

SPECIAL INVITED PAPER

Tempo and mode of flower color evolution¹

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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 examined the tempo and mode of flower color evolution in four clades (Antirrhineae, Iochrominae, Loeseliaceae, Quamoclit) using models that incorporate trait transitions and lineage diversification. Focusing on floral anthocyanin pigmentation, we estimated rates of gain and loss of pigmentation and tested whether these changes occur predominantly through anagenesis or cladogenesis.

KEY RESULTS: We found that the tempo of pigment gains and losses varied significantly across the clades and that the rates of change were 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; floral color; 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 fundamental principle 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 by 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 instance, 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., Pérez et al., 2006; Martén-Rodríguez 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

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act as key innovations, increasing speciation in lineages that possess them (Sargent, 2004; de Vos et al., 2014)?

One 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 et al., 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 (Rauscher, 2006; Campanella et al., 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 Rauscher, 2009; Smith and Rauscher, 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 et al., 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 et al., 2008).

The current 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), Loeseliae (Porter and Johnson, 2000), and *Ipomoea* subg. *Quamoclit* (Miller et al., 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 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

Data set 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 et al., 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). 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 data sets 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 data sets 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 et al., 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 (Paafe et al., 2008; Särkinen et al., 2013), Loeseliae (Porter et al., 2010), and the *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 data sets 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 the program Tracer v.1.6 (Rambaut et al., 2014). Also, each run was repeated twice to ensure similar results. We subsampled the post burn-in trees using the LogCombiner program in BEAST v. 2.1.2 (Bouckaert et al., 2014) 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 Rauscher, 2011), Loeseliae (Harborne and Smith, 1978; Nakazato et al., 2013), and the *Quamoclit* clade of morning glories (Eich, 2008; Des Marais and Rauscher, 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 Loeseliae;

and Smith et al. (2010) for Quamoclit. Color descriptions were verified when possible by examining images or specimens in the Tropicos (www.tropicos.org) and CalFlora (www.calflora.org) databases.

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 et al., 2007; Goldberg and Igić, 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 data sets 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 et al., 2007) as implemented in the R package Diversitree 0.9-7 (FitzJohn, 2012). We incorporated unsampled taxa with the “skeleton tree” approach (FitzJohn et al., 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 Igić, 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 ($\lambda_{111} + \lambda_{110}$).

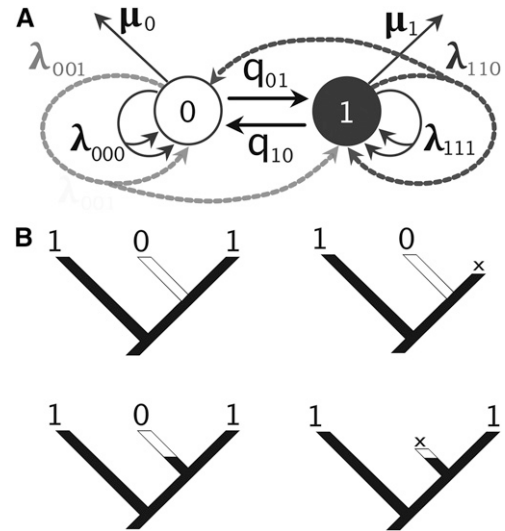


FIGURE 1 The ClaSSE model with cladogenetic and anagenetic changes. (A) The full model including state-dependent diversification is depicted, although a simplified model without was used in our analyses. In the diagram, lineages without floral pigmentation have state 0, and those with pigmentation have 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.

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 data sets. The set of top models for each data set 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 nonnested 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

TABLE 1. Cladogenetic and anagenetic models. Model parameters include speciation rates with no character change ($\lambda_{000}, \lambda_{111}$), speciation rates with character change ($\lambda_{001}, \lambda_{110}$), 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 Materials and methods, Cladogenetic and anagenetic model fitting) 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 ($\mu_0 = \mu_1 = \mu$). When speciation rate does not depend on character state, 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; change can be asymmetric	$\lambda_{111} = \lambda_{000} + \lambda_{001} - \lambda_{110}$	6: $\lambda_{000}, \lambda_{001}, \lambda_{110}, \mu, q_{01}, q_{10}$
2. Clado.sym.Ana.asym Both modes of change possible; only anagenetic change can be asymmetric	$\lambda_{111} = \lambda_{000} = \lambda_N; \lambda_{001} = \lambda_{110} = \lambda_c$	5: $\lambda_N, \lambda_c, \mu, q_{01}, q_{10}$
3. Clado.asym.Ana.sym Both modes of change possible; only cladogenetic change can be asymmetric	$\lambda_{111} = \lambda_{000} + \lambda_{001} - \lambda_{110}; \mu_0 = \mu_1$	5: $\lambda_{000}, \lambda_{001}, \lambda_{110}, \mu, q$
4. Clado.asym Only cladogenetic change possible; change can be asymmetric	$\lambda_{111} = \lambda_{000} + \lambda_{001} - \lambda_{110}; q_{01} = 0; q_{10} = 0$	4: $\lambda_{000}, \lambda_{001}, \lambda_{110}, \mu$
5. Clado.sym.Ana.sym Both modes of change possible; change can only be symmetric	$\lambda_{111} = \lambda_{000} = \lambda_N; \lambda_{001} = \lambda_{110} = \lambda_c; q_{01} = q_{10} = q$	4: $\lambda_N, \lambda_c, \mu, q$
6. Ana.asym Only anagenetic change possible; change can be asymmetric	$\lambda_{111} = \lambda_{000} = \lambda_N; \lambda_{001} = 0; \lambda_{110} = 0$	4: $\lambda_N, \mu, q_{01}, q_{10}$
7. Clado.sym Only cladogenetic change possible; change can only be symmetric	$\lambda_{111} = \lambda_{000} = \lambda_N; \lambda_{001} = \lambda_{110} = \lambda_c; q_{01} = 0; q_{10} = 0$	3: $\lambda_N, \lambda_c, \mu$
8. Ana.sym Only anagenetic change possible; change can only be symmetric	$\lambda_{111} = \lambda_{000} = \lambda_N; \lambda_{001} = 0; \lambda_{110} = 0; q_{01} = q_{10} = q$	3: λ_N, μ, q

