The Effects of Flower Color Transitions on Diversification Rates in Morning Glories (*Ipomoea* subg. *Quamoclit*, Convolvulaceae)

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About the Authors

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Representative Articles

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[4] Baum D A, Smith S D, Donovan S S. The tree-thinking challenge [J]. *Science*, 2005, 310:979-980.

Richard E. Miller is an Associate Professor of Plant Evolutionary Biology at Southeastern Louisiana University located in Hammond, LA. His research interests include the systematics of morning glories, molecular evolution of anthocyanin pathway genes, and evolutionary ecology of life history traits. This research program includes an active group of undergraduate research assistants, as well as masters students. The studies in the Miller lab draw on an extensive collection of morning glory seeds that have been amassed over the past decade. Southeastern is primarily an undergraduate institution; therefore he teaches a wide range of courses including genetics, evolution, and native plants of Louisiana.

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Sarah P. Otto, Professor, Department of Zoology, University of British Columbia; BSc and PhD from Stanford University. Her research has two main themes (i) The evolution of genetic systems (recombination, ploidy level, gene duplications) and (ii) the evolution of mating systems (sexual vs asexual reproduction, sexual selection, floral reproductive strategies). Research approaches include mathematical modeling, phylogenetic analyses, and yeast experimental evolution. She has served as Vice President of the Society for the Study of Evolution, the American Society of Naturalists, and the European Society of Evolutionary Biology, Council member for the Society for the Study of Evolution and the American Genetic Association, and member of the editorial boards of American Naturalist, Biology Letters, Evolution, Genetics, Theoretical Population Biology, and Trends in Ecology and Evolution. Awards include a McDowell Award for Excellence in Research (UBC), a Steacie Fellowship (Natural Sciences and Research Council; Canada), and the Steacie Prize (National Research Council, Canada).

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Richard G. FitzJohn is currently a PhD student at the University of British Columbia. He is interested in understanding what drives variation in both organismal and trait diversity among groups of organisms, and in developing statistical methods for doing this. He is also the author of the R package, "diversitree" (http://www.zoology.ubc.ca/prog/diversitree/).

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Mark D.Rausher, Professor of Biology at Duke University, obtained his PhD from Cornell University. His primary interest is understanding the genetics and evolution of ecologically important traits. In this work, he focuses on the evolution of flower color, an ecologically important trait that mediates interactions between plants and pollinators. In addition, his laboratory is investigating the molecular evolution of genes associated with the anthocyanins pigment pathway, the evolution of metabolic pathways, and the evolution of plant-enemy interactions. He is currently Editor-in-Chief of the journal *Evolution*, the section editor for evolution of *New Phytologist*, and a member of the board of editors of *Biology Letters*. He has also been Editor-in-Chief of *The American Naturalist*, and has served on the editorial boards of *Evolution* and *Ecology*.

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Abstract

As in many clades of flowering plants, the Quamoclit clade of morning glories (*Ipomoea* subgenus *Quamoclit*) exhibits unequal proportions of different flower colors, with pigmented species outnumbering unpigmented species by nearly a factor of 7. We examined three possible macroevolutionary explanations for this pattern: (1) asymmetric transition rates between pigmented and unpigmented flowers; (2) low transition rates preventing the flower colors from reaching equilibrium; and (3) differential diversification. In order to discriminate among these explanations, we employ the newly-developed Binary-State Speciation and Extinction (BiSSE) model, which jointly estimates transition rates among states and the speciation and extinction rates in each state. Using maximum likelihood and Bayesian BiSSE estimation, we find no evidence for asymmetrical transition rates. Also, BiSSE simulations of character evolution demonstrate that there is sufficient time for the estimated transition rates to produce as many or even more white species than we observe in Quamoclit, suggesting that low-transition rates do not fully account for the paucity of white species. In contrast, we find support for the differential diversification hypothesis with the rate of speciation in pigmented lineages estimated as three-fold greater than the rate in unpigmented lineages. Our analyses thus indicate the low frequency of white-flowered morning glory species is due largely to lineage selection. Our analysis also suggests that estimating character transition rates without simultaneously estimating speciation and extinction rates can lead to greatly biased estimates of transition rates.

