

Convergent evolution of floral shape tied to pollinator shifts in *Lochrominae* (*Solanaceae*)*

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Flower form is one of many floral features thought to be shaped by pollinator-mediated selection. Although the drivers of variation in flower shape have often been examined in microevolutionary studies, relatively few have tested the relationship between shape evolution and shifts in pollination system across clades. In the present study, we use morphometric approaches to quantify shape variation across the Andean clade *Lochrominae* and estimate the relationship between changes in shape and shifts in pollination system using phylogenetic comparative methods. We infer multiple shifts from an ancestral state of narrow, tubular flowers toward open, bowl-shaped, or campanulate flowers as well as one reversal to the tubular form. These transitions in flower shape are significantly correlated with changes in pollination system. Specifically, tubular forms tend to be hummingbird-pollinated and the open forms tend to be insect-pollinated, a pattern consistent with experimental work as well as classical floral syndromes. Nonetheless, our study provides one of the few empirical demonstrations of the relationship between flower shape and pollination system at a macroevolutionary scale.

KEY WORDS: Floral syndromes, morphometrics, PGLS, pollinator importance.

Although the variation in form, shape, and color of flowers has often been attributed to pollinator-mediated selection, the nature of this process and its importance in driving divergence remain contentious. Various authors have argued for the pollinator-shift model (the Grant-Stebbins model; Johnson 2006), wherein suites or “syndromes” of floral traits are shaped by “functional” groups of pollinators, and floral diversity arises through shifts among these functional groups (Grant 1949; Stebbins 1970; Fenster et al. 2004). While the pollinator-shift model appears to fit well with the floral variation and pollination systems of many classic radiations (e.g., Wilson et al. 2004; Whittall and Hodges 2007; Tripp and Manos 2008), it remains unclear how well shifts among major groups of pollinators explain diversity more broadly (Van der Niet et al. 2014). For example, many florally diverse groups of plants have radiated while retaining an essentially unchanged

but specialized pollination ecology (De Luca and Vallejo-Marin 2013; Davis et al. 2014). Pollinators may still play a role in floral divergence in these systems, for example through competitive interactions (Armbruster et al. 1994; Muchhala and Potts 2007; Eaton et al. 2012). Equally, many factors beyond pollinators, such as herbivores and climatic features, are known to influence floral evolution (Armbruster 2002; Strauss and Whittall 2006).

Testing the relationship between pollinator shifts and floral trait evolution on a macroevolutionary scale is largely hindered by the lack of comparative quantitative data. Although broad-scale studies often treat pollination system and floral traits as discrete characters (e.g., Ollerton et al. 2009; Rosas-Guerrero et al. 2014), both vary continuously in natural systems (Waser et al. 1996; Barrett and Hodgins 2006; Conner 2006) and are thus best treated as continuous traits. Nonetheless, capturing floral diversity and plant–pollinator interactions as continuous variables amenable to analysis can be challenging. In terms of quantifying the contribution of a pollinator to a plant’s reproduction, metrics such as pollinator efficiency and pollinator importance have been proposed

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to incorporate aspects of both visit frequency (“quantity”) and the amount of pollen deposited (“quality”) (reviewed in Ne’eman et al. 2010). Easily measured floral traits, such as aspects of length and width, have been commonly studied in the context of pollinator shifts across clades (Wilson et al. 2004; Whittall and Hodges 2007) while complex traits such as patterning, reward, and shape have received less attention (but see Armbruster 1990; Perez et al. 2006; Gomez et al. 2008). Nonetheless, recent advances in geometric morphometrics (reviewed in Klingenberg 2010) have reinvigorated the study of floral shape and the role of pollinators in its evolution (van der Niet et al. 2010; Fernandez-Mazuecos et al. 2013; Gomez et al. 2014).

While macroevolutionary tests of pollinator-mediated floral shape evolution remain uncommon, studies of within-species variation provide insight into the mechanisms of selection. For example, pollinators may exhibit differential visitation rates with respect to flower shape variation, resulting in directional selection (e.g., Galen and Cuba 2001; Gomez et al. 2006). This apparent pollinator preference can arise from a variety of factors, including differences in the rate of approach, handling time, or correlated traits, such as nectar reward (Cresswell and Galen 1991; Sutherland and Vickery 1996; Fenster et al. 2006; Temeles et al. 2009). Shape variation may also influence the interaction between the pollinator and the floral reproductive organs, leading to associated variation in pollen deposition or removal. This link between flower shape and pollinator efficiency has well-documented consequences for both male and female components of fitness (e.g., Campbell et al. 1996; Kulbaba and Worley 2013; La Rosa and Conner 2017). Importantly in the context of the pollinator-shift model, different pollinators often exert different selective pressures on flower shape (Campbell 2004; Muchhala 2007; Gomez et al. 2008), suggesting that shifts among pollinators, whether within a single species or across a clade, will lead to corresponding shifts in multiple aspects of shape.

