Tempo and mode of flower color evolution[1]

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ABSTRACT

- *Premise of the Study:* Flower color is one of the best-studied floral traits in terms of its genetic basis and ecological significance, yet few studies have examined the processes that shape its evolution across deep timescales. Advances in comparative methods along with larger phylogenies for floral radiations offer new opportunities for investigating the macroevolution of flower color.
- *Methods:* We examine the tempo and mode of flower color evolution in four clades (Antirrhineae, Iochrominae, Loeselieae, Quamoclit) using models that incorporate trait transitions and lineage diversification. Focusing on floral anthocyanin pigmentation, we estimate rates of gain and loss of pigmentation and test whether these changes occur predominantly through anagenesis or cladogenesis.
- *Key Results:* We found that the tempo of pigment gains and losses varies significantly across the clades and that the rates of change are often asymmetrical, favoring gains over losses. The mode of color shifts tended to be cladogenetic, particularly for gains of color; however, this trend was not significant.
- *Conclusions:* Given that all flowering plants share the same pathway for producing anthocyanins, the marked variation in the tempo of transitions across the four groups suggests differences in the selective forces acting on floral pigmentation. These ecological and physiological factors, together with genetic basis for color, may also explain the bias toward gains of floral anthocyanins. Estimates for cladogenetic and anagenetic rates suggest that color transitions can occur through both modes, although testing their relative importance will require larger datasets.

Key words: anagenetic; anthocyanins; cladogenetic; diversification; macroevolution; pigmentation; speciation; transition rate

Phylogenetic comparative methods are grounded in the notion that observations of present-day variation can be used to make inferences about the past (Harvey and Pagel, 1991). This is the fundamental principle that allows us to build phylogenetic trees from DNA sequences of extant species and infer the characteristics of now-extinct ancestral taxa. In addition to estimating particular evolutionary histories (e.g., trees or ancestral states), we are increasingly using comparative methods to understand the processes that give rise to those outcomes, which include factors such as trait evolution, lineage-splitting, dispersal, and extinction. With the growing availability of large and well-resolved phylogenies, comparative methods have moved to build more complex models and more powerful methods that incorporate a broader array of biological processes (reviewed in O'Meara, 2012; Ng and Smith, 2014).

To date, applications of comparative methods to the history of angiosperms have largely focused on evolutionary outcomes, with less attention to estimating underlying processes. For example, ancestral state reconstructions have been used to trace the origins of a wide range of floral characters, from major morphological features (e.g., Endress, 2011) to fine-scale changes in corolla size and shape (e.g., Perez et al., 2006; Marten-Rodriguez et al., 2010). This morphological diversity arises due to a potentially large number of interacting processes, occurring both within and across lineages. For example, the overall range of forms depends on the rate at which new phenotypes evolve while the frequency of species with those forms is affected by their rates of diversification (Maddison, 2006). Nonetheless, relatively few studies have quantified these key processes in the context of angiosperm diversification. With the exception of floral symmetry (which has been well-studied), we have yet to answer many basic macroevolutionary questions about the tempo, directionality, and mode of floral trait evolution. For example, what is the rate at which different floral characters change along the phylogeny

(Davis et al., 2007; Alcantara and Lohmann, 2011), and are these changes biased toward or away from particular states (Ree and Donoghue, 1999)? Moreover, do some floral traits act as key innovations, increasing speciation in lineages that possess them (Sargent, 2004; de Vos et al., 2014)?

One key floral feature that is amenable to addressing these broad evolutionary questions is flower color. Flower color varies tremendously at a range of taxonomic scales (within and between species, genera, and families), providing power for estimating the rates and directionality of shifts (Perret et al., 2003; Burd et al., 2014). Despite its evolutionary lability, flower coloration arises from only a handful of biochemical pathways: carotenoids, betalains, and, most commonly, anthocyanins (Tanaka, Sasaki, and Ohmiya, 2008). Thus, even though similar flower colors have evolved independently many times (e.g., Wilson et al., 2007), these convergent phenotypes often share an underlying deep homology due to the conservation of the biosynthetic pathways across angiosperms (Rausher, 2006; Campanella, Smalley, and Dempsey, 2014). Moreover, the genetic changes in these pathways that lead to flower color transitions have been studied in detail in many systems (e.g., Streisfeld and Rausher, 2009; Smith and Rausher, 2011; Zhang et al., 2015), creating the potential for connecting the mechanisms of change within species to variation across lineages. Finally, among floral traits, flower color has received a great deal of attention with respect to ecological drivers of divergence. In addition to the canonical mechanism of shifts between pollinator types (Fenster et al., 2004), flower color differences also evolve in response to competition for the same pollinators, as well as abiotic conditions and herbivory (Strauss and Whittall, 2006; Muchhala, Johnsen, and Smith, 2014). Given that the dynamics of flower color evolution often vary across clades, this ecological context provides a

set of testable macroevolutionary hypotheses for these differences (Armbruster, 2002; Smith, Ane, and Baum, 2008).

The present study uses a comparative approach to investigate the processes underlying variation in flower color in four floral radiations: Antirrhineae (Sutton, 1988), Iochrominae (Olmstead et al., 2008), Loeselieae (Porter and Johnson, 2000) and *Ipomoea* subg. *Quamoclit* (Miller, McDonald, and Manos, 2004). We specifically focus on gains and losses of floral anthocyanin pigmentation. Flowers expressing anthocyanins appear in shades of blue, red, pink, and purple, while those without range from white to yellow. Transitions between the presence and absence of anthocyanin pigmentation are common in many clades of angiosperms (Quattrocchio et al., 1999; Whittall et al., 2006; Cooley et al., 2011). However, this study will be among the first to examine the dynamics of these macroevolutionary color transitions (see also Smith et al., 2010). Here, we ask: (1) What is the tempo of changes in pigmentation and how do these rates vary across clades? (2) Are transitions in pigmentation directional, that is, is there a trend toward gains or losses? (3) Do changes in flower color tend to coincide with speciation events (cladogenesis) or do they more often occur within single lineages (anagenesis)? Whether or not the answers to these questions differ across the four radiations will give insight into the generality of macroevolutionary dynamics for this deeply homologous trait.

MATERIALS AND METHODS

Dataset construction - Model-based trait transition and diversification analyses require the input of an ultrametric tree with branches in units of time or proportional to time (Maddison, Midford, and Otto, 2007). We thus selected clades for study that had divergence time estimates, as well as a sufficiently rich taxonomic literature for scoring color for all species (see below). In

order to make our results maximally comparable across the clades, we generated time-calibrated trees ("timetrees") for each clade using existing nuclear and plastid sequence data (Appendix S1; see Supplemental Data with the online version of this article). Our datasets included all previously sampled species in the named clades, with the exception of Antirrhineae. Due to difficulties in assessing taxonomic status and flower color states, we pruned three genera (*Anarrhinum, Kickia,* and *Linaria*) from Antirrhineae and included only the lineage comprising the Maurandya, Chaenorrhinum, Antirrhinum, and Gambelia groups (Vargas et al., 2004). Overall, the datasets contained 52 to 94% of the total species in each clade (Appendix S1). Previous simulation studies suggest that estimates of diversification and transition rates are relatively robust to this level of incomplete sampling (FitzJohn, Maddison, and Otto, 2009).

Timetrees were estimated using Bayesian relaxed-clock methods as implemented in BEAST v. 2.1.2 (Bouckaert et al., 2014). Tree searches used a GTR+gamma model of sequence evolution with parameters unlinked across genes and a relaxed clock log normal model to accommodate rate variation across branches. We chose a birth-death model for trees with a uniform prior on the rates. The trees were dated using secondary calibrations from previous divergence time studies for each group: Antirrhineae (Vargas et al., 2004; Vargas et al., 2009); Iochrominae (Paape et al., 2008; Sarkinen et al., 2013); Loeseliaeae (Porter, Johnson, and Wilken, 2010); and Quamoclit clade of morning glories (Eserman et al., 2014). Normally distributed priors were used for each calibration point, and the standard deviation was adjusted to reflect the level of uncertainty found in the original studies. We chose this approach because the goal of this study was not to re-estimate divergence times or improve phylogenetic resolution for these taxa, but to create comparable sets of trees (samples of the posterior distribution of timetrees) across the four datasets for downstream analyses. BEAST chains were run for 5 to 10

million generations (depending on the number of generations needed for convergence). Convergence and effective sample size (200 or greater) was assessed using Tracer v.1.6. Also, each run was repeated twice to ensure similar results. We subsampled the post-burnin trees using LogCombiner to obtain a set of 100 trees for each clade for downstream analyses.

For analyses of character evolution and diversification, we scored all described species for the presence of anthocyanin pigmentation using empirical studies, taxonomic literature, and online databases. Anthocyanins are flavonoid pigments that are responsible for red, blue and purple coloration in most plants, including those studied here (Harborne, 1994; Winkel-Shirley, 2001). In addition, each of the clades contains several species in which the production of anthocyanin pigments has been studied in detail: Antirrhineae (Martin et al., 1991; Schwinn et al., 2006); Iochrominae (Smith and Rausher, 2011); Loeseliaeae (Harborne and Smith, 1978; Nakazato, Rieseberg, and Wood, 2013); and the Quamoclit clade of morning glories (Eich, 2008; Des Marais and Rausher, 2010). Most species were scored based on species descriptions, with flowers in shades of red to blue indicating the presence of anthocyanins. Species that were polymorphic for pigmentation were scored as "present", and species that were almost entirely lacking in floral anthocyanins except for small regions (<5%) of the corolla, such as the veins, were scored as absent (following Smith et al., 2010). Flower color descriptions were obtained from the literature: Sutton (1988) for Antirrhineae; Smith and Baum (2007) for Iochrominae; Porter (1998), Porter and Johnson (2000), Porter and Steinmann (2009) for Loeselieae; and Smith et al. (2010) for Quamoclit. Color descriptions were verified when possible by examining images or specimens on Tropicos (www.tropicos.org) and CalFlora (www.calflora.org).

Diversification Analyses - Although the focus of this study was to determine the tempo and mode of character evolution, inference of these rates can be compromised if the character

state affects rates of speciation or extinction (Maddison, Midford, and Otto, 2007; Goldberg and Igic, 2008). For example, if lineages with pigmented flowers diversify more rapidly, an analysis that does not account for this state-dependent diversification may mistakenly conclude that gain of pigmentation is more common than loss. Thus, we first used the four datasets to test for significant differences in diversification rates between lineages with and without floral anthocyanins. We estimated speciation rates (λ_0, λ_1) and extinction rates (μ_0, μ_1) in each state (where 0 and 1 denote absence and presence of anthocyanins, respectively) as well as transition rates between states (q_{01}, q_{10}) using the BiSSE model (Maddison, Midford, and Otto, 2007) as implemented in the R package Diversitree 0.9-7 (FitzJohn, 2012). We incorporated unsampled taxa with the "skeleton tree" approach (FitzJohn, Maddison, and Otto, 2009), which assumes that missing species are randomly distributed across the tree. Model parameters were estimated using Markov chain Monte Carlo (MCMC) with 5000 steps on each of the 100 trees. Priors were exponential with rates taken from a short run with a symmetrical model ($\lambda_0 = \lambda_1$; $\mu_0 = \mu_1$). Diversification rates in each state (r_0, r_1) were computed from the MCMC run as the difference between speciation and extinction rates at each step ($r_0 = \lambda_0 - \mu_0$ and $r_1 = \lambda_1 - \mu_1$), and the significance of differential diversification was assessed by testing whether the 95% credibility interval of the difference in diversification rates ($r_0 - r_1$) included zero.

