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**Research Article**

# Punctuated evolution of bill morphology in the largest family of songbirds (Thraupidae)

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## ABSTRACT

We assessed correlations between speciation rate and bill morphology in an ecologically diverse and continental-scale songbird radiation, the tanagers (Thraupidae). Our analyses showed that bill size, shape, and their evolutionary rates are not correlated with speciation rate. However, we did find evidence that each axis of variation in bill morphology diversifies at speciation events interspersed with periods of gradual evolution, consistent with a punctuated equilibrium model of character change. To determine correlations, we incorporated a time-calibrated molecular phylogeny and high-resolution three-dimensional surface scans of bill structure from museum study skins. Overall, our findings suggest that bill size and shape diversify in association with the speciation process, but not through any effect on the rate of speciation. Previous studies have shown other traits, such as song, ecological niche, and plumage do influence speciation and, ultimately, species richness in tanagers.

**Keywords:** ecomorphology, morphometrics, phenotypic evolution, punctuated equilibrium, songbirds, speciation

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## LAY SUMMARY

- Bill morphology is widely considered to play a role in avian speciation and diversification as a result of ecological specialization
- Previous studies on the relationship between bill morphology and speciation have mostly focused on closely related species often in island settings
- We quantify correlations between speciation rate and bill morphology in a diverse and broadly distributed songbird radiation, the tanagers
- We find that bill size, shape, and their evolutionary rates are not correlated with speciation rate
- We did find support for mixed punctuated and gradual evolution of bill morphology
- Our results suggest that bill size and shape diversify in association with the speciation process, but not through any effect on the rate of speciation in the tanagers

# Evolución puntuada de la morfología del pico en la familia más grande de aves canoras (Thraupidae)

## RESUMEN

Evaluamos las correlaciones entre la tasa de especiación y la morfología del pico (tamaño, forma y tasa evolutiva) en una radiación diversa y ampliamente distribuida de aves canoras, los tangaras (Thraupidae). Nuestros análisis mostraron que el tamaño, la forma y las tasas evolutivas del pico no están correlacionadas con la tasa de especiación. Sin embargo, encontramos evidencia de que cada eje de variación en la morfología del pico se diversifica en eventos de especiación intercalados con períodos de evolución gradual, en consonancia con un modelo de equilibrio puntuado de cambio de caracteres. Para determinar las correlaciones, incorporamos una filogenia molecular calibrada en el tiempo y escaneos de superficie de alta resolución en 3D de la estructura del pico a partir de pieles de estudio de museos. En general, nuestros hallazgos sugieren que el tamaño y la forma del pico se diversifican en asociación con el proceso de especiación, pero no a través de ningún efecto en la tasa de especiación. Estudios previos han demostrado que otros rasgos, como el canto, el nicho ecológico y el plumaje, sí influyen en la especiación y, en última instancia, en la riqueza de especies en los tangaras.

**Palabras clave:** aves canoras, ecomorfología, equilibrio puntuado, especiación, evolución fenotípica, morfometría

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# INTRODUCTION

Bill morphology has long been thought to be important to the process of avian speciation (Darwin 1859). For instance, changes in bill depth associated with ecological divergence in resource use has promoted speciation and diversification in *Loxia* crossbills (Benkman 1993, 1999, 2003). Another classic example of the interplay between bill morphology and speciation is the adaptive radiation of Darwin's finches on the Galápagos Islands (Burns et al. 2002, Grant and Grant 2008). Divergent natural selection on bill size and shape variation in Darwin's finches has produced significant feeding diversity that can be tied directly to ecological opportunity and speciation. Darwin's finches are embedded within a larger radiation of songbirds, the tanagers (Thraupidae), which also show a wide diversity of ecologies and bill morphologies (Burns et al. 2002, Mauck and Burns 2009, Vinciguerra and Burns 2021, Demery and Burns 2023).

The avian bill may be associated with the speciation process in three ways. First, species with certain trait values may be more prone to speciate, causing heterogeneity in rates of speciation. Traits, such as body size (Gittleman and Purvis 1998), dispersal ability (Phillimore et al. 2006, Claramunt et al. 2012), and range size (Rosenzweig 1995) have been shown to influence the rate of speciation. Previous studies have suggested that species with finch-shaped bills specialized for eating seeds of various sizes are prone to speciate faster than species with warbler-shaped bills specialized for insectivory (Rundell and Price 2009, Price 2011). Second, trait evolution could occur in association with speciation events ("punctuated evolution" Eldredge and Gould 1972). Punctuated evolution has been implicated in ornamental coloration in *Carduelis* finches (Cardoso and Mota 2008), brain shape in kingfishers (Eliason et al. 2021), and several morphological characters in ratites (Cubo 2003). Third, high rates of trait evolution may result in higher rates of speciation (*sensu* Lanfear et al. 2010). These hypotheses are not mutually exclusive but highlight how traits are associated with the speciation process.