Here, we quantify the diversity of corolla shapes in Iochrominae and assess the relationship between shape variation and pollination system across this clade. Iochrominae comprises roughly 35 Andean species in the tomato family (Solanaceae). It has been traditionally divided among seven small genera and is known for its tremendous diversity of flower shapes, sizes, and colors (Smith and Baum 2006). Previous studies have shown that a wide array of insects and hummingbirds can act as effective pollinators (Smith et al. 2008b). Most of the species of *Iochroma*, the largest genus, are principally pollinated by hummingbirds, while some are pollinated by a mix of hummingbirds and insects and those in closely related genera are often principally insect pollinated (Taura and Laroca 2004; Verçozza et al. 2012). These differences in pollination system are closely associated with reward and display, as species with large displays and high-reward flowers are more likely to be pollinated by hummingbirds (Smith et al. 2008a).

Combining this existing body of information about pollination ecology of *Iochroma* and its relatives with new floral morphometrics and an expanded phylogeny, we test whether pollinator shifts are associated with floral shape evolution. Iochrominae are radially symmetric (or at most weakly bilaterally symmetric) along the dorsal-ventral axis (Knapp 2010), but they vary greatly along the proximal-distal axis, with shapes ranging from widely campanulate to narrowly tubular (Smith and Baum 2007). We hypothesize that this shape variation is tied to transitions between insect, bird, and mixed pollination systems, with narrow flowers being favored in bird-pollinated taxa and open flowers in insect-pollinated taxa. In the present study, we apply geometric morphometrics to quantify corolla shape variation and conduct statistical comparative analyses to estimate its relationship to changes in pollination system. In addition, we use the phylogeny to trace the evolution of shape and determine the extent of shape convergence across the clade.

Materials and Methods

PHYLOGENY

To reconstruct the evolution of flower shape, we updated the existing calibrated phylogeny of Iochrominae (Smith and Goldberg 2015) to include a recently discovered species of *Saracha* (*S. andina* Rob. Fernandez, I. Revilla and E. Pariente). We added *S. andina* sequences from Fernandez-Hilario and Smith (2017) to the existing three-gene alignment, which includes the internal transcribed spacer (ITS), *LFY* intron II, and exons 2 through 9 of *waxy*. In total, we sampled 36 ingroup species (Fig. 1A) and included six additional Solanaceae as outgroups, following Fernandez-Hilario and Smith (2017). We conducted likelihood ratio tests in PAUP 4.0b10 (Swofford 2002) to compare the fit of five models (JC, K2P, HKY, GTR, and GTR+ Γ) and chose the best model (GTR+ Γ) for phylogenetic inference. To obtain ultrametric trees suitable for comparative methods, we estimated the phylogeny using relaxed-clock methods in BEAST v. 2.1.2 (Bouckaert et al. 2014). Following Smith and Goldberg (2015), we used a relaxed-clock log-normal model for rate variation across branches, a birth-death model for trees, a set of three secondary calibrations (one for the root node, one for the Iochrominae-Physalinae clade, and one for Iochrominae) from Särkinen et al. (2013). We completed two runs of 5 million generations, and after evaluating convergence and effective sample size, we removed the first 25% of trees from as burn-in. We computed the maximum clade-credibility (MCC) tree using the TreeAnnotator program in BEAST. We also subsampled 100 trees from the posterior distribution for downstream analyses. Outgroups were pruned from the tree before analyses. The final alignment, MCC tree (Fig. S1) and 100-tree sample are deposited in Dryad (<https://doi.org/10.5061/dryad.5jn7b>).