a symmetric model (for scripts and all input data, see the Dryad database, <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: $\lambda_{001}, \lambda_{110}, q_{01}, q_{10}$) 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 toward either state (Fig. 2; online Appendices S1, S2). 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 the 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). 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

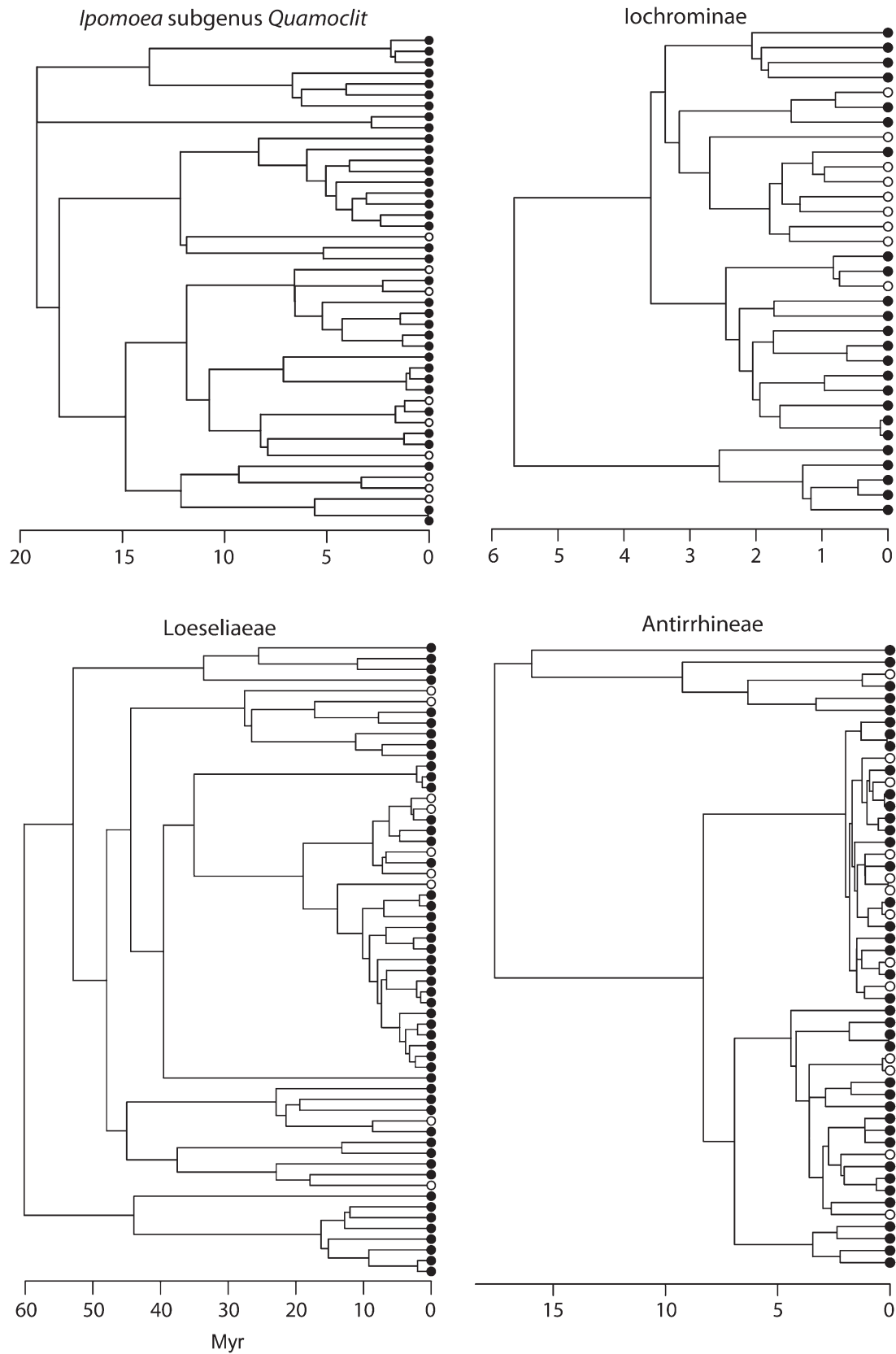


FIGURE 2 Timetrees for four floral radiations. Maximum clade credibility (MCC) trees from relaxed clock analyses. Species with floral anthocyanins shown with filled circles, those lacking floral anthocyanins with open circles.

