

Repeated evolution of vertebrate pollination syndromes in a recently diverged Andean plant clade

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Although specialized interactions, including those involving plants and their pollinators, are often invoked to explain high species diversity, they are rarely explored at macroevolutionary scales. We investigate the dynamic evolution of hummingbird and bat pollination syndromes in the centropogonid clade (Lobelioideae: Campanulaceae), an Andean-centered group of ~550 angiosperm species. We demonstrate that flowers hypothesized to be adapted to different pollinators based on flower color fall into distinct regions of morphospace, and this is validated by morphology of species with known pollinators. This supports the existence of pollination syndromes in the centropogonids, an idea corroborated by ecological studies. We further demonstrate that hummingbird pollination is ancestral, and that bat pollination has evolved ~13 times independently, with ~11 reversals. This convergence is associated with correlated evolution of floral traits within selective regimes corresponding to pollination syndrome. Collectively, our results suggest that floral morphological diversity is extremely labile, likely resulting from selection imposed by pollinators. Finally, even though this clade's rapid diversification is partially attributed to their association with vertebrate pollinators, we detect no difference in diversification rates between hummingbird- and bat-pollinated lineages. Our study demonstrates the utility of pollination syndromes as a proxy for ecological relationships in macroevolutionary studies of certain species-rich clades.

KEY WORDS: Andes, bat pollination, floral morphology, herbarium specimens, hummingbird pollination, neotropics, Ornstein-Uhlenbeck, phylogenetic comparative methods.

The tropical Andes are home to more than 15% of all angiosperm species and encompass the world's most species-rich biodiversity hotspot (Myers et al. 2000). Specialized relationships and shifts between different pollinators are commonly invoked to explain this high level of biodiversity (Gentry 1982; Antonelli and Sanmartín 2011). The more than 550 species in the centropogonid clade of Neotropical bellflowers (Campanulaceae: Lobelioideae) are an ideal system to explore the effect of specialized pollination syndromes on species evolution in the Andes. This morpholog-

ically and ecologically diverse group, composed of the genera *Centropogon*, *Siphocampylus*, and *Burmeistera*, originated approximately five million years ago and is one of the fastest Andean radiations reported to date (Lagomarsino et al. 2016). Its exceptional diversification appears to have been driven by a complex interplay of abiotic and biotic drivers, including Andean uplift, climate change, and biological traits facilitating plant–animal interactions, including pollination by vertebrates (i.e., hummingbirds or bats; Lagomarsino et al. 2016). A similar relationship between

hummingbird pollination and elevated diversification rates has been documented in other Neotropical clades (Kay et al. 2005; Tripp and McDade 2013; Givnish et al. 2014; Roalson and Roberts 2016; Iles et al. 2017; Serrano-Serrano et al. 2017; Tripp and Tsai 2017), though few studies have investigated the impact of bat pollination. More broadly, there is some support for a link between specialized pollination and high species diversity, especially in the tropics (Eriksson and Bremer 1992; Hedges and Arnold 1995; Dodd et al. 1999; Vamosi and Vamosi 2011).

Such specialist pollination systems are often invoked as key factors underlying floral diversification in angiosperms (Darwin 1862; Grant and Grant 1965; Faegri and van der Pijl 1979; Fenster et al. 2004; Rosas-Guerrero et al. 2014). Pollination syndromes, or integrated suites of reproductive characters that match flowers to functional pollinator groups (Faegri and van der Pijl 1979; Fenster et al. 2004), are *prima facie* evidence of the importance of pollinators as agents of natural selection. An underlying assumption of the pollination syndrome concept is that each functional pollinator group (e.g., birds, bees, moths) exerts different selective pressures on plant species. This contributes to divergent floral morphologies among closely related plants with different pollinators, and convergent floral morphology in distantly related plants with shared pollinators (Fenster et al. 2004). The floral characters associated with a particular pollination syndrome ensure effective visitation by the corresponding functional group, thus increasing the probability of successful reproduction. Studies have consistently shown that pollination syndromes can predict pollinator visitation accurately (Pauw 2006; Lázaro et al. 2008; Tripp and Manos 2008; Martén-Rodríguez et al. 2009; Danieli-Silva et al. 2012). Moreover, a recent global meta-analysis supported the pollination syndrome concept, and demonstrated that the ability to predict effective pollinators by floral morphology is especially accurate in tropical regions and for species that depend on animal pollination for reproduction (Rosas-Guerrero et al. 2014).

The pollination syndrome concept is not without controversy, however. Some studies have found evidence against pollination syndromes (Ollerton 1996; Waser et al. 1996; Ollerton et al. 2009), while others have found mixed support (Smith et al. 2008; Fenster et al. 2009). The disagreement between cross-clade analyses (e.g., Ollerton et al. 2009; Rosas-Guerrero et al. 2014) may result from differing methodologies, data, and underlying assumptions (Ollerton et al. 2015). A prominent argument against the usefulness of pollination syndromes is that they are overly simplistic given that many plant species are visited by more than one functional group of pollinators (Waser et al. 1996, Ollerton 1996). However, this overlooks the idea, first proposed by Stebbins (1970), that pollination syndromes reflect selection by the most frequent and effective pollinators; this does not exclude an important role for secondary pollinators (Muchhala 2003; Martén-Rodríguez et al. 2009; Rosas-Guerrero et al. 2014). It has also been

argued that specialization to specific pollinators, as predicted by pollination syndromes, increases variance in reproductive success across years and thus should not be evolutionarily favored (Waser et al. 1996). Despite these criticisms, macroevolutionary investigations of plant–animal interactions rely on such proxies (Rosas-Guerrero et al. 2014) and, in fact, represent a powerful avenue in which to investigate the nature of pollination syndromes. By focusing on clades with multiple pollinator shifts, we can test the predictions that floral traits undergo correlated evolution and that species tend to converge on evolutionary optima that correspond to pollination syndromes.

Shifts between different pollination syndromes are fundamentally interesting evolutionary events that have happened frequently during angiosperm diversification and have resulted in widespread convergent evolution in distantly related species that share pollinator types (Smith 2010; Van der Niet and Johnson 2012). For example, the bird pollination syndrome, characterized by bright, often red floral pigmentation, long tubular corollas, and production of copious dilute nectar from well-protected nectaries, has evolved independently in at least 65 plant families (Cronk and Ojeda 2008). This represents 1% of all angiosperm diversity (Waser and Ollerton 2006) and includes groups as phylogenetically diverse as *Heliconia* (Heliconiaceae: Stiles 1975; Temeles and Kress 2003), *Aquilegia* (Ranunculaceae: Whittall and Hedges 2007), *Mimulus* (Phrymaceae: Schemske and Bradshaw 1999), and *Erythrina* (Fabaceae: Bruneau 1997). Although this broad evolutionary pattern underscores convergent selection pressures operating at deep phylogenetic scales, shifts between pollination syndromes also have an important role in microevolution: they can generate prezygotic reproductive isolation between sympatric plant populations or reinforce it upon secondary contact of divergent allopatric populations, and thus have the potential to promote speciation (Ramsey et al. 2003; Muchhala 2006b; Waterman et al. 2011; Van der Niet and Johnson 2012). Despite this, the effect of repeated, convergent shifts in pollination syndrome on species diversification has seldom been investigated within a narrowly focused plant clade.