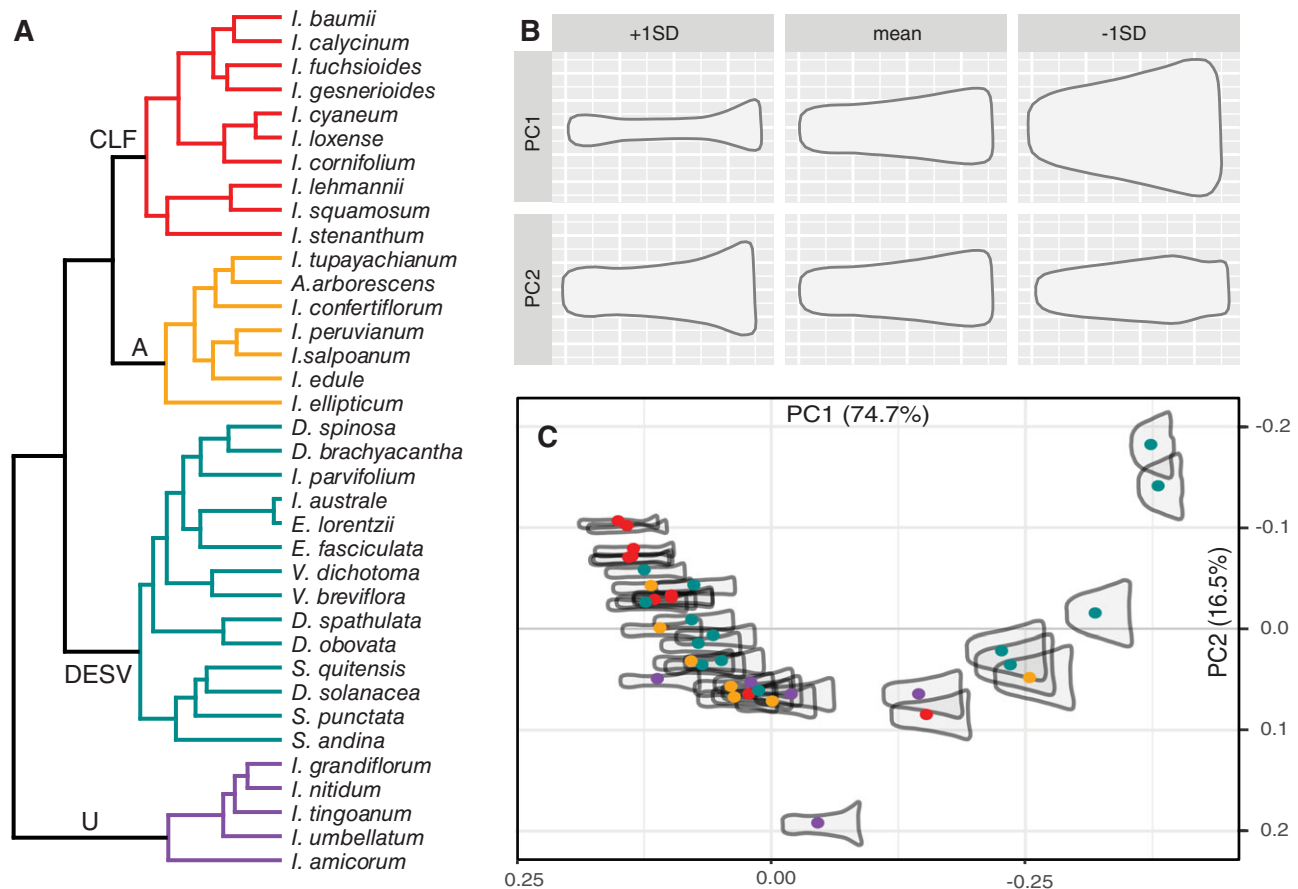


Figure 1. Iochrominae phylogeny and floral morphospace. (A) MCC tree from BEAST analysis of the three-gene dataset for Iochrominae. Abbreviations for genera of Iochrominae are I. = *Lochroma*, A. = *Acnistus*, V. = *Vassobia*, S. = *Saracha*, E. = *Eriolarynx*, D. = *Dunalia*. Outgroups (*Tubocapsicum anomalum*, *Physalis peruviana*, *Witheringia solanacea*, *Leucophysalis grandiflora*, *Cuatresia harlingiana*, and *Larnax sachapapa*) were pruned from the tree. Major clades ("A," "CLF," "DESV," "U") are labeled following Smith and Baum (2006). (B) Shape variation along PC axes. For each of the first two PCs, the mean is shown along with the shapes corresponding to ± 1 standard deviation (SD). (C) Empirical morphospace with outlines for each of the 36 species. Colored dots correspond to clade membership shown in (A).

