ORIGINAL ARTICLE

Complex interactions underlie the correlated evolution of floral traits and their association with pollinators in a clade with diverse pollination systems

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Natural selection by pollinators is an important factor in the morphological diversity and adaptive radiation of flowering plants. Selection by similar pollinators in unrelated plants leads to convergence in floral morphology, or "floral syndromes." Previous investigations into floral syndromes have mostly studied relatively small and/or simple systems, emphasizing vertebrate pollination. Despite the importance of multiple floral traits in plant-pollinator interactions, these studies have examined few quantitative traits, so their co-variation and phenotypic integration have been underexplored. To gain better insights into pollinator-trait dynamics, we investigate the model system of the phlox family (Polemoniaceae), a clade of ∼400 species pollinated by a diversity of vectors. Using a comprehensive phylogeny and large dataset of traits and observations of pollinators, we reconstruct ancestral pollination system, accounting for the temporal history of pollinators. We conduct phylogenetically controlled analyses of trait co-variation and association with pollinators, integrating many analyses over phylogenetic uncertainty. Pollinator shifts are more heterogeneous than previously hypothesized. The evolution of floral traits is partially constrained by phylogenetic history and trait co-variation, but traits are convergent and differences are associated with different pollinators. Trait shifts are usually gradual, rather than rapid, suggesting complex genetic and ecological interactions of flowers at macroevolutionary scales.

KEY WORDS: Adaptive radiation, floral syndromes, phenotypic integration, Polemoniaceae, pollinator shifts, trait correlation.

An example commonly used to illustrate the concept of modification by natural selection has been the "fit" of flowers to their pollinators, including morphological construction and sensory cues (Baker 1963; Grant and Grant 1965; Stebbins 1970; Lloyd and Barrett 1995; Schemske and Bradshaw 1999; Fenster et al. 2004). An extension of this observation is that selection by pollinators can result in reproductive isolation among populations, spurring adaptive radiation and floral diversification (Grant 1994; Lunau 2004; Harder and Johnson 2009; Givnish 2010; van der Niet and Johnson 2012; van der Niet et al. 2014). Reproductive isolation is enhanced by divergence in multiple floral traits, and pollinators rely on multiple cues when foraging (Raguso 2004; Kulahci et al. 2008; Leonard et al. 2011). Therefore, it is expected that

pollinators select for multiple floral traits, including those related to attraction and efficiency of pollen movement (Rosas-Guerrero et al. 2011; Caruso et al. 2019).

Correlated evolution of quantitative floral traits is well-documented (Galen 1989; Mitchell and Waser 1992; Campbell 1996; Campbell et al. 1996; Galen and Cuba 2001; Goodwillie et al. 2006) and is facilitated by polygenic and pleiotropic inheritance of traits (Conner 2002; Goodwillie and Ness 2005; Juenger et al. 2005; Goodwillie et al. 2006; Nakazato et al. 2013; Yuan et al. 2013; Smith 2016). Therefore, a mutation directly effecting one floral trait has the potential to indirectly effect multiple traits. Correlated variation among traits arising from their common genetic, developmental, and functional relationship is

known as phenotypic integration or modularity (Berg 1960; Armbruster et al. 1999, 2014; Ordano et al. 2008; Goswami et al. 2014; Klingenberg 2014). Floral integration is expected to be strong in species that have either highly specialized pollination systems or are predominate selfers. In specialized species, high integration maximizes the efficiency of pollen transfer (Rosas-Guerrero et al. 2011), whereas in selfers it is due to strong linkage disequilibrium (Pérez et al. 2007; Rosas-Guerrero et al. 2011; Fornoni et al. 2016).

Selection on floral traits by pollinators leading to specialized, distinct phenotypes characterized by suites of traits has been termed "floral syndromes" (Grant and Grant 1965; Faegri and van der Pijl 1979; Proctor et al. 1996; Fenster et al. 2004). Fenster et al. (2004, p. 376) explicitly define floral syndromes as "a suite of floral traits, including rewards, associated with the attraction and utilization of a specific group of animals as pollinators." Many studies explicitly testing for floral syndromes have found support for them (Hargreaves et al. 2004; Wilson et al. 2004; Muchhala 2006; Martén-Rodríguez et al. 2009; Johnson 2013; Rosas-Guerrero et al. 2014), whereas others have been skeptical, as species are often visited by many kinds of animals (Ollerton 1996; Waser et al. 1996; Johnson and Steiner 2000; Smith et al. 2008; Ollerton et al. 2009). Two arguments have been used to explain the apparent paradox of why morphological diversity exists if flowers are pollinated by a breadth of vectors. First, although multiple vectors interact with a flower, one of them is much more efficient at making contact with the stigma and anthers (Schemske and Horvitz 1984; Armbruster et al. 2000; Muchhala 2006). Second, selective pressure exists to maintain a somewhat "generalized" floral morphology as a failsafe under changing ecological conditions (Waser et al. 1996; Aigner 2001; Mayfield et al. 2001; Fumero-Cabán and Melendez-Ackerman 2007).

Fenster et al. (2004) highlight three important facets involved in pollinator-mediated natural selection on floral form which are rarely examined in concert: (1) which traits are involved in pollinator shifts, (2) if these traits are correlated or not (i.e., evolve independently), and (3) the role of phylogenetic history in the development of floral syndromes. The expansion of phylogenetic comparative methods has provided tools suitable for addressing these questions. Although previous studies using a phylogenetic context have provided important insights into floral evolution, most have been lacking in three important areas. (1) Most macroevolutionary studies have focused on clades at or below the level of genus (sometimes containing only tens of species), where pollinators categories are few (two to four; Wilson et al. 2004; Whittall and Hodges 2007; Martén-Rodríguez et al. 2010; Eaton et al. 2012; Lagomarsino et al. 2017; Joly et al. 2018; Kriebel et al. 2020; reviewed in Dellinger 2020). Consequently, transitions among pollinator types are also relatively few,

limiting the statistical power of the results. (2) The focus of most studies has been on one or more highly specialized pollinators (especially vertebrates), rather than including a greater number of generalist pollinators (Dellinger 2020; but see Smith et al. 2008 and Gómez et al. 2014). (3) Many studies have focused on few floral traits, often from one whorl, ignoring the importance of multivariate traits in pollinator-mediated selection, the effect of trait correlation, and the impact of traits involved in pollinator attraction versus efficiency in floral evolution (Dellinger 2020).

Here, we seek to fill these gaps in knowledge using the phlox family (Polemoniaceae), a family integral in developing the floral syndrome hypothesis (Grant and Grant 1965; Stebbins 1970, 1971). Polemoniaceae is a family of ∼400 species almost exclusively found in the Americas (Fig. 1; Grant 1959; Porter and Johnson 2000). In their seminal study, Grant and Grant (1965) described the pollination biology of Polemoniaceae based on years of study. They concluded that Polemoniaceae show adaptations to pollinators in a "lock-and-key" relationship by which floral phenotypes exclude many types of visitors. Their hypothesis was based on the close association of morphologically diverse flowers with an array of pollinators (bees, beetles, lepidopterans, hummingbirds, flies, and selfing/autogamy). They hypothesized that temperate Polemoniaceae arose from a bee-pollinated ancestor but did not address the ancestral pollination mode of Polemoniaceae due to limited knowledge of its tropical members. They argued that the occurrence of similar pollinators across many relatively distantly related genera indicated widespread convergence of floral form. Furthermore, based on their understanding of phylogenetic affinities, they diagrammed a hypothesis of pollinator transitions within Polemoniaceae, illustrating how these transitions came about from a bee-pollinated common ancestor (Grant and Grant 1965, fig. 46). This now-classic hypothesis of pollinator shifts in Polemoniaceae has been widely cited and illustrated (e.g., Stebbins 1974), but has not yet been fully tested using a modern understanding of the phylogeny of the family. Landis et al. (2018) studied floral evolution in Polemoniaceae, with a focus on the effect of floral color and breeding system on diversification rates. They included only two quantitative traits (corolla length and width at opening) and reconstructed ancestral breeding system (treated as selfing or outcrossing), rather than ancestral pollinator. Although not the main focus of their study, they examined the correlation of their two quantitative traits as well as their association with pollinators, finding both trait correlation and association. However, a greater number of traits are needed to more robustly tease apart associations of suites of traits with pollinators, examine correlated evolution among floral whorls, and link shifts in traits to shifts in pollinators.