Key Words

Anthocyanins; diversification; *Ipomoea*; transition rate

Introduction

In *On the Origin of Species*, as well as in his other works, Darwin wrote extensively about evolutionary changes within lineages and their causes. As is well-known, the mechanisms he described were focused on individuals (in the case of natural selection) or on mating pairs (in the case of sexual selection). In his discussion of the evolution of neuter castes of social insects, he did however propose a form of group selection, but he wrote little about selection at other levels. Because he knew nothing about the mechanisms of inheritance, he did not conceive of meiotic drive, segregation distortion, or selfish genetic elements. At higher levels, he appears to have given little attention to the possibility of lineage selection.

In the 150 years since *The Origin*, it has become clear that selection at levels below and above the individual do occur (Lewontin 1970; Hurst and

Werren 2001; Jablonski 2008). In particular, at higher levels, there are many reported examples of differential lineage extinction or speciation involving both individual characters and emergent characters of populations or species (Jablonski 2008; Rabosky and McCune 2009). But while lineage selection is an accepted phenomenon, its consequences for macroevolutionary patterns are still poorly understood. In this report, we focus on one macroevolutionary pattern that is potentially influenced by lineage selection: the proportion of species within a clade exhibiting a particular state of a character of interest. As we describe below, this proportion is expected to be determined, on the one hand, by rates of evolutionary transitions between character states, and on the other hand, by state-specific extinction and speciation rates. Here we seek specifically to understand the relative importance of differential speciation, extinction and transition rates in the evolution of an ecologically important trait: flower color.

Flower color is evolutionarily very labile. For example, many species exhibit natural polymorphisms in flower color (e.g. Jones and Reithel 2001; Zufall and Rausher 2003; Streisfeld and Kohn 2005). Above the species-level, it is frequently the case that sister taxa differ markedly in flower color (Campbell *et al.* 1997; Wesselingh and Arnold 2000; Porter *et al.* 2009; Wilson 2004). Despite this widespread capacity for flower color evolution, particular colors often account for a disproportionately large or small number of species in a given group (Perret *et al.* 2003; Tripp and Manos 2008). For example, among the estimated 270 species of penstemons, only 6 are white-flowered (Lodewick and Lodewick 1999; Wilson *et al.* 2004), and similarly, only 5 of the 70 species of *Phlox* produce exclusively white flowers (Wherry 1955). While the representation of different flower colors in a clade is likely to vary by pure chance, there also are macroevolutionary factors that may account for the differential species richness.

One such factor is an asymmetry in rates of transition between flower color states. Assuming no net differences in the diversification of species with different colors, the flower color state that is most likely to change is expected to be rare once a clade achieves an equilibrium distribution of flower colors (Nosil and Mooers 2005; Maddison 2006). If, for example, purple flowers were more likely to change to red flowers than the reverse, red-flowered species would eventually come to outnumber purple-flowered species, or even replace them entirely. Such asymmetrical transition rates could arise due to genetic constraints or to selective forces that favor transitions in one direction but not in the reverse (Zufall and Rausher 2004).

A second macroevolutionary factor is low rates of transition between flower-color states. In particular, if transition rates between flower colors are equal but low relative to the rate of diversification, we would expect that most species would retain the ancestral state for the group as opposed to reaching the equilibrium of equal numbers of species in each state (Schwander and Crespi 2009). Thus, if having pigmented flowers were the ancestral state in penstemons, the rarity of white species might be attributed to a low rate of change to white.

A third factor potentially contributing to differences in flower colors among species in a clade is differential diversification. If increased speciation or decreased extinction is associated with lineages with particular flower colors, those colors will become more common than species with other colors. Flower color transitions are often associated with adaptations to new environments or new pollinators, potentially resulting in a shift in diversification rates (Dodd *et al.* 1999; Fenster *et al.* 2004; Strauss and Whittall 2006). The nectar spurs of *Aquilegia* are a classic example of this phenomenon, where the transition from unspurred to spurred flowers resulted in increased diversification (Hodges and Arnold 1995). This effect has been attributed to the potential for reproductive isolation between populations or species differing in spur length (Whittall and Hodges 2007). A similar scenario could be envisioned with flower color. For example, the transition from pigmented to white flowers could allow exploitation of a new and diverse group of pollinating moths, and trigger an increase in speciation associated to specialization for different moth species (see
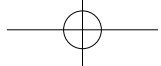
Sargent 2004 for evidence of this effect involving flower symmetry). However, transitions in flower color could also have the opposite effect. If transitions to novel flower colors are associated with, for example, greater pollinator specialization, species with the novel color might be at greater risk of extinction in the face of fluctuations in the pollinator fauna over time (Futuyma and Moreno 1998).