MORPHOMETRIC ANALYSIS

Corolla shape was characterized using images of fresh flowers taken in the field. For each species, a single mature flower from the individual sampled for the phylogeny was photographed in lateral view, providing a two-dimensional representation of the shape along the proximal-distal axis. These images were converted into silhouettes in Adobe Photoshop (San Jose, CA), and the silhouettes were transformed into outlines using the R package Momocs (Bonhomme et al. 2014). Each outline was converted into a list of 200 x,y coordinates describing the polygon and then centered, also using Momocs. Variation in shape was quantified using elliptical Fourier analysis (EFA), which decomposed the outlines into 32 harmonics, each with four coefficients. To avoid twisting of the outlines during the EFA, four landmarks were placed on each outline, one on each side of each tube, and one above and one below in the middle area of each tube. These landmarks were then used to align the outlines

with a Full Generalized Procrustes alignment in Momocs. The 128 coefficients resulting from the EFA were summarized using a principal component analysis (PCA). Species were plotted along the PC axes to visualize their distribution in morphospace. As an indication of phylogenetic structure in these data, we computed Blomberg's K for the PC variables (Blomberg et al. 2003) and used 1000 randomizations of the data across the MCC tree to test if the value was significantly different from $K = 0$ (no phylogenetic signal). Scripts and shape outlines used for all analyses are deposited in Dryad (<https://doi.org/10.5061/dryad.5jn7b>).

ASSOCIATION BETWEEN SHAPE VARIATION AND POLLINATION SYSTEM

We used phylogenetic generalized least squares (PGLS) to test the relationship between shape variation and pollination system. Pollination system was characterized using pollinator importance, a metric that incorporates both visitation rates and effectiveness in

terms of pollen deposition (Waser and Price 1983; Mayfield et al. 2001). Estimates of pollinator importance for both insects and hummingbird visitors are available for 15 species of Iochrominae from previous studies (Smith et al. 2008b). We also included data from Taura and Laroca (2004), who studied the pollination of *Vassobia breviflora* during two flowering seasons. Given the small size of this dataset (16 species), we focused our analyses on relative hummingbird importance, which ranges from 0 to 1, with 1 being 100% bird-pollinated. Since insects constitute the non-hummingbird pollinators, relative hummingbird importance in this case can be considered 1 minus relative insect importance. *V. breviflora* was scored as 0 given that it is exclusively bee-pollinated (Taura and Laroca 2004), and data for the remaining species was taken from Smith et al. (2008b).

We regressed shape PCs from the morphometric analysis against values for pollinator importance with phylogenetic-generalized least squares (PGLS, Martins and Hansen 1997) using the `gls` functions in the `nlme` package in R (Pinheiro et al. 2017). We assumed a correlation structure based on the Ornstein-Uhlenbeck (OU) process, which models stabilizing selection around an optimum (Hansen 1997; Butler and King 2004). The strength of selection is determined by the α parameter, which we estimated during model fitting. When α is zero, the model is equivalent to Brownian motion and as α increases toward infinity, the model converges on a non-phylogenetic “TIPS” model. Given that multiple evolutionary processes can result in the similar patterns of phylogenetic structure in the data (Revell et al. 2008), we consider this approach primarily as a way to adjust for the level of statistical dependence among the trait values due to the phylogeny. The PGLS analyses were repeated for the first two shape PCs using the MCC tree and also the sample of 100 trees from the posterior distribution. The α parameter was optimized for each tree during PGLS model fitting. Distributions for each parameter across the 100 trees were summarized by computing the median and the range.

Given the relatively small number of taxa in this data, we implemented several approaches to examine the robustness of our results. First, we repeated the PGLS analyses excluding *Vassobia breviflora*, the species whose pollination was not studied directly by the first author. It appears as an outlier in the shape analysis (see Results, Morphospace), having a much wider mouth than the other 15 species for which pollinator data is available. Second, we conducted a phylogenetic ANOVA analysis, treating pollination system as a categorical variable. We included six additional taxa for which there were field observations of pollinators (Table S1), but no quantitative study. Pollination systems were categorized as principally hummingbird-pollinated ($\geq 75\%$ relative hummingbird importance for species with quantitative data or only hummingbirds observed for those without), principally insect-pollinated ($\geq 75\%$ relative insect

importance or only insects observed), or mixed (intermediate values of pollinator importance or both insect and hummingbird visits observed). Third, these PGLS and phylogenetic ANOVA analyses were repeated using multiple structures to model the degree of phylogenetic correlation in the residuals (the Brownian motion (BM) model, the OU model, and the TIPS model).

