DISENTANGLING THE MECHANISMS OF SONG EVOLUTION IN TYRANNIDAE FLYCATCHERS

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DISENTANGLING THE MECHANISMS OF SONG EVOLUTION IN TYRANNIDAE

FLYCATCHERS

by

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ABSTRACT

Tyrannidae is the largest family of birds, and its species breed throughout North and South America across broad climatic and ecological ranges. Across species, songs within the Tyrannidae family are highly variable, and as suboscine songbirds, their songs are innate, meaning that their songs are inherited rather than learned. Song variation among suboscine species therefore represents adaptive differences that may be associated with reproductive barriers. The overall goal of this study was to investigate how morphological, ecological, and geographic factors may have affected song evolution and ultimately whether song evolution is linked to the radiation of the Family Tyrannidae. To do this, we used phylogenetically controlled analyses to test the relative influence of morphology (body size, beak size), ecology (habitat, diet, climate) and range overlap on song evolution. Our results indicate that song structure in Tyrannidae appears to be driven by a combination of range overlap, selection on signaling via the environment as well as selection on morphological traits. Environmental properties seem to have the strongest effect on song length, while morphological properties appear to drive song frequency in tyranids. Understanding the ways in which differences in song between related species arise and the role of song divergence in species radiation is an important topic in evolutionary ecology because it addresses a fundamental question in biology-the origin of species.

Thesis Supervisor: Professor Matthew Reudink

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INTRODUCTION

Birds produce a vast array of vocalizations that function as important signals in different contexts, including predation (e.g., warning others of potential threats, recruiting others to assist in mobbing), soliciting food (e.g., nestlings begging), social interactions (e.g., assessing competitors), and mating (e.g., assessing the quality of potential mates). Songs can diverge through both natural selection and sexual selection or as a result of neutral processes like genetic drift (Dynesius and Jansson 2000). Within a species, divergence of songs among related populations can cause assortative mating and lead to reproductive isolation and ultimately, speciation. When songs begin to differ to such an extent that individuals no longer recognize each other as conspecifics, species borders become established and song can function as a premating isolating mechanism (Seddon 2005, Bradbury and Vehrencamp 2001, Mason and Burns 2014, Derryberry et al. 2017). Understanding the ways in which differences in song between related species arise and are maintained by selection is an important topic in evolutionary ecology, as it key to understanding speciation (Seddon 2005).

The environment in which a bird lives can influence song structure due to differences in the sound transmission properties of habitats: a process termed "Sensory Drive" (Endler et al., 1980, Morton 1975). Because sounds do not all propagate equally in different habitats, selection should favour songs that provide the greatest broadcast potential in the environment in which a species live (Morton 1975). For example, acoustic signals in forests are subject to scattering by refraction, reflection, or diffraction from objects as a result of the presence of vegetation (Derryberry et al. 2017, Richards and Wiley 1977). Consequently, the acoustic signals of forest species tend to have a slower pace, purer tones, and lower frequencies than those of species that live in less dense habitats such as grassland areas (Richards and Wiley 1977). This form of sensory drive, often termed acoustic adaptation, has shaped the evolution of bird song in many species. For example, Satin Bowerbirds (*Ptilonorhynchus violaceus*), are found along a wide range of habitat types across the east coast of Australia (Nicholls and Goldizen 2006). Using an analysis controlling for phylogeny, researchers demonstrated that call structure was strongly related to habitat type, and calls from the same habitat type grouped together even if from geographically separate sites. As predicted by the acoustic adaptation hypothesis, Satin Bowerbirds calls in denser habitats (tropical rainforests) utilized lower frequencies and had little or no trill, while calls in open habitats utilized high frequencies and long trills.

The acoustic adaptation hypothesis, however, can be contentious and does not seem to hold true for all songbirds. Recent studies on song frequency across over 5,000 passerine species found that although there is evidence supporting a relationship between song frequency and body mass, there is no generalized relationship between song frequency and habitat (Mikula et al. 2020). These results suggest that variation in passerine song frequency may be primarily driven by selection causing shifts in morphology, rather than habitat-related selection on sound (Mikula et al. 2020). Part of the reason why sensory drive is so contentious is that previous studies are unclear on what portion of the hypothesis they are testing (Cummings and Endler 2018). The sensory drive model, modified from Endler (1992), can be divided into two components: sensory and signaling (Cummings and Endler 2018). The sensory component of the model predicts a strong correlation between receiver sensory detection properties (e.g., sensory system, brain characteristics, and perception of signals) with features within the physical environment (Cummings and Endler 2018). The signaling component describe the evolution of signaling traits as a product of the physical and biotic environment (Cummings and Endler 2018), such as timing and season of courtship, preferred habitat, and signal modes and signal properties.