Tanagers (Thraupidae) are the most species-rich songbird family in the world, with 384 species distributed across Central and South America (Clements et al. 2023). Species in the family inhabit nearly every terrestrial niche, including forest and open habitats, from coastlines to Amazonia to the Andean highlands (Parker et al. 1996). Tanagers are well known for their exceptional diversity in plumage, song, and ecomorphologies (reviewed in Isler and Isler 1999, del Hoyo et al. 2011; Fjeldså 2020). Consequently, many studies have aimed to identify the drivers of speciation in this remarkably diverse radiation, including traits such as climatic niche (Title and Burns 2015), plumage complexity (Price-Waldman et al. 2020), song (Mason et al. 2017), morphology (Demery 2018), and combinations of these traits (Price-Waldman 2019). The well-studied ecomorphological diversity of tanagers sets the stage to investigate the role of the avian bill during speciation at larger geographic and temporal scales.

Studies that have assessed the role of bill morphology in driving speciation in tanagers have reported mixed results. Using linear measurements of bills, Demery (2018) found links between speciation rate and the rate of bill evolution, whereas Price-Waldman (2019) found no support for this relationship when these measurements were combined with other traits in phylogenetic path analysis. However, the analysis of three-dimensional (3D) bill morphology could give a more complete description of bill shape variation than standard linear measurements (length, depth, width). Therefore, 3D morphometrics of bill shapes may provide a more detailed understanding of how species interact with their environment and how this influences speciation.

In this study, we leverage species-level 3D morphometrics and phylogenetic comparative methods to test (1) whether species with particular bill morphologies (i.e., insectivorous warbler-like bills, generalist bills, and granivorous finch-like bills) are inherently associated with higher speciation rates. Next, we test (2) if bill size and shape are diverging at speciation events or whether these traits are better explained by gradual evolution. Finally, we test (3) whether the rate of bill evolution is linked to speciation rate across the tanager phylogeny.

# METHODS

## Phylogeny

We used a species-level timetree of Thraupidae from Burns et al. (2014). The tree was inferred using two mitochondrial (protein-coding genes, cytochrome *b* [Cyt *b*] and ND2) and four nuclear genes (protein-coding gene RAG1 and 3 introns ACO1-19, FGB-15, MB-12). A secondary node calibration (~12 Mya) from Barker et al. (2013, 2015) was used to obtain absolute divergence times. This calibration point agrees with a recent fossil-calibrated phylogeny of passerine families, representing the split between Thraupidae and Mitrospingidae (Oliveros et al. 2019). We pruned the outgroup taxa (*Cardinalis cardinalis*, *Piranga ludoviciana*, *Pheucticus tibialis*, *Passerina ciris*) for comparative analyses.

## Speciation Rate Estimation

We used the cladogenetic diversification rate shift model (ClaDS) to compute branch-specific speciation rates in RPANDA v1.7 (Morlon et al. 2016). ClaDS is a Bayesian method for estimating heterogeneity in species-specific speciation and extinction rates (Maliet et al. 2019). Specifically, we used the ClaDS2 model, which allows changes in diversification rates at speciation events while allowing lineages to vary in extinction rates but with a constant turnover. We ran the Markov chain Monte Carlo (MCMC) for 200,000 iterations, incorporating a sampling fraction of 0.95 (365/384) to account for missing species in the phylogeny. To assess convergence, we ran three independent MCMC chains simultaneously and computed Gelman statistics every 100 iterations. The inference of diversification stopped when the maximum of the Gelman statistics (computed for each parameter) was below 1.05 and the mixing of the chains approached stationarity. We discarded the first 25% of the posterior as burn-in before calculating maximum a posteriori estimates.

## Bill Size and Shape Data

We obtained landmark data describing bill morphology from the supplemental material of Vinciguerra and Burns (2021). These data are based on 3D surface scans from Mark My Bird (markmybird.org). The data consist of landmark and semi-landmark configurations of bill morphology from one adult male specimen per species when available from study skins at the Natural History Museum (NHM), Tring, UK. Our species-level taxon sampling followed the taxonomy of Clements (Clements et al. 2023), except for the inclusion of *Ramphocelus costaricensis*. This taxon is treated as a subspecies by Clements et al. (2023) but treated as a full species in previous checklists (Clements et al. 2013) and is included in the phylogeny from Burns et al. (2014). In total, we sampled 319 species (~83% of the family) present in both the phylogeny and trait datasets. Supplementary Material Table 1 provides a list of species analyzed in this study, including NHM catalog numbers, sex, and species not included. For details on the protocols for scanning, landmarking, and quality control of the 3D data, see Cooney et al. (2017). We used geomorph v3.12 (Adams and Otárola-Castillo 2013, Collyer and Adams 2018) to generate Procrustes-aligned coordinates via generalized Procrustes analysis (Klingenberg 2013). We slid semi-landmarks to minimize bending energy and enforced symmetry to remove differences related to the arbitrary placement of semi-landmarks (Gunz and Mitteroecker 2013). To remove the effects of size on shape, we used a phylogenetic regression to obtain size-adjusted residuals as a measure of allometry-free shape (Adams 2014).