PATTERNS OF CONVERGENCE

Given the relationship between the first corolla shape PC and pollinator importance detected in the PGLS analysis, we applied model-based methods to examine the extent of convergence in this aspect of floral shape. We first constructed a traitgram for PC1 using the MCC tree with the phenogram function in `phytools` (Revell 2012). We then used the `l1ou` R package (Khabbazian et al. 2016) to estimate the number of distinct optima (or selective regimes) across the phylogeny under an Ornstein-Uhlenbeck process using a LASSO (Least Absolute Shrinkage and Selection Operator) procedure (Tibshirani 1996). One of the advantages of `l1ou` is that it does not require the a priori designation of where regime shifts may have occurred. Convergence is inferred with `l1ou` as independent shifts to the same regime. We chose to use the phylogenetic Bayesian information criterion (pBIC) for model selection as it has been shown to minimize the inference of false shifts (Khabbazian et al. 2016). We repeated these analyses across the sample of 100 trees from the posterior distribution to assess the effect of phylogenetic uncertainty on the results.

Results

MORPHOSPACE

The PCA analysis of harmonic coefficients from the elliptic Fourier analysis captured 91.2% of the variation in the first two principal components. Principal component 1 accounts for 74.7% of the variation and describes the narrowness of the tube and to some degree, the extent of constriction of the tube toward the middle (Fig. 1B). The second principal component, comprising 16.4% of the variation, captures the amount of restriction at the mouth, given that some species have strongly flared corollas and other are relatively straight. Tubular shapes with slightly flared mouths appear to be the most common forms and are characteristic of the largest genera, *Iochroma* and *Dunalia*.

Although corolla shapes were clustered to some extent by relatedness, the phylogenetic distribution suggests multiple shifts among the various forms. For example, bowl-shaped flowers are only found in the two sister species of *Vassobia* (Fig. 1C, upper right), but funnel-shaped flowers are found in several clades and traditional genera (*Acnistus*, *Saracha*, and *Iochroma*). Similarly, tubular forms appear in all of the major clades. Accordingly, species with similar flower shapes are often distantly related.

Table 1. Parameter estimates from PGLS analyses.

Model	Tree	Coefficient	SE	α	P	σ
PC1 ~ Poll	MCC	0.29	0.08	0.18	0.004	0.16
	100 trees	0.28 (0.27, 0.32)	0.08 (0.08, 0.09)	1.31 (0.00, 7.21)	0.004 (0.001, 0.006)	0.14 (0.12, 584)
PC2 ~ Poll	MCC	-0.01	0.05	0.00	0.86	
	100 trees	-0.02 (-0.03, 0.00)	0.05 (0.04, 0.05)	0.08 (0.00, 1.34)	0.74 (0.46, 0.98)	330 (0.09, 2255)

“Poll” denotes relative hummingbird importance. For PGLS analyses across the 100 tree samples, median parameter values are presented with 95% intervals in parentheses.

Blomberg’s K suggests little phylogenetic signal ($K = 0.30$, $P = 0.63$) in the first shape PC. The second PC does show significant phylogenetic signal ($K = 0.78$, $P = 0.001$), but this component accounts for a small portion of the variation in shape compared to PC1.

ASSOCIATION BETWEEN SHAPE VARIATION AND POLLINATION SYSTEM

PGLS analyses indicated a strong relationship between pollination system and the first shape PC but not the second. Species with constricted, tubular corollas (higher PC1 scores) tend to be pollinated by hummingbirds while those with open corollas tend to be pollinated by a mix of hummingbirds and insects, or insects exclusively (Table 1; Fig. 2). One species, *I. lehmannii* appears as an outlier with more bird pollination that would be expected based on its shape, suggesting that additional factors are likely at play in this taxon. The significant relationship between PC1 and relative hummingbird importance was recovered in all of the replicated PGLS analyses across the 100 Bayesian trees ($P = 0.001$ – 0.006 , Table 1). By contrast, PC2 was not associated with pollination system in any analysis ($P = 0.46$ – 0.98 , Table 1).

Our sensitivity analyses suggested that these associations are robust, despite the small sample size in the original dataset (16 species). The relationship between shape PC1 and pollinator importance was recovered in PGLS analyses with all three models (BM, OU, TIPS) after removing the *V. breviflora* outlier, albeit with reduced significance levels ($P = 0.01$ – 0.03 , Table S2). The phylogenetic ANOVA with pollination system coded as a qualitative character also returned a significant relationship for PC1 ($P < 0.002$ across the three models). Parallel PGLS and ANOVA analyses with shape PC2 did not result in any significant effects of pollinators on this aspect of shape (Tables S2 and S3), consistent with the findings from the original dataset.