These three macroevolutionary factors, asymmetric transitions, low transition rates, and differential diversification, may, of course, act together to shape the phylogenetic distribution of flower colors. For instance, strongly asymmetric transition rates could be counterbalanced by differential extinction or speciation, resulting in the long-term maintenance of multiple flower colors. Alternately, strong asymmetry in transition compounded by differential diversification could make some character states, such as white flowers in penstemons, exceptionally rare. Indeed, the potential joint effects of asymmetric transition and differential diversification have lead some authors to question studies that have examined character evolution without accounting for state-dependent diversification (Maddison 2006; Goldberg and Igic 2008).

We present here the first attempt to disentangle the effects of asymmetric transitions, low transition rates, and state-dependent extinction and speciation on flower color diversity. We focus on the Quamoclit clade of morning glories (*Ipomoea* subgenus *Quamoclit*, Convolvulaceae). As with many other angiosperms, flower color is largely determined by the type and amount of anthocyanin pigments produced. Flowers that produce anthocyanins are red, pink, purple or blue whereas those that lack anthocyanin pigments appear white, or occasionally yellow. In the Quamoclit clade, multiple independent transitions from pigmented to unpigmented flowers have occurred (Fig. 1), and the unpigmented (white-flowered) species are often specialized for bat or moth pollination (Galetto and Bernardello 2004; Sanchez and Medellín 2007; McMullen 2009). A simple parsimony analysis would suggest at least 7 transitions from pigmented to unpigmented and no reverse transitions (Fig. 1). If transition

rates were indeed asymmetrical, with losses of pigmentation occurring more frequently than the gains, we would expect white species to outnumber pigmented species at equilibrium. However, pigmented species outnumber unpigmented species by roughly a factor of 7 (Appendix 1), suggesting that if transitions favor losses, either this bias is countered by some form of lineage selection, or transition rates are low enough that the clade has not yet reached equilibrium with respect to flower-color diversity.

In order to understand the relative contribution of these three macroevolutionary factors to the evolution of flower color, we undertake three types of analysis. First, we apply traditional transition-rate models that ignore state-dependent diversification and examine the number and directionality of transitions implied by these rates. Second, we employ the newly-developed BiSSE method, which simultaneously estimates transitions rates and state-specific extinction and speciation rates. Finally, we use estimates of transition rates from the BiSSE analysis to simulate character evolution to determine whether evolution of flower color is consistent with the null hypothesis of low transition rates. We use these analyses to address several specific questions: (1) is the forward transition rate (pigmented to white) significantly different from the reverse transition rate (white to pigmented), supporting the transition rate asymmetry hypothesis? (2) does any form of lineage selection, in the form of differential diversification, contribute to the low frequency of white-flowered species?; and (3) in the absence of differential diversification, is the observed number and distribution of white species consistent with estimated transition rates?

1. Methods

1.1 Data Collection

Both traditional and BiSSE analyses of character evolution require two pieces of information. First, we must provide an estimate of the phylogeny (with branch lengths) for the species comprising the Quamoclit clade. Second, we need to score flower color for each species. In this section we

▲ Fig. 1

Phylogeny of 45 of the 87 species in the Quamoclit clade. Relationships were inferred from maximum likelihood analysis of ITS sequences (see Methods). *I. cairica* was used as an outgroup. Species producing no floral anthocyanins are indicated with a white box; species with floral anthocyanin are indicated with a black box.

describe the phylogenetic analysis and the collection of flower color data.

1.1.1 Phylogenetic Inference

The phylogeny of the Quamoclit clade was inferred from internal transcribed spacer (ITS) sequences for 45 of the estimated 87 species. Twenty-one of these sequences were newly obtained for this study and for ongoing systematic studies in morning glories (Eserman and Miller, unpublished) while the remainder were obtained from previous studies (Manos *et al*. 2001; Miller *et al*. 1999, 2004). Genbank numbers and voucher information are available from the authors upon request. The ITS region was sequenced following Miller *et al*. (2004), and the resulting
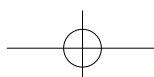
sequences were aligned manually in MacClade 4.0 (Maddison and Maddison 2000) to give a matrix of 689 characters. *I. cairica* was included as an outgroup based on previous studies (Miller *et al*. 2004). The model of sequence evolution for this dataset was selected using hierarchical likelihood ratio tests. Likelihood scores were calculated in PAUP*4.0b10 (Swofford 2002) for a neighbor-joining tree and a range of models (JC, K2P, HKY, HKY+Γ, HKY+Γ+I, GTR+Γ, and GTR+Γ+I, described in Swofford *et al*. 1996). The GTR+Γ+I (a general time-reversible model with gamma-distributed rate variation across sites and a proportion of sites estimated as invariant) was identified as the best-fitting model.