Given that much of the knowledge about plant-pollinator interactions at macroevolutionary scales is vertebrate focused, concerns (relatively) small clades, and examines few traits, we use
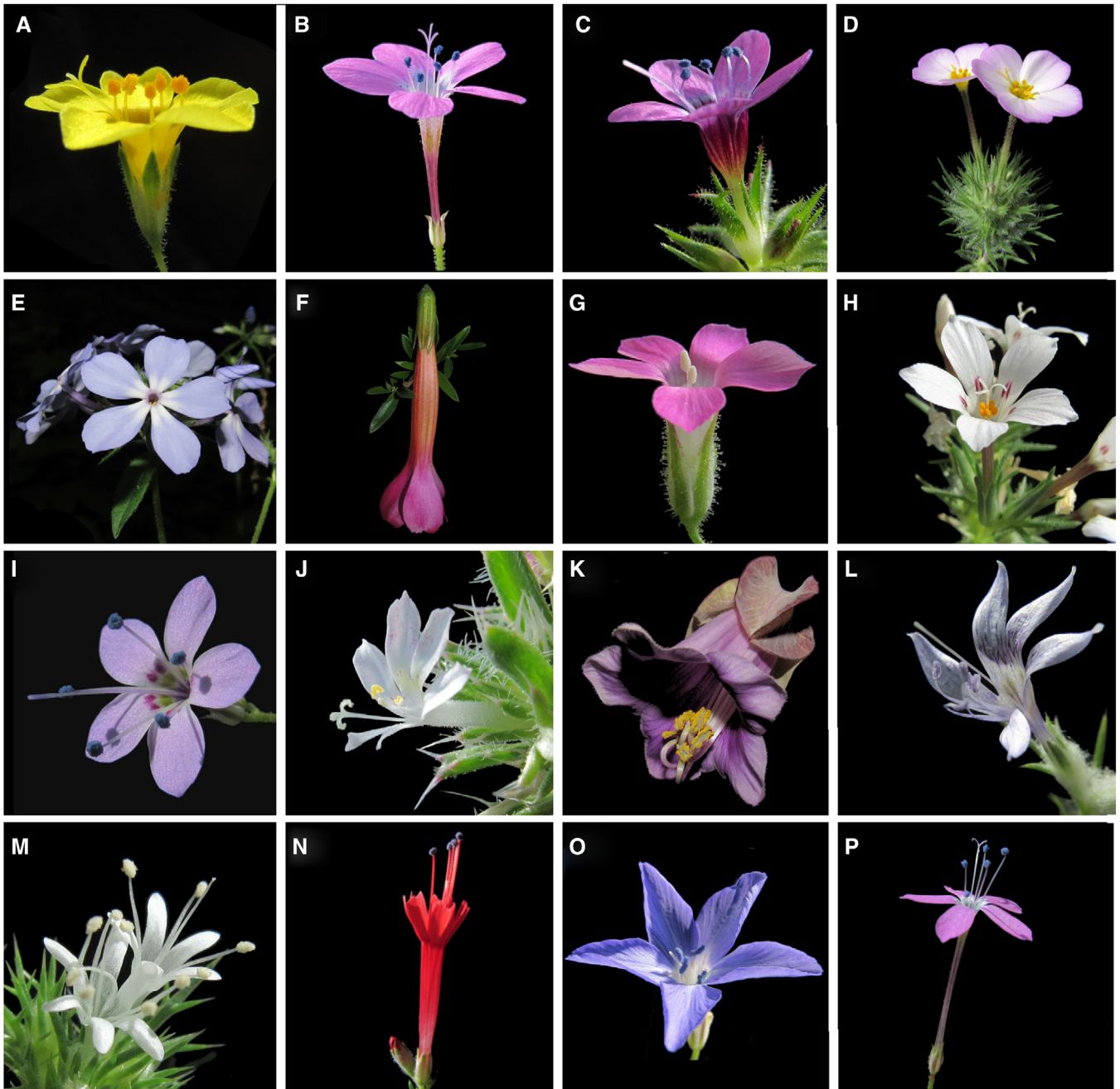
Figure 1. Floral diversity in Polemoniaceae illustrating different pollination systems. (A) *Leptosiphon aureus* subsp. *aureus* (bee); (B) *Saltugilia splendens* subsp. *grantii* (hummingbird and long-tongued fly); (C) *Navarretia hamata* subsp. *hamata* (long-tongued fly); (D) *Leptosiphon montanus* (long-tongued fly); (E) *Phlox divaricata* subsp. *laphamii* (lepidopteran); (F) *Cantua buxifolia* (hummingbird); (G) *Aliciella latifolia* subsp. *latifolia* (selfing); (H) *Linanthus orcuttii* (unknown); (I) *Saltugilia caruifolia* (bee); (J) *Loeseliastrum schottii* (selfing and/or long-tongued fly); (K) *Cobaea scandens* (bat); (L) *Eriastrum eremicum* subsp. *eremicum* (long-tongued fly); (M) *Navarretia leucocephala* subsp. *leucocephala* (bee); (N) *Ipomopsis tenuifolia* (hummingbird); (O) *Dayia scabra* (unknown, probably bee); (P) *Gilia leptantha* subsp. *leptantha* (long-tongued fly). All photos by JPR except (F) by Ricardo Kriebel.

the model system of Polemoniaceae to better understand plant-pollinator interactions. We integrate a comprehensive phylogeny with a large matrix of pollinator observations and morphological traits to test four hypotheses. (1) Consistent with the hypothesis of Grant and Grant (1965), pollinator transitions are predictable and arise through a limited number of pathways. (2) Consistent with the idea of phenotypic integration, floral traits are correlated. (3) Consistent with the floral syndrome hypothesis, differences in suites of floral traits are associated with different pollinator types within and across floral whorls. (4) If floral traits are shaped by

selection from pollinators, shifts in floral traits should occur concomitantly with shifts in pollinators. If convergent evolution is operative, the trait(s) that shift and their "optimal" value(s) should be consistent across independent shifts to the same pollinator.

Materials and Methods

PHYLOGENETIC CONTEXT

We generated a supermatrix phylogeny of Polemoniaceae and its sister family Fouquieriaceae (polemonioids). Sequence data were obtained from GenBank for 14 loci spanning 12 plastid markers, one nrDNA marker, and one low-copy nuclear marker (Data S1). GenBank data were supplemented by newly generated sequences and unpublished sequences from J. Mark Porter (Rancho Santa Ana Botanic Garden) for a total of 490 terminals. Landis et al. (2018) presented a phylogeny of Polemoniaceae based on seven plastid loci and nrDNA for 427 terminals. However, this phylogeny did not include some species for which pollinator data exist, excluded some loci that may help resolve infrageneric relationships, and estimated a posterior distribution of chronograms using a fixed topology.

We included subspecies (usually treated as "races" in Grant and Grant [1965]), because they frequently have different pollinators. If a species contains multiple subspecies but none were identified on the sequence, the sequence was used as a placeholder for the nominal subspecies. Sequences for each locus were aligned using MAFFT (Katoh and Standley 2013), with sequence direction adjusted to account for potentially reversed sequences in GenBank. To check for misidentifications, gene trees for all orthologous sequences were generated using RAxML (Stamatakis 2014) under the GTRGAMMA model. We used the longest correctly identified orthologous sequence from the most common ortholog cluster for each taxon. We obtained sequences for 477 Polemoniaceae and 11 Fouquieriaceae, with two Actinidiaceae as the outgroup (72.2% missing sequences). All terminals had at least ITS represented.

Phylogenetic analysis and divergence time estimation were conducted simultaneously in BEAST version 2.4.8 (Drummond et al. 2012; Bouckaert et al. 2014) on the concatenated, unpartitioned supermatrix of 490 tips and 18,271 aligned nucleotides, as runs from a partitioned matrix would not converge. We used an uncorrelated relaxed lognormal clock and a Yule branching process under the GTR + I + G model. One secondary date and two fossil priors were used as calibration points. First, a normally distributed prior (mean = 98.41 My, sigma = 2.3) was set on the root, a distribution that encompasses the mean and 95% highest posterior density for this node from Magallón et al. (2015). Second, we placed a lognormal prior on stem Actinidiaceae (mean = 1.5, SD = 1.0, offset = 83.6 My) based on *Parasaurauia allonen-*

sis, from the Upper Santonian, broadly assigned to Actinidiaceae (Keller et al. 1996; Sims et al. 1999; Löfstrand and Schönenberger 2015). Third, we placed a prior based on the only known macrofossil of Polemoniaceae, *Gilisenium huberi* from the middle Eocene of Utah (Lott et al. 1998), assigned nearest to extant *Gilia* and previously used to constrain crown tribe Gilieae (Porter et al. 2010; Landis et al. 2018). However, *Gilisenium* is similar to several genera, making its exact placement uncertain (Lott et al. 1998; Grant 2001). Therefore, we conservatively used *Gilisenium* to constrain the crown of subf. Polemonioideae with a lognormal distribution (mean = 1.5, SD = 1.0, offset = 48.8 My) corresponding to a minimum age of the Green River Formation (Machlus et al. 2004). Four separate MCMC runs were conducted each for 2×10^8 generations with sampling every 1000 generations. Convergence was assessed using TRACER version 1.7.1 (Rambaut et al. 2014) after excluding 30% of generations as a burn-in. The posterior distribution of trees was summarized using the maximum sum of clade credibilities (MCC) tree using median heights. To integrate our analyses over phylogenetic uncertainty, we extracted a random sample of 100 trees from the posterior distribution.