Cladogenetic and anagenetic model fitting - As our BiSSE analyses did not demonstrate state-dependent diversification (details below), we created a range of transition and diversification models focused on examining the tempo, mode, and directionality of character change. The Cladogenetic State change Speciation and Extinction or "ClaSSE" model (Goldberg and Igic, 2012), equivalent to the BiSSEness model of Magnuson-Ford and Otto (2012), is an extension of the BiSSE model that allows cladogenetic character changes (Fig. 1A). These

transitions during speciation events may occur either at observed nodes along the reconstructed phylogeny or at hidden nodes where the bifurcation is not observed due to subsequent extinction of one daughter (Fig. 1B). ClaSSE incorporates this cladogenetic change through additional speciation rates, λ_{001} and λ_{110}, in which one of the daughter lineages retains the parent state and the other acquires a new state (Fig. 1A). (We do not consider the scenario of both daughters acquiring states different from the parent, so our analyses all set to zero the other cladogenetic rates, λ_{011} and λ_{100}, of the general model.) Anagenetic character change occurs within single lineages through the q rates (q_{01}, q_{10}), which are shared with BiSSE as well as state-independent models (e.g., Mk2, Lewis, 2001). For this study (based on our initial BiSSE analyses, described below, which do not support state-dependent diversification), the full ClaSSE model was reduced to exclude the effects of flower pigmentation on rates of extinction and speciation by constraining the extinction rates to be equal ($\mu_0 = \mu_1$) and the total speciation in state 0 ($\lambda_{000} + \lambda_{001}$) to be equal to that in state 1 ($\lambda_{111} + \lambda_{110}$).

This model, with six free parameters (Table 1), contains all the processes of interest for our study: rates of flower pigment gain and loss, through both cladogenetic and anagenetic modes. We refer to it as the "full" model even though it is a simplified version of the ClaSSE model. To assess whether any of these processes is not necessary to explain our data, we conducted statistical comparisons among a set of submodels, each formed by applying a set of constraints to the full model. In total, we examined eight models (Table 1): we included or excluded cladogenetic and anagenetic modes of change, and we did or did not allow differing (asymmetric) rates of forward and reverse transitions (pigment gain and loss, respectively). For example, the full model allows asymmetric transition rates for both modes, while the simplest two models (7 and 8, Table 1) allow only symmetric rates of change by only one mode. All eight

of these models were fit with maximum likelihood (ML) methods in Diversitree to each of the 100 trees from the four datasets. The set of top models for each dataset comprised those within two Akaike Information Criterion (AIC) units from the lowest-scoring model (Burnham and Anderson, 2002).

This ML model comparison procedure did not identify a simpler model that sufficed for all clades, and each clade supported multiple non-nested simpler models (details below). We therefore performed our comprehensive model fit with the full model (model 1, Table 1). For our Bayesian analysis on each tree, we completed 5000 MCMC steps, with prior rates determined by a short run of a symmetric model (for scripts and all input data, see Dryad http://dx.doi.org/10.561/dryad.0732.g). The first 1000 steps were discarded as burn-in. The remaining 4000 steps comprise a posterior distribution that captures uncertainty in the rate estimates on that tree. This analysis was conducted on each of 100 phylogenies from the posterior set of trees for the clade. Combining all 400,000 samples for the clade forms a final posterior distribution that additionally incorporates uncertainty in the clade's phylogeny. All comparisons of rate parameters within a clade were based on this distribution.

Within each clade, we compared the individual rate parameters (e.g., q_{01} vs. q_{10}) and also several compound rate parameters, such as the total rate of change (summing across parameters that involve a color transition: λ_{001}, λ_{110}, q_{01}, q_{10}) and the asymmetry of rates of gains and losses, regardless of mode ($\lambda_{001} + q_{01}$ vs. $\lambda_{110} + q_{10}$). Each statistical comparison between two rates, whether individual or compound, was conducted by taking the difference between the two rates (computed for each MCMC sample). The rates were judged significantly different if the 95% credibility interval of their difference did not include zero. These credibility intervals were calculated as the smallest region containing 95% of the samples using the hdr (‘highest density

region') function in Diversitree. All statistical rate comparisons for a clade thus incorporate both within-tree and among-tree uncertainty.

We also used the rate estimates from the full model MCMC to visualize potential histories of character change and compute expected equilibrium state frequencies. We conducted stochastic mapping (SM) with the median parameter values summed for gains ($\lambda_{001} + q_{01}$) and losses ($\lambda_{110} + q_{10}$) as in Smith et al. (2010) to simulate character histories possible with these values. As currently implemented (Bollback, 2006), SM does not allow for cladogenetic change or diversification parameters; thus, this visualization only shows the history that could arise from anagenetic processes. We calculated equilibrium state frequencies (percentage of taxa with and without pigmented flowers at equilibrium) expected given the median rates using the stationary.frequencies.classe function in Diversitree (FitzJohn, 2012).

RESULTS

Distribution of anthocyanin pigmentation - Although the four sampled clades belong to different plant families, all present similar numbers of pigmented species. The proportion of extant pigmented species ranges from 74 to 85%, and the proportion sampled in the phylogenies is similar, suggesting that the taxon sampling was not biased towards either state (Fig. 2; Appendices S1, S2, see Supplemental Data with the online version of this article). In three of the four clades (Antirrhineae, Loeselieae, Quamoclit), the species lacking anthocyanin pigmentation are distributed widely across the phylogeny, nested in clades of taxa with pigmented flowers (Fig. 2). By contrast, most of the species lacking floral anthocyanins in Iochrominae are clustered in a single clade (the "A" clade *sensu* Smith and Baum (2006)). This pattern suggests that different macroevolutionary processes might be at play in Iochrominae.

Diversification analyses - There was an indication of higher diversification in pigmented lineages in Iochrominae, Loeselieae, and Quamoclit, consistent with previous studies (Smith et al., 2010). The pattern was reversed in Antirrhineae, where the distribution for diversification of unpigmented lineages is bimodal, but typically higher than that for pigmented lineages. In all clades, however, the posterior distributions of the difference in two diversification rates (r_0 and r_1) overlapped, and the 95% credibility interval for difference between these rates (r_0 - r_1) across the MCMC steps included zero (Appendix S3, see Supplemental Data with the online version of this article). The same was true for the speciation and extinction rates in each state (Appendix S3). These patterns indicate that anthocyanin pigmentation is not associated strongly or consistently with state-dependent diversification. This conclusion is not compromised by recent concerns about false positives with the BiSSE model (Maddison and FitzJohn, 2015; Rabosky and Goldberg, 2015) because here we report no significant signal of state-dependent diversification.

Rates and mode of flower color transitions - Our maximum likelihood model fitting supported asymmetric anagenetic and cladogenetic change in flower color for all four of the datasets. We estimated all eight models for 76-100% of the trees across the four datasets, and most trees had two or three top models (less than two AIC units different; Appendix S4, see Supplemental Data with the online version of this article). Trees for which all models could not be completed were excluded (24% in Antirrhineae, 8% in Quamoclit, but none in Iochrominae and Quamoclit, Appendix S4). The failure to estimate all models for these trees occurred because some of the less complex models (e.g., ana.sym) do not fit well for the larger datasets (Antirrhineae, Quamoclit). The top models among the trees that completed all eight possible models frequently included asymmetric change, whether through anagenesis, cladogenesis, or

both (Fig. 3). For example, models 4 and 6 (clado.asym and ana.asym) were among the top models for all of the datasets. Between these two models, ana.asym was more commonly supported by trees for Antirrhineae and Loeselieae, while clado.asym was among the top models for a larger number of trees for Quamoclit and Iochrominae (Fig. 3, Appendix S4). Iochrominae was the only dataset with significant support for a simpler symmetrical model (clado.sym; Fig. 3). It is the smallest of the clades, with 35 species, and may thus require fewer transitions and fewer parameters (e.g., no rate asymmetries, or only one mode of change) to describe the variation.

Because these model comparisons did not strongly and consistently support a simpler model across the datasets, we could not conclude that character change has been through only one mode or equally likely in either direction. Furthermore, because multiple non-nested models are compatible with the data for each clade, there is no basis for focusing on any one simpler model for any clade. Thus, in order to assess the relative importance of anagenetic and cladogenetic change and asymmetry as well as overall rates of change, we focused our MCMC analyses on the full model (model 1, Table 1). Comparing the magnitude of rates across clades indicates the extent of variation in tempo, while determining the relative values within clades is informative about the direction of change (e.g., q_{01} vs. q_{10}) and the mode (e.g., q_{01} vs. λ_{001}).

Our estimates of rates of flower color gain and loss indicate significant differences in the tempo of character evolution across the clades. For example, median rates of gain ($\lambda_{001} + q_{01}$) vary roughly eight-fold, with the lowest in Loeselieae (0.04 mya^{-1}; Appendix S5, see Supplemental Data with the online version of this article) and the highest in Antirrhineae (0.34 mya^{-1}; Appendix S5). In a biological context, these rates indicate the expected waiting time for a lineage to transition to a new state, i.e., the propensity to evolve. Thus, a rate of 0.1 mya^{-1} would

translate to one expected transition after ten million years. Taking Loeselieae as an example, with a gain rate of 0.04 mya^{-1}, a lineage lacking anthocyanin pigmentation (state 0) would wait on average 25 mya to transition to state 1. The non-overlapping credibility intervals of the gain rates for Loeselieae and Antirrhineae indicate substantial difference in the tempo of pigment gain between these two clades (Fig. 4A; Appendix S5). Iochrominae and Quamoclit, however, exhibit intermediate gain rates with credibility intervals broad enough that their tempos cannot be distinguished from any of the other clades (Fig. 4A). Very similar patterns were observed for rates of loss (Fig. 4B), again with Loeselieae having low rates, Antirrhineae high and the other two clades intermediate (Fig. 4B, Appendix S5). Stochastic mapping suggests that even the lower rates of change may still lead to multiple forward and reverse transitions along a branch (Appendix S6, see Supplemental Data with the online version of this article).

Comparing the rates of gain and loss within clades, we also observed significant transition asymmetry (directionality of flower color change). All of the clades except Iochrominae (perhaps because of its small size) showed higher median rates of flower color gain than loss. For example, in Antirrhineae, the rate of gain of flower color was roughly four times the rate of loss (Appendix S5). To examine the confidence in this directionality, we computed the transition rate asymmetry across the MCMC samples as $(\lambda_{001} + q_{01}) - (\lambda_{110} + q_{10})$. The credibility intervals for this asymmetry excluded zero for Antirrhineae and Loeselieae (Fig. 4C; Appendix S5). These results effectively reject symmetrical flower color transitions for these two clades and indicate a significant trend toward gains of pigmentation. The tendency toward asymmetrical transitions is consistent with the model comparisons, in which fully symmetric models were rejected for all datasets except for Iochrominae.

We next considered how flower color transitions were partitioned between the anagenetic and cladogenetic modes. Models with exclusively one mode or the other (e.g., clado.asym, ana.asym) were among the top models for most trees in most clades (Fig. 3, Appendix S4), and thus we might expect both modes to contribute to this joint model. Although credibility intervals for all cladogenetic and anagenetic rates excluded zero in all clades except Quamoclit (Appendix S5), many of them reached very low values (10^{-8}) and thus may not be effectively different from zero given the nature of the MCMC sampler. There was a slight trend toward higher rates of cladogenetic than anagenetic change in three of the four clades (all except Iochrominae, Fig. 5A). However, this trend is not significant as the credibility interval for the difference between these rates included zero for all clades (Appendix S5).