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 data sets, and most trees had two or three top models (less than two AIC units different; online Appendix S4). 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) did not fit well for the larger data sets (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 data sets. Between these two models, ana.asym was more commonly supported by trees for Antirrhineae and Loeseliae, 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 data set 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 data sets, we could not conclude that character change has been through only one mode or equally likely in either direction. Furthermore, because multiple nonnested models are compatible with the data for each clade, there is no basis for focusing on any one simpler model for any clade. Thus, 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 8-fold, with the lowest in Loeseliae (0.04 Myr⁻¹; online Appendix S5) and the highest in Antirrhineae (0.34 Myr⁻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 Myr⁻¹ would translate to one expected transition after 10 Myr. Taking Loeseliae as an example, with a gain rate of 0.04 Myr⁻¹, a lineage lacking anthocyanin pigmentation (state 0) would wait on average 25 Myr to transition to state 1. The nonoverlapping credibility intervals of the gain rates for Loeseliae 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 Loeseliae 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 (online Appendix S6).

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 Loeseliae (Fig. 4C; Appendix S5). These results effectively reject symmetrical flower color transitions for

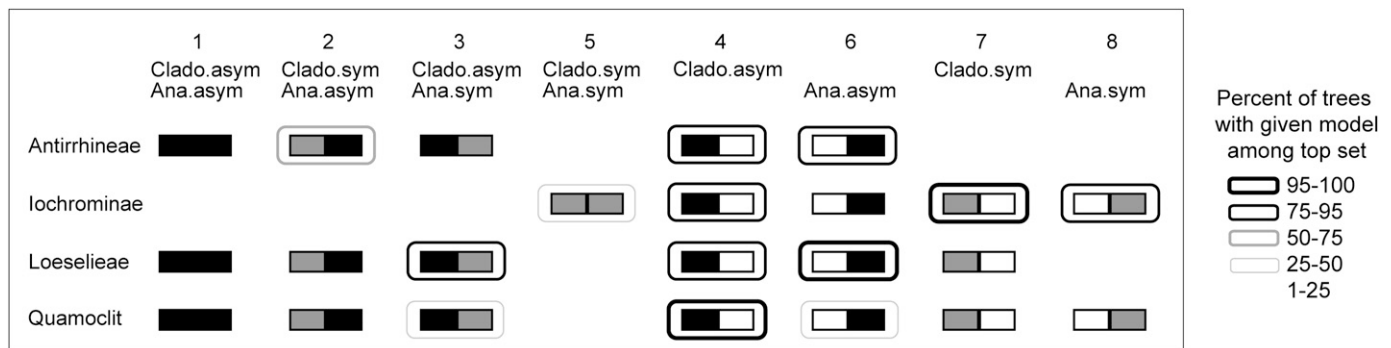


FIGURE 3 Summary of model fitting for the eight possible models and four clades. The two-tone rectangular symbols are visual descriptions of each model. The left side indicates inclusion of cladogenetic change and the right, anagenetic change; black denotes that the change is asymmetric, gray symmetric, and white that the mode (anagenetic or cladogenetic) is not in the model. 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.