Hummingbird and bat pollination are the two major syndromes in our focal group. Across angiosperms, hummingbird pollination is more common than bat pollination (Fleming and Muchhala 2008; Fleming et al. 2009): bats pollinate species in ~250 genera, whereas hummingbirds pollinate species in ~500 genera (Fleming et al. 2009). Together, these two classes of vertebrate pollinators are costlier to their food plants than their insect counterparts: they are large, warm-blooded animals with high energetic demands that require large flowers that produce abundant sugar-rich nectar. However, they also provide distinct pollination benefits: vertebrate pollinators carry large pollen loads over long distances, are relatively long-lived, are generally faithful, and may have a higher cognitive capacity than insects (Fleming and



Figure 1. Bat and hummingbird pollination characterize flowers in the centropogonid clade. (A) *Centropogon nigricans*, a bat pollinated species, is visited by *Anoura fistulata*. (B) *Centropogon umbrosus* is shown with *Eutoxeres condamini*, its obligate sicklebill hummingbird pollinator. Photos by Nathan Muchhala (A) and Christopher Witt (B).

Muchhala 2008; Fleming et al. 2009). Thus, despite the additional cost, there are many cases in which pollination by vertebrates would be selected for, including at high elevations in tropical latitudes where insect diversity declines with increasing elevation (Cruden 1972).

Within the centropogonid clade, species exhibiting bat and hummingbird pollination syndromes share numerous characteristics, including tubular corollas, abundant nectar production, and a highly specific pollen deposition mechanism. However, they differ in many important regards. Bat-pollinated species typically possess night-blooming, dull-colored flowers (e.g., cream-colored or green) with wide openings that produce a fetid odor and are placed away from the main body of the plant (Muchhala 2006b; Fleming et al. 2009; Figs. 1A and 3[1–7]). These flowers attract nectar-feeding bats of the subfamily Glossophaginae (Phyllostomidae), which have small body sizes and long tongues and tend to hover while feeding from flowers during very short visits (i.e., less than a second; Fleming et al. 2009). In contrast, species displaying the hummingbird pollination syndrome typically have brightly colored, odorless flowers that are receptive during the day and have long, narrow corolla tubes (Figs. 1A and 3[8–14]). These flowers attract a diverse assemblage of hummingbirds (Colwell et al. 1974; Stein 1987; Muchhala and Thomson 2010), including various species with straight bills, as well as the two species in a specialized subclade of hermit hummingbirds with extremely curved bills—sicklebills in the genus *Eutoxeres* (Stein 1992). Concomitantly, the flowers visited by sicklebill hummingbirds are extremely curved, matching the bill morphology of their obligate pollinators (Stein 1987, 1992; Fig. 1B).

The pollination biology of many centropogonid species has been studied in detail. Pollination syndromes have been shown to reliably predict pollination in *Burmeistera* (Muchhala 2006b) and closely related *Centropogon* species (Muchhala and Thomson 2009): wide, green-flowered species are most effectively pollinated by bats, while narrow, red-flowered species are exclusively pollinated by hummingbirds. These results are consistent with

pollination observations in other centropogonid subclades, including the bird-pollinated *Centropogon* subgenus *Eucentropogon* (the eucentropogonid clade *sensu* Lagomarsino et al. [2014]), *C. costaricensis*, *C. talamanicensis*, *C. valerioi*, *Siphocampylus betulaefolius*, *S. sulfureus*, and *S. westianus* (Colwell 1973; Colwell et al. 1974; Stein 1987, 1992; Fenster 1991; Sazima et al. 1994, 1999; Buzato et al. 2000). The most important trait in determining effective pollination by bats versus hummingbirds is the floral aperture width: hummingbird pollinated flowers have a narrow opening, whereas bat pollinated flowers are wider, facilitating entrance by hummingbird bills and bat snouts, respectively (Muchhala 2006b). Although this width is thought to impose an adaptive trade-off (Muchhala 2007), wherein gain of pollination services by one pollinator class is accompanied by loss of the other (Muchhala 2006b), secondary pollination by hummingbirds in bat pollinated flowers has been documented in a handful of species (e.g., *Burmeistera ceratocarpa*, *B. tenuiflora*, *S. sulfureus*; Sazima et al. 1994; Muchhala 2003, 2006b).

Here, we explore the evolution of pollination syndromes in this species-rich, Andean-centered clade. We first apply a phylogenetic principal components analysis to determine whether flowers with different putative pollination syndromes, defined based on their flower color, group into separate regions of morphospace, as predicted if color and shape have evolved together in response to distinct pollinators. We validate this approach using linear discriminant analyses in which pollination syndromes for species with unknown pollinators are predicted based on the morphology of species with known pollinators. We then reconstruct the evolutionary history of pollination syndromes in the centropogonid clade and apply Orstein–Uhlenbeck (OU) models to determine whether floral morphology evolves under adaptive regimes corresponding to pollination syndromes. Finally, we apply trait-dependent models to determine if there is a differential effect of pollinator type on diversification rates in this clade. By applying these phylogenetic comparative methods, we investigate patterns of convergent evolution and determine the evolutionary

lability of morphological characters that underlie specialized relationships.

Methods

PHYLOGENETIC RELATIONSHIPS AND FLORAL TRAIT CHARACTERIZATION

The phylogeny of the centropogonid clade was recently resolved with high support (Lagomarsino et al. 2014, 2016). Two of the three genera are nonmonophyletic and are distinguished based solely on their fruit type: *Centropogon* produces berries and *Siphocampylus* produces capsules. A third genus, *Burmeistera*, is monophyletic and also berry-producing; its monophyly is supported by a series of synapomorphies that include a dilated anther opening and isodiametric seeds. We use the phylogeny from Lagomarsino et al. (2016), whose topology was inferred simultaneously with divergence time estimates in BEAST version 2 (Drummond and Rambaut 2007; Bouckaert et al. 2014), as a framework for phylogenetic comparative methods. Taxon sampling includes 191 species that span geographic and morphological diversity in the clade and include representatives of all major taxonomic groupings. Comparative analyses performed here use either the maximum clade credibility (MCC) tree or a subset of 100 trees from the posterior distribution, as specified below. The MCC topology with posterior probabilities can be found in Figure S1.

To characterize floral morphology, 11 quantitative floral traits were measured from herbarium specimens. These included collections from the Harvard University Herbaria (herbarium codes: A, GH), New York Botanical Garden (NY), and the Missouri Botanical Garden (MO), as well as images of herbarium specimens on the JSTOR Plants database (<http://plants.jstor.org/>). Where possible, these specimens were the same as those used for constructing our molecular phylogeny (95/153 samples); when not available, or when the specimen lacked flowers, floral measurements were taken from a different specimen of the same species. When a specimen possessed more than one flower, two flowers were measured and results were averaged. The traits measured were as follows: corolla length, corolla width at three points, anther length and width, floral curvature (calculated using two length measurements of the corolla; Fig. S1 inset), peduncle length, and ovary length and width (Table S1; Fig. S2). Many of these traits were combined into volume, area, or ratios to capture an integrated measure of floral form, including approximate volume of the ovary (treated as a cylinder with the height and radius corresponding, respectively, to the length and half the width of the ovary), area of the anthers, and ratio of various measurements of corolla width (i.e., basal, midpoint, and apical measurements).

Flowers of each species were additionally classified into putative pollination syndromes based on their corolla color (i.e., not using any of the above quantitative traits as a determinant).

Flower color is a key element in traditional descriptions of pollination syndromes (Faegri and van der Pijl 1979), and its usefulness for inferring pollination syndromes in the centropogonid clade is further bolstered by studies of pollination ecology (Muchhala 2006b, 2007). Flower color was determined by field observations, species descriptions, or herbarium label information. Two broad pollination syndromes were represented: bat (Figs. 1A and 3) and hummingbird (Figs. 1B and 3). We additionally recognized two subclasses of hummingbird pollination—by straight-billed hummingbirds (Fig. 3[8–12; 14]) and by sicklebill hummingbirds (Figs. 1B and 3[13]). Sicklebill hummingbird pollinated flowers were identified by their extreme corolla curvature; this coding is supported by field observations of many sicklebill pollinated species (Stein 1987, 1992). Where the data was available, the resulting pollination syndrome codings based on color were consistent with known pollinators (Colwell et al. 1974; Snow and Teixeira 1982; Stein 1987, 1992; Sazima et al. 1994; Muchhala 2003, 2006b, 2008; Muchhala and Potts 2007; Fleming et al. 2009; Muchhala and Thomson 2009; Avalos et al. 2012).