PATTERNS OF CONVERGENCE

Given the apparent relationship between the aspects of shape captured by PC1 and pollination system, we examined the varia-

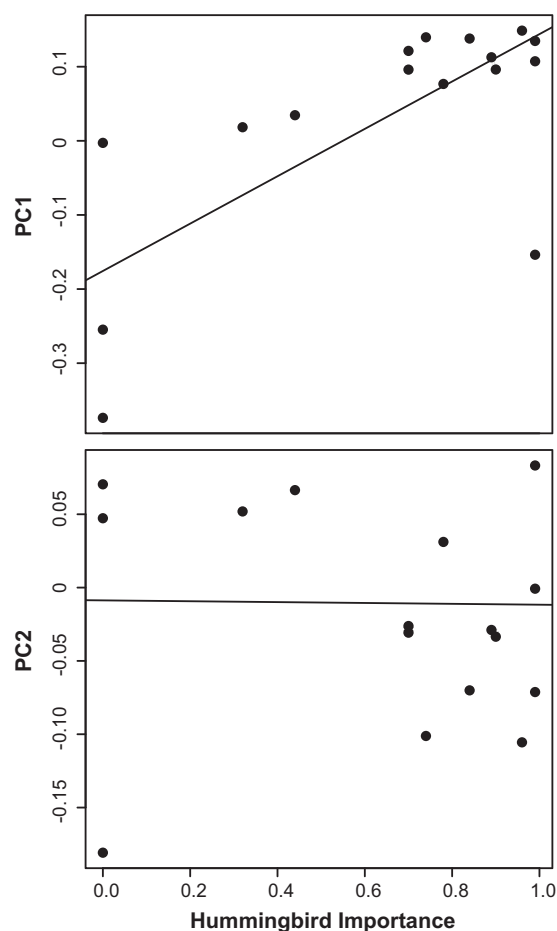


Figure 2. Biplots of hummingbird importance and PC axes. Each point represents one of the 16 species in the analysis; regression line from PGLS analysis. Hummingbird importance ranges from 0 (all insect pollination) to 1 (all hummingbird pollination); see Methods. The top panel shows results for PC1 and the bottom panel for PC2.

tion in PC1 across the phylogeny and tested for significant shifts in shape. Using the MCC tree, seven lineages are inferred to have shifted away from the ancestral regime (Fig. 3, Fig. S2), and many of these fall within the campanulate, bowl-shaped, and

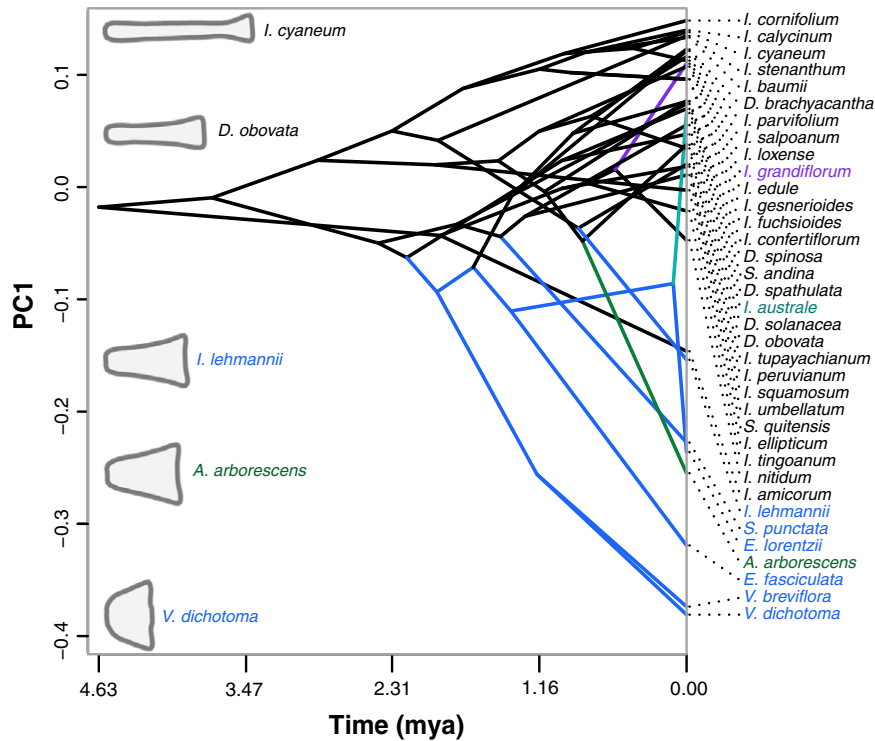


Figure 3. Traitgram for floral shape PC1. Branches are colored by regimes estimated using *l1ou* on the MCC tree. Each color represents a unique regime, a distinct inferred selective optimum for shape PC1. Example floral shapes corresponding to the PC values are shown on the left.