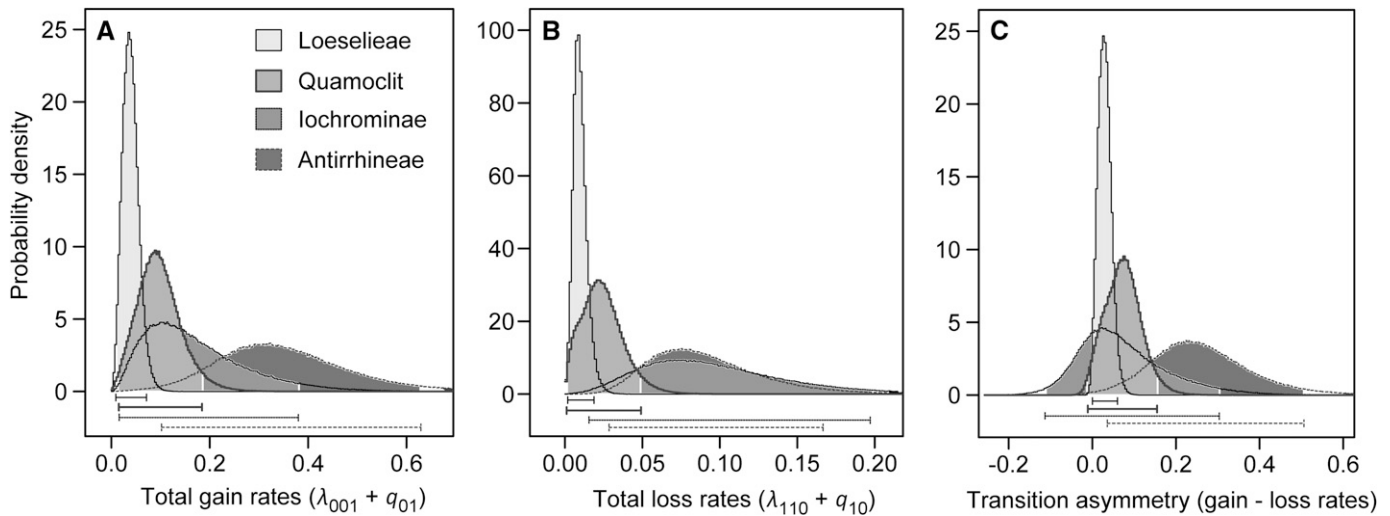


FIGURE 4 Tempo and asymmetry of flower color transitions across clades. Rates of gain and loss are calculated as the sum of changes through both modes: $(\lambda_{001} + q_{01})$ for gains and $(\lambda_{110} + q_{10})$ for losses. The asymmetry (directionality of changes) is the difference between rates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

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 data sets except for Iochrominae.

We next considered how flower color transitions were partitioned between the anagenetic and cladogenetic modes. Models with 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 vs. anagenetic) might vary with the type of change (gain vs. 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 $\lambda_{110} > q_{10}$). Cladogenetic change was indeed more common 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, Loeseliaceae 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), and they indicate that species with pigmented flowers will remain two to four times more common than those with unpigmented flowers given the estimated rates of diversification and transition.

DISCUSSION

Flower color has been a focal trait for the study of evolutionary processes within species because of its selective importance (Rauscher, 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 toward cladogenetic change, particularly for gains of pigmentation. Overall, we observed a trend toward gains of floral pigmentation, a result that runs counter to the notion that transitions will often be biased toward 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