Both ecology and morphology can influence song structure (Derryberry et al. 2017). Morphological adaptation typically results when structural change in an organism provides it with either higher reproductive success, or with a greater chance of survival. For example, the beak, which is typically under strong selection in the context of food and foraging, is used in coordination with syrinx movements to modify sound (Derryberry et al. 2017). Indeed, variation in beak morphology driven by dietary access has been directly linked to variation in song structure and the song performance capabilities of birds (Podos 2001, Wilkens et al. 2013). Morphological selection for beak size therefore represents a potential driver of song divergence (Wilkens et al. 2013). In addition to changes in beak size, changes in body size can also affect song structure: many larger bodied species and species with larger bills have been shown to produce lower frequency and slower paced sounds (Podos 2001).

Range overlap is another possible driver of divergence in mating signals; signalling is predicted to differ between closely related species when their respective geographic ranges overlap (Simpson et al. 2021). If multiple species co-habit the same environment, their song may subsequently become more or less complex to allow them to differentiate species and prevent interbreeding (Simpson et al. 2021). In contrast, allopatric species do not face the same pressures to differentiate their song as they are geographically isolated from their closely related species. Prior research reviewed in Simpson et al. 2021 has revealed that signals such as bird song are more divergent in areas where closely related species co-occur.

Songbirds are the largest group in the largest order in the Class Aves. (Clayton et al. 2009). This order, Passeriformes, accounts for over half of all extant bird species (Clayton et al. 2009). Within this order, the radiation of oscine (Suborder Passeri), and suboscine (Suborder Tyranni) birds began about 65 million years ago (Clayton et al. 2009). Tyrannidae is considered the largest family of passerine birds and its species breed throughout North and South America across broad climatic and ecological ranges (Johnson and Cicero 2002). Across species, songs within Tyrannidae are highly variable. As suboscine songbirds, the tyranids have songs that innate, meaning they develop from inherited information rather than learned information. Song

variation among suboscine species therefore represents genetic differences that may be associated with reproductive barriers (Tobias et al. 2012). Thus, Tyrannidae flycatchers are an interesting group in which to investigate the ecological and morphological mechanisms that contribute to song variation because song differences are not confounded by learning (Tobias et al. 2012).

The radiation of tyranids is unique due to their relatively conservative plumage evolution and high numbers of sibling species—biological species that are almost indistinguishable phenotypically from their close relatives but are nonetheless reproductively isolated. Though sibling species may be morphologically similar, they can be distinguished by their vocalizations, which serve to isolate them reproductively (Johnson 2002). Thus, vocal evolution in Tyrannidae may be intrinsically linked to their speciation. Additionally, sibling species usually occur in distinctive habitats across broad geographic regions. For example, in the tyranid genus *Empidonax*, differing habitat preferences among species are crucial in promoting and maintaining species limits; it has therefore been proposed that the difference in habitat preference is critical to all Tyrannidae speciation (Johnson and Cicero 2002).

The overall goal of this study is to investigate how morphological, ecological, and geographic factors affect song evolution and ultimately how song evolution has contributed to the species radiation within the Family Tyrannidae. To do this, we use phylogenetically controlled analyses to test the relative influence of morphology (body size, beak size), ecology (habitat, diet, climate) and range overlap in order to test the following hypotheses: 1) the Sensory Drive Hypothesis that predicts that acoustic signals vary in association with local environments due to the fact that different habitat conditions produce varied acoustic properties; 2) the Morphological Adaptation Hypothesis that predicts that body morphology is associated with variation in song structure and performance capabilities; and 3) the Species Recognition

Hypothesis which predicts that sympatric overlap may lead to divergence in song among related species.

METHODS

Study Species

The Family Tyrannidae comprises 399 species in 101 genera living in diversity of habitats across North and South America (Winkler et al. 2020). Tyrant flycatchers are the most species rich bird family and makes up one of the three main branches of passerine diversity in the Neotropics. Many of the genera in the family have diversified into many phenotypically similar species. Based on availability of data, this study concentrated on 283 species and 85 genera based on availability of data.