PHYLOGENETIC COMPARATIVE ANALYSES

Validation of pollination syndrome concept using principal components and linear discriminant analyses

If centropogonids can be classified into distinct pollination syndromes based on color, we would expect these classes to fall into distinct regions of morphospace, reflecting the selective pressures pollinators impose on floral form. To test this prediction, we performed a phylogenetically corrected principal components analysis (Felsenstein 1985; Revell 2009) using the quantitative floral traits listed in Table S1, which include both raw measurements (e.g., corolla length) and integrated values (e.g., ratio of corolla widths at different points; anther area); no color information was included. This analysis was performed in the phytools package of R (Revell 2012) using average trait values (or the point estimate when only one flower was measured) and the MCC tree.

To further explore pollination syndromes, results from the phylogenetic principal components analysis were compared to a linear discriminant analysis in which morphological data from species with known pollinators (Table S2) were used to predict the pollinators of the remaining species. This was performed using the *lda* and *predict* functions of the MASS R package (Venables and Ripley 2002).

Ancestral state reconstruction

After validation, ancestral states of pollination syndrome as a categorical trait were inferred. First, a preliminary ancestral state reconstruction was performed using standard maximum-likelihood methods using the equal rates (ER) and all rates different (ARD) models with the *ace* function in ape (Paradis et al. 2004). A likelihood ratio test was subsequently performed to determine the

Table 1. Model comparison for the BiSSE analyses for bat (0) and hummingbird (1) pollinated lineages, with mean parameter estimates for each model.

| Model | NP | Log L | AICc | Δ AIC | λ_0 | λ_1 | μ_0 | μ_1 | q_{01} | q_{10} |
|--|----------|----------------|---------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Null model ($\lambda_0 = \lambda_1; \mu_0 = \mu_1; q_{01} = q_{10}$) | 3 | -311.669 | 629.50 | 2.80 | 2.13 | 2.13 | 1.21 | 1.21 | 0.14 | 0.14 |
| $\lambda_0 \neq \lambda_1; \mu_0 = \mu_1; q_{01} = q_{10}$ | 4 | -311.02 | 630.26 | 3.56 | 1.98 | 2.20 | 1.20 | 1.20 | 0.15 | 0.15 |
| $\lambda_0 = \lambda_1; \mu_0 \neq \mu_1; q_{01} = q_{10}$ | 4 | -311.23 | 630.68 | 3.98 | 2.13 | 2.13 | 1.34 | 1.13 | 0.15 | 0.15 |
| $\lambda_0 = \lambda_1; \mu_0 = \mu_1; q_{01} \neq q_{10}$ | 4 | -309.25 | 626.71 | 0 | 2.12 | 2.12 | 1.20 | 1.20 | 0.30 | 0.05 |
| $\lambda_0 \neq \lambda_1; \mu_0 \neq \mu_1; q_{01} = q_{10}$ | 5 | -310.90 | 632.13 | 5.42 | 1.89 | 2.26 | 1.09 | 1.28 | 1.28 | 1.28 |
| $\lambda_0 \neq \lambda_1; \mu_0 = \mu_1; q_{01} \neq q_{10}$ | 5 | -309.04 | 628.41 | 1.71 | 2.01 | 2.13 | 1.12 | 1.12 | 0.29 | 0.06 |
| $\lambda_0 = \lambda_1; \mu_0 \neq \mu_1; q_{01} \neq q_{10}$ | 5 | -309.20 | 628.72 | 2.02 | 2.12 | 2.12 | 1.19 | 1.20 | 0.31 | 0.05 |
| $\lambda_0 \neq \lambda_1; \mu_0 \neq \mu_1; q_{01} \neq q_{10}$ | 6 | -307.75 | 627.96 | 1.25 | 1.51 | 2.56 | 0.35 | 1.71 | 0.43 | 0.04 |

The best-fitting model is determined via a combination of the lowest corrected Akaike information criterion (AICc), a Δ AIC with 2 units of difference with other models, and likelihood ratio tests (LRT) in case where Δ AIC values are close between models. The best model is in red and bold text.

optimal model. Ancestral character states were then estimated along the MCC tree from 1000 iterations of Bayesian stochastic character mapping using the empirical Bayes method and the optimal model with the *make.simmap* function in the phytools package; this was repeated across 100 trees from the BEAST posterior distribution to account for phylogenetic uncertainty. We then used the *rayDISC* function in the corHMM package of R (Beaulieu et al. 2015) to infer ancestral states using maximum likelihood across the posterior distribution. We also counted number of transitions between each state for each tree in the posterior sample to generate a mean, median, and range of transition numbers.

Identification of evolutionary regimes associated with pollinator syndrome

We tested the hypothesis that quantitative floral characters evolve toward different evolutionary optima that are dependent on pollination syndrome (i.e., bat, straight-billed hummingbird, sicklebill hummingbird) by evaluating the likelihood of Brownian motion (BM) and three OU models of trait evolution (Butler and King 2004; Beaulieu et al. 2012). We tested four models: BM, single-state OU (OU1), two-state OU (OU2), and three-state OU (OU3). Both the BM and OU1 models imply that there is no relationship between the quantitative trait and pollination syndrome; BM suggests that the evolution of the trait follows a random walk, while OU1 is consistent with stabilizing selection. For the OU2 model, we collapsed the subclasses of hummingbirds (straight-billed and sicklebill), allowing for separate trait optima for bat and hummingbird pollination, but no difference within hummingbird pollination. Finally, the OU3 model infers three separate trait optima for bats, straight-billed hummingbirds, and sicklebill hummingbirds. We additionally explored the optima for the first three PC axes of the phylogenetic PCA as dependent on pollination syndrome. All analyses were conducted using the *OUwie* function in the R package OUwie (Beaulieu and O'Meara 2012) on a sample of 100 trees from the posterior distribution. These input trees had

the most likely pollination syndrome reconstructions at the nodes from the *rayDisc* analysis (see above). Model fit was assessed via AICc. We used a model-averaging approach to estimate the value of trait optima (thetas) across the OU models (Burnham and Anderson 2002).

TRAIT-DEPENDENT SPECIES DIVERSIFICATION

We modeled the impact of bat versus hummingbird pollination on species diversification in two ways. In addition to revealing diversification dynamics, the results of these analyses are important in determining whether to account for bias due to state-dependent diversification in ancestral state reconstruction (Goldberg and Igić 2008). We first evaluated eight Binary State-Speciation and Extinction (BiSSE, Maddison et al. 2007) models of increasing complexity in which speciation, extinction, and transition rates were allowed to either vary or remain equal between traits (Table 1). For these analyses, the sicklebill and straight-billed hummingbird pollination syndromes were treated as a single state. A sampling fraction that varied by character state was applied to account for species not sampled in our analyses (hummingbird = 0.405; bat = 0.340; FitzJohn et al. 2009). Analyses were performed using the R package *diversitree* 0.7-6 (FitzJohn 2012). We additionally applied FisSE (Rabosky and Goldberg 2017), a recently described nonparametric method to identify state-dependent diversification, to this binary trait.

We did not implement a multistate speciation-extinction model (MuSSE; FitzJohn 2012) in which sicklebill and straight-billed hummingbird pollination are treated separately because the distribution of our data makes this model inappropriate (Rabosky and Goldberg 2015). Sicklebill pollination in the centropogonid clade arose once, and characterizes all but approximately 10 of the approximately 55 species of that subclade (the eucentropogonids, Stein 1992; Lagomarsino et al. 2014). Statistical power would be lacking to identify any realistic difference in diversification dynamics for this pollination syndrome subclass ("Darwin's scenario," *sensu* Maddison and FitzJohn 2015).