FLORAL TRAIT DATA

Data for 28 quantitative and qualitative floral and reproductive traits were gathered from the literature, primarily from monographic and floristic treatments, at least partially complete for 610 taxa (Data S2). To maximize the number of terminals with complete phylogenetic and morphological data, we reduced our dataset to 10 quantitative and two qualitative traits (Table 1). The phrase "corolla tube" has been ambiguously applied, depending on if the fused corolla has the same width throughout or if it flares broadly in the distal portion to form a "throat." Here, corolla tube refers to the entire fused corolla. When possible, mean values were recorded but ranges are more often reported, so we used the midpoint of the range of values given (excluding any outlying values noted in the literature) as a proxy for the mean. Incomplete data from the literature were supplemented with measurements from cultivated plants or herbarium specimens. Measurements were derived from flowers photographed when fresh, preserved in 70% ethanol, or from specimens rehydrated by boiling in water for 20 s followed by fixing and clearing in 70% ethanol. We only used specimens whose identity was either verified by the first author or another Polemoniaceae taxonomist.

Corolla tube shape was quantified using elliptical Fourier analysis (EFA) in Momocs version 1.2.9 (Bonhomme et al. 2014). A total of 772 scanned images of corolla tubes from the literature or photographed flowers were imported into GIMP version 2.8 (http://www.gimp.org) and converted to black and white outlines, with the base of the corolla at the point of pedicel attachment to the left of the image. Images were converted into 8-bit

Table 1. The 12 morphological traits examined in this study including a description of what was measured for reproducibility. Daggers (†) indicate qualitative traits.

Trait	Description
Calyx length	Total length (mm)
Calyx tube length	Total length of fused portion (mm)
Corolla shape	First principal component score from elliptical Fourier analysis
Corolla length	Total length (mm)
Corolla width	Width of corolla limb (mm)
Corolla tube length	Length of fused portion (mm)
Corolla tube width	Width halfway along fused portion (mm)
Corolla lobe length	Total length (mm)
Corolla lobe width	Maximum width (mm)
Corolla lobe color[†]	Blue/pink, red, white, or yellow
Mean androecium length	Total length of vascular trace (mm), (averaged for heterantherous taxa)
Anther length	Total length (mm)
Pollen color[†]	Blue, white, or yellow/orange
Style length	Total length (mm)

JPG images using Fiji (Schindelin et al. 2012) and imported into R. Three landmarks were placed on the corolla to aid in alignment of the shapes: one at the point of pedicel attachment and one each on either side of the distal end of the corolla tube. Harmonic amplitudes were calculated for the outlines using EFA. EFA attempts to fit trigonometric functions (harmonics) to the outlines, each composed of four coefficients (amplitudes). The function calibrate_harmonicpower identified nine harmonics as the smallest number that accurately describes the shape. Mean shapes for each species were calculated using the MSHAPES function. Principal components analysis (PCA) of the 36 amplitudes (4 coefficients × 9 harmonics) was performed to determine which harmonics were most important in describing the variation in corolla tube shape. The first principal component (PC) explained 75.7% of the variation in corolla shape and PC2 explained 10.5% (Fig. S1). PC1 mostly explained corolla tubularity from tubular (low scores) to bell-shaped (high scores) corollas, whereas PC2 explained corolla throat flare from gradual (low scores) to abrupt (high scores). Because the vast majority of variation in corolla shape was described in PC1, we used this axis as a quantitative variable to describe shape.

POLLINATOR DATA

Pollinator data were gathered from the literature, or in rare cases our own field observations (Data S3). Gathering pollinators from the literature is difficult given issues inherit in the heterogenous or incomplete nature of the data. In addition, it is difficult to quantify efficiency of visitors or fully observe all ecological interactions that a flower has (Mayfield et al. 2001; Ollerton et al. 2015; van der Niet 2020). We incorporated both knowledge and uncertainty about pollinators in our reconstruction of ancestral pollination type by including hypothesized primary and secondary pollinators. We scored primary pollinators based on noted frequency of visitation and, when possible, efficiency as indicated by any pollen removal and/or stigmatic contact. Major secondary pollinator was scored based on frequency of visitation. Cases in which pollinators were anecdotally mentioned were not recorded. Pollinators were assigned to one of eight groups based on Fenster et al. (2004), except short- and long-tongued bees were lumped together to conform better to the hypothesis of Grant and Grant (1965). An additional group was made for self-pollination. As it is difficult to distinguish facultative from obligate self-pollination, pollen-ovule ratios (P:O) < 100 were mostly used as a second line of evidence to justify self-pollination (Cruden 1977; Plitmann and Levin 1990) but served as the only line of evidence to score self-pollination in two species.

Most methods of ancestral state reconstruction (ASR) are limited in that ancestors can only occupy a single state, tips can only occupy a single state or be treated as missing data, and any state can be reconstructed at each node (Beaulieu et al. 2013). Because pollination systems are often more complex, we evaluated ancestral pollinators across the MCC tree using Bio-GeoBEARS version 1.1.2 (Matzke 2012, 2013) to implement a Markov-k (Mk) model applicable for a standard unordered character model with equal rates character evolution (Kriebel et al. 2019). To infer ancestral pollinators, we modified the BAYAREA-ALIKE model by setting anagenetic range switching (a) to be a free parameter. We assessed a model in which only primary pollinators were allowed and one including secondary pollinators. We allowed one (primary pollinators only) or two (with secondary pollinators) states at internal nodes, made no a priori assumptions about frequency of transitions among pollinators, and conducted a time-stratified analysis to penalize against pollinators at certain times. First, we did not allow bird-pollination to be inferred earlier than 47.4 My, which is the lower bound of the error bar for the divergence of hummingbirds and swifts (McGuire et al. 2014). Second, Neotropical nectivorous bats are restricted to family Phyllostomidae (Fleming et al. 2009), so we did not allow bat-pollination to be inferred earlier than 32.0 My, which is the lower bound of the error bar for the divergence of nectivorous Phyllostomidae from subf. Desmodontinae (Datzmann et al. 2010). Transitions among pollinators types were counted with 500 stochastic maps. Ancestral P:O was inferred using the function phylopars in Rphylopars version 0.2.12 (Goolsby et al. 2017) under an Ornstein-Uhlenbeck (OU) model of trait evolution based on the result of a likelihood ratio test against a Brownian motion (BM) model (Table S1).

FLORAL TRAIT ANALYSIS

Variation and association with pollinators

To visualize the distribution of floral traits in multivariate space, we conducted PCA on all 12 traits using the package PCAmix-data version 3.1, which allows for analysis of mixed variable types (Chavent et al. 2014). A phylogenetic PCA has been proposed, but relies on a BM model (Revell 2009) and may negatively impact analyses if the underlying model of trait evolution is more complex (Uyeda et al. 2015; Bastide et al. 2018). To visualize the location of the ancestral floral morphology in morphological space, we overlaid our MCC tree on a scatterplot of the first two PCs using the phylomorphospace function in phytools version 0.6.72 (Revell 2012). The amount of morphological space occupied by each pollination system was calculated as the area of a convex hull polygon.

To test for differences among pollination systems for all quantitative traits, we conducted a phylogenetic ANOVA (Garland et al. 1993) on PC1 and each natural log-transformed linear trait using the phylANOVA function in phytools that we modified to accept a broader range of models of trait evolution than BM ("phylANOVA2," available in the Supporting Information). We first tested to see if a BM or one-optimum OU model was a better fit for each quantitative trait, which allows us to discriminate between models where trait change is due to gradual evolution (BM) versus a model in which there is selection on a trait toward and "optimum" value (OU). The fit of each model was calculated using fitContinuous in geiger version 2.0.6.1 (Harmon et al. 2008) and the best model was selected using a likelihood ratio test. An OU model of trait evolution was favored for all traits (Table S1). For each tree, the optimal values of the OU parameters alpha (α), sigma (σ^2), and theta (θ) estimated by fitContinuous were used in phylANOVA2 with 10,000 simulations for the MCC tree and 1000 simulations for each posterior tree. Post hoc testing employed the Benjamini and Hochberg (1995) correction for multiple comparisons.

Correlation and temporal evolution

ASR of corolla lobe color was conducted on the MCC tree using the rayDISC function in corHMM version 1.22 (Beaulieu et al. 2013) with the re-rooting method of Yang (2006). We tested equal rates, symmetric rates, and all rates different models of trait evolution using the Bayesian information criterion (BIC) to select among models.

To examine if floral traits are evolving independently of the phylogeny (i.e., wholly driven by factors other than relatedness), phylogenetic signal in natural log-transformed quantitative traits values was assessed using Blomberg's *K* (Blomberg et al. 2003) and Pagel's Lambda (Pagel 1999) with the phylosig function in phytools. Both statistics compare the observed signal in a trait on a phylogeny to the signal under a BM model of trait evolution. A

value of 1 indicates that the trait values of the tips are correlated as expected under BM, whereas 0 indicates no correlation. The statistical significance of K or λ was evaluated using a null model ($K/\lambda = 0$) of shuffling taxa across the tips of the phylogeny 1000 times. We performed tests for phylogenetic signal on the MCC and posterior trees. For floral color, we scored phylogenetic signal using the inferred value of λ from fitDiscrete in geiger under the best fitting model inferred from the ASR of color.