funnel-shaped groups. Three lineages (*Eriolarynx* + *Vassobia*, *S. punctata*, and *I. lehmannii*) are estimated to have shifted independently to the same regime, and one clade (that includes *D. spinosa*) appears to have subsequently reverted to the ancestral tubular shape (Fig. 3, Fig. S2). Three lineages (*I. grandiflorum*, *I. australe*, and *A. arborescens*) are estimated to be evolving toward unique optima despite the fact that their values for PC1 are similar to taxa in other regimes. This result likely relates to the short length of the subtending branch relative to the observed trait value. For example, since *I. australe* has experienced such a large change in shape over a short period of time, the optimum toward that it is evolving is inferred to be different than the other black-colored lineages with similar present-day values. Given this interaction between branch length variation and inferred shifts, we compared the number and placement of shifts across all of the 100 posterior trees. We found a slightly lower number of inferred shifts on average (4.83 ± 1.22 SD compared to 7 on the MCC tree) and also fewer convergent shifts (2.28 ± 0.50 SD compared to 4 on the MCC tree) (Fig. 3, Fig. S2). Still, the overall patterns were similar across the analyses. For example, *I. australe* appeared as a shift in 94 of the 100 trees and this shift was consistently inferred to correspond to a distinct regime (Tables S3). Also, the reversion of the clade formed by *D. spinosa*, *D. brachyacantha*, and *I. parvifolium* to the ancestral regime was inferred in the 61% of the trees (Table S4).

Discussion

The pollinator-shift model suggests that macroevolutionary transitions between groups of pollinators will lead to changes in multiple floral traits. Although this concept is long standing in the literature (Johnson 2006), empirical tests of this hypothesis, particularly with quantitative measures of pollination system, remain few (reviewed in Rausher 2008; Smith 2010). Some of the strongest evidence for a tight relationship between pollination system and floral trait evolution comes from intraspecific studies or studies of species pairs (e.g., Johnson and Steiner 1997; Perez-Barrales et al. 2007; Streisfeld and Kohn 2007). However, studies of entire clades have the potential to capture multiple independent transitions, providing greater power for hypothesis testing. Moreover, macroevolutionary analyses are crucial for addressing how often pollinator shifts are tied to speciation events (van der Niet and Johnson 2012).

Our results suggest that changes in corolla shape are often linked to changes in pollination system, consistent with pollinator shift model. In Iochrominae, species with tubular corollas tend to be pollinated primarily by hummingbirds, those with open corollas (bowl-shaped or campanulate) by insects, and those with funnel-shaped corollas by a mixture. The insect pollinators are principally bees except in *A. arborescens* and *I. ellipticum*, which have generalized systems with contributions from multiple

hymenopterans, dipterans, and lepidopterans (Smith et al. 2008b). A wide variety of pollinators has been shown to exert selection on corolla shape in microevolutionary studies (e.g., Galen 1996; Gomez et al. 2006; Kulbaba and Worley 2013). Nonetheless, our study appears to be the first to examine this relationship on a macroevolutionary scale, treating both pollination system and floral shape as quantitative traits (see Gomez et al. (2015) for a discrete example).

The pattern of transitions inferred in Iochrominae reinforces the theme of reversibility in pollination ecology and associated traits (Vamosi et al. 2014). Iochrominae belongs to the Physalid clade of the tomato family, which is inferred to be ancestrally open-flowered and bee-pollinated (Knapp 2010). Tubular flowers have evolved many times in the family outside of the Physalids, and at least in some cases, these taxa are documented to be pollinated by hummingbirds, hawkmoths, or both (Sazima et al. 1995; Ippolito et al. 2004; Gubitz et al. 2009). Our reconstruction of shape evolution in Iochrominae demonstrates the potential for reevolution of open flowers with bee or generalist insect pollination in tubular clades (Fig. 3). This transition may be facilitated by the retention of at least some degree of insect pollination in most of the tubular taxa (Fig. 2). In Iochrominae, the exclusively insect-pollinated taxa (*V. breviflora*, *A. arborescens*, *I. ellipticum*) are found at lower elevations (Smith 2006), and thus the reversal to open flowers is likely related to range expansions from the Andes into adjacent regions. Similar lability of floral traits and pollination systems has been documented in many clades (Perret et al. 2003; Tripp and Manos 2008; Marten-Rodriguez et al. 2010) although the relationship to range evolution is largely untested (Herrera et al. 2006).

