajectories of song and plumage in Tyrannidae. The variable patterns of plumage and song among Tyrannidae genera suggest multiple signal modalities, possibly including other aspects of morphology, are needed to recognize conspecifics (Seddon 2005, Kirschel et al. 2009, Uy et al. 2009).

Overall, our large-scale phylogenetic analysis examines factors influencing Tyrant flycatcher plumage coloration evolution, focusing on the underlying processes of radiation in the family. We identified migration behavior, climate, and diet as the main predictors of plumage coloration and did not find strong support for a latitudinal gradient or for light environment effects (although see Appendix 2). Family-level differences in color were consistent with expectations under drift, which contrasts our findings from Part I of our study, however some genera showed support for the species recognition hypothesis, and we posit species delineations are maintained through multimodal signaling including song and other aspects of morphology. To better understand the

mechanisms driving dichromatism in Tyrannidae, future studies could investigate whether dichromatism results from a loss or gain of plumage elaboration due to migratory behavior, a pattern observed in Icteridae orioles and Parulidae warblers (Friedman et al. 2009, Simpson et al. 2015), as well as formally testing the relationship between Tyrant flycatcher color and song.

Data Availability:

Data used in this manuscript are available as appendices.

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Appendix 1. Data used in analyses.

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Appendix 2. Red Chromaticity and Methods

To account for the fact that carotenoid-based coloration leads to elaborate plumage ornamentation through different physiological mechanisms, we calculated chromaticity values using the red and blue color scores extracted from the RGB values provided by Dale et al. (2015). Chromaticity values provide an estimate of the relative contribution of short and long wavelengths to the expressed color and can act as a reliable metric for measuring variation in carotenoid and structural plumage coloration separately (Dey et al. 2015). To calculate red chromaticity we used the equation: R/(R+G+B), where R is the red value, G is the green value, and B is the blue value (Dey et al. 2015). Many Tyrannidae taxa exhibit carotenoid plumage (Fig. 1 main text) and few express structural, so we focused on red chromaticity here (Gray 1996). We previously used chromaticity values to test biogeographic macroevolutionary processes of passeriform color evolution (Oud et al. *in revision*). In that work, we provided evidence from Thraupidae that distributions of female (n=346) and male (n=346) red and blue chromaticity values from 10 plumage patches do not overlap, indicating the values captured separate aspects of the color signal. We also showed the values are consistent with visual color estimates determined by a single, independent observer (Oud et al. *in revision*, Fig. S1). We then used PGLS models following methods in the main text to test the effect of climatic and ecological variables on female and male red chromaticity.

Red Chromaticity Results

Female and male red chromaticity were significantly predicted by forest cover (females: X^2=8.22, p=0.004, ϕ=0.14 [95% CI: 0-0.24]; males: X^2=4.25, p=0.04, ϕ=0.09 [95% CI: 0-0.20]; Appendix 2, Table 1).

Appendix 2, Table 1.

	Variable	df	X^2	p	Estimate
	Latitude	1,392	0.07	0.795	0.001
Female red	Diet breadth	1,392	0.21	0.648	0.001
chromaticity	**Forest cover**	**1,392**	**8.22**	**0.004**	**0.007**
(λ=0.82)	Annual precipitation	1,392	0.61	0.434	-0.002
	Max. Temperature	1,392	0.32	0.571	-0.001

	Migration behavior	1,392	2.52	0.113	-0.004
Male red chromaticity (λ=0.83)	Latitude	1,392	0.00	0.96	0.000
	Diet breadth	1,392	0.17	0.68	0.001
	Forest cover	**1,392**	**4.25**	**0.04**	**0.005**
	Annual precipitation	1,392	0.01	0.91	0.000
	Max. Temperature	1,392	0.89	0.35	-0.002
	Migration behavior	1,392	0.84	0.36	-0.002

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Appendix 3. Phylogenetic Path Analysis model selection results testing the effect of climatic and ecological factors on Tyrannidae color variation. Candidate models were built from final PGLS models. The top path model appears in bold and multiple competing models (< 2 ΔCICc) were averaged. Climate variables were sourced from BIOCLIM. Maximum temperature of the warmest month (BIO5, ºC); Annual precipitation (BIO12, mm).

Please click here to download file 'appendix3.xlsx'.