We tested for correlated evolution of all quantitative traits using phylogenetic generalized linear regression (PGLS) on natural log-transformed values using the phylolm function in phylolm version 2.6 (Ho and Ané 2014a). We conducted this analysis on the MCC and posterior trees assuming an OU model of evolution with a random root.

Finally, we examined the location and timing of trait shifts in the history of polemonioids to gain insight on the evolutionary history of floral traits, as well as evidence for the association of particular traits or combinations of traits with shifts in pollinators. A variety of methods exist for inferring an OU model with one or multiple trait optima (Butler and King 2004; Beaulieu et al. 2012), but many of these methods do not specifically indicate where along the phylogeny such shifts occur. Additionally, model selection usually relies on AICc, which may overestimate the number of shifts when their positions are unknown (Ho and Ané 2014b). To specifically test where selective optima of traits have changed across the phylogeny, we used l1ou version 1.41 (Khabbazian et al. 2016) that estimates the location and number of shifts. l1ou can fit models for both univariate and multivariate cases, with the multivariate case assuming that all traits are independent. Bastide et al. (2018) proposed the scalar OU model to deal with correlated traits. However, the scalar OU model assumes that all traits reach their optimum value at the same rate, which may not be biologically appropriate. We therefore tested each of nine log-transformed quantitative traits separately (dropping total corolla length, which is decomposed into tube and lobe) on the MCC tree with a random root OU model, detection of up to 50 shifts in the model, and model selection (best number of shifts) using pBICess (Ané 2008; Khabbazian et al. 2016). We also tested for shifts to similar trait optima (regimes), a process that models convergent evolution. This step conducts both forward and backward searches and collapses similar OU regimes. We used the backward search modified from SURFACE (Ingram and Mahler 2013) in l1ou using BIC to penalize against collapsing too many shifts into the same regime.

Results

PHYLOGENY

The BEAST analysis recovered a MCC tree similar in topology to previous studies but with higher support values for
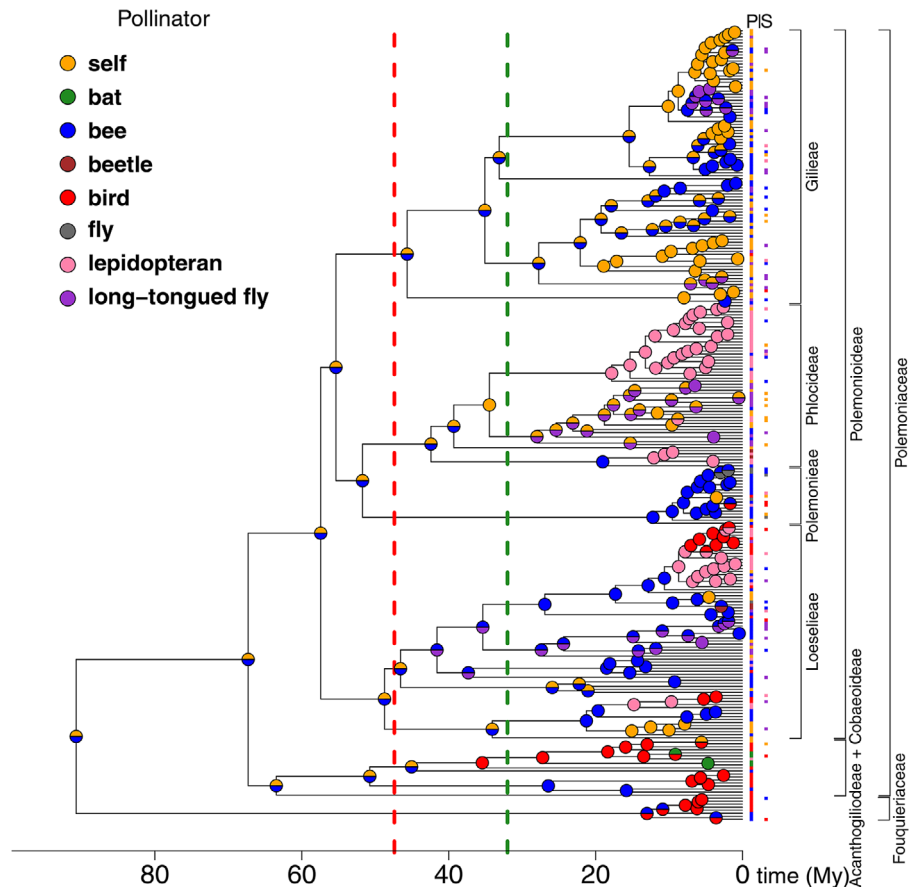
Figure 2. Ancestral state reconstruction of pollination system in Fouquieriaceae and Polemoniaceae. Node pies represent the most probable state. Tip states represents primary pollinator (P) and secondary pollinator (S), if present. Clade labels reflect familial, subfamilial, and tribal classifications. The red vertical line is the stem age of hummingbirds from McGuire et al. (2014) and the green vertical line is the stem age of Neotropical nectivorous bats from Datzmann et al. (2010). See Figure S3 for the full ancestral sate reconstruction of pollination system.

relationships within genera and including 50 more Polemoniaceae than previous studies (Fig. S2). The basal split within Polemoniaceae was between subf. Acanthogilioideae + Cobaeoideae and subf. Polemonioideae ($PP = 0.54$). Tribe Loeselieae was sister to the remainder of Polemonioideae ($PP > 0.99$). *Aliciella* was sister to the remainder of Loeselieae ($PP = 0.94$). Despite increased support for infrageneric relationships, uncertainty remained within *Gilia*, *Phlox*, and *Polemonium*. Polemoniaceae diverged from Fouquieriaceae 90.7 My (79.0–101.0 My, 95% highest posterior density), with crown Polemoniaceae originating 67.3 My (56.6–77.8 My, 95% highest posterior density).

POLLINATOR SHIFTS

We obtained pollinator data for 278 polemonioids. Of these species, 262 matched the phylogeny. Reconstruction of pollination system in polemonioids with primary and secondary pollinators showed multiple transitions among pollination systems (Figs. 2, 3, and S3) with a mean of 152.2 transitions inferred

across all stochastic maps (Fig. 3). The ancestral state of Polemoniaceae was reconstructed as either self + bee-pollinated ($P = 0.72$) or bee + long-tongued fly-pollinated ($P = 0.08$). Self + bee-pollination was reconstructed along most internal nodes of Polemoniaceae until ~40 My, although bee + long-tongued fly-pollination was also reconstructed at lower probabilities. Loeselieae excluding *Aliciella* and *Giliastrum* switched from self + bee-pollination to bee + long-tonged fly-pollination ($P = 0.45$). Ancestral pollination system for the common ancestors of *Cantua* and *Cobaea* was ambiguous, although bird and/or bee-pollination had the highest probability at each crown. The crowns of the *Ipomopsis aggregata* clade and *Phlox* were reconstructed as lepidopteran pollinated ($P = 0.51$ and 0.86, respectively). Several deep transitions to self-pollination were reconstructed in *Collomia* and *Gilia* ($P = 0.72$ and 0.89, respectively) with transitions back to outcrossing.

Using only primary pollinator had an effect on the inferred reconstruction (Figs. S4 and S5). Although bee-pollination was inferred for the deepest nodes, reconstructions toward the tips
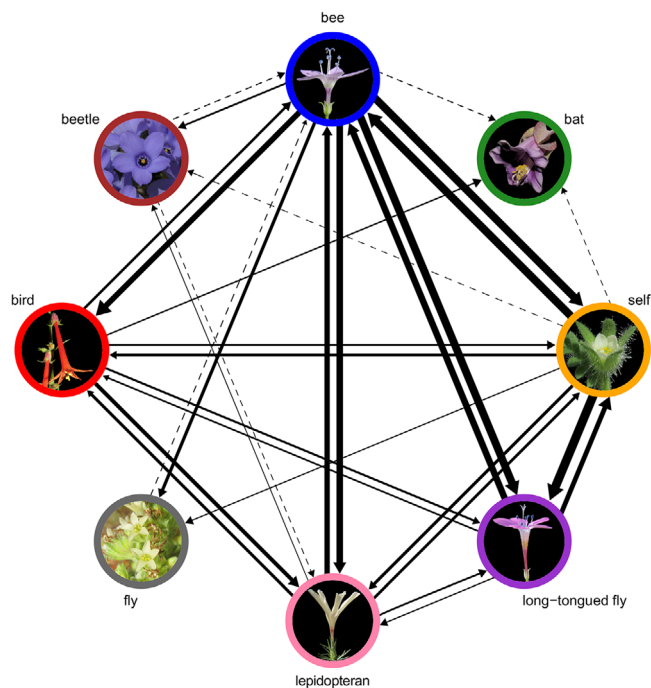
Figure 3. Transitions among pollination systems in Fouquieri-
aceae and Polemoniaceae inferred from 500 stochastic maps in
BioGeoBEARS. Lines connecting pollination systems show the di-
rectionality of transitions and line thickness is proportional to the
mean number of transitions across all stochastic maps. Only tran-
sitions with a mean value of ≥ 1 across all stochastic maps on the
maximum clade credibility tree are shown. Solid lines show transi-
tions that are recovered assuming the most probable state at each
node is the "true" reconstruction, whereas dashed lines show less
probable transitions. Flowers within circles are exemplars for each
pollination system.

differed markedly, including strong signal for self-pollination at
most internal nodes of Gilieae and deeper nodes in *Ipomopsis*. In
addition, long-tongued fly-pollination was inferred at the crown
of *Leptosiphon + Phlox*.