Phylogenetic analysis was conducted using both likelihood and Bayesian methods. The maximum likelihood (ML) analysis was conducted in RAxML-7.0.4 (Stamatakis 2006) using the GTR+Γ+I model. This single best estimate of the topology and branch-lengths was used for the ML BiSSE analyses (see section 1.3).

Bayesian analyses were performed to obtain a sample set of trees for testing the effects of phylogenetic uncertainty on the BiSSE results. The ITS data were analyzed in MrBayes version 3.1.1 (Ronquist and Huelsenbeck 2003), with 5 million generations, sampling every 1000 generations, providing 5000 trees. We conducted two independent runs, each comprising four linked chains with temperature set to 0.05, which gave acceptance rates for swapping between adjacent chains in the range of 10 to 70%. The runs showed evidence of convergence with the average standard deviation of split frequencies dropping below 0.01 after the first 1.5 million generations and produced identical majority-rule consensus topologies with posterior probabilities for clades differing less than 5% between the two runs. Given the similarity of the two runs, we used only trees from the first run. The first 2000 trees (2 million generations) were conservatively discarded as burnin, and the remaining 3000 trees were subsampled uniformly (every 30th) to provide a set of 100 trees for BiSSE analysis.

Trees from the likelihood and Bayesian analyses were ultrametricized prior to BiSSE analyses using r8s (Sanderson 2003). This process was necessary because the trees from RAxML and Mr-Bayes (with branch lengths in terms of expected numbers of substitutions per site) are not ultrametric, i.e., the root-to-tip distance is not equal for all taxa. The trees were made ultrametric using the penalized likelihood method with the truncated Newton (TN) algorithm and with the optimal smoothing parameter (1, for these data) identified using cross-validation. The root node was arbitrarily fixed to 100. The ITS dataset and the ultrametric ML and Bayesian trees for the Quamoclit clade are available through Treebase (Study #SN4830, Pin# 11026).

This rescaling of the tree depth must be taken into account when interpreting the rate parameters we estimate (see section 2). For example, when the tree depth is 100 and the estimated transition rate from white to pigmented flowers is 0.01, we could expect 100 × 0.01 or 1 transition along a branch that spans the root-to-tip distance starting in the white-flowered state. Although we arbitrarily fixed the root node (the split between the outgroup *I. cairica* and the rest) at 100, we only use the ingroup depth for BiSSE so the value will be slightly less (87 in the case of the ML phylogeny).

1.1.2 Flower Color Data Collection

We scored all 87 Quamoclit species for anthocyanin pigmentation based on descriptions from the taxonomic literature and herbarium specimens. These descriptions and their sources are provided in Appendix 1. BiSSE requires that the character of interest is coded as binary (0/1) variable. Accordingly, we recognized two states for flower color: 0 denoting absence of anthocyanin pigmentation and 1 denoting presence. Species that were polymorphic for pigment production were coded as 1. In 2 of the 13 species coded as lacking anthocyanins, small amounts of anthocyanins sometimes occur in the interplicae (the five narrow zones between the five large folds (plicae) of petal tissue comprising the limb), but not in the rest of the corolla (the throat and the plicae). Also, one of the "white" species, *I. jicama*, is typically white but can produce small
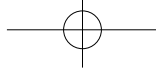
amounts of anthocyanins in the limb, giving it a pale lavender color. The remaining 10 species coded as white produce no floral anthocyanins. By contrast, all species coded as containing anthocyanins produce abundant anthocyanins throughout the corolla limb and usually in the throat as well.

1.2 Transition Rate Analysis Using Traditional ML Approach.

We applied the widely-used transition rate models developed by Pagel (1994) to estimate flower color transition rates and test for significant rate asymmetry. These methods model the evolution of discrete binary (0/1) traits as a continuous-time Markov process where the transitions between states are governed by two instantaneous rate parameters (q_{01} and q_{10}) and where probability of change depends only the character state at that time (Pagel 1994). We used the Multistate program in the BayesTraits Package (Pagel *et al*. 2004) to estimate q_{01} and q_{10} with the 45-taxon maximum likelihood tree (Fig. 1). We compared two models in Multistate: one in which the rates were constrained to be equal (the "mk1" model) and another in which the two rates were free to vary (the "mk2" model). The significance of the difference in likelihood was determined using a likelihood ratio test with 1 degree of freedom.