While our study supports a close association between floral shape and pollination system, additional studies would be needed to determine the underlying drivers of this relationship. Pollinators could exert selection on shape either through their rates of visitation or through their effectiveness in pollen removal and deposition during those visits. Manipulative experiments in the principally bee-pollinated *Penstemon strictus* have shown that artificially constricting the corolla tube results in fewer bee visits (Zung et al. 2015), a shorter duration of these visits, and reduced pollen removal (Castellanos et al. 2004). The latter effects are likely related to aspects of mechanical fit, but the reduced visitation suggests that visual cues may also be important. Nectar guides are presumably more visible in flowers with a wider opening, and these patterns invoke innate preferences in bees (Lehrer et al. 1995). In this context, it is notable that the bowl-shaped and campanulate corollas in Iochrominae present dramatic patterns on the inside surface of the corolla while the tubular species are uniformly colored or at most, slightly patterned on the lobes. Experimental manipulations of these corolla patterns (as in Koski and Ashman 2014) as well as of the floral tube would help to

tease apart these trait interactions and determine how the aspects of shape variation captured in the geometric morphometric analysis alter interactions with pollinators in Iochrominae.

Together with previous work in this system, corolla form appears to be one of several floral traits, including nectar reward and floral display size, which fit the pollinator-shift model in Iochrominae. Although not quantitatively examined, floral scent may also belong to this suite of traits, as scent appears to be present only in the insect or mixed pollination taxa (Kaiser 2000; S. D. Smith, pers. obs.). Nonetheless, other traits, most notably flower color, do not correlate well with pollinator shifts (Smith et al. 2008a). Although the three red-flowered *Iochroma* species are principally pollinated by hummingbirds, the remaining hummingbird-pollinated taxa exhibit other colors (white, green, purple, yellow), and the geographical distribution of this color variation is consistent with a competition-driven model of floral divergence (Muchhala et al. 2014). These results support the notion that pollination syndromes are not likely to be universal (Ollerton et al. 2009; Ollerton et al. 2015), that is, that they are not likely to explain floral diversity across all traits or clades. In this context, the core question for future studies should not be whether the pollinator-shift model applies, as clearly it does in some cases, but rather when and why it is likely to apply. Addressing this question will require both microevolutionary studies to measure selective forces acting within lineages as well as macroevolutionary studies to detect broad patterns and overarching processes.

AUTHOR CONTRIBUTIONS

S.D.S. and R.K. designed the research and conducted the analyses. S.D.S. wrote the manuscript with revisions by R.K.

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DATA ARCHIVING

All input data (shape outlines, sequence alignments, phylogenetic trees) and scripts for analysis are available at Dryad repository (<https://doi.org/10.5061/dryad.5jn7b>).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Timetree of Iochrominae. Maximum clade credibility tree from BEAST analysis.

95% highest posterior densities for each node depth are shown with the light blue bars.

Branches with less than 95% posterior probability (PP) are colored in gray and those with greater PP values are in black. Time is indicated in millions of years (mya) along the *x*-axis.

Figure S2. Estimate of regime shifts on MCC tree. Significant shifts are indicated with black asterisks and can be either transitions to new shape regimes or reversals to the ancestral regime. Distinct regimes are indicated with different colors; here there are 5 estimated regimes. Analysis is based on PC1 values, plotted on the right.

Table S1. Pollinator data for Iochrominae taxa. Quantitative measures of relative hummingbird importance are listed where available. For the remaining taxa, principal pollinator was coded from field observations. Voucher specimens are listed for personal observations.

Table S2. Parameter estimates from PGLS analyses. "Poll" denotes relative hummingbird importance and the PCs correspond to the shape PCs (Fig. 1). The "No Vb" analysis has the outlier *Vassobia breviflora* removed from the dataset (see text).

Table S3. Phylogenetic ANOVA with pollinator system as a categorical variable. "Poll" denotes relative hummingbird importance and the PCs correspond to the shape PCs (Fig. 1).

Table S4. Inclusion in a regime shift. Each species is listed along with the frequency with which it was associated with a shift across the 100-tree sample.

Table S5. Distinct convergent regimes estimated by I1ou across the 100-tree sample. These include both cases where independent lineages acquired the same derived regime (e.g., the blue lineages in Fig. S2) and where lineages reverted to the ancestral (background) regime (e.g., the *D. spinosa*+*D. brachyachantha*+*I. parvifolium* clade in Fig. S2).