Stochastic mapping on the reconstruction with secondary
pollinators indicated that the most frequent transitions were from
self, bee, lepidopteran, and long-tongued fly-pollination (Fig. 3).
Although transitions among pollination systems were labile,
some transitions were more frequent than others (e.g., switches
among self-pollination, bee-pollination, and long-tongued fly-
pollination), whereas other transitions did not occur in our dataset
(e.g., switches from bat-pollination, beetle to bird-pollination),
and there was directionality within transitions that did occur (e.g.,
bee to bird-pollination occurred twice as many times as the op-
posite) (Fig. 3).

Ancestral P:O (Fig. S6) suggested a facultatively selfing
ancestor of Polemoniaceae (P:O = 354), and ancestral P:O re-
mained constant along the backbone of Polemonioideae. Transi-
tions to low P:O occurred throughout Polemoniaceae, especially

in *Aliciella*, but the deeper nodes reconstructed as obligately self-
ing in the reconstruction of pollination system all had P:O > 180.
Obligate xenogamy evolved few times, most notably in *Phlox*
(P:O = 1845).

TRAIT VARIATION AND CORRELATION
After matching the dataset of 12 traits to the phylogeny, we ob-
tained complete data for 380 terminals, including 239 with pol-
linators (Data S4). Test for phylogenetic signal yielded values of
K and $\lambda < 1$ for all floral traits, which differed significantly from
0, indicative of phylogenetic signal in the data. This result was
robust across the posterior sample of trees (Table 2).

PGLS found that linear traits were significantly correlated
but shape were not significantly and/or strongly correlated to lin-
ear measurements (Figs. S7 and S8). Overall, there was signifi-
cantly positive allometry (but not isometry) among linear mea-
surements, and this was robust across the posterior sample of
trees (Table S2).

PCA explained a total of 57.18% of the variation in the
dataset within the first two PCs: 44.41% by PC1 and 12.77% by
PC2 (Fig. 4). PC1 was explained mostly by trait size (especially
corolla and androecium lengths) and PC2 by corolla shape and
trait colors. The phylomorphospace based on the first two PCs
suggested that the common ancestor of Polemoniaceae had a flo-
ral morphology intermediate between present day bird, bee, and
lepidopteran-pollinated species (Fig. 4).

TRAIT ASSOCIATION WITH POLLINATORS
Color-coding taxa in the PCA by primary pollinator suggested
differentiation and convergence in floral traits by pollinator type,
as evidenced by the overlapping branches of the MCC tree where
unrelated species with identical pollination systems occupied
similar areas of morphospace (Fig. 4). Each pollinator group oc-
cupied a distinct area of morphospace, but this area may be par-
tially overlapping or wholly included in other groups (Fig. 4;
Table S3). Bat-pollinated species (*Cobaea*) occupied the smallest
(0.10 PC units^2) and most distinct part of morphospace, only par-
tially overlapping bird-pollinated species. Self-pollinated species
occupied the largest area of morphospace (44.08 PC units^2) and
overlapped all other groups with the exception of bat-pollinated
species. Bird, bee, and lepidopteran-pollinated species also oc-
cupied large areas of morphospace. Bee-pollinated species over-
lapped most groups. Bird and lepidopteran-pollinated species
shared a large amount of morphospace, with 75% of the area
of lepidopteran-pollinated species nested within bird-pollinated
species (Table S3).

Phylogenetic one-way ANOVAs on each trait showed sig-
nificant differences in trait means among many pollinator groups
(Figs. 5 and S9; Table S4). When considering all ANOVAS,
groups were often differentiated based on multiple traits, with

Table 2. Phylogenetic signal using Blomberg's *K* and Pagel's Lambda (λ) for quantitative traits and corolla lobe color in Fouquieriaceae and Polemoniaceae.

Trait	*K*	λ
Tube PC1	0.30 (0.23 ± 0.051)	0.90 (0.90 ± 0.014)
Calyx length (mm)	0.31 (0.20 ± 0.062)	0.93 (0.92 ± 9.3 × 10^{-3})
Corolla length (mm)	0.19 (0.13 ± 0.038)	0.90 (0.90 ± 0.011)
Corolla tube length (mm)	0.16 (0.11 ± 0.031)	0.89 (0.80 ± 0.014)
Corolla tube width (mm)	0.66 (0.43 ± 0.14)	0.92 (0.91 ± 9.0 × 10^{-3})
Corolla lobe length (mm)	0.26 (0.19 ± 0.044)	0.93 (0.93 ± 9.8 × 10^{-3})
Corolla lobe width (mm)	0.27 (0.18 ± 0.054)	0.90 (0.89 ± 0.014)
Anther length (mm)	0.43 (0.30 ± 0.087)	0.91 (0.90 ± 0.013)
Androecium length (mm)	0.22 (0.16 ± 0.041)	0.89 (0.89 ± 0.013)
Style length (mm)	0.19 (0.15 ± 0.032)	0.88 (0.89 ± 0.018)
Corolla lobe color	N/A	0.83 (0.82 ± 0.070)

Values outside of the parentheses are based on the maximum clade credibility tree. Numbers in parentheses represent mean values and standard deviation based on the sample of 100 trees from the posterior distribution. For both statistics, values close to 1 indicate a distribution of traits as expected under Brownian motion. For all quantitative traits, the hypothesis that the statistic is equal to 0 (random distribution of tip states) is rejected at $\alpha = 0.05$.
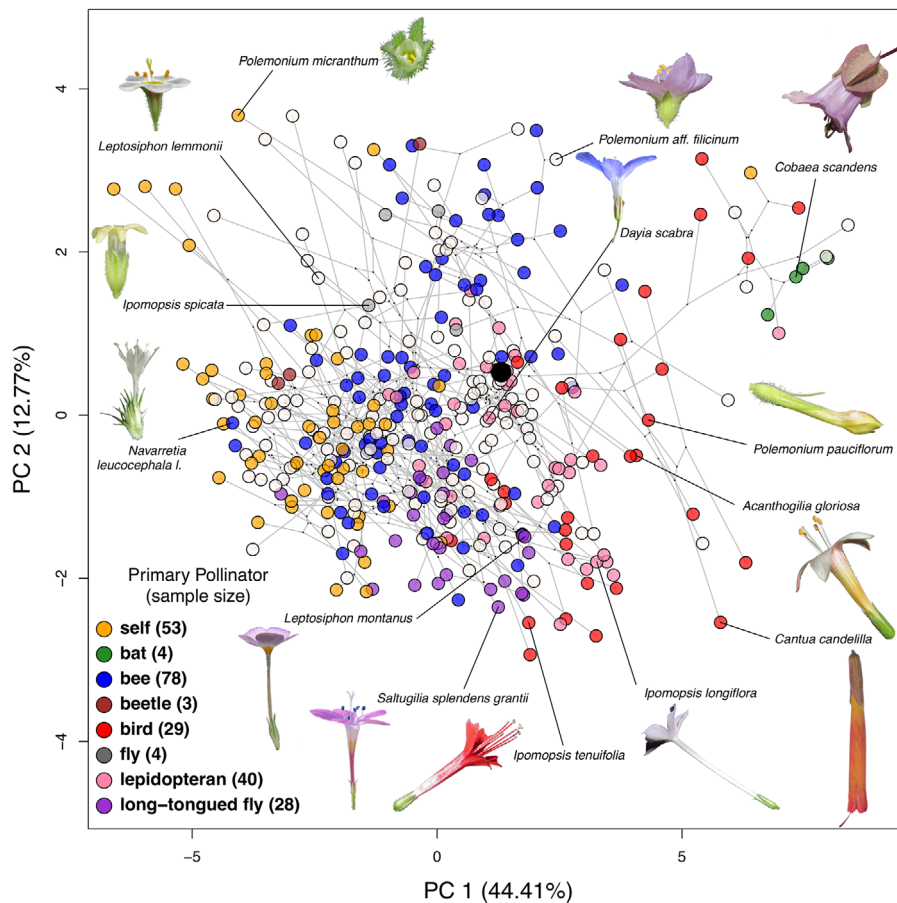