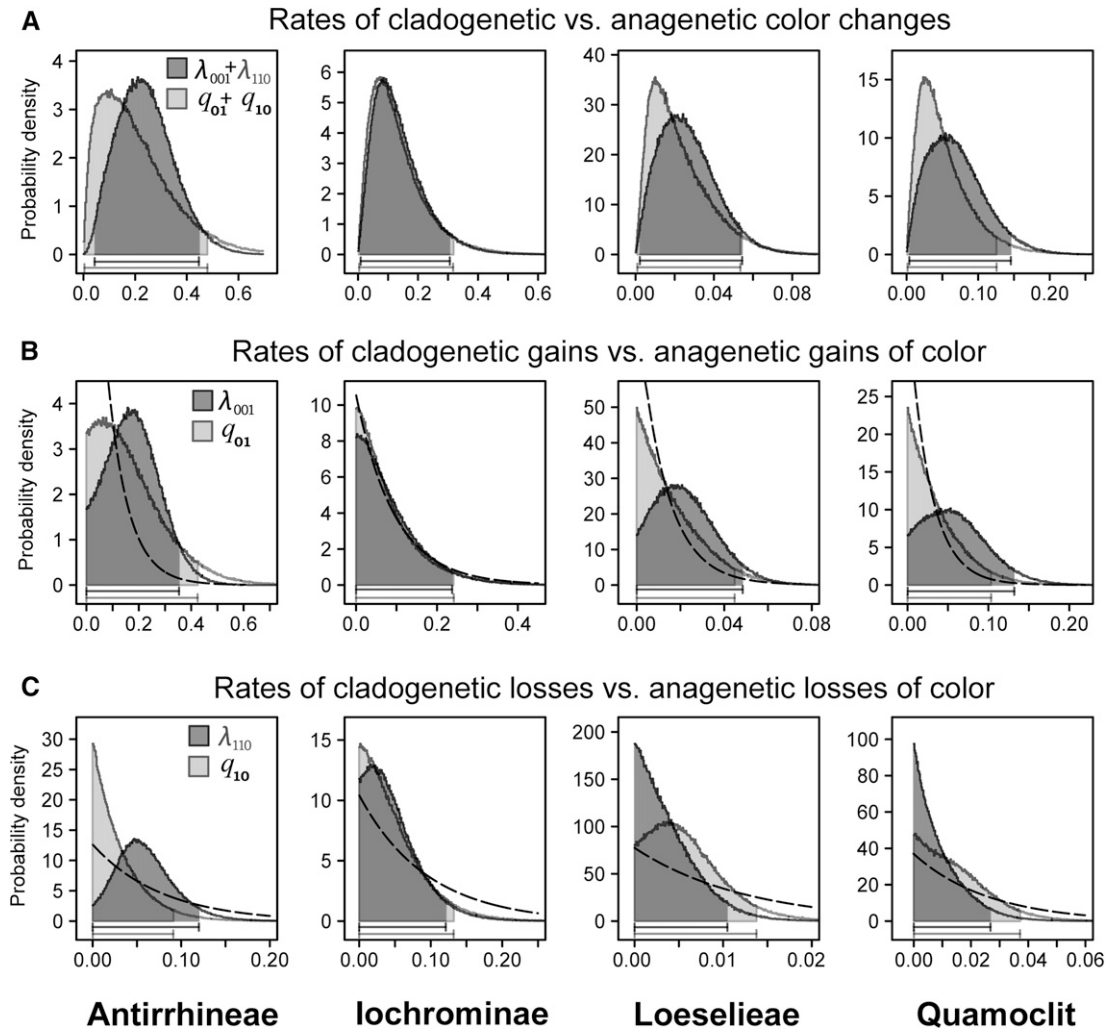


FIGURE 5 Mode of flower color gains and losses across clades. (A) Rates of total cladogenetic and anagenetic changes are the sums ($\lambda_{001} + \lambda_{110}$) and ($q_{01} + q_{10}$), respectively. These total rates are divided into gains of pigmentation (λ_{001} , q_{01}) in (B) and losses (λ_{110}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

quantitative variation in flower color than for other floral traits (Smith et al., 2008; McEwen and Vamossi, 2010; Muchhala et al., 2014), although low signal alone is insufficient to conclude high rates of evolution (Revell et al., 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 et al., 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 et al., 2010). By contrast, *Ipomoea* subgenus *Quamoclit* is a group of neotropical vines pollinated by hummingbirds and insects (McDonald, 1991; Miller et al., 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, floral pigmentation may be gained 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 the anthocyanin pathway are the predominant mechanism responsible for gains of floral anthocyanin pigmentation (Cooley et al., 2011; Streisfeld et al., 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 et al., 2013). Losses of floral pigmentation can occur 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 blue to red transitions involving switches to hummingbird pollination in *Penstemon* (Wilson et al., 2006). Transitions from unpigmented to pigmented flowers, as suggested by our study, could be favored by a range of selective forces, from pollinator preference (Lunau and Maier, 1995) to thermoregulation (Lacey et al., 2010) or herbivory (Irwin et al., 2003). Overall, bias in favor of gains vs. losses of pigmentation provides a viable explanation for the high frequency of species with floral pigmentation (Fig. 2) as this directionality should lead to the predominance of pigmented taxa at equilibrium (Nosil and Mooers, 2005).

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

To the extent that flower color plays a role in speciation events, it is important to 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 issue 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 nonfloral, 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 data sets, 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 et al., 2015) and the ecological factors that may exert selection on this segregating variation (Strauss and Whittall, 2006; Rausher, 2008; Muchhala et al., 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 scales. The potential for these flower color changes to be commonly and directly involved with cladogenesis is less clear, and thus future comparative studies could 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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LITERATURE CITED