Recordings

Publicly available songs were acquired from the Cornell Lab of Ornithology's Macaulay Library (https://www.birds.cornell.edu/home/), and from xeno-canto.org. To avoid recordings from non-breeding, migrating birds, only songs that provided specific location metadata, and songs that were recorded during the breeding season (May, June and July for non-migratory birds and February to April for migratory birds) were included. Song characteristics of each subspecies were quantified (425 species, 5 songs per species). Songs for 116 species were omitted due to lack of data, resulting in a total of 283 species within 85 genera analysed using Raven Pro (Cornell Lab of Ornithology).

Data collection

To examine the relationship between song characteristics and bioclimatic variables, the frequency, and temporal characteristics (Appendix A, table 3) of our collected songs were

analyzed following Podos (2001) and song structure was summarized using principal components analysis (PCA). Following methods from Derryberry et al. (2017), we chose precipitation and temperature as bioclimatic variables because they are typically expected to impact sound transmission due to their strong influence on vegetation growth patterns. We retained four temperature and five precipitation variables for initial analysis: annual mean temperature (Bio1), mean diurnal range (Bio2), isothermality (day to night temperature oscillation) (Bio3), temperature annual range (Bio7), annual precipitation (Bio12), precipitation of driest month (Bio14), precipitation seasonality (Bio15), precipitation of warmest quarter (Bio18), and precipitation of coldest quarter (Bio19). Precipitation of the warmest and coldest quarter and mean diurnal range were removed from the models due to intercorrelation and insignificance, and multiple models were run separately for isothermality and temperature annual range due to intercorrelation. To examine the relationship between song characteristics and morphological variables, we gathered bill morphology data from Pigot et al. (2020) and body mass data from Veale (unpublished data). Both beak and body predicting variables were summarized using PCA. For the body size data, tarsus length, wing length, kipp distance (wingtip length), hand-wing index, tail length, and mass loaded heavily and positively on PC1, while for Beak PC1 culmen beak length, nares beak length, beak width, and beak depth loaded heavily (Appendix A). To test the morphological adaptation, sensory drive, and species recognition hypotheses, we obtained range maps for each species from the IUCN (International Union for Conservation of Nature) and climate data from the PRISM database (https://prism.oregonstate.edu/), and habitat data (primary habitat type) from Tobias et al. (2022).

Phylogenetic Methods

To control for phylogenetic non-independence in the analysis, we downloaded 1000 potential phylogenies from birdtree.org (Jetz et al. 2012; 2014) for the 283 Tyrannidae species for which we had sufficient data. TreeAnnotator in BEAST v1.10.1 (Suchard et al. 2018) was used 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), in the nlme package (Pinheiro et al. 2018). This technique uses knowledge of phylogenetic relationships to estimate the expected covariance in cross-species data (Symonds and Blomberg 2014). When using this technique, closely related species are assumed to have similar traits due to their shared ancestry (Symonds and Blomberg 2014), so phylogenetic relationships are controlled for within the analysis. Using a stepwise model reduction, based on Akaike Information Criterion (AIC), we examined the factor(s) that best explained song structure (PC1 and PC2). To do so, we first built a full model that included song (PC1 and PC2) as the response variable, with habitat, bill and body structure, and bioclimatic variables as the main effects. We then performed a model reduction by testing all possible models using the StepAIC function in the MASS package, and selecting the top model based on the change in AIC between the full model and each reduced model. (AIC, Burnham and Anderson 2003). ΔAIC values within 4 units were considered competitive and the final model was chosen based on the lowest AIC (Burnham and Anderson 2003). To assess the relationship between acoustic distance and geographic distance, we constructed pairwise distance matrices between all comparisons of species' range centroids and their song variables. To calculate acoustic distances, we used the *vegdist* function in the Vegan package for R, using Euclidean distances (Oksanen et al. 2013). We then calculated geographic distances using linear distance (km) between all comparisons of range centroids. We subsequently conducted a Mantel test using R under the hypothesis that song distances would be greater if species ranges were closer (i.e., that trait distance is negatively related to geographic distance). A second, partial Mantel test was then conducted with a third matrix accounting for environment. This test considers the assumption that other factors can change with space, including environmental similarity and subsequently removes confounding factors of the full Mantel test.