We used principal component analyses (PCA) on the user-averaged, Procrustes coordinates to identify, describe, and analyze major axes of bill shape variation. We extracted species scores from the first two principal components to analyze the main axes of variation in the dataset. We also obtained bill size measurements from the landmark configurations and body mass from the EltonTraits database (Wilman et al. 2014). To obtain an index of bill size, we used centroid size, defined as the square root of the sum of squared Euclidean distances of the landmarks from their centroid (Klingenberg 2016). We used phylogenetic generalized least squares (Grafen 1989) residuals of bill size against body mass as a proxy for bill size relative to body size. In total, we analyzed three bill traits in comparative tests: (1) bill shape PC1, (2) bill shape PC2, and (3) relative bill size (hereafter referred to as bill size in the text). Bill shape PCs, log bill centroid size, log body mass, and relative bill size measurements are provided in Supplementary Material Table 2.

## Comparative Tests

### Hypothesis 1: Trait-dependent speciation

We used a tip-rate correlation approach (Harvey and Rabosky 2017) to test whether a particular bill size or shape influenced speciation rates. Specifically, we tested whether speciation rates were correlated with bill size and shape by determining whether the correlation significantly deviated from a simulated null distribution of associations between speciation rates and trait values. This approach has high statistical power and is robust to false positives. We used speciation rates from ClaDS because studies have shown that model-based diversification rate metrics are more accurate than non-model-based metrics (Maliet et al. 2019, Title and Rabosky 2019). We generated the null distribution by simulating 1,000 Brownian motion (BM) character histories on the Maximal Clade Credibility (MCC) tree in phytools (Revell 2012). To simulate the null distribution, we used the root state and diffusion rate ($\sigma^2$) parameters from the best-fit model of trait evolution based on the empirical trait dataset in geiger v2.0.6.4 (Supplementary Material Table 3; Harmon et al. 2008, Pennell et al. 2014). To account for tip variance unrelated to phylogeny (i.e., measurement error), we added a nuisance parameter to each model of trait evolution (Silvestro et al. 2015, Clavel et al. 2018).

### Hypothesis 2: Punctuated evolution

We estimated whether evolutionary changes in bill size and shape were the result of gradual or punctuated evolution using the $\psi$ model (Ingram 2010). The $\psi$ model quantifies the extent to which trait change occurs at speciation events. Specifically, the parameter $\psi$ quantifies the proportion of interspecific divergence (punctuated + gradual) that is attributable to punctuated change. Values of $\psi$ range between 0 and 1, whereby 0 reflects BM trait change that is proportional to time and a value of 1 corresponds to traits that diverge in concert with speciation events (punctuated evolution).

To test whether bill size and shape were better explained by gradual or punctuated evolution, we fitted four models in a maximum likelihood framework: (1) the BM model of only gradual evolution, (2) the $\psi$ model of gradual and punctuated evolution, (3) multi-BM (O'Meara et al. 2006, Thomas et al. 2006), and (4) multi-$\psi$ model of gradual and punctuated evolution (Ingram et al. 2016). We incorporated multi-BM and multi-$\psi$ models to allow rates to vary in two parts of the phylogeny where diversification rate shifts were identified in Burns et al. (2014), the clades subtending the Darwin's finches and *Sporophila* seedeaters. If clades with faster speciation also have faster BM trait evolution, Rabosky (2012) showed that this pattern can mimic the signal of punctuated evolution. We assessed the relative support of each model using Akaike Information Criterion (AIC) and AIC weights ($w_i$). We used the R package MOTMOT v2.1.3 (Puttick et al. 2020) to fit models. Although we do not account for measurement error in these model comparisons, studies have shown that the issue of variance at the tips unrelated to phylogeny is of concern when the Ornstein-Uhlenbeck (OU) model is compared to other trait evolution models (Cooper et al. 2016), with the OU model being artefactually favored.

### Hypothesis 3: Rates of Trait Evolution and Speciation Rate

We estimated rates of bill size and shape evolution using the variable rates model (Venditti et al. 2011) in BayesTraits v3.0.2 (https://www.evolution.reading.ac.uk/). We ran a single-chain MCMC run for $10^9$ generations, sampling every $10^5$ generations, removed the first 25% of the posterior as burn-in, and assessed convergence in Tracer v1.7.1 (Rambaut et al. 2018). From the output of the variable rates model, we computed species-specific rates of trait evolution following the approach of Cooney et al. (2019). We assessed the correlation between rates of trait evolution and speciation rates using the tip-rate correlation method (Harvey and Rabosky 2017). Parameter estimates from the best-fit model of trait evolution that we used to simulate the null distribution are reported in Supplementary Material Table 4. We added a nuisance parameter to each model to account for measurement error.