- Alcantara, S., and L. G. Lohmann. 2011. Contrasting phylogenetic signals and evolutionary rates in floral traits of Neotropical lianas. *Biological Journal of the Linnean Society* 102: 378–390.
- Armbruster, W. S. 2002. Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. *Journal of Evolutionary Biology* 15: 468–486.
- Bollback, J. P. 2006. SIMMAP: Stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* 7: 88.
- Bouckaert, R., J. Heled, D. Kuhnert, T. Vaughan, C. H. Wu, D. Xie, M. A. Suchard, et al. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10: e1003537.
- Bradshaw, H. D., S. M. Wilbert, K. G. Otto, and D. W. Schemske. 1995. Genetic mapping of floral traits associated with reproductive isolation in monkey-flowers (*Mimulus*). *Nature* 376: 762–765.
- Burd, M., C. T. Stayton, M. Shrestha, and A. G. Dyer. 2014. Distinctive convergence in Australian floral colours seen through the eyes of Australian birds. *Proceedings of the Royal Society, B, Biological Sciences* 281: 20132862.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multi-model inference: A practical information-theoretic approach, 2nd ed. Springer, New York, New York, USA.
- Campanella, J. J., J. V. Smalley, and M. E. Dempsey. 2014. A phylogenetic examination of the primary anthocyanin production pathway of the Plantae. *Botanical Studies (Taipei, Taiwan)* 55: 10.
- Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology* 70: 1–9.
- Coberly, L. C., and M. D. Rausher. 2003. Analysis of a chalcone synthase mutant in *Ipomoea purpurea* reveals a novel function for flavonoids: Amelioration of heat stress. *Molecular Ecology* 12: 1113–1124.
- Coburn, R. A., R. H. Griffin, and S. D. Smith. 2015. Genetic basis for a rare floral mutant in an Andean species of Solanaceae. *American Journal of Botany* 102: 264–272.
- Cooley, A. M., J. L. Modliszewski, M. L. Rommel, and J. H. Willis. 2011. Gene duplication in *Mimulus* underlies parallel floral evolution via independent trans-regulatory changes. *Current Biology* 21: 700–704.
- Davis, C. C., M. Latvis, D. L. Nickrent, K. J. Wurdack, and D. A. Baum. 2007. Floral gigantism in Rafflesiaceae. *Science* 315: 1812.
- de Vos, J. M., C. E. Hughes, G. M. Schneeweiss, B. R. Moore, and E. Conti. 2014. Heterostyly accelerates diversification via reduced extinction in primroses. *Proceedings of the Royal Society, B, Biological Sciences* 281: 20140075.
- Des Marais, D. L., and M. D. Rausher. 2010. Parallel evolution at multiple levels in the origin of hummingbird pollinated flowers in *Ipomoea*. *Evolution* 64: 2044–2054.
- Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. *Nature* 400: 354–357.
- Eich, E. 2008. Solanaceae and Convolvulaceae: Secondary metabolites. Springer, Berlin, Germany.
- Endress, P. K. 2011. Evolutionary diversification of the flowers in angiosperms. *American Journal of Botany* 98: 370–396.
- Eserman, L. A., G. P. Tiley, R. L. Jarret, J. H. Leebens-Mack, and R. E. Miller. 2014. Phylogenetics and diversification of morning glories (tribe Ipomoeae, Convolvulaceae) based on whole plastome sequences. *American Journal of Botany* 101: 92–103.
- Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics* 35: 375–403.
- FitzJohn, R. G. 2012. Diversitree: Comparative phylogenetic analyses of diversification in R. *Methods in Ecology and Evolution* 3: 1084–1092.
- FitzJohn, R. G., W. P. Maddison, and S. P. Otto. 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. *Systematic Biology* 58: 595–611.
- Goldberg, E. E., and B. Igić. 2008. On phylogenetic tests of irreversible evolution. *Evolution* 62: 2727–2741.
- Goldberg, E. E., and B. Igić. 2012. Tempo and mode in plant breeding system evolution. *Evolution* 66: 3701–3709.
- Gould, S. J. 1970. Dollo on Dollo's Law: Irreversibility and the status of evolutionary laws. *Journal of the History of Biology* 3: 189–212.
- Harborne, J. B. 1994. Introduction to ecological biochemistry, 4th ed. Academic Press, San Diego, California, USA.
- Harborne, J. B., and D. M. Smith. 1978. Chemosystematics of Polemoniaceae 4. Correlations between anthocyanin chemistry and pollination ecology in Polemoniaceae. *Biochemical Systematics and Ecology* 6: 127–130.
- Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford, UK.
- Hopkins, R., and M. D. Rausher. 2011. Identification of two genes causing reinforcement in the Texas wildflower *Phlox drummondii*. *Nature* 469: 411–414.
- Irwin, R. E., S. Y. Strauss, S. Storz, A. Emerson, and G. Guibert. 2003. The role of herbivores in the maintenance of a flower color polymorphism in wild radish. *Ecology* 84: 1733–1743.
- Lacey, E. P., M. E. Lovin, S. J. Richter, and D. A. Herington. 2010. Floral reflectance, color, and thermoregulation: what really explains geographic variation in thermal acclimation ability of ectotherms? *American Naturalist* 175: 335–349.
- Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50: 913–925.
- Lunau, K., and E. J. Maier. 1995. Innate color preferences of flower visitors. *Journal of Comparative Physiology, A, Sensory Neural and Behavioral Physiology* 177: 1–19.
- Maddison, W. P. 2006. Confounding asymmetries in evolutionary diversification and character change. *Evolution* 60: 1743–1746.
- Maddison, W. P., and R. G. FitzJohn. 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. *Systematic Biology* 64: 127–136.
- Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a binary character's effect on speciation and extinction. *Systematic Biology* 56: 701–710.
- Magnuson-Ford, K., and S. P. Otto. 2012. Linking the investigations of character evolution and species diversification. *American Naturalist* 180: 225–245.
- Maloof, J. E., and D. W. Inouye. 2000. Are nectar robbers cheaters or mutualists? *Ecology* 81: 2651–2661.
- Martín-Rodríguez, S., C. B. Fenster, I. Agnarsson, L. E. Skog, and E. A. Zimmer. 2010. Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytologist* 188: 403–417.
- Martin, C., A. Prescott, S. Mackay, J. Bartlett, and E. Vrijlandt. 1991. Control of anthocyanin biosynthesis in flowers of *Antirrhinum majus*. *Plant Journal* 1: 37–49.