RESULTS

Factors associated with Tyrannidae song evolution:

Song PC1 (Song Frequency)

The top model for Song PC1 (song frequency) included Beak PC1 (F = 16.69, p = 0.0001), Body PC1 (F = 8.17, p = 0.005), driest month precipitation (F=0.49, p=0.48), and precipitation seasonality (F = 3.47, p = 0.06). Frequency was strongly negatively correlated with Beak PC1 and Body PC1, and marginally negatively correlated with precipitation seasonality. It was positively correlated, however, with driest month precipitation.



Fig. 1. PC1 variation in Tyrannidae (n=283) is related to body and beak characteristics. Left panel: Song frequency is significantly lower as body PC1 increases (F=8.17, p=0.046). Right panel: Song frequency is significantly lower as beak PC1 increases (F=16.69, p=0.0001).

Song PC2 (Song Length)

The top model for Song PC2 (song length) included habitat (F = 4.11, p = 0.0006), annual mean temp (F = 3.94, p = 0.048), Isothermality (F = 2.41, p = 0.12), annual precipitation (F = 2.16, p = 0.14), and driest month precipitation (F = 8.67, p = 0.004). Specifically, longer songs were positively correlated with annual mean temperature, isothermality, annual precipitation, and driest month precipitation



Fig 2. PC2 variation in Tyrannidae (n=283) related to classified habitat types. Song length changes significantly depending on habitat type (F=4.11, p=0.0006).



Fig 3. PC2 variation in Tyrannidae (n=283) is related to precipitation and mean temperature characteristics. Left panel: Songs are significantly longer in wetter habitats as represented by levels of precipitation in the driest month. (F=8.67, p= 0.004). Right panel: Songs are significantly longer in warmer habitats as represented by annual mean temperature levels. (F=3.94, p= 0.048).

Mantel tests (Range Overlap)

Consistent with the species recognition hypothesis, a significant negative relationship was found between song distance and geographic distance (r = -0.074 [95% CI: -0.087, -0.066)], p=0.003). These results indicate that song is more different among species that are geographically closer.



Fig 4: Correlation between song difference and geographic distance. A significantly negative relationship is observed (p=0.003), indicating song is more different among species that are geographically closer.

Results from the partial Mantel test accounting for environmental similarity found a negative, but slightly weaker, relationship between song and geographic distance ((song distance~geo distance+climate distance): r=-0.02, P=0.3 (95% CI: -0.04, -0.004).

Table 1.	AIC selected model results	s demonstrating the	e effect of each	fixed effect a	and interactions	on PC1	(song frequency)	and PC2 (song
length).	Significant results are indic	cated in bold text.						

variable	Fixed Effects	Model df	Residual d	f F	р
PC1	Beak PC1	1	277	16.69	0.0001
	Body PC1	1	277	8.17	0.0046
	driest month precipitation	1	277	0.49	0.48
	precipitation seasonality	1	277	3.47	0.06
PC2	habitat	6	271	4.11	0.0006
	annual mean temp	1	271	3.94	0.048
	isothermality	1	271	2.41	0.12
	annual precipitation	1	271	2.16	0.14
	driest month precipitation	1	271	8.67	0.0035

Table 2. Full and reduced top model selection results to assess the relationships between biological, climatic, and ecological variables on PC1 (song frequency) and PC2 (song length).

Variable	Model	Model Terms	K	AICc	ΔAICc	AICcWt	Cum.Wt	LL
PC1	Тор	Beak PC1 + Body PC1 + driest month precip. + precip.seasonality	9	1911.49	0	0.66	0.66	-946.42
	Full	Habitat + migration +trophic level + range size + Beak PC1 + primary lifestyle + Body PC1 + annual mean temp + isothermality + annual precip. + driest month precip. + precip seasonality	20	1937.02	25.52	0	1	-946.90
PC2	Тор	habitat + annual mean temp + isothermality + annual precipitation+diest month precipitation	12	1900.98	0	0.48	0.48	-937.91
	Full	Habitat + migration +trophic level + range size + Beak PC1 + primary lifestyle + Body PC1 + annual mean temp + isothermality + annual precip. + driest month precip. + precip seasonality	20	1913.67	12.69	0	1	-935.22

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

DISCUSSION

Song structure in Tyrannidae appears to be driven by a combination of direct selection on signal design via transmission properties of the environment, indirect selection on song characteristics as a result of selection on morphological traits, and by range overlap of species. Environmental properties seem to have the strongest effect on song length, while morphological properties appear to drive song frequency in tyranids. Taken together, these results provide support for both the sensory drive, morphological adaptation, and species recognition hypotheses.