Although the estimated rates give some sense for the directionality and magnitude of character transitions, simulations are necessary to determine the patterns expected from a given set of rates. We explored patterns of flower color change along the 45-taxon ML tree using stochastic mapping as implemented in the program SIMMAP (Bollback 2006). Polytomies in this tree were randomly resolved with zero length branches as required by the program. Stochastic mapping, originally described in Huelsenbeck *et al*. (2003), relies on the same underlying transition rate model as BayesTraits although the two rates (q_{01} and q_{10}) are instead specified with an evolutionary rate parameter (μ) and bias parameters (π_0, π_1). Note that this evolutionary rate parameter (μ) is distinct from the extinction rate parameter (μ) of the BiSSE model. In stochastic mapping, the rate from 1 to 0, q_{10}, is equal to the bias (π_0) multiplied by μ, and the reverse q_{01} is

equal to π_1 (or 1- π_0) multiplied by μ. Thus, by specifying one of the bias parameters and evolutionary rate, we can conduct stochastic simulations of character change along the phylogeny with rates equivalent to those inferred by Multistate. We carried out 1,000 simulations in SIM-MAP 1.0 using the asymmetric mk2 rates, and, for each simulation, we recorded the number of transitions in each direction.

1.3 Estimating the BiSSE Model with Maximum Likelihood

Whereas traditional transition rates analyses such as those above assume that the rates of diversification do not vary between the two character states, the BiSSE model estimates state-dependent speciation and extinction rates along with the rates of transition between states.

The BiSSE model, originally described in Maddison *et al*. (2007), has six parameters: the speciation rates of lineages in states 0 and 1 (λ_0 and λ_1), extinction rates of lineages in states 0 and 1 (μ_0 and μ_1), and the rates of trait evolution in the forward and reverse directions (q_{10} and q_{01}). Although the original method required a fully-resolved and complete phylogeny (where all the species were included), Fitzjohn *et al*. (2009) developed an extension that allows for the analysis of incomplete phylogenies, such as our 45-taxon sample of the Quamoclit clade. Incomplete sampling can be accounted for using either the "skeleton tree" approach, in which a random subset of the taxa were sampled, or the "terminally unresolved tree" approach where all taxa were included but the tree is incompletely resolved so that some species are present only in terminally unresolved clades (Fitzjohn *et al*. 2009). Simulations have shown that the unresolved approach provides more accurate and precise parameter estimates than the skeleton tree approach for a given amount of phylogenetic resolution. For this reason, we have used the unresolved tree approach here.

In order to prepare the phylogeny for BiSSE analysis, we converted the fully-resolved 45-taxon ML tree into a terminally unresolved tree that includes all the unsampled taxa. The Quamoclit clade and related morning glories have

been the subject of several phylogenetic and taxonomic studies (McDonald 1991; Austin and Huáman 1996; Miller *et al*. 2004). The existing sampled taxa (Fig. 1, Appendix 1) include members of each of the 11 taxonomic sections within Quamoclit, and for small sections, such as Microsepalae, all known species are present. The unsampled taxa were added to their sections, and these clades were collapsed to represent the uncertainty in the placement of these taxa (Fig. 2). In practice, the tree was pruned to leave a single representative for each unresolved clade, which provides the depth of the clade. This procedure resulted in a tree with 16 tips representing all 87 species in the Quamoclit clade (as shown in Fig. 2). In estimating the rate parameters for this unresolved tree, BiSSE integrates over all possible resolutions that could have given rise to the observed numbers of white and pigmented species

(Fitzjohn *et al*. 2009).