Finally, we examined how the mode of change (cladogenetic versus anagenetic) might vary with the type of change (gain versus loss). Given that total cladogenetic rates were higher, one possible explanation is that one or both types of changes tend to occur through cladogenetic modes (i.e., $\lambda_{001} > q_{01}$ and/or $\lambda_{110} > q_{10}$). This was the case for Antirrhineae, where both gains and losses were, on average, three to six times more likely through cladogenesis than through anagenesis (Fig. 5B,C; Appendix S5). By contrast, Loeselieae and Quamoclit showed conflicting patterns for the two types of changes. In both, the rate of gains was higher through the cladogenetic mode ($\lambda_{001} > q_{01}$) while the rate of losses was higher through the anagenetic mode ($\lambda_{110} < q_{10}$) (Fig. 5B, C; Appendix S5). Nonetheless, all distributions were broadly overlapping and credibility intervals for the differences in these rates included zero (Appendix S5). Thus, we cannot conclude that any particular mode predominates for either gains or losses.

Our equilibrium calculations suggest that the inferred processes of character evolution in these clades will result in pigmented taxa continuing to outnumber pigmented lineages over

longer evolutionary timescales. The estimated equilibrium frequencies for the two states are similar to the observed frequencies for most clades (Appendix S7, see Supplemental Data with the online version of this article), and they indicate that species with pigmented flowers will remain twice to four times more common than those with unpigmented flowers given the estimated rates of change.

DISCUSSION

Flower color has been a focal trait for the study of evolutionary processes within species because of its selective importance (Rausher, 2008) and high variability (Warren and Mackenzie, 2001). Nonetheless, few studies have examined the macroevolution of flower color to estimate the tempo, directionality, and mode of transitions at the species level. Focusing on one class of flower color changes (those involving floral anthocyanin pigmentation), we found that rates of change vary significantly across clades, with the highest rates of both gains and losses in Antirrhineae. These transitions appear to occur through both modes of character evolution (cladogenetic and anagenetic), with a slight bias towards cladogenetic change, particularly for gains of pigmentation. Overall, we observed a trend toward gains of floral pigmentation, a result which runs counter to the notion that transitions will often be biased towards losses and that trait losses are irreversible (Gould, 1970). Below we discuss the implications of these findings for understanding the process of flower color evolution.

Tempo and directionality of flower color evolution - Flower color is considered one of the most evolutionarily labile traits. Sister species often differ in color (Bradshaw et al., 1995; Wesselingh and Arnold, 2000), and many species exhibit fixed differences across populations (Streisfeld and Kohn, 2007; Cooley et al., 2011). Previous studies examining the tempo of flower

color evolution have largely focused on continuous variation, such as changes in hue and brightness across species. These studies typically find lower phylogenetic signal for quantitative variation in flower color than for other floral traits (Smith, Ane, and Baum, 2008; McEwen and Vamosi, 2010; Muchhala, Johnsen, and Smith, 2014), although low signal alone is insufficient to conclude high rates of evolution (Revell, Harmon, and Collar, 2008). A few studies have examined the tempo of discrete changes in flower color, such as gains or losses of pigmentation (Wilson et al., 2007; Smith et al., 2010), but the use of different methods (ML and parsimony) makes comparing the results across clades difficult. By using the same methods and model for all four clades, we can directly compare the inferred rates of change, which we find to vary roughly 8-fold (Fig. 4; Appendix S5). This variation in rate may be due to intrinsic genetic factors or extrinsic selective forces, as a macroevolutionary transition requires both the appearance of new mutations and their spread within a species. The biochemical pathway involved in anthocyanin production is conserved across all angiosperms (Rausher, 2006; Campanella, Smalley, and Dempsey, 2014), to some degree limiting the explanatory potential of intrinsic factors. By contrast, the external forces shaping the evolution of these clades are likely to vary markedly as they differ widely in environment, geography, and pollination biology. For example, Antirrhineae are largely bee-pollinated herbs, which have radiated in Mediterranean habitats in Europe and western North America (Sutton, 1988; Oyama, Jones, and Baum, 2010). By contrast, *Ipomoea* subgenus *Quamoclit* is a group of Neotropical vines pollinated by hummingbirds and insects (McDonald, 1991; Miller, McDonald, and Manos, 2004). Thus, inferred differences in the evolutionary history of flower color among these clades may be more likely to reflect ecological factors than genetic limitations. Analogous analyses of other clades, ideally coupled with field

studies, could help to reveal the particular ecological factors associated with the tempo of flower color evolution.

Our analysis also suggests that gains of floral anthocyanin pigmentation occur at a higher rate than losses (Fig. 4C). This pattern would seem counterintuitive as trait losses are commonly posited to occur at higher rates than trait gains (Dollo's Law, Gould, 1970). However, gains of floral pigmentation may be facilitated by the production of anthocyanins in other tissues, such as stems and leaves. In addition to their role in floral pigmentation, anthocyanins are involved in physiological responses to UV stress and drought, as well as fruit coloration (Chalker-Scott, 1999; Winkel-Shirley, 2001). This range of functions may explain the deep conservation of the pathway across flowering plants. Thus, gaining floral pigmentation may occur through activation of this existing pathway in petals as opposed to re-evolution of the entire pathway *de novo*. Recent studies suggest that changes in the R2R3 MYB transcription factors that regulate thee anthocyanin pathway are the predominant mechanism responsible for gains of floral anthocyanin pigmentation (Cooley et al., 2011; Streisfeld, Young, and Sobel, 2013). For example, the evolution of red flowers in *Mimulus aurantiacus* from a yellow-flowered ancestral state is due to a *cis*-regulatory mutation at the *MaMyb2* locus, which leads to upregulation of at least three anthocyanin biosynthesis genes and the production of floral anthocyanins (Streisfeld, Young, and Sobel, 2013). Losses of floral pigmentation can arise through mutations that cause loss of expression or loss of function in anthocyanin pathway genes, however, the pleiotropic effects of these mutations may limit the extent to which they rise to fixation (Coberly and Rausher, 2003; Streisfeld and Rausher, 2011).

In addition to these genetic factors, pigmentation gains may occur at a higher rate than losses if they are more commonly favored by selection. Such directionality has been posited for

366 blue to red transitions involving switches to hummingbird pollination in *Penstemon* (Wilson et
367 al., 2006). Transitions from unpigmented to pigmented flowers, as suggested by our study, could
368 be favored by a range of selective forces, from pollinator preference (Lunau and Maier, 1995) to
369 thermoregulation (Lacey et al., 2010) or herbivory (Irwin et al., 2003). Overall, bias in favor of
370 gains versus losses of pigmentation provides a viable explanation for the high frequency of
371 species with floral pigmentation (Fig. 2) as this directionality should lead to the predominance of
372 pigmented taxa at equilibrium (Nosil and Mooers, 2005).

373 ***Flower color and speciation*** - One motivation for this study was to determine the extent
374 to which changes in floral pigmentation occur at lineage-splitting events, consistent with a role in
375 speciation. Previous studies have implicated flower color shifts in speciation (Bradshaw et al.,
376 1995; van der Niet and Johnson, 2012) although none have statistically tested their involvement
377 across whole clades. Moreover, the observation of sister species differing in flower color does
378 not by itself implicate the change at speciation, as other characters could have caused the
379 divergence with flower color evolving later along branches (anagenetically). Our results suggest
380 that flower color changes may occur through both modes although they are largely inconclusive
381 as to which is more common. We observed a trend of higher rates of cladogenetic change overall
382 and for gains of pigmentation specifically, but neither pattern was statistically significant. These
383 results could relate to the limited sizes of the datasets, and indeed similarity of the posterior
384 distributions to the priors in some cases (Fig. 5) is consistent with low power. However, it is
385 possible that the results reflect biological factors (e.g., truly similar rates of cladogenetic and
386 anagenetic change, heterogeneity of processes across the tree).

387 To the extent that flower color plays a role in speciation events, it is important to
388 determine what evolutionary forces underlie its divergence. Studies within lineages commonly

find that flower color variation is shaped by selection (Schemske and Bierzychudek, 2007; Streisfeld and Kohn, 2007; Rausher, 2008), although the agents of selection may be diverse (Strauss and Whittall, 2006). As an example, we will consider the scenario of a gain of floral anthocyanin pigmentation during a speciation event. From an ancestral white-flowered lineage lacking floral anthocyanins, we could imagine a pollinator-mediated scenario where a subpopulation disperses to a new region with a different pollinator fauna that select for colored flowers (Waser and Campbell, 2004). Other biotic agents such as herbivores or nectar-robbers that differ between the ancestral range and the new region could similarly alter the selective regime for flower color (Maloof and Inouye, 2000; Irwin et al., 2003). The appearance of a gain of pigmentation mutant in the ancestral population could also lead to the formation of a new lineage if this trait allows or even promotes dispersal to a new region (Ng and Smith, 2014). In addition, sympatric speciation (i.e., not involving a change in geographic range) could be associated with a change in flower color, but this process would require strong selection and assortative mating based on color (Dieckmann and Doebeli, 1999). Determining the geographic distribution of color variation within species would provide an initial assessment of the possible role of flower color in dispersing to new habitats or contributing to assortative mating within populations.

A related challenge in testing the role of flower color or any other trait in speciation is role of the trait in taxonomy. Investigations that aim to test the relationship between a trait and speciation, whether using micro- or macroevolutionary approaches, must begin with well-defined species as units of study. If the species have been defined by the trait, then there is the potential for circularity. In the context of this study, if flower color was used as a taxonomic character to delimit species, all flower color changes would be, by definition, cladogenetic. While it is the

case that many sister species differ in flower color, taxonomic practice in the clades targeted here has been to use multiple characters, often non-floral, for species delimitation (e.g., Sutton, 1988; Porter and Johnson, 2000). Moreover, the concepts allow for variation in flower color within species. For example, roughly half of the Antirrhineae are polymorphic (e.g., pink to white, Appendix S2). For this study, we scored those species as floral anthocyanins present because they have the capacity to produce pigments. However, this frequent segregating variation in flower color may function as the fuel for flower color shifts. With larger datasets, it would be interesting to consider polymorphism as a third state to directly test this question.

CONCLUSIONS

A major challenge for evolutionary biologists is to determine how processes acting within and among lineages interact to shape patterns across the tree of life, such as the range of phenotypic variation, the frequencies of different traits, and the distribution of species richness across clades. In the case of flower color, microevolutionary studies have begun to reveal the genetic changes that give rise to variation in pigment production (e.g., Hopkins and Rausher, 2011; Coburn, Griffin, and Smith, 2015) and the ecological factors that may exert selection on this segregating variation (Strauss and Whittall, 2006; Rausher, 2008; Muchhala, Johnsen, and Smith, 2014). Phylogenetic comparative analyses are well positioned to complement these studies and to test the generality of patterns they may suggest. For example, evolutionary genetic studies increasingly support the possibility of regain of floral anthocyanin pigmentation following loss (Cooley et al., 2011; Sobel and Streisfeld, 2013), and our study finds that on average, gains are more likely than losses over broad evolutionary time. The potential for these flower color changes to be commonly and directly involved with cladogenesis is less clear, and

thus comparative studies have the potentially to contribute significantly to this lingering question. However, given the complexity of the relevant models, large floral radiations with well-documented color variation and densely sampled phylogenies will be required for precise and robust inferences.