Sensory Drive Hypothesis

The Sensory Drive hypothesis predicts that acoustic signals vary in association with local environments because different environmental conditions produce different acoustic properties. Specifically, the hypothesis predicts acoustic signals in dense habitats will become lower, simpler, and longer in order to maximize their transmission (Boncoraglio & Saino, 2007, Morton 1975), compared to open habitats where high frequency sounds travel further. My results indicate that although song length was significantly correlated with climatic features associated with habitats, song frequency was not.

A systematic review of sensory drive studies over 25 years (Cummings & Endler, 2018) found that of 132 studies, 86% found support for the hypothesis. Of the studies that focused on auditory signals in birds, 21 (80.7%), found support for the signaling aspect of the hypothesis and one found support for the sensory components in the sensory drive hypothesis. Notably, among the studies supporting auditory sensory drive in birds, a study by Francis et al. (2010) found evidence for the signaling component of sensory drive in ash-throated flycatchers. Because our study focused on song frequency and length, our results therefore also provide evidence for the signaling components in the sensory drive hypothesis rather than the sensory

components. Our results indicate that song length is associated with habitat features, and song frequency is marginally (although not significantly) associated with habitat. Thus, our results provide evidence to suggest that song signaling varies in association with the local environment, thus providing evidence supporting the sensory drive hypothesis.

Outside of habitat, bioclimatic variables are also strongly associated with song length. Annual mean temperature and driest month precipitation had the strongest effect on song length, followed by isothermality and annual precipitation. Specifically, warmer temperatures and higher precipitation (both for annual and for driest month) were associated with longer songs. Likewise, the higher the percentage of isothermality, the longer the length of song tended to be.

Precipitation may indirectly influence song traits due its role in driving vegetation density (Medina & Francis, 2012), a factor known to influence sound transmission. Attenuation is the decrease of signal intensity, and dense vegetation typically increases attenuation (Boncoraglio and Saino 2007). One possibility is that higher precipitation levels result in higher vegetation density and thus a larger increase in attenuation (Boncoraglio and Saino 2007). As mentioned, the sensory drive hypothesis predicts these dense habitats will produce lower, simpler, slower, and longer to maximize their transmission (Boncoraglio & Saino, 2007, Morton 1975). Our results indicate that Tyrannidae song does in fact appear to be longer in habitats with denser vegetation, consistent with the sensory drive hypothesis (Fig 2. Fig 3.).

Song frequency (PC2) was not associated with habitat and only weakly associated with precipitation—findings that are inconsistent with the sensory drive and acoustic adaptation hypotheses. This result, though surprising, is not unheard of, as a similar study on all passerines also found that song length, but not song frequency, was associated with differences in habitat (Mikula et al. 2020). Taken together, our results suggest that the environment appears to have a greater effect on song length than on song frequency in Tyrannidae.

Songs within human modified habitats diverged most from all other habitats, a finding consistent with recent studies of bird song in urban environments (Slabbekoorn and den Boer-Visser 2006, Francis et al. 2010) Because anthropogenic noise is typically louder and more continuous than sounds in natural environments, it provides a potential force influencing the ecology and evolution of species (Francis et al, 2010). This trend has been observed in many studies on oscine birds (Francis et al. 2010), but relatively little is known regarding suboscine responses to anthropogenic environments. Given that suboscine song develops in the absence of learning, suboscines typically are considered to have little vocal plasticity (Francis et al. 2010); however, the results in this study indicate that Tyranids within human modified habitats did in fact have a great divergence in their song. It should be noted that only 6 species of the 283 in this study are described as settled in anthropogenic habitats, which could indicate only species whose vocalizations already can be successfully heard in human modified areas are able to settle anthropogenic habitats (Francis et al. 2010).

Oscine songbirds can adjust their songs through plasticity in song development, allowing them to compensate for the increased low frequency noise from urbanization (Francis et al. 2010). However, any changes to suboscine song would likely indicate adaptive change. Considering our evidence, future studies could include ancestral state reconstruction of the song of tyranids found in human-modified habitats to investigate whether these differences are adaptive or whether birds with longer songs may simply be better able to colonize urban habitats.

Morphological Adaptation Hypothesis

The morphological adaptation hypothesis predicts that morphological variation is associated with variation in song structure and performance capabilities in birds. Consistent with this hypothesis, both beak and body size traits predicted variation in song frequency, suggesting that variation in Song PC1 (song frequency) may be an indirect result of selection on beak and body morphology. Our finding that as body and beak size increases, frequency decreases, aligns with the morphological adaptation hypothesis that suggests that vocal performance capabilities will vary as a result of vocal tract and beak morphology (Podos 2001).