## RESULTS

Speciation rates varied both through time and among lineages (Figure 1). Three general patterns of speciation on the phylogeny were apparent: (1) a rapid burst of speciation at the base of Thraupidae leading to the majority of subfamilies, (2) an increased rate of speciation in *Sporophila* seedeaters, and (3) rapid speciation in the Darwin's finches. Species-specific rates of speciation ranged from 0.087 to 2.604 species per

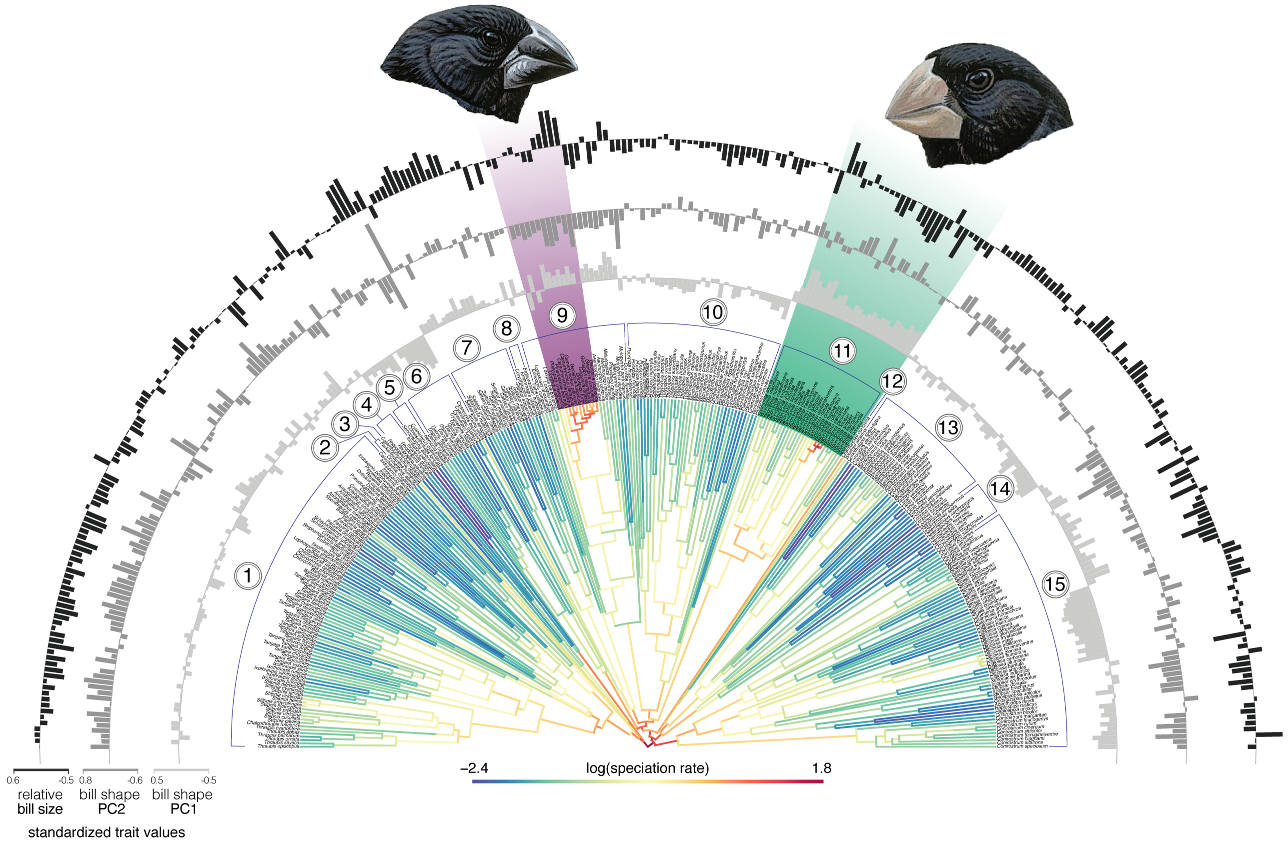

**FIGURE 1.** Speciation rates and phylogenetic distribution of variation in bill size and shape (PC1 and PC2) in tanagers. Branches are colored according to the rate of speciation: warm colors indicate higher rates of speciation and cold colors represent lower rates. Bill size and shape are represented as standardized trait values. Clade numbers refer to tanager subfamilies: (1) Thraupinae, (2) Orchesticinae, (3) Catamblyrhynchinae, (4) Porphyrospizinae, (5) Nemosiinae, (6) Dacninae, (7) Saltatorinae, (8) Emberizoidinae, (9) Coerebinae, (10) Poospizinae, (11) Sporophilinae, (12) Charitospizinae, (13) Tachyphoninae, (14) Hemithraupinae, and (15) Diglossinae. Darwin's finches are representative of the subfamily Coerebinae (clade 9) and *Sporophila* seedeaters are representative of Sporophilinae (clade 11). Bird illustrations are reproduced with permission from Billerman et al. (2020).

million years (median = 0.186, mean = 0.293 species per million years).

