DISENTANGLING THE MECHANISMS OF PLUMAGE COLOUR EVOLUTION IN TYRANNID FLYCATCHERS

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DISENTANGLING THE MECHANISMS OF PLUMAGE COLOUR EVOLUTION IN

TYRANNID FLYCATCHERS

by

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ABSTRACT

In birds, plumage coloration is an important visual signal involved in communication and survival that varies greatly across species. Plumage colour evolution is influenced by a variety of factors including phylogeny, predation pressure, sexual selection, light and resource availability, and ecological context. Tyrant flycatchers are an interesting group because although there are many monomorphic species with drab plumage, some species are quite colourful. Given the complexity of factors involved, the mechanisms influencing plumage colour evolution in Tyrant flycatchers are likely complex, involving multiple drivers. To account for the fact that differences in the availability of resources and light will result in differences in the acquisition and transmission of plumage signals, we calculated red and blue chromaticity values from red, blue, green scores originally extracted from digitally scanned images from the Birds of the World. Next, we used phylogenetically controlled analyses to assess the influence of a variety of ecological factors on plumage colouration, and dichromatism. Finally, we examined the extent of range overlap among species to test if plumage varies to a greater extent in overlapping species due to reinforcement of pre-mating isolating mechanisms (i.e., for species recognition). We found that diet, precipitation, and temperature all influence colour expression, that precipitation and latitude influence the extent of dichromatism, and finally that the extent of range overlap affects plumage divergence between species. Taken together, our results highlight the complexity of plumage evolution in Tyrant flycatchers and demonstrate that the evolution of plumage colouration is driven by a combination of ecological, geographic, and biological factors.

Thesis Supervisor: Professor Matthew Reudink

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INTRODUCTION

In birds, feather colours and patterns are important visual signals that vary among and within species (Andersson 1994). Across species, the factors influencing plumage evolution are many and varied and can be driven by both adaptive (natural and sexual selection) and neutral processes. For example, plumage characteristics of birds may be under selection to match specific environments to increase camouflage (Wolf 2015). In addition to crypsis, differences in plumage colouration may influence thermoregulation. Gloger's rule (1833), which has received ample empirical support (Delhey 2019, reviewed by Zink and Remsen 1986), 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 (Chui and Doucet 2009). Still, support for Gloger's rule is widespread among those studying birds (Review by Zink and Remsen 1986).

Ornamental colours can evolve through sexual selection when individuals prefer specific, and/or bright colours, leading to 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 and McGraw 2006). 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., though sensory bias, Hill 2015). Males with elaborate colours may be perceived as higher quality because the acquisition of pigments (e.g.,

carotenoids) can be costly, meaning only individuals in good physiological condition during moult can produce the most colourful displays (Hill 2015, Hill and McGraw 2006). Regardless of the mechanism, if colourful males are more successful at obtaining mates and females prefer more colourful males, more elaborate plumage can quickly evolve (Hill 2015).

Additionally, sexual 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 that results in more elaborate ornamentation in that sex (Bailey 1978, Simpson *et al.* 2015). Alternatively, dichromatism can arise through the loss of elaborate ornaments in one sex due to relaxation of sexual selection and/or increased selection for crypsis (Simpson *et al.* 2015). This change in sexual dichromatism is seen in New World woodwarblers (Parulidae). A study looking into the mechanism driving the variation in female ornamentation found that more colourful female plumage is lost when there is reduced social selection pressure on females and an increase in predation risk with migration. The results of this study show that social and natural selection are driving the alternative plumage in female woodwarblers, as opposed to sexual selection (Simpson *et al.* 2015).

At a broader scale, 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 matting success can diverge, leading to reproductive isolation (Lande 1981). When many species are living in sympatry and competition is high, plumage signal divergence is magnified and can result in radiation events; when the signals diverge. Reproductive isolation separates species based on mate choice (Seddon *et al.* 2013).

Birds that are closely related often have similar plumage colour and patterns due to shared ancestry; however, when these closely related species have overlapping ranges, their signals are more likely to diverge (Sætre *et al.* 1997, Seddon 2005). This phenomenon could be explained by the species recognition hypothesis, which states that species living in sympatry are more likely to have signals that diverge, allowing individuals of the same species to recognize one another and avoid hybridization with other species (Simpson *et al.* 2021). In their study of wood warblers, Simpson *et al.* found that warblers with overlapping ranges exhibited more plumage divergence. However, they also found that an increased number of species in one area will limit the signal divergence since there is only a certain number of signals that wood-warblers can display and recognize. Thus, when there are too many species living in the same area, limitations on the number of signals that can be displayed and recognized, thus create more overlap of signals (Simpson *et al.* 2021).