Results

PHYLOGENETIC RELATIONSHIPS AND FLORAL TRAIT CHARACTERIZATION

Floral measurements collected from herbarium specimens are presented in Table S1 and are deposited on Dryad. In total, quantitative measurements were made from 153 species; we were not able to access floral material for the additional 38 species in our phylogeny. These taxa were not included in the phylogenetic comparative analyses that relied on quantitative data (i.e., phylogenetic PCA, OU modeling). Average trait values for each pollination syndrome are presented in Table 2.

PHYLOGENETIC COMPARATIVE ANALYSES

Validation of pollination syndromes using principal components and linear discriminant analyses

The first three axes of the phylogenetically corrected PCA explained 66.5% of the variance in floral traits across species (Fig. 2; Table 3). As is typical for PCAs of morphological data, the majority of the variation captured by the first PC axis reflected overall size: ovary volume, anther size, and lengths of the peduncle, corolla, and corolla tube (Table 3). There was no clear association between PC1 and pollination syndrome as inferred by color (Fig. 2). In contrast, PC2 captured both size and shape variation, and largely separates species according to pollination syndrome (Fig. 2). The major loadings of PC2 include ratio of the bottom to the middle of the corolla (i.e., presence of basal corolla constriction, e.g., Fig. 3[9, 11–12]), ratio of bottom to top of the corolla (i.e., corolla flare), corolla tube length, and anther area (Table 3). PC3 further separated the sicklebill hummingbird pollinated species (Fig. 2). The primary loadings for this PC were corolla curvature and the ratio of the width at the middle and top of the corolla (i.e., distal inflation of the corolla, e.g., Fig. 1B; Table 3). Overall, our phylogenetic PCA placed species with different pollination syndromes, inferred via their flower color, in distinct areas of morphospace.

Results of the LDA, which used the morphology of species with known pollinators to predict pollination syndromes of the other species, agreed well with our color-defined pollination syndromes. Most taxa (91.5%) were assigned to the same syndrome using these two methods (Fig. S3; Table S3). Among the 13 taxa that differed of 153 total, two lacked statistical support (Table S3) and at least two are generalists that are pollinated by both hummingbirds and bats (i.e., *Centropogon incanus* and *C. mandonis*; L. P. Lagomarsino and N. Muchhala, unpubl. data).

Ancestral state reconstruction

Both Bayesian stochastic character mapping (Fig. 3) and maximum likelihood ancestral state reconstruction (Fig. S4) support pollination by straight-billed hummingbirds as ancestral within

Table 2. Average values and SDs for each quantitative floral trait used in comparative analyses for all species within a pollination syndrome.

| | Ratio— bot:mid | Ratio— mid:top | Ratio— top:bot | Peduncle (L) | Corolla (L) (L) | Corolla tube (L) | Ovary (Vol) | Anther (A) | Stamen (L) | Curvature (ratio) |
|--------------------------------|-------------------|-------------------|-------------------|-------------------|--------------------|---------------------|-----------------------|-------------------|-------------------|----------------------|
| Straight-billed hummingbird | 0.58 (±0.22) | 0.85 (±0.18) | 0.48 (±0.18) | 53.5 (±31.25) | 41.34 (±10.69) | 30.63 (±9.12) | 380.35 (±439.22) | 19.11 (±13.12) | 43.72 (±11.84) | 0.996 (±0.01) |
| Sicklebill hummingbird | 0.92 (±0.23) | 0.49 (±0.06) | 0.44 (±0.09) | 23.55 (±9.92) | 37.53 (±7.92) | 25.48 (±10.21) | 268.86 (±130.97) | 19.88 (±7.05) | 37.13 (±11.40) | 0.90 (±0.045) |
| Bat | 1.44 (±0.59) | 0.58 (±0.23) | 0.75 (±0.25) | 89.37 (±48.44) | 36.45 (±14.72) | 18.70 (±8.90) | 1070.34 (±1328.37) | 45.36 (±29.49) | 38.49 (±13.54) | 1.00 (±0.002) |

L = length, values in mm; A = area, values in mm²; Vol = volume, values in mm³.