Bill size (centroid size on a log scale) varied from 3.07 (*Sporophila hypoxantha*) to 4.21 (*Saltator atriceps*) with an average bill size of 3.59 (± 0.24), which is typical of species in the genus *Tangara* (Supplementary Material Table 2). Larger-bodied species tended to have larger bills whereas smaller-bodied species tended to have smaller bills ($\beta = 0.34$, $p < 0.005$). However, many smaller-bodied species had large bills for their size (e.g., *Cyanerpes*, *Geospiza*, and *Sporophila*), whereas some larger-bodied species had small bills relative to their size (e.g., *Sericossypha albocristata*).

The primary axis of bill shape in tanagers (PC1: 57%) described variation in linear dimensions of the bill (length, width, and depth) and curvature of the culmen and tomium (Figure 1). Low PC1 scores corresponded to species with long, thin, and slightly curved bill morphologies (e.g., *Cyanerpes*, *Diglossa*). In contrast, high PC1 scores described species with finch-like bills that are short and stout with strongly curved culmens (e.g., *Geospiza*, *Sporophila*). The second axis of bill shape (PC2: 31%) differentiated species with narrow bills, such as *Rowettia* (low PC2 scores) from species with wide bills, such as *Tersina* and some *Tangara* species (high PC2 scores). Further details on the principal components analyses are reported in Vinciguerra and Burns (2021).

We found no support for our first hypothesis of trait-dependent speciation. Specifically, we did not find a significant relationship between speciation rate and bill size or shape across the tanager phylogeny (Figures 2A, 3A, and 3C). Because the Darwin's finches and *Sporophila* seedeaters show rapid rates of speciation relative to the tanager background rate, we performed additional analyses to explore the effect of removing these clades. When we removed *Sporophila*, there was no effect on our results (Supplementary Material Figure 1C). However, when we removed the Darwin's finches (Supplementary Material Figure 1B) or both the Darwin's finches and *Sporophila* (Supplementary Material Figure 1D), we found a significant inverse relationship between speciation rate and bill size. Our bill shape finding was robust to the removal of Darwin's finches and *Sporophila* (Supplementary Material Figures 2 and 3).

Our second hypothesis, that bill size and shape are associated with speciation events (i.e., punctuated evolution) was supported. For each trait, the best model was a scenario of mixed punctuated and gradual evolution; the $\psi$ model outperformed BM and multi-rate BM models based on ΔAIC and AIC weights (Table 1). When a model of punctuated evolution was fitted to the phylogeny, we estimated that the punctuated signal ($\psi$ parameter) accounted for 76% of the total evolutionary rate for bill size and more than 50% of the total evolutionary rate for bill shape (PC1 and PC2); the remainder

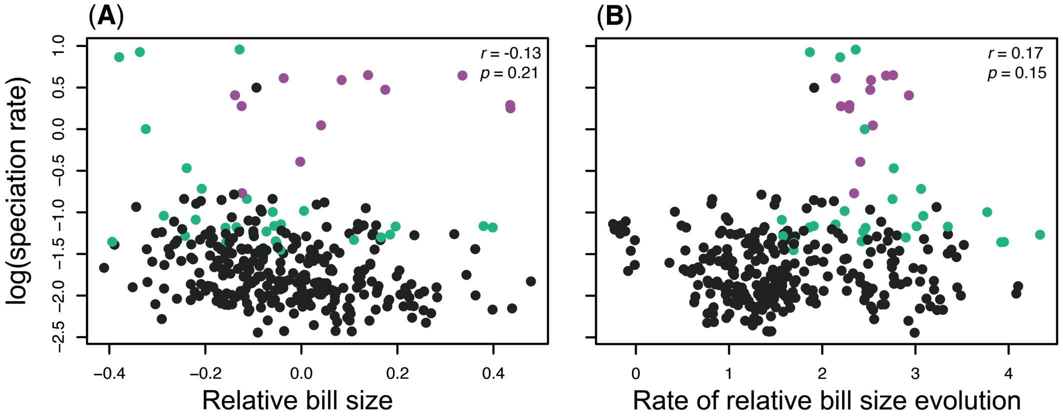

**FIGURE 2.** Correlations between log speciation rate and (**A**) relative bill size and (**B**) rate of relative bill size evolution. Darwin's finches (purple), *Sporophila* seedeaters (green), and remaining tanager species (black).