Finally, other ecological aspects of a species' environment may influence colour evolution. The sensory drive hypothesis states that systems of communication will change based on environmental factors (Boughman 2002). In the case of plumage colouration these environmental factors can include resource availability, which may restrict the nutrients needed for colour expression (Hill and McGraw 2006) and light availability, which may affect signal transmission of (Endler 1993). For example, birds that display carotenoid-based colours are unable to synthesize these pigments and must obtain them from the diet, metabolically convert the compounds, and ultimately deposit them in feathers. Carotenoid compounds will absorb and then emit certain wavelengths of light, resulting in red, orange, and yellow plumage colouration (Badyaev and Hill 2003, Hill and McGraw 2006). Another way that plumage colour 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, these nanostructures interact with light and transmit blue, green, and iridescent colours (Hill and McGraw 2006). For both carotenoid- and structural-based plumage, the environment in which those traits are displayed (e.g., open grassland vs. forest

understory) can influence signal transmission through differences in both resource availability and the available wavelengths of light, thus influencing signal efficacy.

Tyrant flycatchers are an interesting group with which to study the evolution of visual signals because although there are many monomorphic species with drab plumage, some species are quite colourful. The mix of conserved, drab plumage traits among many species, combined with high elaboration in other, raises fundamental questions about the factors driving plumage evolution in Tyrannidae. These flycatchers breed throughout North, South, and Central America, across broad climatic and ecological gradients. Because they occur across different ecological contexts, the factors that contribute to their plumage evolution are likely complex and difficult to disentangle. In this study, we will use chromaticity values to quantify carotenoid-based and structural-based colouration separately, to investigate how ecological variables affect the signal divergence in flycatchers, and gain insight on how these differences arise and are maintained. Using a phylogenetically controlled analysis, we will test whether both carotenoid-based and structuralbased colouration have been shaped by ecological and geographical factors. Additionally, we will use range extents to test the species recognition hypothesis. We predict that overall, plumage will vary among species (Mahoney et al. 2020) and that we will find more variability in plumage colouration with varying habitats, breeding ranges, climatic conditions, and location of breeding grounds. We expect to find support for the sensory drive hypothesis and in cases where there is range overlap, we expect to find support for the species recognition hypothesis (Simpson et al. 2021).

METHODS

Data Collection

Tyrannidae plumage colour data was obtained for 399 species from Dale *et al.* (2015). Dale *et al.* used digitally scanned images from the *Handbook of the Birds of the World* to score plumage colour on males and females of each species from six patches (crown, forehead, nape, throat, upper breast, and lower breast). The patches used were found to be consistently included in the illustrations and important for communication. Colour patch values were measured by Dale *et al.* from 400 randomly selected pixels to obtain Red-green-blue (RGB) values. These values were then corrected using species with similar colouration and averaged to provide a single plumage colour score for males and females of each Tyrannidae species. These scores were used to provide a measurement for the general degree of colour elaboration in each species and sex and are ideally suited to large-scale comparative studies (Dale *et al.* 2015).

To account for the fact that carotenoid-based colouration and structural-based colouration leads to elaborate plumage ornamentation through different physiological mechanisms, we calculated chromaticity values using the red and blue colour 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 colour 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), and to calculate blue chromaticity we used the equation: B/(R+G+B), where R is the red value, G is the green value, and B is the blue value (Dey *et al.* 2015).

We calculated red and blue dichromatism values to represent the level of dichromatism in each Tyrannidae species. Red dichromatism was calculated by subtracting the female red chromaticity score from the male chromaticity score, and blue dichromatism was calculated by subtracting the female blue chromaticity score from the male blue chromaticity score.

To examine the factors influencing plumage colouration, we collected data on diet, habitat type, and geographic region (data from Pageau *et al.* (2020), based on Wilman *et al.* (2014), Barçante *et al.* (2017), and ["BirdLife Data Zone" n.d.]).