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Table 1. Cladogenetic and anagenetic models. Model parameters include speciation rates with no character change (λ_{000}, λ_{111}), speciation rates with character change (λ_{001}, λ_{110}), extinction rates (μ_0, μ_1) and rates of anagenetic character change (q_{01}, q_{10}). See Fig. 1. All models have equal total diversification in states 0 and 1 (see text) although constraints differ as needed to vary the mode and symmetry of transitions. The bottom seven models are all nested within the full model (model 1: Clado.asym.Ana.asym). Models 6 and 8 are commonly referred to as the Markov 2-rate (mk2) and Markov 1-rate (mk1) models, respectively. All models have the additional constraint of state-independent extinction ($\mu_0 = \mu_1 = \mu$). For simplicity, we use λ_C to refer to speciation that involves cladogenetic character change and λ_N for speciation that does not.

Model	Constraints	Free parameters
1. Clado.asym.Ana.asym Both modes of change possible and change can be asymmetric	$\lambda_{111} = \lambda_{000} + \lambda_{001} - \lambda_{110}$, $\mu_0 = \mu_1 = \mu$	6: λ_{000}, λ_{001}, λ_{110}, μ, q_{01}, q_{10}
2. Clado.sym.Ana.asym Both modes of change possible; only anagenetic change can be asymmetric	$\lambda_{111} = \lambda_{000} = \lambda_N$, $\lambda_{001} = \lambda_{110} = \lambda_C$, $\mu_0 = \mu_1 = \mu$	5: λ_N, λ_C, μ, q_{01}, q_{10}
3. Clado.asym.Ana.sym Both modes of change possible; only cladogenetic change can be asymmetric	$\lambda_{111} = \lambda_{000} + \lambda_{001} - \lambda_{110}$, $\mu_0 = \mu_1$, $q_{01} = q_{10} = q$	5: λ_{000}, λ_{001}, λ_{110}, μ, q
4. Clado.asym Only cladogenetic change possible; change can be asymmetric	$\lambda_{111} = \lambda_{000} + \lambda_{001} - \lambda_{110}$, $\mu_0 = \mu_1$, $q_{01} = 0$, $q_{10} = 0$	4: λ_{000}, λ_{001}, λ_{110}, μ
5. Clado.sym.Ana.sym Both modes of change possible; change can only be symmetric	$\lambda_{111} = \lambda_{000} = \lambda_N$, $\lambda_{001} = \lambda_{110} = \lambda_C$, $\mu_0 = \mu_1$, $q_{01} = q_{10} = q$	4: λ_N, λ_C, μ, q
6. Ana.asym Only anagenetic change possible; change can be asymmetric	$\lambda_{111} = \lambda_{000} = \lambda_N$, $\lambda_{001} = 0$, $\lambda_{110} = 0$, $\mu_0 = \mu_1$	4: λ_N, μ, q_{01}, q_{10}
7. Clado.sym Only cladogenetic change possible; change can only be symmetric	$\lambda_{111} = \lambda_{000} = \lambda_N$, $\lambda_{001} = \lambda_{110} = \lambda_C$, $\mu_0 = \mu_1$, $q_{01} = 0$, $q_{10} = 0$	3: λ_N, λ_C, μ

8. Ana.sym Only anagenetic change possible; change can only be symmetric	$\lambda_{111} = \lambda_{000} = \lambda_N$, $\lambda_{001} = 0$, $\lambda_{110} = 0$, $\mu_0 = \mu_1$, $q_{01} = q_{10} = q$	3: λ_N, μ, q

FIGURE LEGENDS

Fig. 1. The ClaSSE model with cladogenetic and anagenetic changes. (A) The full model including state-dependent diversification (SDD) is depicted, although a simplified model without SDD was used in our analyses. In the diagram, lineages without floral pigmentation have state 0 and those with pigmentation have state 1. Each speciation event gives rise to two daughters, either in the same state (at rates λ_{000}, λ_{111}) or in different states (λ_{001}, λ_{110}). Thus, changes in pigmentation can occur through the anagenetic (q_{01}, q_{10}) or cladogenetic pathway (λ_{001}, λ_{110}). Extinction rates in each state are represented by μ_0 and μ_1. (B) Examples of the events portrayed in the model shown in (A). Each involves two speciation (lineage-splitting events) but differ by the character changes and extinction events. Top row (left to right): one cladogenetic loss of color; one cladogenetic loss of color followed by an extinction event. Bottom row (left to right): one anagenetic loss of color; one anagenetic loss of color followed by an extinction event.

Fig. 2. Timetrees for four floral radiations. Maximum clade credibility (MCC) trees from relaxed clock analyses. Species with floral anthocyanins shown with filled circles and those lacking floral anthocyanins with open circles.

Fig. 3. Summary of model fitting for the eight possible models and four clades. The two-tone rectangular symbols are visual descriptions of each model. The left side indicates inclusion of cladogenetic change and the right, anagenetic change; black denotes that the change is asymmetric and grey symmetric. The model symbol appears in the row for a clade only if it was present among the top models (less than 2 AIC units different from the best model with lowest AIC). Lines drawn around the symbols show the percentage of trees that included that model

among the top models. Thus, darker lines indicate stronger support for the given model across trees, whereas an absent symbol indicates no support for the model in that clade.

Fig. 4. Tempo and asymmetry of flower color transitions across clades. Rates of gain and loss are calculated as the sum of changes through both modes: ($\lambda_{001} + q_{01}$) for gains, and ($\lambda_{110} + q_{10}$) for losses. The asymmetry (directionality of changes) is the difference between rates of gain and rates of loss, (($\lambda_{001} + q_{01}$) – ($\lambda_{110} + q_{10}$)). 95% credibility intervals are shown below the curves.

Fig. 5. Mode of flower color gains and losses across clades. (A) Rates of total cladogenetic and anagenetic changes are the sums ($\lambda_{001} + \lambda_{110}$) and ($q_{01} + q_{10}$), respectively. These total rates are divided into gains of pigmentation (λ_{001}, q_{01}) in (B) and losses (λ_{110}, q_{10}) in (C). Dashed lines show prior distributions for individual parameters, and 95% credibility intervals are shown below the curves.