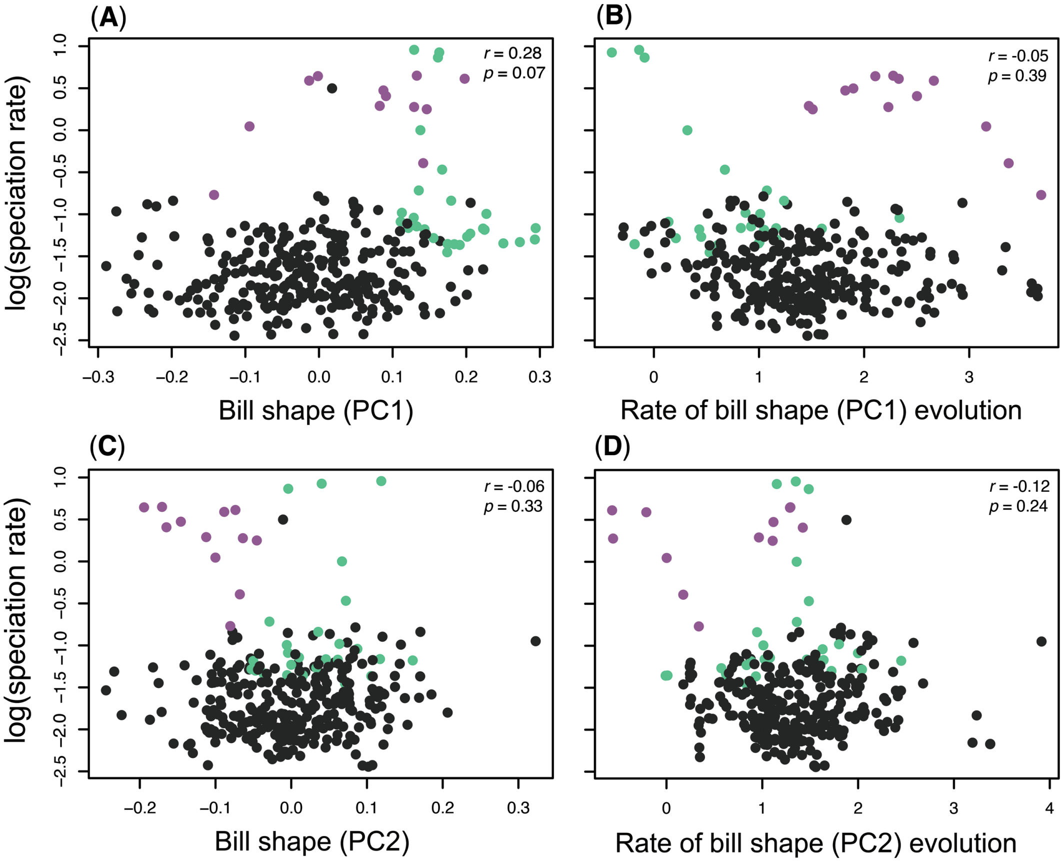

**FIGURE 3.** Correlations between log speciation rate and (**A, C**) bill shape and (**B, D**) rate of bill shape evolution. Darwin's finches (purple), *Sporophila* seedeaters (green), and remaining tanager species (black).

of the total evolutionary rate was attributed to BM gradual evolution. When we partitioned the analysis according to the location of diversification rate shifts identified by Burns et al. (2014), the punctuated signal in all three traits remained. However, clade-specific ψ values for Darwin's finches and *Sporophila* ranged from 0 to near 1 in most traits, suggesting limited power or flat likelihood surfaces when the tree is partitioned this way.

We found no support for our third hypothesis: there was no evidence for a significant relationship between the rate of speciation and the rate of bill evolution (Figures 2B, 3B, and 3D). For each trait (bill size, bill shape PC1 and 2), the rate of bill size and shape evolution was uncorrelated with speciation rates across the phylogeny. For all but one analysis, the lack of any relationship between bill size or shape remained after removing *Sporophila* or removing both Darwin's finches and *Sporophila*. When we removed only the Darwin's finches from the correlation between speciation rate and bill shape (PC1) evolution, we found a significant correlation (Supplementary Material Figure 5B). However, these correlations can be sensitive to outliers (Harvey and Rabosky 2017), and after removing both the Darwin's finches and *Sporophila*, the other

**TABLE 1.** Models of punctuated evolution (ψ) and gradual evolution (BM) fit to bill size and shape. Maximum likelihood parameter estimates (σ, ψ) for each character and the number of parameters (*n*) for each model are shown. Models with clade-specific rates are shown as (background, Darwin's finches, and Sporophila seedeaters). Log-likelihood (logL), Akaike's information criterion ($\text{AIC}_c$), delta AIC ($\Delta\text{AIC}_c$), and Akaike weights ($w_i$) are shown for each model. Best-fitted models for each trait are bolded.