Phylogenetic Methods

To control for non-independence of related species, we downloaded 1000 potential phylogenies from birdtree.org (Jetz *et al.* 2012; 2014) for all Tyrannidae species. We then used TreeAnnotator in BEAST v1.10.1 (Suchard *et al.* 2018) to construct a maximum clade credibility tree using 1% burn in and mean node heights.

Statistical Analysis

All analyses were performed in R 3.5.3 (R Core Team 2017) using phylogenetic generalized least squares (PGLS) analyses in the *nlme* package (Pinheiro *et al.* 2018). We used a series of PGLS models to ask how plumage colouration (red and blue chromaticity) and dichromatism (red and blue) are affected by diet, habitat, latitude, range overlap, and geographic region. To determine which variables, or combination of variables, best explained variation in colouration, we used stepwise model reduction based on Akaike Information Criterion (AIC). First, we constructed a full model which included either red or blue chromaticity or dichromatism as the response variable, and the latitude of the centroid range, feeding guild, habitat type, annual precipitation, and the max temperature of the warmest month as the main effects. Separate models were constructed for each sex. Next, we carried out model reduction for all possible models using the StepAIC function in the MASS package and compared candidate models based on AIC (Burnham, and Anderson 2003).

Changes in AIC values within 4 are considered to be competitive and the final model was chosen based on the lowest AIC (Burnham and Anderson 2003).

Range overlap

Range extents (centroid latitudes, and longitudes) of each species were taken from the International Union for Conservation of Nature species lists (<u>iucn.org</u>). To assess the relationship between plumage distance and geographic distance, we constructed pairwise distance matrices between all comparisons of species' range centroids and their chromaticity variables (red and blue chromaticity). To calculate plumage distances, we used the *vegdist* function in the Vegan package for R, which calculates Euclidean distances (Oksanen *et al.* 2008). Geographic distances were calculated using linear distance (km) between all comparisons of range centroids. We then conducted a Mantel test in R, under the hypothesis that chromaticity distances would be larger if species' ranges overlapped more (i.e., trait distance is negatively related to geographic distance).

RESULTS

Effects of ecological factors on chromaticity scores

The top performing model for male red chromaticity (Table 1) included dietary guild (F=6.57, P=0.002), habitat (F=6.57, p=0.004), and the maximum temperature of the warmest month (F=7.17, p=0.0078, Table 2). Likewise, the top model for female red chromaticity included dietary guild (F=7.26, p=0.0008), habitat (F=13.57, p=0.0003, Table 2), and the maximum temperature of the warmest month (F=6.23, p=0.013). Next, we found that the top model (Table 1) for male blue chromaticity included dietary guild (F=6.03, p=0.003) and annual precipitation (F=8.42, p=0.004, Table 2). The top model for female blue chromaticity included dietary guild (F=5.92, p=0.0029), and annual precipitation (F=11.31, p=0.0008, Tables 1, 2).

Variable	Sex	Model	Model terms	K	AICc	∆AICc	AICcWt	Cum.Wt	LL
	Male	Тор	Dietary Guild + Annual Precipitation + Maximum temperature of the warmest month	6	-1206.51	0	0.96	0.96	609.37
Red		Full	Latitude + Dietary guild + Habitat + Annual precipitation + Maximum temperature of the warmest month	10	-1200.12	6.38	0.04	1	610.36
Chromaticity	Female	Тор	Dietary Guild + Annual Precipitation + Maximum temperature of the warmest month	6	-1256.09	0	0.96	0.96	634.16
		Full	Latitude + Dietary guild + Habitat + Annual precipitation + Maximum temperature of the warmest month	10	-1249.96	6.13	0.04	1	635.28
		Тор	Dietary guild + Annual precipitation	5	-995.07	0	0.87	0.87	502.61
Blue	Male	Full	Latitude + Dietary guild + Habitat + Annual precipitation + Maximum temperature of the warmest month	10	-991.25	3.81	0.13	1	505.92
Chromaticity		Тор	Dietary guild + Annual precipitation	5	-1020.93	0	0.94	0.94	515.55
	Female	Full	Latitude + Dietary guild + Habitat + Annual precipitation + Maximum temperature of the warmest month	10	-1015.28	5.65	0.06	1	517.94

Table 1. Full and top model results to evaluate the relationship between ecological and geographical variables on red and blue chromaticity in male and female Tyrant flycatchers.