Figure 1
Click here to download Figure: Fig1_ClasseModel3.tif

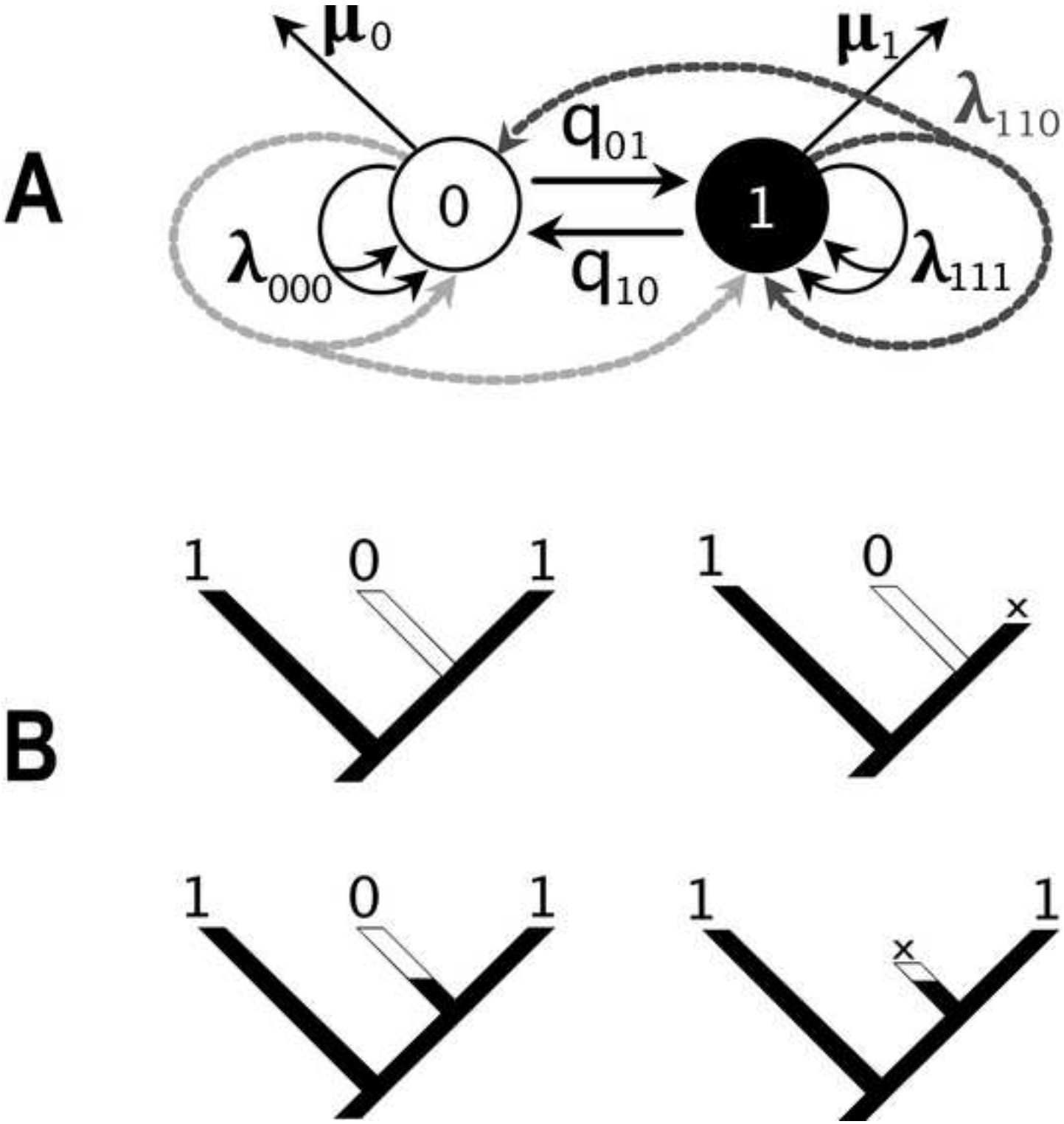

Figure 2

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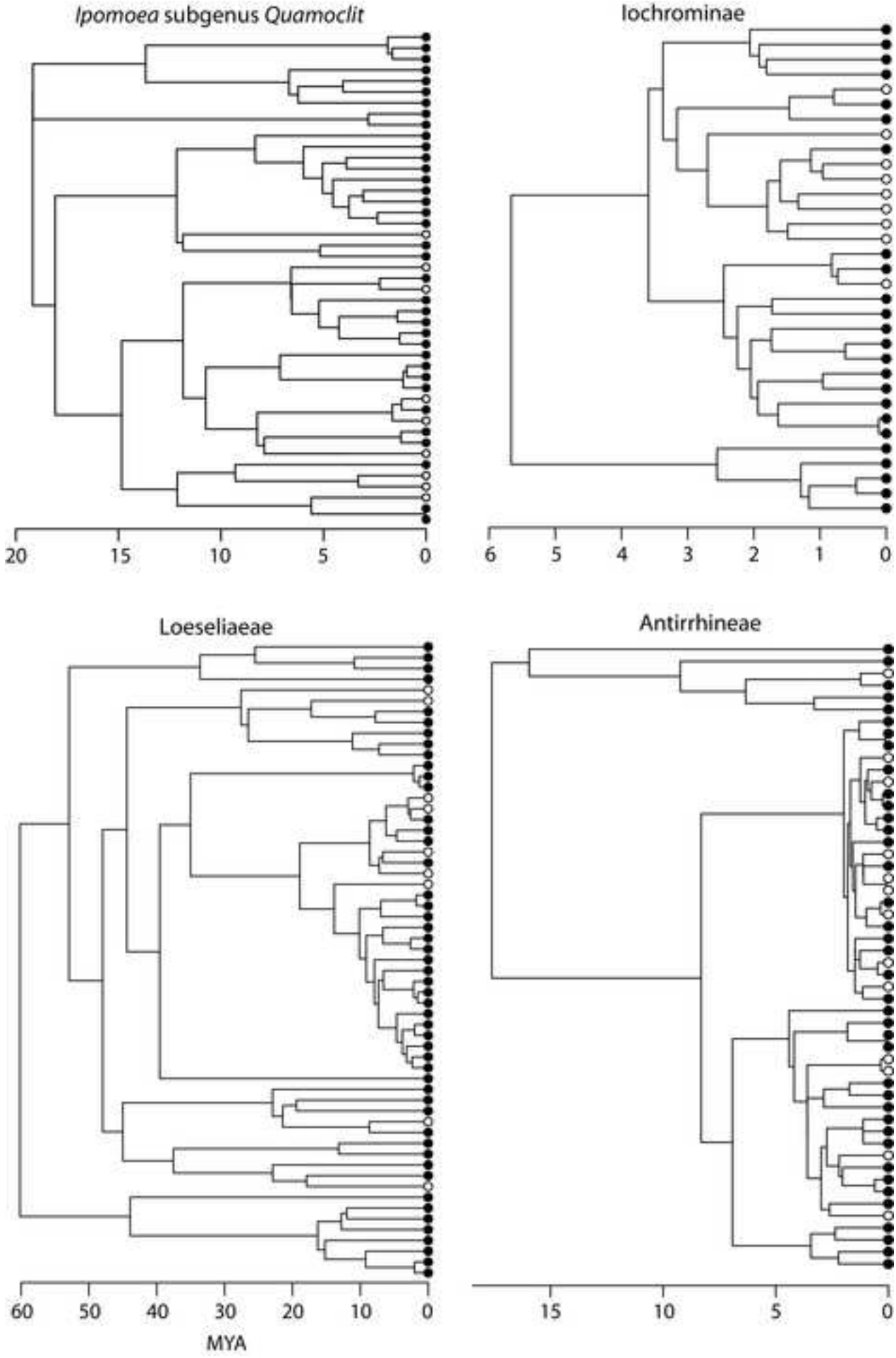

Figure 3
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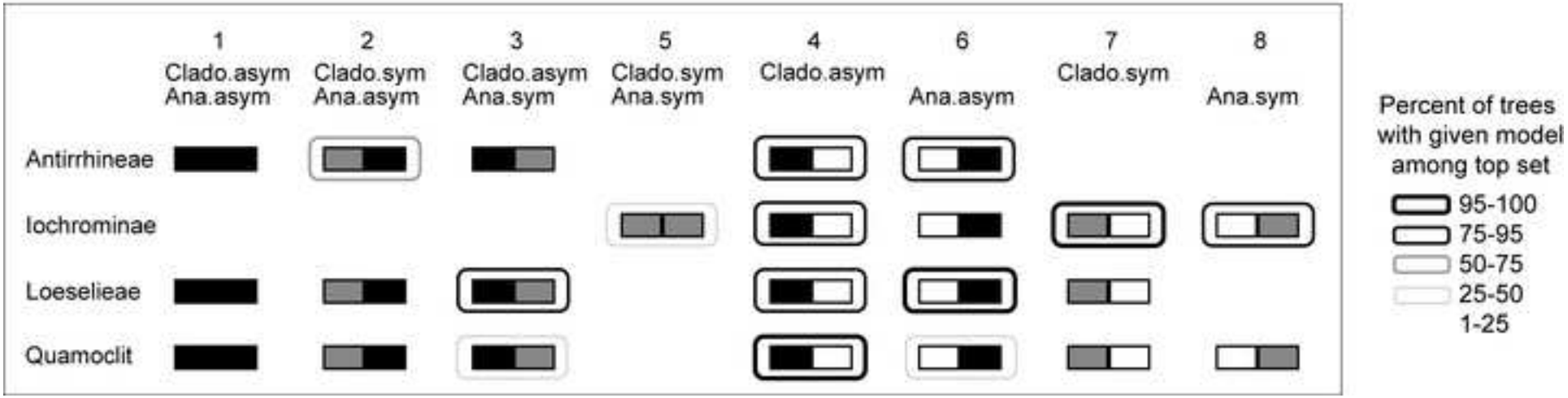

Figure 4
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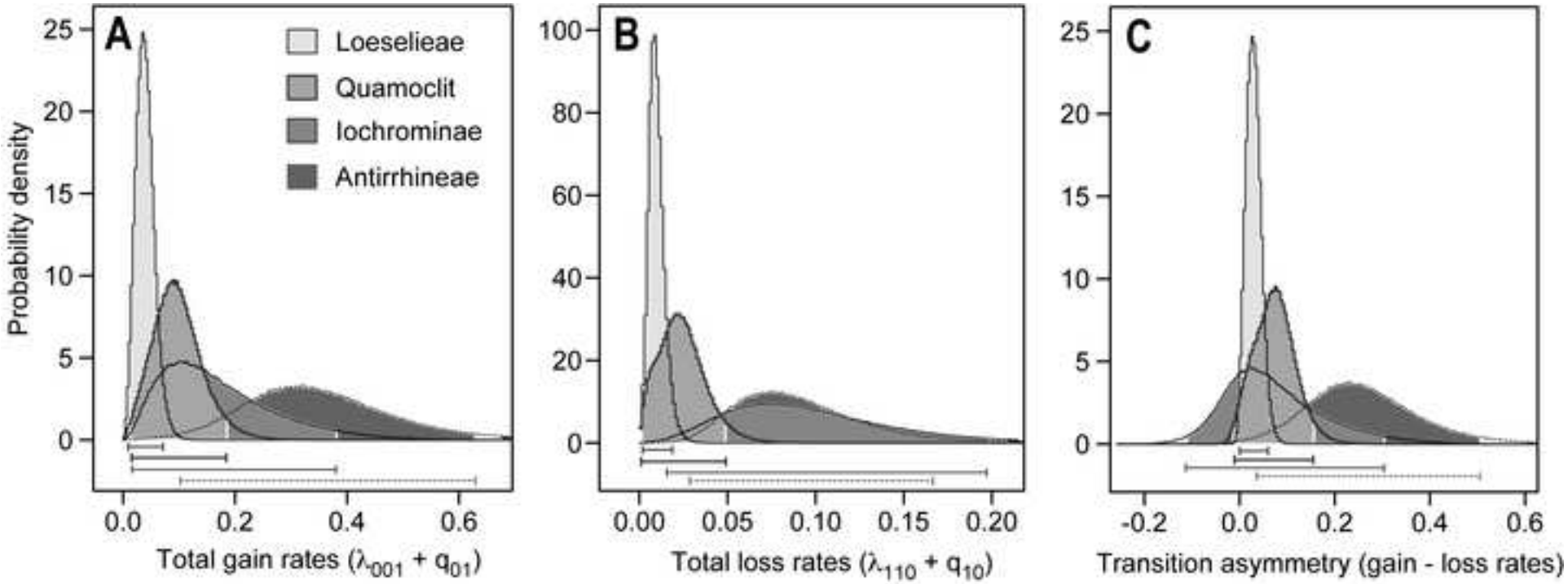

Figure 5
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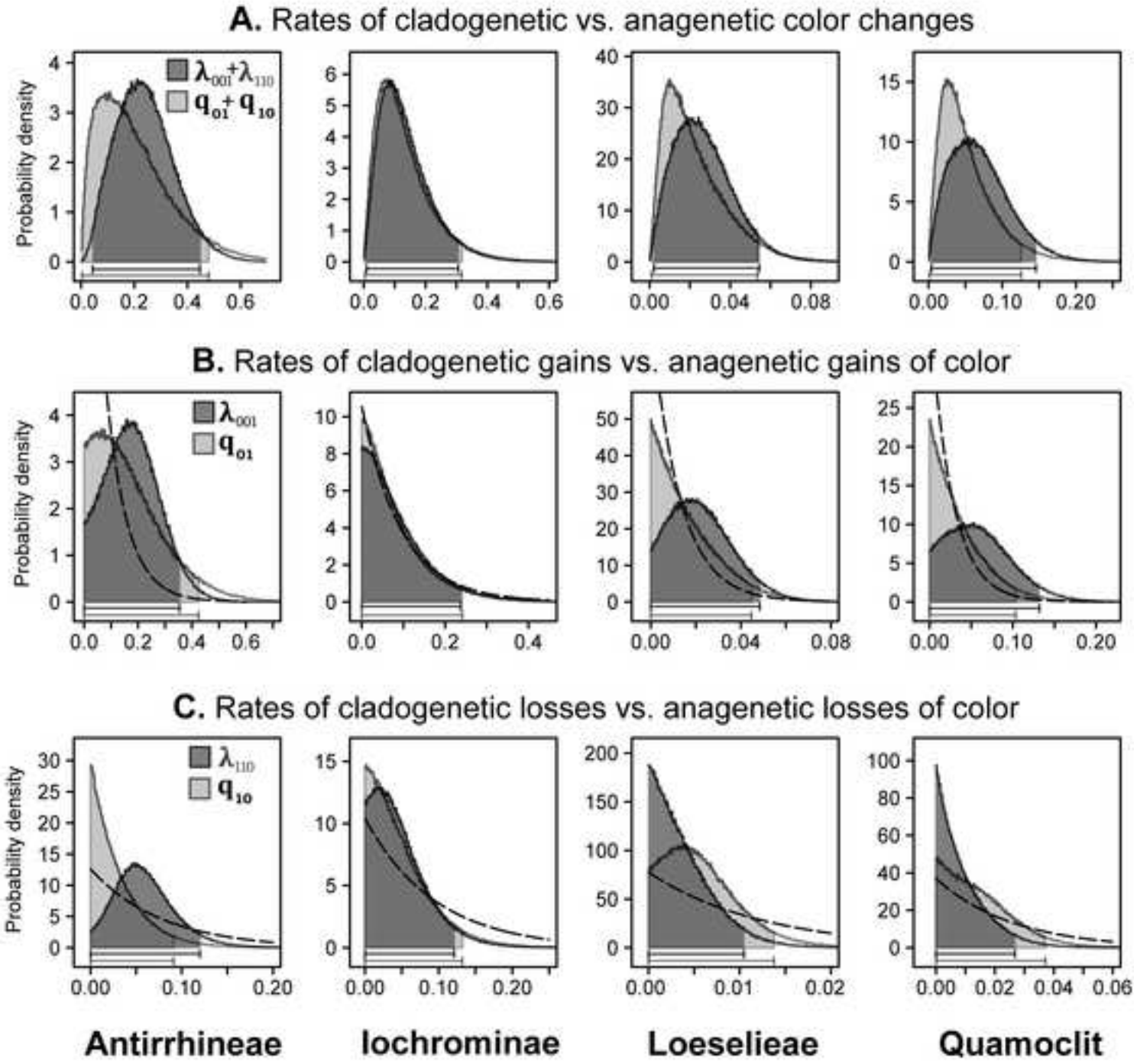


Appendix S1. Taxon sampling for the four datasets. The numbers of extant species estimated to belong in each clade were taken from the sources listed. Scoring of taxa is described in the text and in Appendix S2. Datasets for each clade are available through Dryad (http://dx.doi.org/10.561/dryad.0732.g)

Taxon	Number of extant species	Number of species included	Percentage of extant species pigmented	Percentage of included species pigmented	Loci for phylogenetic inference	Sources
Antirrhineae*	87	52	83.9%	76.9%	ITS, *trnK-matK*	Sutton 1988; Vargas et al. 2004; Oyama and Baum 2004; Vargas et al. 2009
Iochrominae	35	33	74.3%	72.7%	ITS, *leafy*, *waxy*	Smith and Baum 2006; Muchhala et al. 2014
Loeselieae	98	59	84.7%	84.7%	ITS, *trnL-trnF*	Johnson and Weese 2000; Johnson 2007, Johnson et al. 2008; Porter and Johnson 2000; Porter and Steinmann 2009; Porter et al. 2010
Quamoclit clade of *Ipomoea*	87	45	85.1%	80%	ITS	Smith et al. 2010

*Antirrhineae was pruned to the subclade containing the genera *Acanthorrhinum*, *Albraunia*, *Antirrhinum*, *Chaenorrhinum*, *Galvezia*, *Gambelia*, *Holzneria*, *Howelliella*, *Misopates*, *Mohavea*, *Neogarrhinum*, *Psuedomisopates*, and *Sairocarpus*.