| Trait | Model | σ | Ψ | *n* | log*L* | $\text{AIC}_c$ | $\Delta\text{AIC}_c$ | $w_i$ |
|---|---|---|---|---|---|---|---|---|
| Relative bill size | BM | 0.01 | – | 2 | 45.60 | –87.17 | 279.53 | 0.00 |
| | Ψ | 0.00 | 0.76 | 3 | 186.39 | –366.70 | **0.00** | **0.76** |
| | Multi-BM | (0.01, 10.08, 8.05) | – | 4 | 108.86 | –209.60 | 157.10 | 0.00 |
| | Multi-ψ | 0.00 | (0.79, 0.00, 0.62) | 5 | 187.28 | –364.37 | 2.32 | 0.24 |
| Bill shape (PC1) | BM | 0.00 | – | 2 | 333.00 | –661.96 | 148.81 | 0.00 |
| | Ψ | 0.00 | 0.59 | 3 | 408.42 | –810.76 | **0.00** | **0.72** |
| | Multi-BM | (0.00, 16.05, 0.95) | – | 4 | 387.20 | –766.28 | 44.48 | 0.00 |
| | Multi-ψ | 0.00 | (0.58, 0.00, 1.00) | 5 | 409.52 | –808.85 | 1.91 | 0.28 |
| Bill shape (PC2) | BM | 0.00 | – | 2 | 319.43 | –634.83 | 173.93 | 0.00 |
| | Ψ | 0.00 | 0.53 | 3 | 407.42 | –808.76 | **0.00** | **0.80** |
| | Multi-BM | (0.00, 4.09, 3.56) | – | 4 | 339.70 | –671.27 | 137.49 | 0.00 |
| | Multi-ψ | 0.00 | (0.51, 0.01, 0.76) | 5 | 408.10 | –806.02 | 2.74 | 0.20 |

clade with high speciation rates, we found no significant relationship (Supplementary Material Figure 5D).

## DISCUSSION

Studies of avian bill morphology have provided important insights into speciation theory (Darwin 1859, Lack 1947, Price 2007, Grant and Grant 2008). However, our current understanding of how variation in bill morphology plays a role in species formation across broad geographic and temporal scales remains limited. In this study, we showed that bill shape and its evolutionary rate did not influence the rate of speciation across an ecologically and morphologically diverse songbird radiation, the tanagers. However, we did find evidence for a pattern of mixed punctuated and gradual bill evolution, with a particularly strong signal of diversification in bill morphology (size and shape axes) associated with speciation events. These results suggest that changes in bill size and shape are associated with the speciation process but not through any effect on the rate at which new species form in tanagers.

In contrast to our first hypothesis that finch-shaped bills would lead to faster rates of speciation relative to warbler-shaped bills, we found no support for trait-dependent speciation. We found that stout-billed granivorous species (higher PC1 scores) were not associated with higher speciation rates (Figure 3). Thus, the hypothesis that species with finch-like bills are prone to higher rates of speciation than species with warbler-like or nectivorous bill shapes (Lovette et al. 2002, Rundell and Price 2009, Price 2011) is not supported by our results. This result conflicts with a recent study by Conway and Olsen (2019) who showed an association between bill shape and speciation rate across the nine-primaried oscines, including tanagers, suggesting the drivers of speciation may vary at different taxonomic scales.

Although tanagers with finch-shaped bills were not correlated with higher speciation rates as a whole, two lineages with primarily finch-shaped bills (the Darwin's finches and *Sporophila* seedeaters) showed accelerated rates of speciation compared to the background rate of tanagers. Several studies have demonstrated how divergent selection on bill shape is an important driver of speciation in the Darwin's finches (Lack 1947, Grant 1999). In contrast, the relationship between bill morphology and speciation is relatively unexplored in *Sporophila* seedeaters. Bill sizes and shapes show considerable variation in *Sporophila* (Supplementary Material Table 2; del Hoyo et al. 2011, Mason and Burns 2013, Demery and Burns 2023), including some species with bills that are specialized for feeding on bamboo seeds (Areta et al. 2009). This variation is highlighted by their taxonomic history, as several *Sporophila* species were originally classified as other genera (e.g., *Dolospingus*, *Neorhynchus*, *Oryzoborus*) largely due to bill differences (de Schauensee 1952, Stiles 1996). More detailed work on bill morphology in *Sporophila* is needed. Although the *Sporophila* clade and the Darwin's finch clade show accelerated speciation rates, other tanager clades with finch-shaped bills do not have accelerated rates. Therefore, there is no broad correlation across tanagers between bill shape and speciation rate. In contrast, we found elevated rates of speciation in tanagers with smaller bills relative to species with larger bills in some of our analyses (Supplementary Material Figure 1B and 1D). However, bill size is correlated with body size, thus it is unclear whether body size, bill size, or aspects of both traits predict speciation rates in the tanagers. Gittleman and Purvis (1998) suggested that higher metabolic rates and shorter generation times associated with smaller-bodied species may be associated with higher diversification rates. Although this hypothesis was not supported across all tanagers, our finding of smaller-bodied species with smaller bill sizes having higher speciation rates (when Darwin's finches were removed) is consistent with this idea.