Figure 4. Phylomorphospace of Fouquieriaceae and Polemoniaceae based on scores along the first two principal components from a mixed principal components analysis of 12 floral traits. Points represent terminals color coded by primary pollinator. White circles are terminals with unknown pollination biology. Gray lines are branches of the maximum clade credibility tree overlain on the scatter, with small black circles depicting internal nodes. The most recent common ancestor of Polemoniaceae alone is denoted with a large black circle and is inferred to have a morphology similar to present day bee, bird, and lepidopteran-pollinated species.
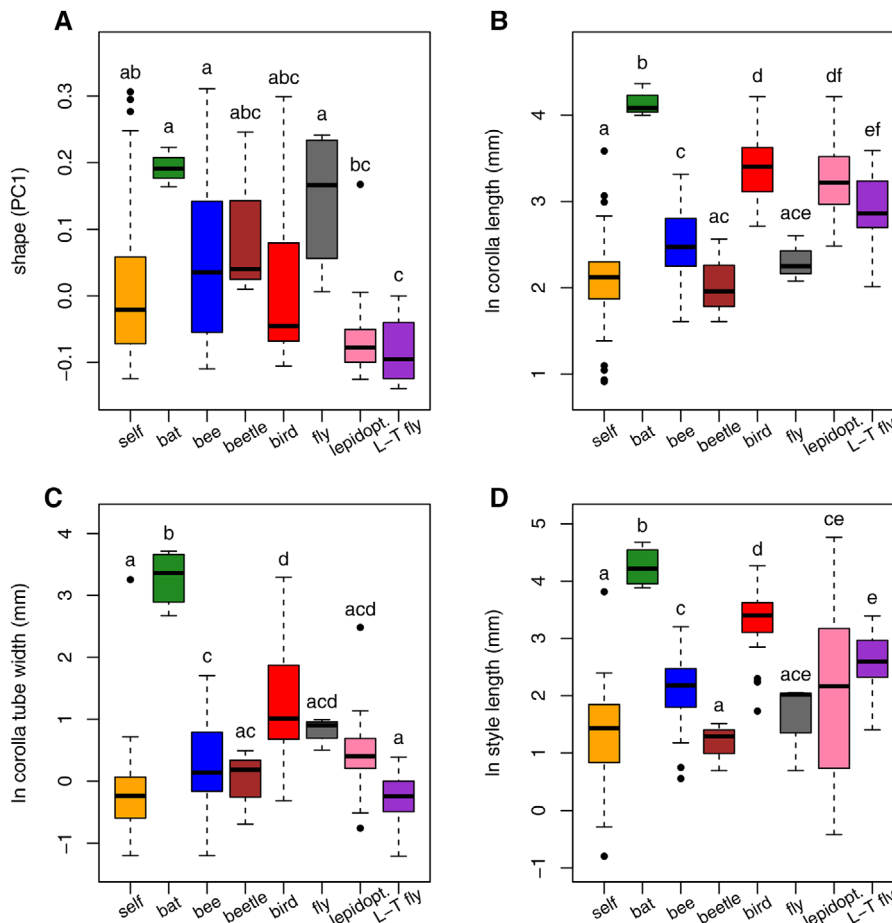
Figure 5. Boxplots showing the distribution of select morphological traits across pollination systems in Fouquieriaceae and Polemoniaceae. (A) Corolla shape. (B) Total corolla length. (C) Corolla tube width. (D) Style length. Black points represent outliers. Letters above boxes represent statistically significant groups based on post hoc testing following a phylogenetic ANOVA under an Ornstein-Uhlenbeck model of trait evolution and using the maximum clade credibility tree.

a different mean value of at least one trait in 25 of 28 (89%) of all possible pairwise comparisons of pollination system (Fig. 5; Table S5). The most consistent significant differences among pollinator groups were in total corolla length, corolla tube length, corolla lobe length, and androecium length (Table S5). Trait differentiation was robust across trees, with significant differences found using the MCC tree also found in all or the vast majority of posterior trees. Some trait comparisons not recovered as significantly different in the MCC tree were significantly different in a majority of posterior trees (Table S4).

Bird, lepidopteran, and long-tongued fly-pollinated flowers were tubular and bat and bee-pollinated flowers were bell-shaped (Fig. 5A). Corolla tube width was narrow in lepidopteran and long-tongued fly-pollinated flowers (Fig. 5C). Bat and bird-pollinated flowers were large and with long styles (Fig. 5D), whereas self and bee-pollinated flowers were small (Fig. 5B).

TRAIT SHIFTS

Ancestral corolla lobe color was reconstructed as blue/pink with multiple shifts to red, white, and yellow (Fig. S10). Modeling quantitative trait shifts showed one to 21 shifts in optimum value per trait for a total of 42 shifts distributed across 32 unique branches of the MCC tree, with 21 shifts in floral shape (Figs. 6 and S11; Table S6). Assuming the most probable ancestral state of pollinators at each node is the "true" reconstruction, 145 shifts in pollinator occurred in the morphology tree (Fig. 6). Of the branches in the morphology tree for which there is at least one trait shift, a total of nine out of 32 (28%) also showed a shift in pollinator, whereas 94% of shifts in pollinator occurred without a trait shift. Tests for trait convergence showed that shifts represented one to eight distinct OU regimes depending on the trait (Fig. S11). Although many clades were convergent for individual traits, none showed a similar convergence pattern across multiple traits.
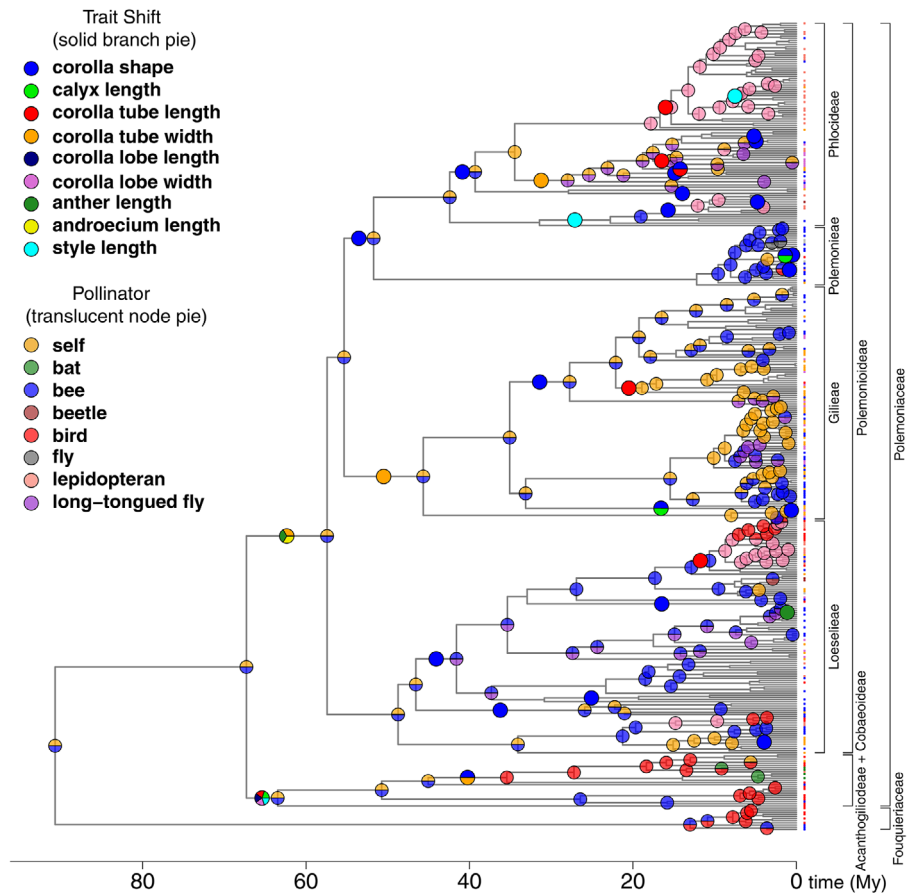
Figure 6. Summary of inferred shifts in trait values throughout the phylogenetic history of Fouquieriaceae and Polemoniaceae. Branch pies show the location of shifts, arbitrarily placed at the midpoint of that branch. Segments of pies indicate which trait(s) shift along the edge. Pies below represent the ancestral state reconstruction of pollination system for nodes found in both the morphology dataset and pollinator dataset. Nodes without a reconstruction of pollination system are present in the morphological dataset but absent from the pollinator dataset. Tip boxes represent primary pollinator, if known. Clade labels reflect familial, subfamilial, and tribal classifications.