A negative relationship between body size and song frequency, in which larger species produce lower-frequency sounds, has been documented across many groups including insects, fishes, amphibians, reptiles, mammals, and birds (Mikula et al. 2020). In birds it is typically suggested that body size influences the functional aspects of the vocal apparatus, such as the size of the vibratory structures, the vocal tract, and the size of the labia in the syrinx (Podos 2001, Riede & Goller, 2010, Mikula et al. 2020). Additionally, in previous studies, it has been found that songbirds with relatively larger and stronger beaks will face performance constraints on their vocal tract; as jaws become more adapted for strength, for example to crush hard seeds, they will be less able to perform the rapid movements required for high pitched trills (Podos 2001). This hypothesis is supported by our results that song frequency became significantly lower as beak size increased. Overall, the results from this study indicate that the evolution of song frequency in Tyrannidae is primarily constrained by morphology over any other factor, as predicted by the morphological adaptation hypothesis.

Species Recognition Hypothesis

The species recognition hypothesis predicts that sympatric overlap may lead to greater divergence in song in closely related species (Seddon 2005, Simpson et al. 2021). Mantel tests showed weakly negative, but significant, effects of distance—meaning that song is more different among species that are geographically closer. This result is consistent with findings from previous work on species recognition (Seddon 2005, Simpson et al. 2021), in which results

suggest species located in close proximity to each other face pressure to differentiate themselves in some way. This also aligns with traditional theory that predicts selection will favour traits that reliably signal species identity and augment assortative mating (Liou and Price 1994. Seddon 2005). The results of our study therefore demonstrate that the need for species recognition provides a driving force in vocal evolution in Tyrannidae. Although we don't have any data on whether song divergence was accompanied by morphological divergence, work done by Mahoney et al (2020) found that at the subspecies level in Tyrannidae, song is more associated with species recognition than is plumage, indicating that song is a better indicator of species limits in sympatry.

Conclusion

There are some limitations to our experimental approach that may have resulted in mixed results. First, we lack detailed information on the habitat and vegetation density; classifying preferred habitats as "forest", "grassland", etc. does not account for any within-habitat variation relevant to the transmission of sound (Mikula et al. 2020). Second, this study doesn't account for any other factors in the environment; for example, other bird species may influence background noise in the environment. Finally, our data was limited to publicly available songs, which naturally limited the number of species we could use. Future work regarding song evolution in Tyrannidae should focus on a more detailed approach to characterizing habitat features that may affect song evolution, as well as looking into the effect of environmental properties on other various acoustic signals, including song complexity.

In conclusion, after controlling for phylogeny, we gained several major insights into the evolution of song in Tyrannidae. We found that: (1) Song length is positively correlated with temperature and precipitation, suggesting that living in more densely vegetated habitats may result in longer songs, as suggested by the sensory drive hypothesis; (2) Song frequency is

weakly correlated with precipitation variables but doesn't provide significant evidence supporting aspects of the sensory drive hypothesis regarding frequency; (3) Selection on morphology is likely the main driver in the evolution of song frequency in Tyrannidae; (4) Song is most different in tyranids living in human modified habitats, indicating that some tyranids may be able to adapt their songs to anthropogenic settings. (5) Song is more different among species that are geographically closer, providing supporting evidence for the species recognition hypothesis. This study thus provides significant support for the morphological adaptation and species recognition hypothesis but challenges a main component of the sensory drive hypothesis. Overall, we conclude that song evolution in Tyrannidae is shaped by a combination of selection on morphological traits driving song frequency, range overlap of heterospecifics, and environmental properties driving song length.

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APPENDIX A

Contrast	estimate	SE	df	t. ratio	p. value
Forest – Grassland	5.153	2.61	271	1.977	0.4316
Forest - Human Modified	-7.441	1.96	271	-3.801	0.0033
Forest – Rock	-4.120	3.60	271	-1.146	0.9132
Forest – Shrubland	1.548	1.07	271	1.446	0.7764
Forest – Wetland	-0.228	3.96	271	-0.058	1.0000
Forest -Woodland	0.112	1.21	271	0.093	1.0000
Grassland- Human Modified	-12.594	3.08	271	-4.090	0.0011
Grassland-Rock	-9.273	4.46	271	-2.080	0.3678
Grassland-Shrubland	-3.605	2.64	271	-1.363	0.8210
Grassland-Wetland	-5.382	4.45	271	-1.208	0.8906
Grassland-Woodland	-5.041	2.81	271	-1.797	0.5512
Human Modified – Rock	3.321	4.08	271	0.814	0.9834
Human Modified - Shrubland	8.989	2.00	271	4.498	0.0002
Human Modified - Wetland	7.213	4.30	271	1.676	0.6325

Table A1: Results of Post-hoc test on habitat for significant categories.