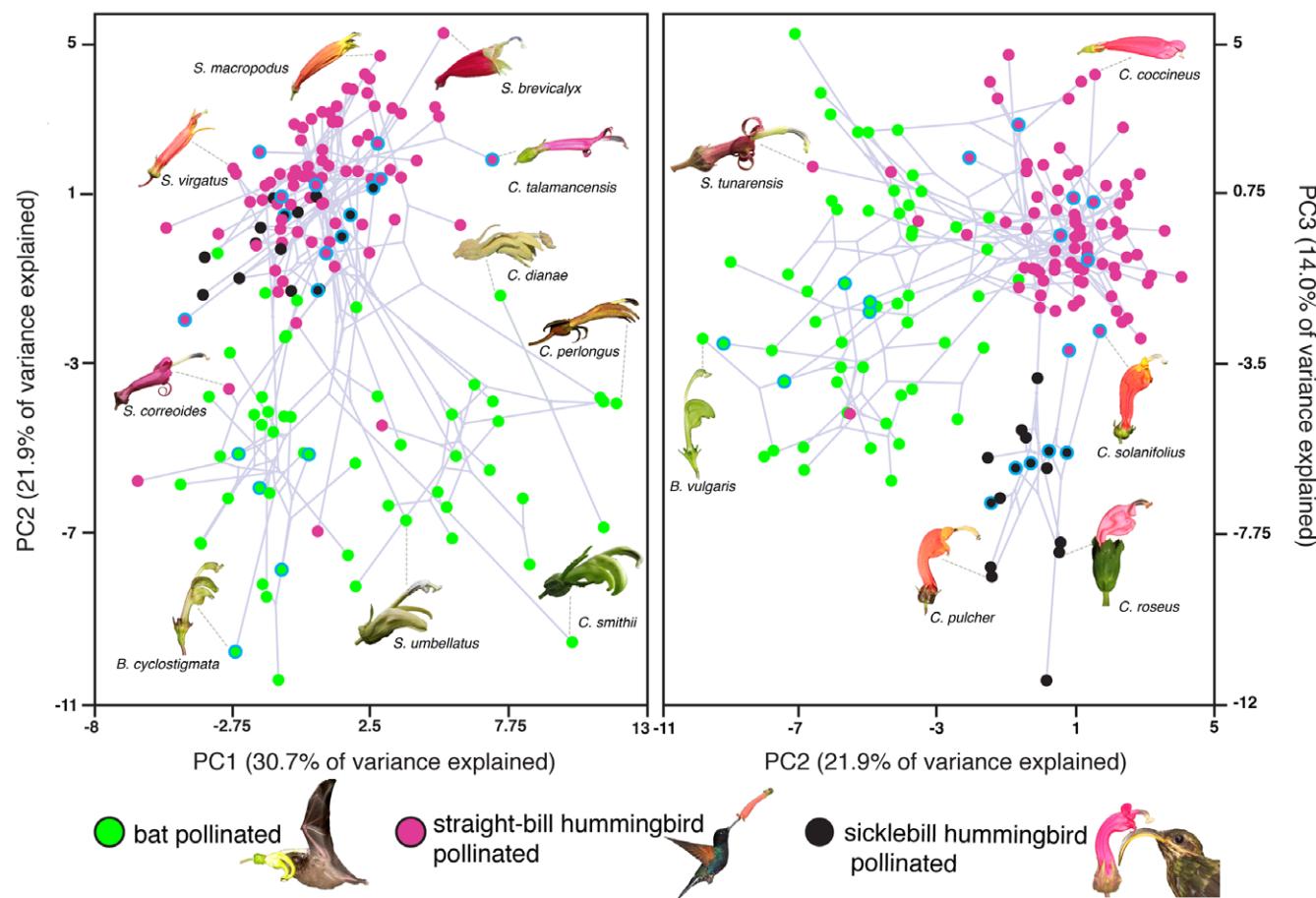


Figure 2. Phylogenetic principal components analysis of floral morphology. Scores of the first three principal component axes of floral morphology in 153 species of the centropogonid based on a phylogenetic PCA, explaining 66.5% of the variance. Flower color (our proxy for pollination syndrome) is indicated by the color of points (green [bat pollination proxy] = green, cream, or white flowers; pink and black [our proxies for hummingbird pollination] = red, orange, yellow, or pink flowers). Black points represent species known or hypothesized to be pollinated by specialized sicklebill hummingbirds, whereas pink points are known or hypothesized to be pollinated by more generalist straight-billed hummingbirds. Blue circles around an individual point indicate a species whose pollination biology has been studied in the field. MCC tree topology is depicted in light gray lines connecting individual points. Representative flowers are included to show the diversity depicted along the axes. The loadings for the PC axes are provided in Table 3. The match between flower color and morphology validates the presence of pollination syndromes in the centropogonid clade, which is corroborated by our LDA analysis (Table S3). Bat photo by NM, flower photos by LPL.

Table 3. Loadings for each trait for the first three PC axes from a phylogenetic principal components analysis of floral morphology.

| Trait | PC1 (30.67%) | PC2 (21.87%) | PC3 (13.99%) |
|---|--------------|---------------|--------------|
| Bottom: middle corolla width (basal constriction) | -0.090 | -0.752 | -0.333 |
| Middle: top corolla width (distal inflation) | -0.09 | 0.210 | 0.876 |
| Bottom: top corolla width (corolla flare) | -0.070 | -0.681 | 0.391 |
| Peduncle length | 0.623 | -0.431 | 0.183 |
| Corolla length | 0.853 | 0.275 | -0.042 |
| Corolla tube length | 0.651 | 0.562 | -0.005 |
| Ovary volume | 0.693 | -0.416 | -0.025 |
| Anther area | 0.718 | -0.510 | 0.087 |
| Stamen length | 0.712 | 0.321 | -0.085 |
| Corolla curvature | 0.039 | -0.026 | 0.563 |

The traits with the highest loadings for each PC axis (>0.5) are indicated in bold text.

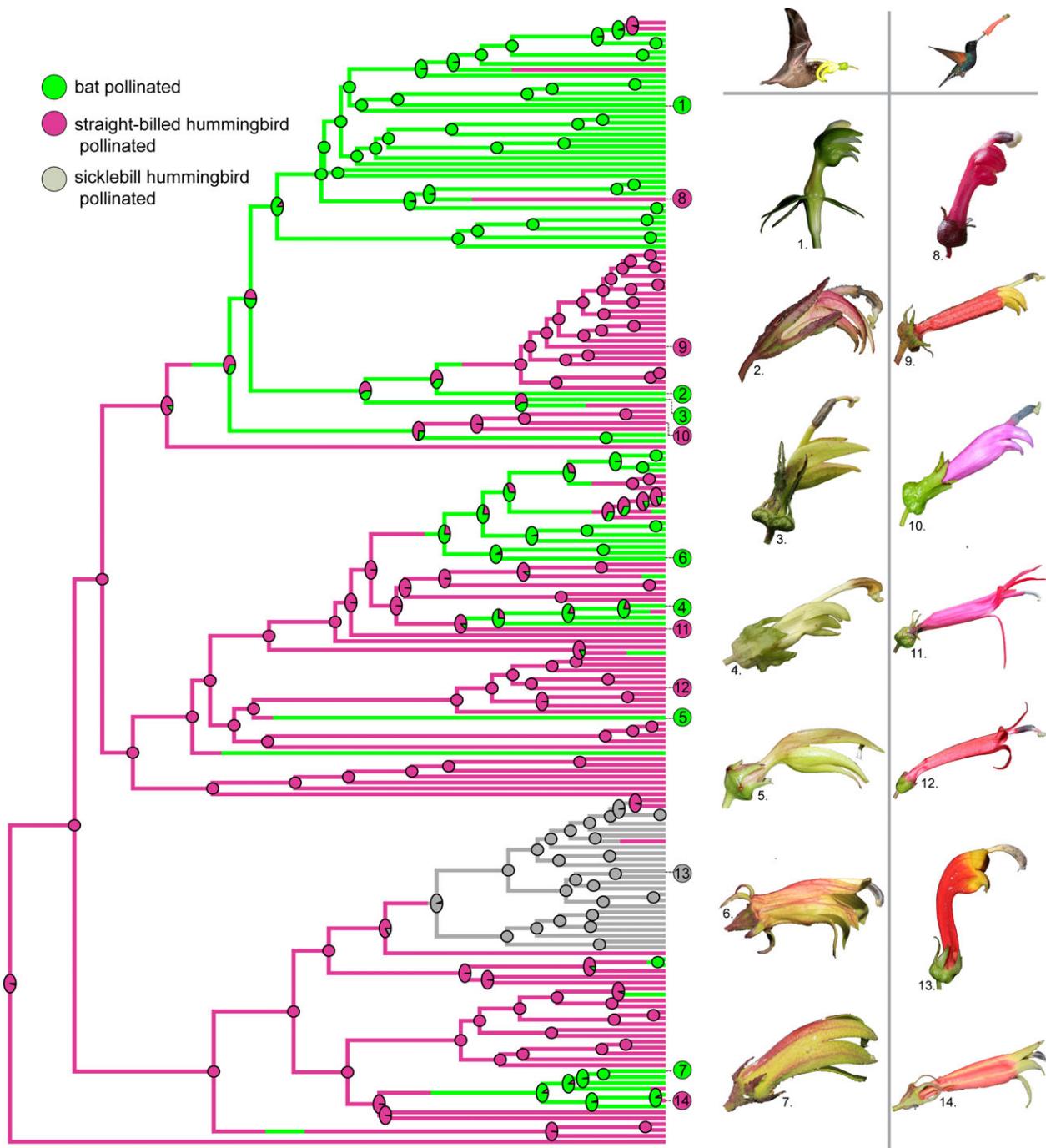


Figure 3. Bayesian stochastic character mapping of pollination syndrome evolution. Pie charts at nodes along the MCC chronogram of the centropogonid clade represent ancestral states that were calculated as the marginal posterior probability of bat pollination (green), straight-billed hummingbird pollination (pink), and sicklebill hummingbird pollination (gray), whereas colors along branches represent a single instance of stochastic character mapping, which allows state transitions to occur along branches. Colored circles with numbers placed at tips correspond to the numbered pictures of flowers to the right of the phylogeny, which represent bat (left; 1–7) or hummingbird (right; 8–14) pollination syndromes. According to the reconstruction presented, each bat-pollinated flower pictured represents an independent evolution of that pollination syndrome, while two hummingbird-pollinated flowers (8, 14) represent reversals to hummingbird pollination (the remainder are plesiomorphically hummingbird pollinated). These photos illustrate the repeated evolution of gross morphology while simultaneously demonstrating that the end result of this convergent evolution is not completely identical flowers. Results from a maximum likelihood reconstruction are supplied in Figure S4. Photos 1–7, 9, 11–14 by LPL, 8 and 10 by A. Antonelli (1. *Burmeistera tenuiflora*, 2. *Siphocampylus jelskii*, 3. *S. matthiae*, 4. *S. tunicatus*, 5. *S. smilax*, 6. *S. rusbyanus*, *S. actinothrix*, 8. *B. cf. fuchsioides*, 9. *Centropogon argutus*, 10. *C. aequatorialis*, 11. *S. boliviensis*, 12. *C. leucocarpus*, 13. *C. granulosus*, 14. *S. antonelli*).