Discussion
PHYLOGENY

As the focus of this study is on trait and pollinator data, we briefly discuss our phylogeny, which is most comprehensive time-calibrated phylogeny of polemonioids and accounts for error in topology and divergence time estimation. Although the topology is largely similar to previous studies (Johnson et al. 2008; Landis et al. 2018), we find persistent areas of uncertainty, including the relationships of Acanthogilioideae, Cobaeoideae, and Polemonioideae, and the placement of Loeselieae. However, subfamilial relationships were also supported in Landis et al. (2018) and are strongly supported by 350 nuclear loci and nearly complete plastomes (J. Rose unpubl. data). The impact of uncertain phylogeny on our results is minimized by concordance with larger genetic datasets, as well as our accounting of topological uncertainty in many analyses. Node ages, with the exception of *Phlox* (younger in this study), appear to be robust, and are in close

agreement with those of Landis et al. (2018), despite a differing number and placement of calibration points.

FREQUENT AND UNPREDICTABLE SHIFTS IN POLLINATORS

Based on relationships derived from morphological and cytological data, Grant and Grant (1965) hypothesized that temperate Polemoniaceae arose from a bee-pollinated ancestor, later extended to the entire family (e.g., Stebbins 1974). They hypothesized that transitions between pollination systems occurred only from bee-pollinated ancestors, with the exception of self-pollination and various kinds of lepidopteran-pollination. Apart from finding a bee-pollinated common ancestor of Polemoniaceae, we find little support for this hypothesis (Figs. 2–4). Instead, we show that pollination systems are extremely labile and heterogenous, that is, there are frequent shifts among pollination systems and a particular pollination system may originate

from ancestors with one of several pollination systems (Fig. 3; Table S7). Lability and heterogeneity in pollination systems is not only found across angiosperms (van der Niet and Johnson 2012), but frequently characterizes the evolution of pollination systems within clades diverse in their pollination ecology (Johnson et al. 1998; Valente et al. 2012; Ollerton et al. 2019). Although our reconstructions with and without secondary pollinators differ markedly for several key nodes (Figs. 2 and S3–S5; Tables S7 and S8), both analyses clearly demonstrate lability, heterogeneity, and convergence in pollination systems. One limitation of our analysis including secondary pollinators is that we are unable to give more weight to primary pollinators. However, we argue that this represents a better hypothesis of ancestral states in Polemoniaceae because it incorporates uncertainty and reconstructs ancestral selfing at fewer deeper nodes than the analysis with primary pollinators only (see below).

Unsurprisingly, given their predominance as pollinators in Polemoniaceae, we find that 53% of all transitions involve movement to or from bee-pollination with a mean of at least one transition from bee-pollination to each of the other systems. The ability of such "generalist" pollination systems to transition to most other systems is well-documented in the literature (van der Niet and Johnson 2012). However, we provide another case study demonstrating shifts from highly specialized to more generalized pollination systems, including the transition from bird to bee-pollination (Tripp and Manos 2008; Martén-Rodríguez et al. 2010; Mast et al. 2012; van der Niet and Johnson 2012; Toon et al. 2014; Serrano-Serrano et al. 2017; Kriebel et al. 2019, 2020).

We find no transitions away from bat-pollination, although it evolved only once (*Cobaea*; Fig. 2). Muchhala (2007) suggested that the extremely specialized floral morphology of bat-pollinated species with large, wide corollas may prevent even inefficient pollination by other animals. This implies a limited ability of bat-pollinated lineages to switch pollinators, an idea largely supported by macroevolutionary studies (Tripp and Manos 2008; Martén-Rodríguez et al. 2010; Joly et al. 2018; Dellinger et al. 2019a; but see Duchen and Renner 2010 and Lagomarsino et al. 2017).

Excluding bee-pollination, 54% of the remaining transitions involve selfing. Although a large number of transitions are toward selfing (an average total of 24.8), the majority of transitions are away from it (34.7) and are to bee or long-tongued fly pollination (Table S7). It has been hypothesized that once a species evolves obligate selfing, deleterious mutations accumulate as homozygosity increases. The outcome is an inability to adapt to changing ecological conditions, loss of traits that attract pollinators, and no reversals in pollination system (Stebbins 1957; Barrett et al. 1996; Takebayashi and Morrell 2001; Barrett 2013; Igic and Busch 2013). However, Landis et al. (2018) argued that Polemoniaceae shows transitions from obligate selfing, especially within

Gilieae. Although our reconstruction of primary pollinator alone closely matches their reconstruction, our reconstruction including secondary pollinators finds that transitions to obligate selfing occurred much more recently, in *Collomia* and *Gilia*. In addition, there are an average of six fewer transitions from selfing in our analysis including secondary pollinators (an average total of 40.1 vs. 34.7; Tables S7 and S8). In either case, although our reconstruction represents the most complete hypothesis of pollination system evolution in Polemoniaceae, it should not be overinterpreted, given uncertain infrageneric relationships within Gilieae, nonrandom/nonrepresentative pollinator data, and the possibility that some species may not be obligately selfing (Goodwillie et al. 2005; Igic and Kohn 2006). Our P:O dataset is more randomly distributed across Polemoniaceae and suggests that most ancestors in Gilieae were facultatively selfing (Fig. S6). Apart from rare exceptions, we argue against "rescue" from obligate selfing in Polemoniaceae.

PHYLOGENY SHAPES CORRELATED TRAIT EVOLUTION

We asked if changes in pollination system have been accompanied by changes in floral morphology, possibly with correlated evolution of traits, while accounting for the confounding factor of phylogeny. We find significant phylogenetic structure to the distribution of floral traits in Polemoniaceae: that is, species resemble each other more than expected by chance (Revell et al. 2008; Table 2).

We also find that there is positive correlation among linear traits, but traits are not evolving isometrically (Fig. S7). A nonisometric relationship among floral traits is consistent with previous work in Polemoniaceae (Campbell 1996; Lendvai and Levin 2003; Goodwillie et al. 2006) and is expected because floral traits are polygenic and inherited from multiple linkage groups (Goodwillie et al. 2006; Nakazato et al. 2013; Campitelli et al. 2018). However, few studies have explored this concept at a macroevolutionary scale (Dellinger 2020; but see Pérez et al. 2007, Dellinger et al. 2019b, and Kriebel et al. 2020). Our study represents an important step forward in understanding macroevolutionary trait correlations.

Our finding of correlation among floral traits is indicative of an overall pattern of phenotypic integration (i.e., synorganization, modularity; Armbruster et al. 1999; Klingenberg 2014). The magnitude of regression slopes also suggests that floral traits are decoupled within and among whorls. The most isometric correlations appear within corolla size traits, particularly lengths, as well as between corolla and androecium length, but the latter is expected as the androecium is fused to the corolla in Polemoniaceae (but not Fouquieriaceae). Trait decoupling is most apparent across floral whorls, with style and especially calyx length showing relatively small regression slopes when comparted to corolla

traits. Within the corolla, tube width is notably decoupled from other traits, which explains the near-zero relationship between corolla shape and size (Fig. S7). Decoupling of floral size and shape has been documented previously, although perhaps less elegantly, when "shape" has been defined based on ordination analysis of linear measurements (Pérez et al. 2007; Serrano-Serrano et al. 2015).

In addition to the genetic linkage of floral traits, decoupling among traits may result from antagonistic selection regimes among traits (Armbruster 1996; Armbruster et al. 1999; Galen and Cuba 2001; Mayfield et al. 2001; Strauss and Irwin 2004; Strauss and Whittall 2006; Campbell and Powers 2015). Alternatively, our analysis of correlations within the entire dataset may not capture trends in specific clades or functional groups, and floral integration may be high in specialized or selfing groups but lower in generalized groups (Anderson and Busch 2006; Pérez et al. 2007; Rosas-Guerrero et al. 2011; Klingenberg 2014; Fornoni et al. 2016; Dellinger et al. 2019b).

STRONG EVIDENCE FOR FLORAL SYNDROMES
Despite the influence of phylogenetic history on morphology, we find morphological differentiation in species with different pollination biology (Figs. 5 and S9). Although it is tempting to hypothesize that these trait differences are due to direct selection by pollinators, other hypotheses are possible, including indirect selection on correlated floral traits, selection by herbivores, or abiotic factors (Campbell et al. 1994; Alexandersson and Johnson 2002; Strauss and Whittall 2006; Caruso et al. 2019). However, traits related to pollination efficiency are more likely to be under direct selection by pollinators (Rosas-Guerrero et al. 2011; Caruso et al. 2019).

We have shown that there are convergent shifts in pollination system in Polemoniaceae and these shifts in pollination system are associated with significant differences in trait means. Moreover, as inherent in the definition of floral syndromes (Fenster et al. 2004), we find that species within a pollination system usually differ from other groups in multiple floral traits (i.e., suites or modules), and that different pollination systems differ from each other in unique combinations of traits (i.e., suites/modules differ across pollination systems). For example, bat and bird-pollinated flowers differ from most other groups in nearly all traits, but bee-pollinated species only differ from the remaining groups in up to half of all traits (Table S5). This may reflect the extent to which bat and bird-pollination represent highly specialized systems with high integration of floral traits (Muchhala 2007; Rosas-Guerrero et al. 2011). Moreover, it may explain why studies investigating clades pollinated exclusively by vertebrates and/or with only bee and vertebrate-pollination have found clear evidence for floral syndromes (Martén-Rodríguez et al. 2009; Lagomarsino et al. 2017; Kriebel et al. 2020), whereas the evidence is less con-

vincing in insect-pollinated clades (Smith et al. 2008; Martén-Rodríguez et al. 2009; Gómez et al. 2014).