- McDonald, J. A. 1991. Origin and diversity of Mexican Convolvulaceae. *Anales del Instituto de Biología Universidad Nacional Autónoma de México serie Botánica* 62: 65–82.
- McEwen, J. R., and J. C. Vamasi. 2010. Floral colour versus phylogeny in structuring subalpine flowering communities. *Proceedings of the Royal Society, B, Biological Sciences* 277: 2957–2965.
- Miller, R. E., J. A. McDonald, and P. S. Manos. 2004. Systematics of *Ipomoea* subgenus *Quamoclit* (Convolvulaceae) based on its sequence data and a Bayesian phylogenetic analysis. *American Journal of Botany* 91: 1208–1218.
- Muchhala, N., S. Johnsen, and S. D. Smith. 2014. Competition for hummingbird pollination shapes flower color variation in Andean Solanaceae. *Evolution* 68: 2275–2286.
- Nakazato, T., L. H. Rieseberg, and T. E. Wood. 2013. The genetic basis of speciation in the *Giliopsis* lineage of *Ipomopsis* (Polemoniaceae). *Heredity* 111: 227–237.
- Ng, J., and S. D. Smith. 2014. How traits shape trees: New approaches for detecting character state-dependent lineage diversification. *Journal of Evolutionary Biology* 27: 2035–2045.
- Nosil, P., and A. O. Mooers. 2005. Testing hypotheses about ecological specialization using phylogenetic trees. *Evolution* 59: 2256–2263.
- O'Meara, B. C. 2012. Evolutionary inferences from phylogenies: A review of methods. *Annual Review of Ecology Evolution and Systematics* 43: 267–285.
- Olmstead, R. G., L. Bohs, H. A. Migid, E. Santiago-Valentin, V. F. Garcia, and S. M. Collier. 2008. A molecular phylogeny of the Solanaceae. *Taxon* 57: 1159–1181.
- Oyama, R. K., K. N. Jones, and D. A. Baum. 2010. Sympatric sister species of Californian *Antirrhinum* and their transiently specialized pollinators. *American Midland Naturalist* 164: 337–347.
- Paape, T., B. Igic, S. D. Smith, R. Olmstead, L. Bohs, and J. R. Kohn. 2008. A 15-Myr-old genetic bottleneck. *Molecular Biology and Evolution* 25: 655–663.
- Pérez, F., M. T. K. Arroyo, R. Medel, and M. A. Hershkovitz. 2006. Ancestral reconstruction of flower morphology and pollination systems in *Schizanthus* (Solanaceae). *American Journal of Botany* 93: 1029–1038.
- Perret, M., A. Chautems, R. Spichiger, G. Kite, and V. Savolainen. 2003. Systematics and evolution of tribe Sinningieae (Gesneriaceae): Evidence from phylogenetic analyses of six plastid DNA regions and nuclear *ncpGS*. *American Journal of Botany* 90: 445–460.
- Porter, J. M. 1998. *Aliciella*, a recircumscribed genus of Polemoniaceae. *Aliso* 17: 23–46.
- Porter, J. M., and L. A. Johnson. 2000. A phylogenetic classification of Polemoniaceae. *Aliso* 19: 55–91.
- Porter, J. M., L. A. Johnson, and D. Wilken. 2010. Phylogenetic systematics of *Ipomopsis* (Polemoniaceae): Relationships and divergence times estimated from chloroplast and nuclear DNA sequences. *Systematic Botany* 35: 181–200.
- Porter, J. M., and V. W. Steinmann. 2009. Two new *Loeselia* (Polemoniaceae) species from Michoacan, Mexico. *Systematic Botany* 34: 730–736.
- Quattrocchio, F., J. Wing, K. van der Woude, E. Souer, N. de Vetten, J. Mol, and R. Koes. 1999. Molecular analysis of the *anthocyanin2* gene of petunia and its role in the evolution of flower color. *Plant Cell* 11: 1433–1444.
- Rabosky, D. L., and E. E. Goldberg. 2015. Model inadequacy and mistaken inferences of trait-dependent speciation. *Systematic Biology* 64: 340–355.
- Rambaut, A., M. A. Suchard, and A. J. Drummond. 2014. Tracer v1.6. Available at <http://tree.bio.ed.ac.uk/software/tracer>.
- Rausher, M. D. 2006. The evolution of flavonoids and their genes. In E. Grotewold [ed.], *The science of flavonoids*, 175–212. Springer, New York, New York, USA.
- Rausher, M. D. 2008. Evolutionary transitions in floral color. *International Journal of Plant Sciences* 169: 7–21.
- Ree, R. H., and M. J. Donoghue. 1999. Inferring rates of change in flower symmetry in asterid angiosperms. *Systematic Biology* 48: 633–641.
- Revell, L. J., L. J. Harmon, and D. C. Collar. 2008. Phylogenetic signal, evolutionary process, and rate. *Systematic Biology* 57: 591–601.
- Sargent, R. D. 2004. Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society of London, B, Biological Sciences* 271: 603–608.
- Särkinen, T., L. Bohs, R. G. Olmstead, and S. Knapp. 2013. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree. *BMC Evolutionary Biology* 13: 214.
- Schemske, D. W., and P. Bierzychudek. 2007. Spatial differentiation for flower color in the desert annual *Linanthus parryae*: Was Wright right? *Evolution* 61: 2528–2543.
- Schwinn, K., J. Venail, Y. J. Shang, S. Mackay, V. Alm, E. Butelli, R. Oyama, et al. 2006. A small family of MYB-regulatory genes controls floral pigmentation intensity and patterning in the genus *Antirrhinum*. *Plant Cell* 18: 831–851.
- Smith, S. D., C. Ané, and D. A. Baum. 2008. The role of pollinator shifts in the floral diversification of *Ichroma* (Solanaceae). *Evolution* 62: 793–806.
- Smith, S. D., and D. A. Baum. 2006. Phylogenetics of the florally diverse Andean clade Iochrominae (Solanaceae). *American Journal of Botany* 93: 1140–1153.
- Smith, S. D., and D. A. Baum. 2007. Systematics of Iochrominae (Solanaceae): Patterns in floral diversity and interspecific crossability. *Acta Horticulturae* 745: 241–254.
- Smith, S. D., R. E. Miller, S. P. Otto, R. G. FitzJohn, and M. D. Rausher. 2010. The effects of flower color transitions on diversification rates in morning glories (*Ipomoea* subg. *Quamoclit*, Convolvulaceae). In M. Long, H. Gu, and Z. Zhou [eds.], *Darwin's heritage today*, 202–226. Higher Education Press, Beijing, China.
- Smith, S. D., and M. D. Rausher. 2011. Gene loss and parallel evolution contribute to species difference in flower color. *Molecular Biology and Evolution* 28: 2799–2810.
- Sobel, J. M., and M. A. Streisfeld. 2013. Flower color as a model system for studies of plant evo-devo. *Frontiers in Plant Science* 4: 321.
- Strauss, S. Y., and J. B. Whittall. 2006. Non-pollinator agents of selection on floral traits. In S. C. H. Barrett and L. D. Harder [eds.], *The ecology and evolution of flowers*, 120–138. Oxford University Press, Oxford, UK.
- Streisfeld, M. A., and J. R. Kohn. 2007. Environment and pollinator-mediated selection on parapatric floral races of *Mimulus aurantiacus*. *Journal of Evolutionary Biology* 20: 122–132.
- Streisfeld, M. A., and M. D. Rausher. 2009. Altered trans-regulatory control of gene expression in multiple anthocyanin genes contributes to adaptive flower color evolution in *Mimulus aurantiacus*. *Molecular Biology and Evolution* 26: 433–444.
- Streisfeld, M. A., and M. D. Rausher. 2011. Population genetics, pleiotropy, and the preferential fixation of mutations during adaptive evolution. *Evolution* 65: 629–642.
- Streisfeld, M. A., W. N. Young, and J. M. Sobel. 2013. Divergent selection drives genetic differentiation in an R2R3-MYB transcription factor that contributes to incipient speciation in *Mimulus aurantiacus*. *PLOS Genetics* 9: e1003385.
- Sutton, D. A. 1988. A revision of the tribe Antirrhineae (Scrophulariaceae). Oxford University Press, London, UK.
- Tanaka, Y., N. Sasaki, and A. Ohmiya. 2008. Biosynthesis of plant pigments: Anthocyanins, betalains and carotenoids. *Plant Journal* 54: 733–749.
- van der Niet, T., and S. D. Johnson. 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution* 27: 353–361.
- Vargas, P., E. Carrio, B. Guzman, E. Amat, and J. Guemes. 2009. A geographical pattern of *Antirrhinum* (Scrophulariaceae) speciation since the Pliocene based on plastid and nuclear DNA polymorphisms. *Journal of Biogeography* 36: 1297–1312.
- Vargas, P., J. A. Rossello, R. Oyama, and J. Guemes. 2004. Molecular evidence for naturalness of genera in the tribe Antirrhineae (Scrophulariaceae) and three independent evolutionary lineages from the New World and the Old. *Plant Systematics and Evolution* 249: 151–172.
- Warren, J., and S. Mackenzie. 2001. Why are all colour combinations not equally represented as flower-colour polymorphisms? *New Phytologist* 151: 237–241.
- Waser, N. M., and D. R. Campbell. 2004. Ecological speciation in flowering plants. In U. Dieckmann, M. Doebeli, J. A. J. Metz, and D. Tautz [eds.], *Adaptive speciation*, 264–277. Cambridge University Press, Cambridge, UK.