Table 4. Inferred number of transitions between pollination syndromes in the centropogonid clade.

| Ancestor | Descendant | Mean | Median | Range |
|-----------------------------|-----------------------------|-------|--------|-------|
| Straight-billed hummingbird | Bat | 13.31 | 13 | 8–16 |
| Straight-billed hummingbird | Sicklebill hummingbird | 1.00 | 1 | 1–1 |
| Bat | Straight-billed hummingbird | 10.92 | 11 | 8–19 |
| Bat | Sicklebill | 0.00 | 0 | 0–0 |
| Sicklebill hummingbird | Straight-billed hummingbird | 2.13 | 2 | 2–4 |
| Sicklebill hummingbird | Bat | 0.00 | 0 | 0–0 |

Values calculated from ancestral state reconstructions on a random subset of 100 trees from the BEAST posterior distribution, performed using an "ARD" model with the rayDISC function in the corHMM package. Sicklebills are a subset of hummingbirds in the genus *Eutoxeres*.

the centropogonid clade (marginal likelihood = 0.98 and 0.65, respectively), whose monophyly is well-supported (PP = 1.0; Fig. S1). Further, after correcting for phylogenetic uncertainty, we find that evolution of pollination syndromes has been dynamic (Fig. 3; Table 4): bat pollination has evolved from straight-billed hummingbird pollination an average of 13.3 times (range across trees: eight to 16), and the reverse occurred an average of 10.9 times (range: eight to 19). Pollination by sicklebills evolved once from straight-billed hummingbird pollinated ancestors, with an average of two reversions to straight-billed pollination (range: two to four). There were no transitions between bat and sicklebill pollination. Results are consistent between maximum likelihood (Table 4) and Bayesian (Table S4) analyses.

Identification of evolutionary regimes in pollination syndromes

OU models of trait evolution were a significantly better fit than simple BM models for all floral traits and PC axes (Table 5). OU2 and OU3 models were the best-fit for all floral traits (5/10 for each OU2 and OU3), which supports our hypothesis of the correlated evolution of floral traits within pollination syndromes. The OU2 model, in which sicklebill and straight-billed hummingbird pollination were collapsed into a single character state, was the best fit model for the following five characters: ratio of the bottom to middle corolla width (i.e., basal constriction), ratio of the bottom to top corolla width (i.e., corolla flare), corolla length, ovary volume, and anther area (Table 5). The OU3 model, which modeled separate trait optima for bat, straight-billed hummingbird, and sicklebill pollination, was the best fit model for the following five characters: ratio of the middle to top corolla width (i.e., distal inflation), peduncle length, corolla tube length, corolla curvature, and stamen length (Table 5). Mean theta values (i.e., trait optima) and their associated SEs can be found in Table S5.

Additionally, we found that two of the three PC axes that we explored were also fit best by our OU3 model (PC2–3). This is consistent with the results of our phylogenetic PCA: these are the axes that best separated species according to their pollination syndrome (Fig. 2). Conversely, PC1, which largely captured size

variation, was best modeled in an OU1 framework, suggesting a global optimum for overall floral size across the centropogonid clade rather than separate optima for each pollination syndrome. Again, this is consistent with our phylogenetic PCA, given that the first PC axis did not separate species on the basis of pollination syndrome.

TRAIT-DEPENDENT DIVERSIFICATION

The best-fitting model for the BiSSE analysis shows no difference in speciation or extinction rate between hummingbird and bat pollinated lineages. However, transition rates are asymmetric: our estimate is ~5.6 higher for hummingbird to bat transitions than the reverse (0.305 vs. 0.0543; Table 1). Although SSE-based diversification models are known to produce high Type I error rates (i.e., detecting an association between a given trait and diversification rate when none exists; Rabosky and Goldberg 2015), our analysis, in which no association was found, cannot represent such an error. BiSSE is also known to estimate speciation rates more accurately and precisely than extinction rates under certain scenarios (Davis et al. 2013), and, more generally, it has been argued that extinction rates should not be estimated from molecular phylogenies alone (Rabosky 2010). These criticisms about the extinction rate parameter are mitigated in two ways in our analyses. First, the centropogonids are known to represent a rapid radiation, and so relatively little time has elapsed for extinction rates to significantly impact inferences. Second, our BiSSE results are corroborated by a nonparametric FiSSE test ($P = 0.332$). These results suggest no expected bias in our ancestral state reconstructions (Goldberg and Igić 2008).

Discussion

SUPPORT FOR POLLINATION SYNDROMES IN THE CENTROPOGONID CLADE

Pollination syndromes are a convenient measure by which to predict effective pollinator visitation. The concept is not without controversy, however, and has received both support (Fenster et al. 2004; Martén-Rodríguez et al. 2009; Rosas-Guerrero et al. 2014)

Table 5. Mean Akaike information criterion (AICc) weights representing the relative likelihood of each fitted model of trait evolution.

| Trait | BM | OU1 | OU2 | OU3 |
|---|----------|------------------|-------------------|-------------------|
| Bottom: middle corolla width (basal constriction) | 0.00 (0) | 0.00 (0) | 1.00 (100) | 0.00 (0) |
| Middle: top corolla width (distal inflation) | 0.00 (0) | 0.00 (0) | 0.00 (0) | 1.00 (100) |
| Bottom: top corolla width (corolla flare) | 0.00 (0) | 0.00 (0) | 0.71 (94) | 0.29 (6) |
| Penduncle length | 0.00 (0) | 0.00 (0) | 0.14 (8) | 0.86 (92) |
| Corolla length | 0.00 (0) | 0.14 (0) | 0.44 (61) | 0.42 (39) |
| Corolla tube length | 0.00 (0) | 0.00 (0) | 0.31 (10) | 0.69 (90) |
| Ovary volume | 0.00 (0) | 0.04 (0) | 0.80 (99) | 0.16 (1) |
| Anther area | 0.00 (0) | 0.00 (0) | 0.84 (99) | 0.16 (1) |
| Stamen length | 0.00 (0) | 0.12 (0) | 0.27 (1) | 0.61 (99) |
| Corolla curvature | 0.00 (0) | 0.00 (0) | 0.00 (0) | 1.00 (100) |
| PC1 | 0.00 (0) | 0.49 (73) | 0.30 (8) | 0.21 (19) |
| PC2 | 0.00 (0) | 0.00 (0) | 0.47 (36) | 0.53 (64) |
| PC3 | 0.00 (0) | 0.00 (0) | 0.00 (0) | 1.00 (100) |

Values were calculated after fitting models to maximum likelihood reconstructions of pollination syndrome on 100 trees. The number of trees supporting each model (i.e., the highest weight for each tree) is presented in parentheses. Bold values indicate the best-fit models for each trait. BM = Brownian motion; OU = Ornstein–Uhlenbeck model.

and criticism (Waser et al. 1996; Smith et al. 2008; Ollerton et al. 2009). In this study, we demonstrate that members of the centropogonid clade are morphologically well defined within their hypothesized pollination syndromes. By using a single key character as a proxy for pollination syndrome (i.e., flower color) that is independent of the traits examined in our analyses, we avoided the common criticism of circularity in studies of morphological evolution of pollination syndromes while providing strong support for the concept in the centropogonid clade. We found that the color-defined groups cluster in distinct regions of morphospace in the phylogenetic PCA. Furthermore, an LDA that predicted pollinators of species without known pollinators based on the morphology of those species with known pollinators had high agreement with our color-based definitions (91.5%). Finally, OU models for all floral traits detected two to three evolutionary optima corresponding to pollination syndrome (either bat and hummingbird; or bat, straight-billed, and sicklebill hummingbirds; see below for further discussion). Our results are consistent with recent studies confirming the predictive nature of pollination syndromes in diverse taxa (Temeles et al. 2000; Martén-Rodríguez et al. 2009; Rosas-Guerrero et al. 2014) and are bolstered by empirical and field-based pollination studies within this clade (Muchhala 2006b).