K=number of parameters, Likelihood=model likelihood, LL=log likelihood, Cum.Wt=cumulative model weight

Variable	Sex	Fixed effects	Model df	Residual df	F	р
		Dietary guild	2	374	6.57	0.0016
	Mala	Annual precipitation	1	374	8.35	0.0041
	wate	Maximum temperature of				
Red		the warmest month	1	374	7.17	0.0078
Chromaticity	Female	Dietary guild	2	375	7.26	0.0008
		Annual precipitation	1	375	13.57	0.0003
		Maximum temperature of				
		the warmest month	1	374	6.23	0.0130
	Mala	Dietary guild	2	374	6.03	0.0026
Blue Chromaticity	Male	Annual precipitation	1	374	8.42	0.0039
	Г 1	Dietary guild	2	375	5.92	0.0029
	remale	Annual precipitation	1	375	11.31	0.0008

Table 2. Effect tests of best fit models explaining chromaticity values of Tyrant flycatchers after a stepwise AIC reduction procedure. Significant terms are bolded.

We found that herbivores had the highest red chromaticity scores, while invertivores and omnivores had the highest blue chromaticity scores (Figure 1). Both males and females showed a positive relationship between annual precipitation and red chromaticity, and a negative relationship between annual precipitation and blue chromaticity (Figure 2). We found a positive relationship between maximum temperature of the warmest month and red chromaticity, but the relationship between maximum temperature of the warmest month and blue chromaticity was not significant (Figure 3).



Figure 1. Effects of dietary guild on the red and blue chromaticity scores for male and female Tyrant flycatchers.



Figure 2. Effects of precipitation on the red and blue chromaticity scores for male and female Tyrant flycatchers.



Figure 3. Effects of the maximum temperature of the warmest month on the red chromaticity scores for male and female Tyrant flycatchers.

Effects of ecological factors on dichromatism

When looking at sexual dichromatism in red chromaticity, the top model included habitat (F=2.46, p=0.062), annual precipitation (F=9.25, p=0.003), and the latitude of the centroid of the breeding range (F=5.85, p=0.016; Table 3). For dichromatism in blue chromaticity, the top model included the maximum temperature of the warmest month (F=2.67, p=0.1030), habitat (F=2.11, p=0.099), and the latitude of the centroid of the breeding range (F=8.56, p=0.004, Tables 3, 4).

Variable	Model	Model terms	Κ	AICc	∆AICc	AICcWt	Cum.Wt	LL
	Тор	Habitat + Annual precipitation + Latitude	7	3380.64	0	0.95	0.95	-1683.17
Red dichromatism	Full	Latitude + Dietary guild + Habitat + Annual precipitation + Maximum temperature of the warmest month	10	3386.50	5.86	0.05	1.00	-1682.95
Blue	Тор	Maximum temperature of the warmest month + Habitat + Latitude	7	3203.55	0.00	0.9	0.9	-1594.63
dichromatism	Full	Latitude + Dietary guild + Habitat + Annual precipitation + Maximum temperature of the warmest month	10	3207.90	4.34	0.1	1.0	-1593.65

Table 3. Full and top model selection results to evaluate the relationship between ecological and geographical variables on dichromatism in Tyrant flycatchers.

K=number of parameters, Likelihood=model likelihood, LL=log likelihood, Cum.Wt=cumulative model weight

Variable	Fixed effects	Model df	Residual df	F	р
Red	Habitat Annual	3	373	2.46	0.0624
dichromatism	precipitation	1	373	9.25	0.0025
	Latitude	1	373	5.85	0.0160
	Maximum temperature of the				
Blue	warmest month	1	373	2.67	0.1030
dichromatism	Habitat	3	373	2.11	0.0985
	Latitude	1	373	8.56	0.0036

Table 4. Results of best fit models explaining chromaticity values of Tyrant flycatchers after a stepwise AIC reduction procedure. Significant terms are bolded.

We found a positive relationship between annual precipitation and red dichromatism (Figure 4) and that there was not a significant relationship between annual precipitation and blue dichromatism. There is a positive relationship between latitude and both red and blue dichromatism (Figure 5).



Figure 4. Effect of precipitation on the level of dichromatism via red chromaticity in Tyrant flycatchers.



Figure 5. Effects of latitude on the level of red and blue dichromatism in Tyrant flycatchers.

Effects of range overlap on Tyrannidae colour evolution

When looking at the effect of range overlap on plumage divergence we found a negative relationship between chromaticity distance and geographical distance (r=-0.039 (95% CI: -0.046, -0.031), p=0.009).