Appendix S2. Flower color scoring for Antirrhineae, Iochrominae, Loeselieae, and Quamoclit. Sources for flower color descriptions are given in the main text. Information for Quamoclit was taken from Smith et al. 2010.

ANTIRRHINEAE

Species	Included in phylogeny (has ITS and/or *matK*)	Presence of floral anthocyanins	Flower color description
Acanthorrhinum ramosissimum	y	1	White to Pink
Albraunia foveopilosa	y	1	Pink to Purple
Albraunia fugax	n	1	Blue to Purple
Albraunia psilosperma	n	1	Blue to Purple
Antirrhinum australe	y	1	Pink to Purple
Antirrhinum controversum	y	1	Pink to Purple, or white
Antirrhinum braun_blanquetii	y	1	White with pink
Antirrhinum charidemii	y	1	Pink or white
Antirrhinum cirrhigerum	y	1	Pink
Antirrhinum graniticum	y	1	Pink
Antirrhinum grosii	y	0	White to Yellow
Antirrhinum hispanicum	y	1	White to Pink
Antirrhinum latifolium	y	0	Yellow
Antirrhinum linkianum	y	1	Pink
Antirrhinum litigiosum	y	1	Pink
Antirrhinum lopesianum	y	0	White
Antirrhinum majus	y	1	Pink to Purple
Antirrhinum martenii	n	0	Yellow
Antirrhinum meonanthum	y	0	Yellow
Antirrhinum microphyllum	y	0	White
Antirrhinum molle	y	1	White to Pink
Antirrhinum mollissimum	y	1	White to Pink
Antirrhinum pertegasii	y	0	White

Antirrhinum pulverulentum	y	0	Yellow
Antirrhinum sempervirens	y	1	White with purple
Antirrhinum siculum	y	1	Yellow with pink
Antirrhinum subbaeticum	y	1	Pink
Antirrhinum tortuosum	y	1	Pink
Antirrhinum valentinum	y	1	White with pink
Chaenorrhinum calycinum	n	1	Pink
Chaenorrhinum crassifolium	y	1	Pink to yellow
Chaenorrhinum cryptarum	n	1	White with pink
Chaenorrhinum flexuosum	n	1	Pink
Chaenorrhinum foroughii	n	1	Yellow with purple
Chaenorrhinum glareosum	n	1	Pink to yellow
Chaenorrhinum grandiflorum	n	1	Blue to Purple
Chaenorrhinum grossecostatum	n	1	Blue to Purple
Chaenorrhinum huber-morathii	n	1	Blue to Purple
Chaenorrhinum johnstonii	n	1	Blue to Purple
Chaenorrhinum litorale	n	1	Purple
Chaenorrhinum macropodum	y	1	Lilac
Chaenorrhinum minus	y	1	Yellow to pink
Chaenorrhinum origanifolium	n	1	Purple to pink
Chaenorrhinum reticulatum	n	1	Blue to Purple
Chaenorrhinum robustum	n	1	Blue
Chaenorrhinum rubrifolium	n	1	Blue to Purple, rarely white
Chaenorrhinum rupestre	n	1	Pink
Chaenorrhinum serpyllifolium	n	1	Lilac
Chaenorrhinum tenellum	y	0	White
Chaenorrhinum tuberculatum	n	1	Purple
Chaenorrhinum villosum	n	1	Lilac with yellow
Galvezia fruticosa	y	1	Red
Galvezia leucantha	n	0	White
Galvezia lanceolata	n	1	Red

Galvezia ballii	n	1	Red
Gambelia juncea	y	1	Red
Gambelia glabrata	n	1	Red
Gambelia rupicola	n	1	Red
Gambelia speciosa	y	1	Red
Holzneria microcentron	n	1	White with pink and brown
Holzneria spicata	y	1	White with pink or lilac
Howelliella ovata	y	0	White
Misopates salvagense	n	1	Pink
Misopates oranense	n	1	White to Pink
Misopates chrysothales	n	0	Yellow
Misopates calycinum	y	1	White to Purple
Misopates marraicum	n	1	Purple to pink
Misopates orontium	y	1	Pink
Misopates microcarpum	n	1	Pink
Mohavea confertiflora	y	1	Yellow
Mohavea breviflora	y	0	Yellow
Neogarrhinum strictum	y	1	Purple
Neogarrhinum filipes	y	0	Yellow
Neogarrhinum kelloggii	n	1	Purple
Pseudomisopates rivas-martinezii	y	1	Pink
Sairocarpus coulterianus	y	1	White or Blue
Sairocarpus pusillus	n	1	Blue or White
Sairocarpus kingii	y	0	White
Sairocarpus watsonii	y	1	Blue
Sairocarpus costatus	y	1	Blue to Purple
Sairocarpus multiflorus	y	1	Pink
Sairocarpus nuttallianus	y	1	Pink to Purple
Sairocarpus virga	y	1	Red to Purple
Sairocarpus cornutus	y	1	Blue
Sairocarpus subcordatus	y	1	White with pink

Sairocarpus vexillocalyculatus	y	1	Blue
Sairocarpus breweri	n	1	White to lilac
	(52 total in tree)	(87 extant spp., 72 pigmented)	

IOCHROMINAE

Species	**Included in phylogeny *(leafy, waxy* and/or ITS)**	**Presence of floral anthocyanins**	**Flower color description**
Acnistus arborescens	y	0	White
Dunalia brachyacantha	y	1	Purple
Dunalia obovata	y	1	Purple
Dunalia solanacea	y	0	Yellow to tan
Dunalia spathulata	y	1	Purple
Dunalia spinosa	y	1	Purple
Eriolarynx fasciculata	y	1	Purple with white
Eriolarynx lorentzii	y	1	Purple
Eriolarynx iochromoides	n	1	Purple
Iochroma amicorum	y	1	Purple or white
Iochroma australe	y	1	Purple
Iochroma baumii	y	1	Blue
Iochroma calycinum	y	1	Blue
Iochroma confertiflorum	y	0	White
Iochroma cornfolium	y	1	Blue
Iochroma cyaneum	y	1	Blue
Iochroma edule	y	1	Red to orange

Iochroma ellipticum	y	0	White
Iochroma fuchsioides	y	1	Red
Iochroma gesnerioides	y	1	Red
Iochroma grandiflorum	y	1	Purple
Iochroma lehmannii	y	0	Yellow
Iochroma loxense	y	0	White
Iochroma nitidum	y	1	Purple
Iochroma parvifolium	y	1	Blue
Iochroma peruvianum	y	0	Orange
Iochroma salpoanum	y	0	Yellow
Iohroma stenanthum	n	1	Pink
Iochroma tingoanum	y	1	Purple-brown with green
Iochroma tupayachianum	y	0	White
Iochroma umbellatum	y	1	Purple, brown or green
Saracha punctata	y	1	Purple to brown with yellow
Saracha quitensis	y	1	Purple to brown with yellow
Vassobia breviflora	y	1	Purple
Vassobia dichotoma	y	1	Purple to burgundy
	(33 total in tree)	(35 extant spp., 25 pigmented)	

LOESELIEAE

Species	Included in phylogeny (has ITS and/or trnL-trnF)	Presence of floral anthocyanins	Flower color description
Aliciella caespitosa	y	1	Red
Aliciella formosa	n	1	Pink to purple
Aliciella haydenii	n	1	Pink to purple
Aliciella heterostyla	n	1	White to pink
Aliciella humillima	n	1	White to pink
Aliciella latifolia	y	1	Pink
Aliciella leptomeria	y	1	White to blue
Aliciella lottiae	n	1	White to pink
Aliciella mcvickerae	y	1	Blue
Aliciella micromeria	n	1	White to pink
Aliciella nyensis	n	1	Pink to purple
Aliciella penstemonoides	n	1	Blue
Aliciella pinnatifida	n	1	White to blue
Aliciella ripleyi	n	1	White to blue
Aliciella sedifolia	n	1	Blue
Aliciella stenothrysa	n	1	White to blue
Aliciella subacaulis	n	0	White
Aliciella subnuda	y	1	Red
Aliciella tenuis	y	1	White to blue
Aliciella triodon	y	1	White to pink
Aliciellia hutchinsifolia	y	1	White to blue

Bryantiella_glutinosa	y	0	White
Bryantiella_palmeri	y	1	Purple
Dayia grantii	y	1	Blue
Dayia scabra	y	1	Blue
Eriastrum abramsii	n	1	Yellow with blue
Eriastrum brandegeae	n	0	White or yellow
Eriastrum densifolium	y	1	Blue
Eriastrum diffusum	n	1	Blue
Eriastrum eremicum	n	1	Blue
Eriastrum filifolium	n	0	White
Eriastrum hooveri	n	0	White
Eriastrum luteum	n	0	Yellow
Eriastrum pleuriflorum	n	1	Blue
Eriastrum sapphirinum	n	1	Yellow or blue
Eriastrum signatum	y	1	Blue
Eriastrum sparsiflorum	n	1	Blue
Eriastrum tracyi	n	1	Blue to white
Eriastrum virgatum	n	1	Blue
Eriastrum wilcoxii	y	1	Blue to white
Gilia polyantha whitingii	y	1	Purple
Giliastrum acerosum	n	1	Blue
Giliastrum castellanosii	n	1	Blue
Giliastrum foetidum	y	1	Pink
Giliastrum gypsophilum	n	1	Blue
Giliastrum incisum	n	1	Lavender
Giliastrum insigne	n	1	Blue
Giliastrum ludens	y	1	Blue