Although pollination syndromes are useful for inferring the primary pollinator of a plant (Stebbins 1970), many studies have shown that floral morphologies conforming to a pollination syndrome do not necessarily exclude pollinators from other guilds from providing pollination services (e.g., Muchhala 2003; Reynolds et al. 2009). We were unable to test the importance of secondary pollinators in our study, although they have been shown to be important to many plant species, both

within the centropogonid clade (Sazima et al. 1994; Muchhala 2003) and in distantly related taxa (Sanmartín-Gajardo and Sazima 2005; Martén-Rodríguez et al. 2009, 2010; Muchhala et al. 2009). The zone of incomplete separation of bat and bird flowers in morphospace in our phylogenetic PCA may represent morphologies that can be effectively pollinated by both hummingbirds and bats. Additionally, there are several species that are brightly colored that fall within the bat cluster of PC morphospace, including *C. mandonis*, a red-flowered species for which recent fieldwork has confirmed effective bat pollination (Lagomarsino and Muchhala, unpubl. data). The anthocyanin pigmentation in the corollas of these species may result from pleiotropy or play a protective role (Armbruster 2002; Ng and Smith 2016). The latter seems likely, for example, in *S. tunarensis* (pictured in Fig. 1), an early successional tree whose wide, distally inflated flowers, predicted to be bat pollinated in our LDA (Table S3), are borne on its exposed crown and thus may be more susceptible to UV damage than flowers in the more typical understory centropogonid species.

Echoing a common sentiment, we stress that the pollination biology of many plant groups remains understudied, particularly in the tropics. Although flower color predicts pollination syndrome in the centropogonid clade, this is not true in other groups (Smith et al. 2008), nor is it always expected, especially as flower color serves multiple, sometimes conflicting ecological roles (Armbruster 2002; Muchhala et al. 2014). Additionally, it is likely that specialized pollination systems and discrete syndromes will be present in some clades of flowering plants, but not in others. More and better studies of focused pollination ecology will continue to bolster clade-level macroevolutionary studies of floral evolution, and will improve our understanding of the generality of

pollination syndromes. These two scales of investigation should be viewed as complementary, with a shared goal of deeper insight into the evolution of plant–animal interactions.

WIDESPREAD CONVERGENCE: THE LABILITY AND BIDIRECTIONALITY OF POLLINATION SYNDROME EVOLUTION

Pollination syndrome evolution has been extraordinarily labile in the centropogonid clade: transitions from hummingbird to bat pollination, as well as the reverse, have both occurred numerous times in the clade's history, resulting in an average of 27 estimated pollinator shifts. Transitions from hummingbird to bat pollination are most frequent, consistent with evidence suggesting that the bulk of bat-pollinated flowers in the Neotropics evolved from hummingbird-pollinated ancestors (Sanmartin-Gajardo and Sazima 2005; Perret et al. 2007; Tripp and Manos 2008; Fleming et al. 2009; Martén-Rodríguez et al. 2009). However, transitions from bat to hummingbird pollination are still very common; they have occurred \sim 11 times (mean = 10.9, range = 8–19; Table 4). Although our study is not unique in documenting exceptionally labile pollination syndrome evolution, it documents more transitions away from bat pollination than in any previous taxon-specific investigation (Tripp and Manos 2008; Martén-Rodríguez et al. 2009). Further, within hummingbird-pollinated centropogonids, multiple transitions from sicklebill to straight-billed hummingbird pollination are inferred, despite the extremely specialized nature of the ecological relationship between sicklebills and curved flowers. That floral morphology can evolve relatively easily and yet is associated with specific morphological traits during pollinator shifts is suggestive of strong pollinator-mediated selection (Davis et al. 2014). The frequency of these pollinator shifts may further be facilitated by the relatively minor floral morphological changes necessary to make a large ecological impact (Schemske and Bradshaw 1999; Whittall and Hodges 2007; Rausher 2008).

CORRELATED TRAIT EVOLUTION IN POLLINATION SYNDROMES

Our results illuminate the traits that are most important in discriminating flowers that are adapted to pollination by hummingbirds versus bats. Further, our OU analyses, in which all floral traits were found to evolve under selective regimes corresponding to pollination syndrome (i.e., OU2 or OU3 models), reveal a global pattern of correlated trait evolution. All traits identified by the phylogenetic PCA and OU analyses have putative adaptive significance discussed below.

The width of the corolla opening is a key aspect of both the bat and hummingbird pollination syndromes. Inflated, wide corolla openings are a landmark character of bat pollination (Martén-Rodríguez et al. 2009), while hummingbird visitation is most effective at narrow flowers (Temeles et al. 2002). Our results

confirm the importance of corolla width across the centropogonid clade: corolla inflation (i.e., the middle-top corolla width ratio) is a key trait for distinguishing bat-pollinated flowers from hummingbird-pollinated flowers and is best-fit by an OU3 model. The degree of corolla inflation is most extreme in bat-pollinated flowers, followed by sicklebill-pollinated flowers, while it is minimal across straight-billed hummingbird pollinated flowers (Tables 2 and S4). Wide corolla openings match the shape of bat snouts (e.g., Fig. 1 from Muchhal 2006b), and have been empirically shown to be the most important character in determining effective bat pollination to the exclusion of hummingbird pollination within the centropogonid clade (Muchhal 2006b). They may also aid in echolocation (Martén-Rodríguez et al. 2009). Sicklebill pollinated flowers share inflated corolla openings with bat flowers. Here, they facilitate the idiosyncratic pollination style of sicklebills, which insert their entire face into the flower while perching on the vegetative body of the plant (Stein 1987, 1992). In contrast, the narrow corolla apertures that characterize straight-billed hummingbird pollinated species facilitate effective pollination not only in the centropogonid clade (Muchhal 2006b), but, more generally: within mechanical constraints, hummingbirds are most precise and most likely to come into contact with reproductive organs of flowers with narrower openings (Temeles et al. 2002; Muchhal 2006b).

We also find that the presence or absence of a basal corolla constriction is another key component of corolla morphology. This trait was best fit by an OU3 model (Table 5), with straight-billed hummingbirds having the most extreme corolla constrictions (i.e., low values for bottom-middle corolla width ratio) and bat flowers generally lacking one (Tables 2 and S4). This morphology is hypothesized to play the dual role of guiding hummingbird bills into the flower and protecting against mechanical damage to the ovary during pollination (Grant 1950; Cronk and Ojeda 2008). This constriction is often associated with an externally visible groove (e.g., Fig. 3[9, 11–12]) with internally thickened tissue. In Antillean Gesneriaceae, such a basal corolla constriction was also found to promote effective hummingbird pollination by flowers otherwise demonstrating the bat pollination syndrome in generalist flowers (Martén-Rodríguez et al. 2009, 2010), and it is thought to facilitate hummingbird pollination in *Heliconia* (Wolf and Stiles 1989) and *Drymonia* (Clark et al. 2015). The basal corolla constriction may additionally function as an “anti-bee” mechanism, as seen in *Drymonia* (Clark et al. 2015) and *Penstemon* (Zung et al. 2015), though bee pollination has not been documented in the centropogonid clade.

Although color signals are important to attract visually oriented hummingbirds, morphology that facilitates effective sound reflectance is critical to echolocating bats (Helversen and Helversen 1999; Simon et al. 2011). Among centropogonids, this is accomplished, at least in part, via peduncle length: long

peduncles allow flowers to be held away from the body of the plant, greatly reducing background "clutter echoes" that reduce flower detectability (Muchhala and Serrano 2015; Schoner et al. 2016). Peduncle length was found to be best fit by an OU3 model, with bat flowers having much longer peduncles than either class of hummingbird flower (Tables 2 and S4). Peduncles of bat-pollinated flowers are likely also thicker; we did not measure this trait across species, but thicker peduncles may be important in reducing mechanical damage during bat visitation in *Burmeistera* (Muchhala 2006b).