DISCUSSION

Plumage colouration is an important visual signal that aids in communication, mate choice, predator avoidance and species recognition. With plumage colouration playing such an important role in many facets of a bird's reproduction and survival, the mechanisms driving plumage colour evolution within the large Family Tyrannidae are complex. Our phylogenetic comparative analysis indicates that variation in plumage colouration among flycatchers is associated with diet and climate, both of which may influence resource availability for growing ornamental feathers. Overall, herbivores have the highest red chromaticity values (likely due to high expression of carotenoid-based plumage), while invertivores and omnivores have the highest blue chromaticity values (associated with structural plumage) (Figure 1). Higher levels of precipitation were positively associated with red chromaticity and negatively associated with blue chromaticity (Figure 2). Additionally, we found that there was a negative relationship between climates with warmer temperatures and red chromaticity (Figure 3), and that there was no significant relationship between temperature and blue chromaticity. These patterns appear to be consistent across both males and females. In addition, higher levels of precipitation were associated with a greater amount of red dichromatism (Figure 4), and at higher latitudes there was greater blue and red dichromatism (Figure 5). Lastly, we found that species with greater range overlap have a greater chromaticity distance, meaning that species living closer to each other are more likely to look different. Overall, our results are consistent with the sensory drive hypothesis, Gloger's rule and the species recognition hypothesis.

The sensory drive hypothesis proposed by Endler (1992) suggests that environmental conditions alter the expression of signals to better match their environment. Based on this, we predicted that we would find divergence in plumage colouration with changes in environmental factors (diet, precipitation, temperature). Support for this hypothesis was found by Marchetti

(1993) who observed that *Phylloscopus* warblers were brighter when living in darker habitats and duller when living in habitats with more light (Gomez and Théry 2004). Our findings that plumage colouration was influenced by diet, precipitation, and temperature are consistent with the sensory drive hypothesis and suggest that in Tyrant flycatchers, plumage signals are influenced by environmental factors. However, it can be challenging to disentangle the mechanisms by which these environmental factors influence plumage expression or efficacy. In both females and males, we found that herbivores had the highest red chromaticity values (Figure 1), which could be attributed to resource availability. Carotenoid-based pigments cannot be synthesized by birds and thus need to be obtained through the diet. Herbivorous species that are gaining carotenoid pigments directly from the plants they eat may be better able to acquire sufficient dietary pigments to be able to modify and synthesize sufficient carotenoids into growing feathers to create more elaborate ornaments (Peneaux *et al.* 2021).

Overall, we suggest that our metric representing carotenoid-based colouration—red chromaticity—is likely directly influenced by resource availability though diet. Similarly, blue chromaticity may be directly influenced by diet, because although birds can synthesis melanin themselves, the precursors (phenylalanine and cysteine) come from their diet (Galván and Solano 2016). Again, this demonstrates how resource availability (specifically, food availability) can influence the plumage colours of birds. For example, in Anna's hummingbirds (*Calypte anna*), where it was found that varying protein levels in the diet influenced iridescent colour expression, with protein richer diets resulting in birds with more colourful feathers than those characteristics of the birds subjected to a diet lower in protein (Meadows *et al.* 2012). With protein being a source of the precursors to melanin, we can assume that the protein content in the diet of tyrant flycatchers is influencing their plumage colouration.

Similarly, we predict that precipitation has an influence on colour though resource availability, where habitats with more rainfall will have the ability to produce more of the resources needed to support bright plumage (Ward et al. 2021). A study done by Reudink et al. (2015) found that more rainfall was associated with the production of more highly saturated colours in American redstarts (Setophaga ruticilla), which the authors suggested was driven by an increase in available insect biomass during moult. In our study we found a similar pattern, with more rainfall being associated with higher red chromaticity scores (Figure 2). When looking at blue chromaticity and rainfall we found that areas with lower levels of precipitation were associated with higher blue chromaticity scores (Figure 2). A possible explanation for this is that flycatchers living in drier habitats use structural colours as a more efficacious signal (Medina I et al. 2017). A study done on mountain bluebirds (Sialia currucoides) found that along with precipitation, temperature also increased the production of bright, structurally coloured feathers (Ward et al. 2021). From this we can assume that temperature could also be indirectly affecting the relationship we have found between diet and chromaticity (Ward et al. 2021). Our results show that there is a negative relationship between red chromaticity and temperature (Figure 3), and that temperature did not have a significant effect on blue chromaticity. One possibility is that the negative relationship found between red chromaticity and temperature could be due to the stress that is added when temperatures become too hot, causing a decrease in foraging activity (Edwards et al. 2015).