Giliastrum purpusii	y	1	Pink
Giliastrum rigidulum	y	1	Purple
Ipomopsis aggregata	y	1	Red
Ipomopsis arizonica	y	1	Red
Ipomopsis congesta	y	0	White
Ipomopsis effusa	y	1	White to pink
Ipomopsis gossipifera	y	1	Pink
Ipomopsis gunnisonii	y	1	Pink
Ipomopsis guttata	y	1	Pink
Ipomopsis havardii	y	1	Pink
Ipomopsis laxiflora	y	1	Blue
Ipomopsis longiflora	y	1	Blue
Ipomopsis macombii	y	1	Blue
Ipomopsis macrosiphon	y	1	Pink
Ipomopsis monticola	y	1	Red
Ipomopsis multiflora	y	1	Pink
Ipomopsis pinnata	y	1	Yellow with purple
Ipomopsis polyantha	y	1	Pink
Ipomopsis polycladon	y	0	White
Ipomopsis pringlei	y	1	Purple
Ipomopsis pumila	y	1	Lavender
Ipomopsis roseata	y	0	White
Ipomopsis rubra	y	1	Red
Ipomopsis sanctispiritus	y	1	Pink
Ipomopsis sonorae	y	1	White to pink
Ipomopsis spicata	y	1	Purple to white
Ipomopsis tenuifolia	y	1	Red

Ipomopsis tenuituba	y	1	White to pink
Ipomopsis thurberi	y	1	Blue
Ipomopsis tridactyla	y	0	White
Ipomopsis wendtii	y	1	Pink
Ipomopsis wrightii	y	0	White
Langloisia setosissima	y	1	Blue
Loeselia amplectens	n	1	White to pink
Loeselia caerulea	y	1	Blue
Loeselia ciliata	y	1	Blue
Loeselia cordifolia	n	1	White to pink
Loeselia glandulosa conglomerata	y	1	Pink
Loeselia grandiflora	n	1	White to pink
Loeselia greggii	n	1	Blue
Loeselia involucrata	y	0	White
Loeselia mexicana	n	1	Red
Loeselia pumila	y	1	Purple to blue
Loeselia purpusii	n	1	Pink
Loeselia rupestris	n	1	Lilac
Loeselia rzedowskii	n	0	White
Loeseliastrum depressum	y	0	White
Loeseliastrum matthewsii	y	1	Pink
Loeseliastrum schottii	n	1	White to pink
Microgilia minutifolia	y	0	White to blue
	(59 total in tree)	(98 extant spp., 83 pigmented)	

QUAMOCLIT

Species	Included in phylogeny (has ITS)	Presence of floral anthocyanins	Flower color description
Ipomoea alba	y	0	White, greenish banded
Ipomoea ampullacea	y	0	White
Ipomoea ancisa	y	0	White
Ipomoea aristolochiifolia	y	1	Limb sky-blue or pink, throat white
Ipomoea barbatisepala	y	1	Light-rosy-purple
Ipomoea bracteata	n	1	Magenta or rarely lavender or greenish
Ipomoea capillacea	n	1	Limb purple, throat pink, basal tube white
Ipomoea cardiophylla	y	1	Dark blue, throat white, tube interior yellow
Ipomoea caudata	n	1	Red-purple
Ipomoea chamelana	y	0	Yellow
Ipomoea chenopodiifolia	y	1	Magenta
Ipomoea cholulensis	y	1	Orange-red
Ipomoea coccinea	y	1	Orange-red or red with yellow tube
Ipomoea collina	n	1	Purple
Ipomoea costellata	y	1	Limb blue, tube white
Ipomoea cristulata	n	1	Orange red
Ipomoea decemcornuta	n	1	Violet
Ipomoea dubia	n	1	Red
Ipomoea dumetorum	y	1	Pink to dark lavender

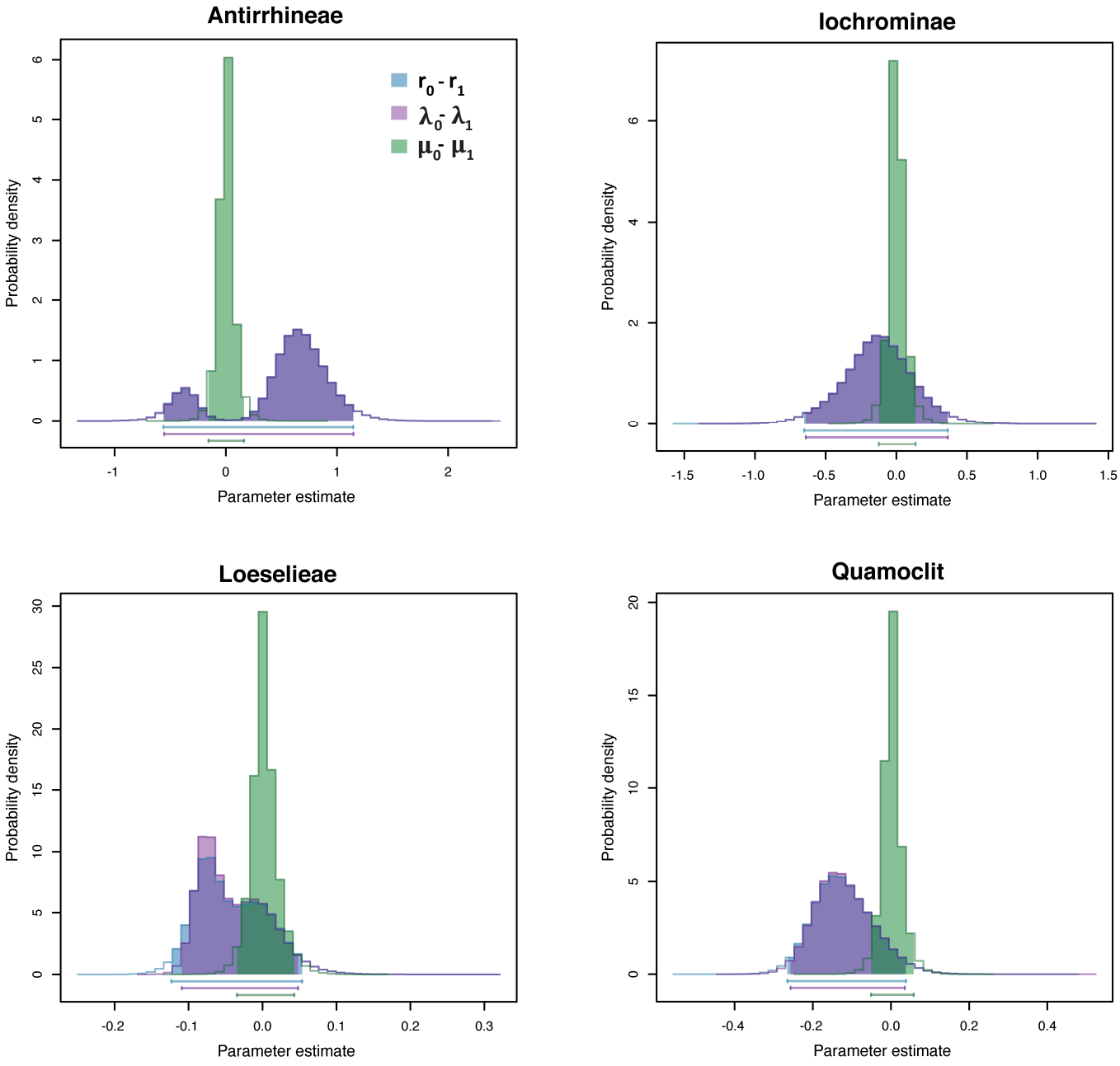
			limb, tube paler
Ipomoea dumosa	y	1	Mauve
Ipomoea elongata	n	1	Red-purple limb
Ipomoea emetica	n	1	Scarlet
Ipomoea eximia	n	1	Purple
Ipomoea expansa	y	1	Pale lavender-blue
Ipomoea fissifolia	n	1	Dark bronzy red or green with faint red tinge
Ipomoea funis	y	1	Limb orange-red
Ipomoea gloverae	n	1	Distal portion striate pigmentation, maroon
Ipomoea hastigera	y	1	Red or orange
Ipomoea hederifolia	y	1	Red or yellow-red
Ipomoea ignava	n	1	Rose or purple
Ipomoea indica	y	1	Limb blue, tube whitish
Ipomoea indivisa	n	1	Red or orange-red
Ipomoea jacalana	n	1	Pink purple
Ipomoea jamaicensis	n	1	Bright crimson to magenta
Ipomoea jicama	n	0	White tube, pale lavender or white limb
Ipomoea laeta	n	1	Purple
Ipomoea lindheimeri	y	1	Lavender, sometimes with white center
Ipomoea lobata	y	1	Red, later becoming whitish or pale yellow
Ipomoea lutea	n	1	Purple
Ipomoea madrensis	n	1	Limb blue-purple, tube pink

Ipomoea magniflora	n	0	White
Ipomoea mairetii	y	1	Pink limb, white tube
Ipomoea marginisepala	y	1	Limb sky blue, throat white, interior tube yellow
Ipomoea mcvaughii	n	1	White tube, pink limb
Ipomoea meyeri	y	1	Limb sky-blue, interior yellow
Ipomoea microsepala	y	0	Yellow
Ipomoea minutiflora	y	0	Yellow
Ipomoea miquihuanensis	n	1	Purple
Ipomoea monticola	n	1	Rose
Ipomoea muricata	y	1	Limb lilac, interior violet
Ipomoea neei	y	1	Yellow or violet or yellow with purple or violet markings
Ipomoea neurocephala	y	0	Whitish
Ipomoea nil	y	1	Blue, purple or almost scarlet, throat often white
Ipomoea noctulifolia	n	1	Red-purple limb, white tube
Ipomoea orizabensis	y	1	Limb magenta-purple, tube white or rose
Ipomoea parasitica	n	1	Limb blue-purple, tube white
Ipomoea perpartita	n	1	Purple inside, limb white
Ipomoea piurensis	n	1	White or with a rose limb and darker tube
Ipomoea plummerae	n	1	Limb purple, tube pink

Ipomoea praematura	y	1	Tube greenish pink, limb alternating pink and orange
Ipomoea pubescens	y	1	Limb blue, tube white
Ipomoea puncticulata	n	0	White or pale pink along interplicae
Ipomoea purga	y	1	Magenta
Ipomoea purpurea	y	1	Limb blue and purple, tube white or rose
Ipomoea quamoclit	y	1	Crimson or white
Ipomoea rubriflora	n	1	Red
Ipomoea santillanii	y	0	White
Ipomoea sawyeri	n	1	Limb lavender, tube white
Ipomoea schaffneri	n	1	Rose
Ipomoea seducta	y	1	Mauve
Ipomoea sescossiana	y	1	Purple
Ipomoea simulans	n	1	Magenta
Ipomoea spectata	n	1	Red or orange
Ipomoea stans	y	1	Purple
Ipomoea subrevoluta	n	1	Lavender or purple
Ipomoea suffulta	n	1	Red-purple or white limb, white tube
Ipomoea tastensis	n	0	White or pale pink along interplicae
Ipomoea temascaltepecensis	n	1	Limb purple, tube white or pale pink
Ipomoea tenuiloba	n	1	White, pink, or purple
Ipomoea ternifolia	y	1	Limb bluishm lavender

			or cream, throat white or yellow inside
Ipomoea thurberi	n	1	Purple
Ipomoea tricolor	n	1	Limb sky blue, throat white, interior tube yellow
Ipomoea tuboides	y	0	White
Ipomoea uhdeana	y	1	Red
Ipomoea variabilis	y	1	Blue or purple, tube white
Ipomoea velardei	n	1	Violet-blue, greenish within
Ipomoea villifera	n	1	Purple
	(45 total in tree)	(87 extant spp., 74 pigmented)	

Appendix S3. Posterior distributions for the differences between BiSSE rate parameters. For each clade and each step in the BiSSE MCMC analysis, the difference was calculated between speciation, extinction, and diversification rates in each state (λ_0 and λ_1, μ_0 and μ_1, r_0 and r_1, respectively). The two parameters are not judged as significantly different if the 95% credibility interval (shown with brackets below each distribution) includes zero. Because extinction rates were similar in each state (0 and 1), the distribution for the speciation rate difference (in purple) and the diversification rate difference (in blue) closely overlaps for all clades.