We did not find evidence for a link between faster-diverging bills and higher speciation rates. Many studies have investigated the drivers of speciation rate in tanagers: climatic niche (Title and Burns 2015), vocalizations (Mason et al. 2017), plumage (Price-Waldman et al. 2020), and morphology (Demery 2018, Reaney et al. 2020). Demery (2018) analyzed linear dimensions of the bill and reported significant correlations between rates of bill evolution and speciation, a result we were not able to corroborate. We analyzed the major PC axes of bill morphology that take into account more variation than is captured by linear dimensions alone. Moreover, Price-Waldman (2019) found that evolutionary rates from linear dimensions of the bill were not associated with speciation rates but that plumage, song, and ecological niche did correlate with speciation rates. Despite methodological differences (model-based versus non-model-based speciation rate metrics) and dataset choice (3D vs linear morphometrics), our results, based on a comprehensive dataset of 3D bill morphology, agree with the findings of Price-Waldman (2019) in showing the rate of bill evolution is independent of speciation rate in the tanagers.

Our alternative hypothesis that bill size and shape would show a signal of punctuated evolution was supported. More than half of the total evolutionary rate for each trait was estimated to occur at speciation events. A signal of punctuated evolution could result from various evolutionary processes that are not mutually exclusive. For instance, bill divergence in allopatry or character displacement upon secondary contact may allow for speciation and coexistence (Tobias et al. 2014, Pigot et al. 2018). Divergence in bill characters could also be a consequence of habitat or climatic differences where adaptation to different niches may increase the rate of speciation (Cadena et al. 2016). However, changes in bill morphology appear to evolve independently of climatic niche evolution in tanagers, whereas changes in diet and song are correlated with changes in bill morphology (Demery et al. 2021, Vinciguerra and Burns 2021, Demery and Burns 2023).

Early in the history of tanagers, there was a rapid burst of both species and bill types (Vinciguerra and Burns 2021). An early burst (EB) model of character change is consistent with the idea of adaptive radiation and the filling of morphological space (Schluter 2000, Harmon et al. 2010). This is also one explanation for why we did not find correlations between speciation rates and bills and their evolutionary rate: most of the evolutionary change in bill morphology occurred early in the radiation (*sensu* Crouch and Ricklefs 2019). It is possible that our new finding represents a scenario where a "punctuated EB" model would be a reasonable explanation for bill shape evolution, where most of the evolution of bill shape early in the radiation occurs during speciation, but the magnitude of divergence has declined over time. It is not possible with existing comparative methods to simultaneously fit punctuated evolution and a slowdown in rates of trait evolution to phylogenies; however, such methods may be necessary to fully describe the evolution of bill shape in the tanagers. Nevertheless, it appears that most of the divergence in bill morphology occurs at speciation events, either as a byproduct or in response to ecological processes, and this happened rapidly and early in the history of the tanager radiation.

Like all statistical methods, phylogenetic comparative methods do not come without caveats. For example, the estimation of $\psi$ can be affected if speciation rates vary across the phylogeny (Rabosky 2012). We attempted to account for this by allowing rates to vary. Likewise, the $\psi$ model attempts to model hidden speciation events, either because of incomplete taxon sampling or extinction and not accounting for this may cause confounding results. However, our sampling was robust and we assume that extinction has had minimal influence because the tanagers are a relatively recent radiation (~12 Mya). Of more recent concern, Louca and Pennell (2020) call for caution when using extant phylogenies to assess diversification dynamics in the absence of fossils. However, they did not consider models where diversification rates can vary within lineages, as in ClaDS, Bayesian Analysis of Macroevolutionary Mixtures (BAMM), or any state-dependent speciation and extinction (SSE) model. In addition, they show that diversification rates at present time are identifiable. Because we focus on present-day speciation rates (i.e., tip rates), we think our approach is justified.

Our findings suggest that changes in bill size and shape are associated with the speciation process but not through any influence on speciation rate. Ultimately, these results indicate that the avian bill does not play a role in explaining broad-scale patterns of species richness in the tanagers, whereas other traits like song, ecological niche, and plumage have greater influence (Title and Burns 2015, Mason et al. 2017, Price-Waldman et al. 2020).

## Supplementary material

Supplementary material is available at *Ornithology* online.

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## Ethical statement

The coauthors complied with all ethics and integrity policies mandated by Ornithology.

## Conflict of interest statement

None to declare.

## Author contributions

N.T.V. conceived the study, analyzed the data, and wrote the manuscript. K.J.B. contributed to the conceptualization, co-wrote the manuscript, and supervised the research. J.M.M. contributed to the conceptualization and co-wrote the manuscript.

## Data availability

Analyses reported in this article can be reproduced using the data provided by Vinciguerra et al. (2024).

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