Human Modified - Woodland	7.553	2.24	271	3.375	0.0146
Rock – Shrubland	5.668	3.73	271	1.518	0.7336
Rock – Wetland	3.891	5.35	271	0.727	0.9908
Rock – Woodland	4.232	3.80	271	1.114	0.9236
Shrubland – Wetland	-1.777	3.98	271	-0.447	0.9994
Shrubland – Woodland	-1.436	1.41	271	-1.016	0.9502
Wetland – Woodland	0.340	4.06	271	0.084	1.000

Table A2: Defining model terms used in phylogenetic generalised least square analysis.

Term	Definition	Units
Habitat	Natural environment. categorised	N/A
	as either forest, grassland,	
	shrubland, wetland, rocky,	
	human modified, or woodland	
Migration	Seasonal movement	N/A
Trophic level	Position occupied in the food	N/A
	web	
Range size	Spacial area that the species is	Kilometres
	found	
Primary lifestyle	Predominant locomotory niche	N/A
Body PC1	First principle component that	N/A
	accounts for variation of all	
	variables related to the body.	
	Includes tarsus length, wing	
	length, kipp distance, hand-wing	
	index, tail length, and mass	
Beak PC1	First principle component that	N/A
	accounts for variation of all	
	variables related to the beak.	
	Includes culmen beak length,	

	nares beak length, beak width, and beak depth	
annual mean temperature	The annual mean temperature	Degrees Celcius
isothermality	Quantifies how large the day to- night temperatures oscillate relative to the summer to-winter (annual) oscillations.	Percent
annual temperature range	A measure of temperature variation over a given period.	Degrees Celcius
annual precipitation	The sum of all total monthly precipitation values.	Millimetres
driest month precipitation	The total precipitation that prevails during the driest month.	Millimetres
precipitation seasonality	a measure of the variation in monthly precipitation totals over the course of the year. This index is the ratio of the standard deviation of the monthly total precipitation to the mean monthly total precipitation (also known as the coefficient of variation)	Percent

Table A3: Tyrannidae PPCA eigenvalues and loadings.

	PC1	PC2
Notes	0.01191361511	-0.4594104814
Duration	0.04165883048	-0.8416858006
Meanfreq	-0.8515455038	0.2928865322
Sd	-0.4931393171	-0.4022629981
freq.median	-0.8115428197	0.3100663475
freq.Q25	-0.5920234376	0.460180642
freq.Q75	-0.9139229464	0.1148630983
freq.IQR	-0.4856843637	-0.3548854044

time.median	0.07957699378	-0.8312877176
time.Q25	0.08482968311	-0.7661674013
time.Q75	0.05539665473	-0.8502556812
time.IQR	0.02087921345	-0.8032114926
Skew	0.3109978819	-0.009620571149
kurt	0.2171928955	-0.04665098863
sp.ent	-0.5021210471	-0.572222648
time.ent	-0.2785904395	-0.08239861428
Entropy	-0.5251562591	-0.4796519257
Sfm	-0.4544272522	-0.4331792628
Meandom	-0.8009469146	0.3208223127
Mindom	-0.2131325233	0.3208223127
Maxdom	-0.7997174084	-0.3205469538
Dfrange	-0.5278258541	-0.5563910496
Modindx	-0.2363260783	-0.688573086
Startdom	-0.465705151	0.07321834248
Enddom	-0.497444919	0.1556883072
Dfslope	-0.257722373	0.09567994895
Meanpeakf	-0.7224899933	0.2995887657
PPCA standard deviations	2.59558731	2.52559110

Principal Component	Variation
PC1	5.564470e+01
PC2	2.682378e+01
PC3	1.012682e+01
PC4	6.118339e+00
PC5	5.552505e-01
PC6	2.156658e-01
PC7	1.554144e-01

Table A4: Principal component axis variation for axes 1-7