- Wesselingh, R. A., and M. L. Arnold. 2000. Pollinator behaviour and the evolution of Louisiana iris hybrid zones. *Journal of Evolutionary Biology* 13: 171–180.
- Whittall, J. B., C. Voelckel, D. J. Kliebenstein, and S. A. Hodges. 2006. Convergence, constraint and the role of gene expression during adaptive radiation: Floral anthocyanins in *Aquilegia*. *Molecular Ecology* 15: 4645–4657.
- Wilson, P., M. C. Castellanos, A. D. Wolfe, and J. D. Thomson. 2006. Shifts between bee- and bird-pollination among penstemons. In N. M. Waser and J. Ollerton [eds.], *Plant–pollinator interactions: From specialization to generalization*, 47–68. University of Chicago Press, Chicago, Illinois, USA.
- Wilson, P., A. D. Wolfe, W. S. Armbruster, and J. D. Thomson. 2007. Constrained lability in penstemon flowers: Counting convergent origins of hummingbird pollination in *Penstemon* and *Keckiella*. *New Phytologist* 176: 883–890.
- Winkel-Shirley, B. 2001. Flavonoid biosynthesis: A colorful model for genetics, biochemistry, cell biology and biotechnology. *Plant Physiology* 126: 485–492.
- Zhang, B., C. Liu, X. Yao, F. Wang, J. Wu, G. J. King, and K. Liu. 2015. Disruption of a *CAROTENOID CLEAVAGE DIOXYGENASE 4* gene converts flower colour from white to yellow in *Brassica* species. *New Phytologist* 206: 1513–1526.