Gloger's rule predicts that brighter coloured individuals will be found at lower latitudes, where there is more precipitation and warmer temperatures. We did not find a significant relationship between red and blue chromaticity and latitude; however, we did find a positive relationship between latitude and both red and blue dichromatism (Figure 5). Based on previous studies that found increased dichromatism at higher latitudes (Friedman *et al.* 2009, Simpson *et al.* 2015), we predicted that latitude would be positively associated with both red and blue

dichromatism in Tyrannidae. Our results confirm that an increase in latitude was associated with an increase in sexually dimorphic species, for both red and blue chromaticity. This increase in dichromatism with latitude could be caused by an increase in intrasexual competition, or an increase in predation risk with migration (Badyaev and Hill 2003). Friedman *et al.* 2009 examined the effects of migration on sexual dichromatism in New world orioles (genus *Icterus*), independent of sexual selection, and found that sexual dichromatism is 23 times more likely to evolve in migratory species, caused by a reduction in female elaboration. To better understand the evolution of dichromatism in Tyrannidae, future studies should use ancestral state reconstructions to determine whether dichromatism results from a loss or gain of elaboration and whether this pattern is associated with gains of migratory behaviour.

The species recognition hypothesis states that differences among species in plumage elaboration have evolved to reduce hybridization, by allowing birds to recognize individuals that are not of the same species (Sætre *et al.* 1997, Seddon 2005). As such, we predicted that species living in closer proximity would display more divergence in their plumage elaboration. Consistent with our hypothesis, we found a negative relationship between red and blue chromaticity distance and geographic distance; however, the relationship was relatively weak. This weak relationship suggests that there is likely another aspect of flycatcher appearance or behaviour that has a stronger influence on species recognition. A study focusing on chestnut-bellied flycatchers (*Monarcha castaneiventris*) found that both plumage and song are used in species recognition, implying that we would likely also find multiple factors influencing species recognition if we examined this at the family level (Uy *et al.* 2009). A study done on another group of flycatchers (*Empidonax traillii*) by Mahoney *et al.* 2020 found that when looking at the subspecies level, song, not plumage, is strongly associated with species recognition. Thus, song may in fact, be a better delineator of species' boundaries in at least some members of this family.

Overall, this large-scale phylogenetic analysis examines the factors influencing the evolution of Tyrant flycatcher plumage colouration, focusing on the underlying processes that have allowed the plumage to evolve and diverge. Our study showed that plumage colouration was influenced by a complex combination of ecological, biological, and geographical factors. Plumage colouration appear to be strongly influenced by diet and weather, though the extent of dichromatism was most strongly influenced by latitude. 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 behaviour—a pattern observed in orioles and warblers (Friedman *et al.* 2009, Simpson *et al.* 2015). Although we did find that species living closer together are more likely to have dissimilar plumage, the relationship was weak and we predict that song, instead of plumage, may be more important for maintaining species boundaries.

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APPENDIX

Variable	Sex	Guild	emmean	SE	df	t.ratio	p-value
v ariable	Bex	Herbiyore	0 447	0.0482	374	9.280	<.0001
	Male	Invertivore	0.381	0.0452	374	8.446	<.0001
		Omnivore	0.394	0.0456	374	8.647	<.0001
Red chromaticity		Herbivore	0.444	0.0451	374	9.829	<.0001
	Female	Invertivore	0.382	0.0423	374	9.023	<.0001
		Omnivore	0.397	0.0427	374	9.283	<.0001
		Herbivore	0.185	0.0638	375	2.907	0.0039
	Male	Invertivore	0.266	0.0597	375	4.450	<.0001
Blue chromaticity		Omnivore	0.254	0.0604	375	4.207	<.0001
		Herbivore	0.191	0.0617	375	3.097	0.0021
	Female	Invertivore	0.265	0.0577	375	4.594	<.0001
		Omnivore	0.25	0.0583	375	4.293	<.0001

Table 5. Post hoc test results examining the effects of dietary guild on red and blue chromaticity. Bolded terms are significant.

emmean = estimated marginal means, SE = standard error, df = degrees of freedom, t.ratio = test statistic