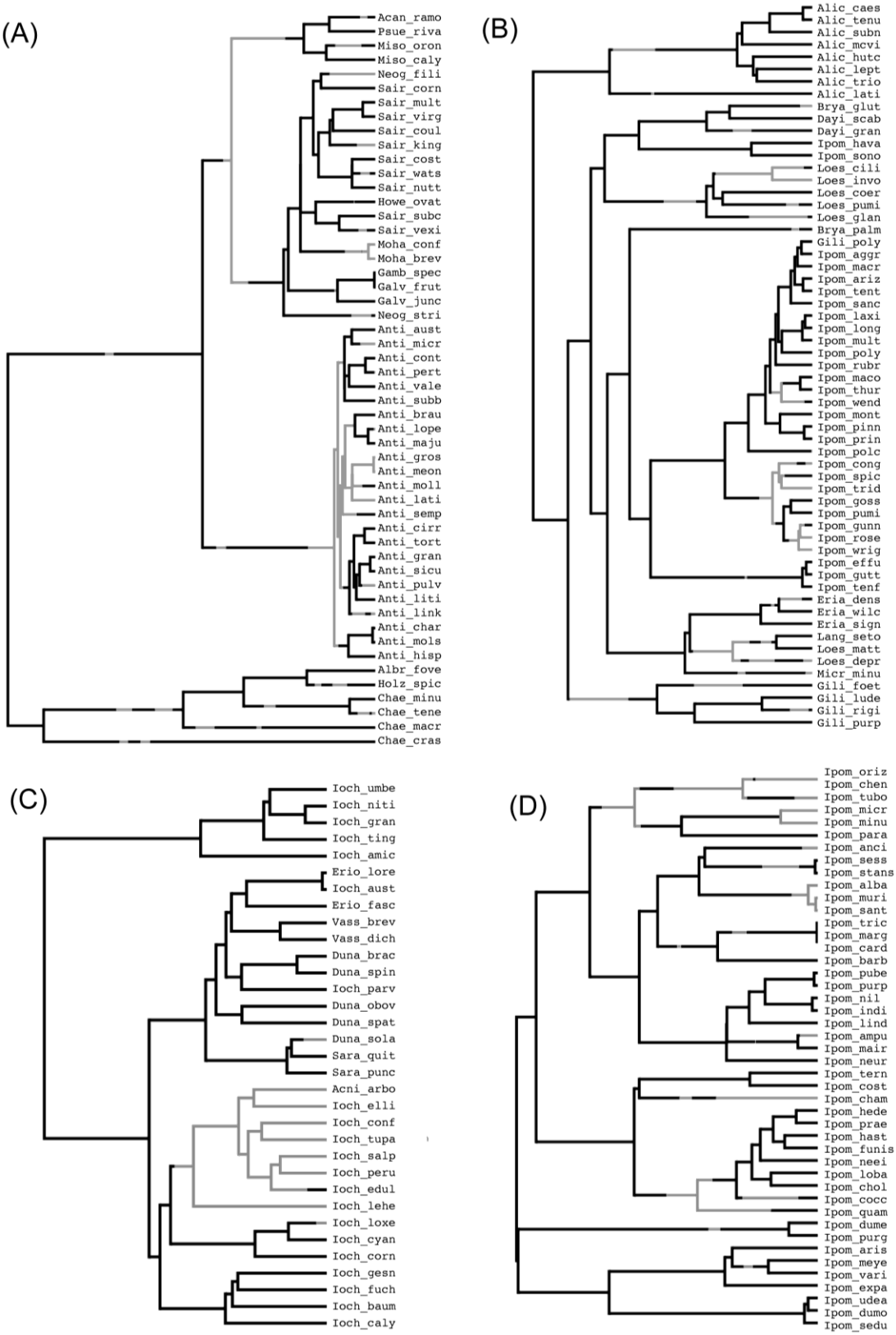
Appendix S4. Best models of flower color evolution across the four datasets. The eight models in Table 1 were fit for up to 100 Bayesian timetrees from each clade. Top models are those that have difference of less than 2 in AIC score; thus each tree could have multiple models among the top models. The top models excluded were not among the top models for any of the trees. The best models (those with the lowest AIC score) are ranked by the number of trees having that model as the best. For example, 50 of the 76 Antirrhineae trees that were fit with all eight models gave the lowest AIC score to the Ana.sym model.

Clade	% of trees completed	Range of # top models Per Tree	Mean # Top Models Per Tree	Top Models Included (# of trees with the model)	Top Models Excluded	Best models across trees (# trees)
Antirrhineae	76	1 to 4	2.54	Clado.asym.Ana.asym (3), Clado.sym.Ana.asym (48), Clado.asym.Ana.sym (3), Clado.asym (68), Ana.asym (71)	Clado.sym.Ana.sym, Clado.sym, Ana.sym	Ana.asym (50), Clado.asym (24), Clado.sym.Ana.asym (2)
Iochrominae	100	2 to 5	3.36	Clado.sym.Ana.sym (36), Clado.asym (93), Ana.asym (19), Clado.sym (99), Ana.sym (78)	Clado.asym.Ana.asym, Clado.sym.Ana.asym, Clado.asym.Ana.sym	Clado.sym (88), Ana.sym (16), Clado.asym (2)
Loeselieae	100	1 to 4	2.60	Clado.asym.Ana.asym (2), Clado.sym.Ana.asym (1), Clado.asym.Ana.sym (75), Clado.asym (80), Clado.sym (1), Ana.asym (100)	Clado.sym.Ana.sym, Ana.sym	Ana.asym (92), Clado.asym (7), Clado.asym.Ana.sym (1)
Quamoclit	91	1 to 5	1.90	Clado.asym.Ana.asym (2), Clado.sym.Ana.asym (2), Clado.asym.Ana.sym (40), Clado.asym (90), Ana.asym (27), Clado.sym (5), Ana.sym (6)	Clado.sym.Ana.sym	Clado.asym (83), Ana.asym (6), Clado.asym.Ana.sym (2)

Appendix S5. Parameter estimates from full model (model 1, Clado.asym.Ana.asym, Table 1). This model has six free parameters: λ_{000}, λ_{001}, λ_{110}, μ, q_{01}, q_{10}. See text for description. Compound parameters (e.g. total transition rate) were computed from these individual parameters as shown in the table. Median parameter estimates with 95% credibility intervals from MCMC analysis.

Clade	Antirrhineae	Iochrominae	Loeselieae	Quamoclit
Speciation, λ_{000}	0.24 (0.04, 0.47)	0.46 (0.22, 0.74)	0.06 (0.02, 0.09)	0.13 (0.04, 0.23)
Extinction, μ_0	0.12 (1.7×10^{-6}, 0.35)	0.02 (6.6×10^{-7}, 0.11)	0.01 (1.5×10^{-7}, 0.04)	0.01 (4.9×10^{-8}, 0.05)
Total gain rate, $\lambda_{001} + q_{01}$	0.34 (0.10, 0.62)	0.15 (0.02, 0.38)	0.04 (0.01, 0.07)	0.1 (0.02, 0.18)
Total loss rate, $\lambda_{110} + q_{10}$	0.09 (0.03, 0.17)	0.09 (0.03, 0.20)	0.01 (0.002, 0.02)	0.02 (0.001, 0.05)
Transition asymmetry, $(\lambda_{001} + q_{01}) - (\lambda_{110} + q_{10})$	0.25 (0.04, 0.51)	0.06 (-0.11, 0.30)	0.03 (0.0001, 0.06)	0.07 (-0.01, 0.15)
Total cladogenetic rate, $\lambda_{001} + \lambda_{110}$	0.23 (0.04, 0.45)	0.12 (0.01, 0.31)	0.03 (0.002, 0.05)	0.07 (0.004, 0.15)
Total anagenetic rate, $q_{01} + q_{10}$	0.17 (0.003, 0.48)	0.11 (0.004, 0.32)	0.02 (0.001, 0.05)	0.04 (0.001, 0.13)
Asymmetry in mode, $(\lambda_{001} + \lambda_{110}) - (q_{01} + q_{10})$	0.06 (-0.36, 0.39)	0.007 (-0.26, 0.26)	0.006 (-0.04, 0.05)	0.02 (-0.10, 0.13)
Cladogenetic gain rate, λ_{001}	0.15 (4.3×10^{-6}, 0.34)	0.07 (5.1×10^{-7}, 0.24)	0.02 (2.1×10^{-6}, 0.05)	0.06 (6.6×10^{-6}, 0.13)
Cladogenetic loss rate, λ_{110}	0.05 (0.0003, 0.12)	0.04 (1.0×10^{-6}, 0.12)	0.003 (6.0×10^{-8}, 0.01)	0.007 (5.2×10^{-8}, 0.03)
Anagenetic gain rate, q_{01}	0.13 (3.4×10^{-6}, 0.42)	0.06 (2.4×10^{-7}, 0.24)	0.01 (1.1×10^{-7}, 0.04)	0.03 (-4.8×10^{-7}, 0.10)
Anagenetic loss rate, q_{10}	0.02 (1.0×10^{-7}, 0.09)	0.04 (8.6×10^{-7}, 0.13)	0.005 (6.7×10^{-8}, 0.01)	0.01 (-2.8×10^{-8}, 0.04)
Asymmetry in mode of gains, $(\lambda_{001} - q_{01})$	0.03 (-0.37, 0.35)	0.005 (-0.23, 0.24)	0.008 (-0.04, 0.05)	0.03 (-0.09, 0.13)
Asymmetry in mode of losses, $(\lambda_{110} - q_{10})$	0.03 (-0.07, 0.12)	0.002 (-0.13, 0.12)	-0.002 (-0.01, 0.01)	-0.004 (-0.04, 0.03)

Appendix S6. Visualizations of potential character histories with stochastic mapping (SM). Median gain and loss rates (Appendix S5) were used to simulate histories with SIMMAP 1.0 (Bollback, 2006). A single representative history is shown per clade: (A) Antirrhineae, (B) Loeselieae, (C) Iochrominae, (D) Quamoclit. Taxon names are abbreviated with the first four letters of the genus name, underscore, and first four letters of specific epithet. See Appendix S2 for full taxon list.



Appendix S7. Equilibrium frequencies expected from estimated models. These expectations were computed from median values for parameter estimates (Appendix S5) using the stationary.freq.classe function in the Diversitree package.

Clade	**Observed proportions of unpigmented vs. pigmented flowers**	**Equilibrium ratio of unpigmented:pigmented flowers**
Antirrhineae	16% vs. 84%	20% vs. 80%
Iochrominae	26% vs. 74%	38% vs. 62%
Loeselieae	15% vs. 85%	21% vs. 79%
Quamoclit	15% vs. 85%	25% vs. 75%