Surprisingly, given its importance in some systems (Smith and Kriebel 2018; Kriebel et al. 2020; but see Gómez et al. 2014), corolla shape shows relatively little differentiation across pollination systems, perhaps due to its variability in bee-pollinated species (Fig. 5A). However, some trends in corolla shape match expectations (e.g., plants with linear corollas are pollinated by long-tongued animals). Little differentiation in corolla shape among pollination systems reinforces the idea of decoupling of floral shape and size, and different combinations of size and shape may be important in floral diversification. Additionally, our broad coding of "bee-pollination" may miss specialization within this category (Fenster et al. 2004; van der Niet 2020).

We find that selfing is accompanied by a reduction in size (Figs. 5 and S9; Stebbins 1970; Lloyd 1979). Selfing species are well-differentiated from most pollination systems, suggesting high levels of floral integration (Pérez et al. 2007; Fornoni et al. 2016), but do not differ much from fly and beetle-pollinated species (Table S5). These nonsignificant results represent cases where pollination systems have evolved few times, so we may lack statistical power to discern differences. Alternatively, these (and other) pollination systems may be differentiated by traits not sampled in this study including scent, corolla color/nectar guides, and/or nectar sugar (Galen and Stanton 1989; Knudsen and Tollsten 1993; Meléndez-Ackerman et al. 1997; Chess et al. 2008; Bischoff et al. 2014, Bischoff et al. 2015).

LITTLE EVIDENCE FOR CONCERTED SHIFTS IN MORPHOLOGY AND POLLINATORS
The majority of shifts in floral traits involve corolla length and tube width, style length, and especially corolla shape (Table S6). All these traits differ among at least some pollination systems (Fig. 5) and are largely related to pollinator efficiency (Rosas-Guerrero et al. 2011). The number of shifts in shape are surprising given that differences in shape are not consistently associated with pollinator type. One explanation is that pollinator association with shape is contingent on size: that is, the selective advantage of a particular floral shape depends on size.

Despite strong evidence for an association between floral traits and pollinators, we find little evidence that shifts in morphology are associated with shifts in pollinators or that traits shift synchronously (Figs. 6 and S11), although we note difficulty in associating trait shifts with pollinator shifts as a result of uncertainty in pollinator reconstruction, incomplete matching between pollinator and morphological data, and difficulty in placing trait shifts on branches (Ho and Ané 2014b; Bastide et al. 2017). Asynchrony in trait shifts is consistent with our finding of a nonisometric relationships among variables. However, our finding that traits are associated with pollinators but that 94% of

pollinator shifts are not associated with a shift in morphology is paradoxical, but four points bear consideration. (1) These analyses have different goals, with ANOVAs comparing extant species and our tests for shifts identifying the location of past changes. (2) An OU model may be favored as a better fit for the data, but a shift in OU regime may only characterize one clade. (3) At low values of the phylogenetic half-life parameter, α (the time to move halfway from the ancestral state to the optimum trait value, $\alpha = 0$ under BM [Hansen 1997]), results under an OU model may be scarcely distinguishable from BM (Cooper et al. 2016). Therefore, a more gradual (i.e., BM or nearly so) model may better represent the majority of trait evolution in Polemoniaceae. (4) Our counting of pollinator shifts includes shifts with a complete transition in pollinator as well as shifts where a descendant either gains or loses another pollinator. Large shifts in floral morphology are not expected in the latter case. Smith et al. (2008) discussed several reasons why trait shifts may not mirror pollinator shifts, including nonadaptation and nonequilibrium. We argue that nonequilibrium dynamics help explain this paradox, where the pleiotropic and polygenic nature of inheritance of quantitative floral traits, trade-offs from antagonistic selection regimes, and/or periods of inconsistent or interrupted selection results in a gradual response of floral traits to abrupt shifts in pollinator niche.

Although the analysis of trait convergence detected convergent evolution in most traits with greater than one OU regime, we failed to find suites of traits that are consistently convergent across the same clades. Current tools for statistically defining convergent selection regimes operate under the assumption that these regimes have an identical optimal trait value (Khabbazian et al. 2016; Bastide et al. 2018), yet in a biological sense, this criterion may be too stringent. Biologically, suites of convergent traits may not be expected in all cases, as phylogenetic history and constraint (Joly et al. 2018; Kriebel et al. 2020) and/or trade-offs from antagonistic selection regimes, including mixed pollination (Aigner 2001; Sahli and Conner 2011; Kulbaba and Worley 2013) may result in multiple combinations of "compromise" morphologies.

Conclusions

The iconic work of Grant and Grant (1965) has influenced countless biologists in diverse subdisciplines. Although prior studies have examined Polemoniaceae in a phylogenetic framework, none have tested their hypothesis of the predictability and directionality of pollinator transitions. Although we do not reject the hypothesis of diversification from a bee-pollinated ancestor of Polemoniaceae, the radiation of pollination systems is far more complex and less predictable than hypothesized (Fig. 3). Inde-

pendent origins of the same pollination system are associated with convergence in multiple floral traits, or floral syndromes (Faegri and van der Pijl 1979; Fenster et al. 2004), but phylogenetic history also plays an important role in floral evolution. Despite an association with different pollinators, floral traits have largely diverged gradually, in a manner more in line with a BM model of evolution (Fig. 6). Future studies should continue to robustly investigate the association of shifts in floral morphology with shifts in pollinators, focusing on developing methods that provide a statistical test for the correspondence of sets of shifts on a phylogeny.

DATA ARCHIVING
All alignments, trees, input files, data matrices, and R scripts are deposited in Dryad (https://doi.org/10.5061/dryad.95x69p8k4).

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Supporting Information
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Table S1. Summary of likelihood ratio tests of a Brownian motion (BM) versus a 1-optimum Ornstein-Uhlenbeck (OU) model of trait evolution for all quantitative floral traits across polemonioids. Ornstein-Uhlenbeck models were a better fit for the data for all traits.

Table S2. Summary of p-values from phylogenetic generalized least squares regression (PGLS) for all pairwise comparisons between all 10 quantitative floral traits across polemonioids.

Table S3. Morphospace dynamics of flowers visited by different groups of primary pollinators based on the first two PCs of the PCA.

Table S4. Summary of p-values from phylogenetic ANOVAs for all pairwise comparisons between pollinator groups for all 10 quantitative floral traits across polemonioids.

Table S5. Summary of statistically significant differences among pollination modes in quantitative floral trait values in polemonioids.

Table S6. Summary of Ornstein-Uhlenbeck shifts and phylogenetic half-life (α; the time it takes to move halfway from the ancestral state to the primary optimum) across 9 quantitative traits in polemonioids.

Table S7. Mean number of transitions between pollination systems inferred from 500 stochastic maps using the modified BAYAREALIKE model in BioGeoBEARS with primary and secondary pollinators.

Table S8. Mean number of transitions between pollination systems inferred from 500 stochastic maps using the modified BAYAREALIKE model in BioGeoBEARS with primary pollinators only.

Figure S1. Shape diversity in the polemonioids colored by genus as described by elliptical Fourier analysis.

Figure S2. Maximum clade credibility tree for polemonioids and outgroups (Actinidiaceae).

Figure S3. Ancestral state reconstruction of pollinator type in polemonioids including primary and secondary pollinator using the modified BAYAREALIKE model in BioGeoBEARS.

Figure S4. Ancestral state reconstruction of pollinator type in polemonioids including primary pollinators only using the modified BAYAREALIKE model in BioGeoBEARS.

Figure S5. Ancestral state reconstruction of pollinator type in polemonioids including primary pollinators only using the modified BAYAREALIKE model in BioGeoBEARS.

Figure S6. Ancestral state reconstruction of pollen to ovule ratio in polemonioids.

Figure S7. Trait correlation in Fouquieriaceae and Polemoniaceae for all pairwise comparisons of quantitative variables. Numbers are the slope of the regression line (β) inferred using phylogenetic generalized least squares regression and Ornstein-Uhlenbeck model of trait evolution on the maximum clade credibility tree.

Figure S8. Matrix plot of natural log-transformed values for all quantitative trait across the polemonioids without any phylogenetic correction.

Figure S9. Boxplots of the six quantitative traits not depicted in Fig. 6.

Figure S10. Ancestral state reconstruction of corolla lobe color in the polemonioids under and all rates different model of trait evolution.

Figure S11. Ornstein-Uhlenbeck shifts for each of the 9 quantitative floral traits.