We also find that bat-pollinated flowers produce larger anthers (and by extension, more pollen) than hummingbird-pollinated flowers (Tables 2 and S4). Anther area is best fit by an OU2 model (Table 5), suggesting that flowers adapted to straight-billed and sicklebill hummingbirds share an evolutionary optimum that differs from bat-pollinated flowers. This result is consistent with our knowledge of pollination in the centropogonid clade. It has been experimentally shown that bats can hold incrementally more pollen in their fur, thus linearly increasing male fitness of the plant with linear increases in pollen production. Hummingbird feathers, on the other hand, can only hold modest amounts of pollen and thus there is little increase in male fitness for the plant with increased pollen production (Muchhala and Thomson 2010). Anther size may be related to another trait that we find is best fit by an OU2 model: ovary size (Table 5). Hummingbird-pollinated plants tend to have significantly smaller ovaries (i.e., fruit precursors) than bat-pollinated plants (Tables 2 and S4). This is potentially explained by the smaller amount of pollen that is transported by hummingbirds, which selects for fewer seeds per fruit, ultimately resulting in smaller ovaries with fewer ovules.

Finally, we find that centropogonid species that are pollinated by hummingbirds tend to have longer corolla tubes than bat-pollinated species (Tables 2 and S4). This is likely a result of the long bills of many hummingbird species that visit centropogonids, though it is important to note that hummingbirds exhibit substantial variation in bill length (Temeles et al. 2002). The corolla length of a particular species, whether it is bat or bird pollinated, is likely a product of the local pollinator pool (Stiles 1981), which can be particularly complex in species-rich communities with a high degree of interspecies interaction, such as the Andean cloud forests where centropogonids thrive. Long corolla tubes are not an exclusive trait of hummingbird-pollinated flowers. In fact, some of the longest centropogonid flowers are bat-pollinated: *Centropogon nigricans*, whose 10-cm long flower is a result of an evolutionary arms race with the nectar bat *Anoura fistulata* (Fig. 1A; Muchhala 2006a; Muchhala and Thomson 2009). Similar cases of extreme specialization have been documented within hummingbird pollination (Abrahamczyk et al. 2014), and is apparent in the abruptly curved flowers adapted to sicklebill

pollination in the centropogonid clade. The two floral traits associated with this obligate relationship, a dilated corolla opening and extreme floral curvature (Table 3), are best fit by an OU3 model (Table 5) and facilitate an effective match between plant and pollinator.

We have documented that a few key traits underlie shifts between pollination syndromes, which have been frequent in the evolutionary history of the centropogonids. This combination makes the group ideal for future studies examining the genetic and genomic changes necessary for transitions between vertebrate pollination syndromes. Although many of the floral characters we identified likely have complex genetic underpinnings, there may be relatively few genomic regions that control morphological shifts from bat to bird pollination, as suggested in the Antillean Gesneriaceae (Alexandre et al. 2015). Future work will seek to determine if evolution of pollination syndrome follows predictable developmental and genomic changes.

THE EFFECT OF VERTEBRATE POLLINATION SYNDROMES ON ANDEAN DIVERSIFICATION

Despite the high frequency of shifts between bat and hummingbird pollination in the centropogonid clade, we find no significant difference in species diversification rate (i.e., neither speciation nor extinction rate). This is true even though there is strong evidence that vertebrate pollination, as a whole, is associated with significantly increased diversification rates in the Neotropical bellflowers (Lagomarsino et al., 2016). We hypothesize that frequent shifts between pollination syndromes within this actively diversifying clade, coupled with lability within a pollination syndrome (Fig. 2), may contribute substantially to its overall high rates of diversification (i.e., 1.83 events/Ma/lineage; Lagomarsino et al., 2016), as recently tested in Gesneriaceae (Serrano-Serrano et al. 2017). The overall high diversification rates accompanied by frequent pollinator shifts may partially be explained by speciation via floral isolation, in which prezygotic reproductive isolation is achieved via an interplay of floral morphology and pollinator behavior (Fulton and Hedges 1999; Muchhala 2003; Schiestl and Schlüter 2009). It is more likely, however, that reproductive isolation rarely evolves via *in situ* floral specialization, and instead is more frequently a by-product of allopatry (Armbruster and Muchhala 2009). This does not mean, however, that pollinator shifts are not important to the process of speciation or morphological diversification: many centropogonid species have sympatric distributions that appear to have been reinforced by either pollinator shifts (Muchhala 2006b) or character partitioning of floral traits within a pollination syndrome, allowing closely related species to share pollinators (Muchhala and Potts 2007). Further, it is clear that the niche of a lineage changes concomitant with a pollinator shift, permitting it to explore a new adaptive zone, which can lead to additional species diversification. This has happened

repeatedly within the centropogonids, and is associated with the origin of radiations within the larger clade that do not experience frequent pollinator shifts (e.g., *Burmeistera*; Uribe-Convers et al. 2017).

Our results further suggest that ecological specialization is not an evolutionary dead end, even when that specialization is obligate (i.e., sicklebill pollination). Instead, adaptation to local ecological contexts may drive the evolution of traits associated with different pollinators as the selective advantage of a particular ecological relationship changes across a landscape. The determinants of these selection gradients are likely often related to the abiotic environment (i.e., temperature, relief), supporting an interaction of multiple ecological and historical factors in driving speciation, as documented recently in the centropogonid clade (Lagomarsino et al. 2016).

Shifts between specialist pollination syndromes and relationships with vertebrate pollinators may partially explain the high species diversity of Andean cloud forests. Supporting this assertion, the evolutionary histories of many other species-rich Neotropical plant clades are characterized by shifts between pollinators including hummingbirds and bats, suggesting that the pattern we have identified here may apply more broadly. Such groups include Gesneriaceae (Sanmartín-Gajardo and Sazima 2005; Perret et al. 2007; Martén-Rodríguez et al. 2009; Serrano-Serrano et al. 2015), Acanthaceae (Tripp and Manos 2008), Solanaceae (Knapp 2010, but see Smith et al. 2008), Passifloraceae (Abrahamczyk et al. 2014), and Bromeliaceae (Sazima et al. 1999; Givnish et al. 2014). In the future, cross-clade studies can further elucidate the role that pollination syndromes have played in generating the extraordinary species richness of Andean cloud forests, as well their stunning morphological diversity. Although we emphasize the need for focused pollination fieldwork in the montane wet tropics, we also encourage a broad macroevolutionary perspective that integrates pollination syndrome as a reasonable first pass approximation for understanding plant–pollinator mutualisms.

AUTHOR CONTRIBUTIONS

LPL and CCD conceived of study. LPL collected data. LPL and EJF performed analyses. All authors participated in writing the manuscript and approved the final version.

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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. Phylogeny of the centropogonid clade.

Figure S2. Depiction of measurements of floral traits taken herbarium specimens.

Figure S3. Distribution of training set for predictive discriminant analysis of centropogonid species based on 11 floral traits.

Figure S4. Maximum-likelihood reconstruction of pollination syndromes in the centropogonid clade.

Table S1. Trait values of floral characters used in phylogenetic comparative methods for all species sampled.

Table S2. List of taxa and their known pollinators used as a training set in predictive linear discriminant analyses.

Table S3. Predictions of pollination syndrome from two linear discriminant analyses based on morphological measurements from species with known pollinators (see Table S2), using both raw morphological measurements and PC loadings from a phylogenetic PCA.

Table S4. Inferred mean number of transitions between pollination syndromes in the centropogonid.

Table S5. Estimates of functional trait optima (mean θ values) and their SEs for species with different pollination syndromes from OUwie analysis.