We explored a range of constrained BiSSE models to test hypotheses regarding differential diversification of pigmented and white-flowered lineages. After first estimating the full six-parameter model with the single ML tree, we asked whether the full model was a significantly better fit than models in which speciation rates, extinction rates or transition rates were equal. Because the BiSSE constrained models are nested within the full model, the significance of differences in model fit was judged with likelihood ratio tests. It is worth noting that a constrained model in which speciation and extinction rates are equal but transition rates are allowed to vary is equivalent to the mk2 (asymmetric rates) model in the BayesTraits analyses and a model with all rates equal is equivalent to the mk1 (symmetric rates)
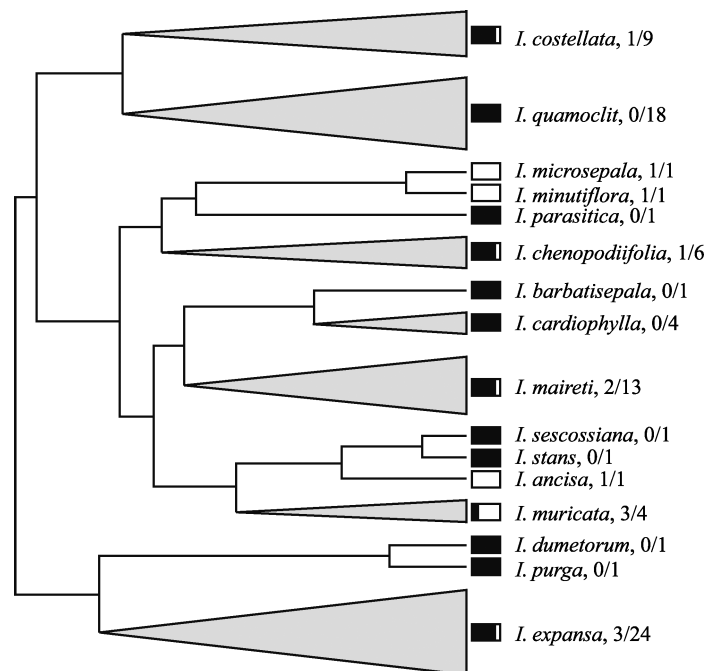
▲ **Fig. 2**

Unresolved tree including all 87 species in the Quamoclit clade. The tree is resolved to the greatest extent possible given current taxonomic knowledge and sampling. A single exemplar is specified for each unresolved clade. The proportion of white species is displayed as the amount of white space in rectangle before the species name and as a fraction after the name (number of white species out of the total number of species).
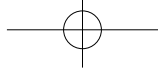
model. However, the estimates of transition rates (q_{01}, q_{10}) are likely to vary between Multistate and BiSSE because BiSSE includes all the taxa in an unresolved tree and Multistate uses only the 45-taxa for which we have sequence data.

1.4 Simulation of Character Evolution

We use the simulation functions in the "diversitree" R package (http://www.zoology.ubc.ca/prog/diversitree/) to examine the distribution of flower colors expected from the ML BiSSE parameters. Specifically, we used the simulations to determine whether transition rates alone are low enough to explain the low proportion of white-flowered species in Quamoclit. If the ancestral state for Quamoclit were pigmented, we might expect few white species simply due to low transition rates. However, if the evolution of such few white species requires differential diversification, we would expect to see the observed proportions only when differential speciation or extinction are included. In order to test this question, we compared two sets of simulations: one set in which we used equal transition, speciation and extinction rates and one set in which the speciation rates were set to those estimated by the ML BiSSE analyses. We focused on equal transition rates and on differential diversification due to differences in speciation rates instead of differences in extinction rates because of the results obtained with full and constrained ML BiSSE models (details in section 2.2). In both sets of simulations, we started with a pigmented ancestral state and simulated a phylogeny of 87 taxa. For each of 1,000 simulations, we recorded the proportion of white species produced.

1.5 Estimating the BiSSE Model with Bayesian Methods

In addition to these ML BiSSE analyses, we conducted Bayesian BiSSE analyses, which provide estimates of the posterior distribution for each of the six parameters. The Bayesian BiSSE approach, when applied across a sample of trees from Bayesian analysis, incorporates uncertainty in both the phylogeny and in the BiSSE parameters. The posterior distributions of the six parameters were estimated using Markov chain Monte Carlo (MCMC) as described in Fitzjohn *et al*. (2009). The priors for each parameter fol-

lowed an exponential distribution with the mean of $2r$ where r is the rate of diversification (λ-μ) that would produce all 87 species over the time span of the tree (following FitzJohn *et al*. 2009). We ran MCMC chains for the 100 trees sampled from the Bayesian phylogenetic analysis using slice sampling (Neal 2003) for parameter updates. Each chain began with the ML values for the six parameters and proceeded for 10,000 steps. Sliding window analyses showed no trends in the likelihoods explored during these chains, except for a very short period at the beginning. We conservatively discarded the first 2,500 steps of each chain, and the last 7,500 steps from all 100 trees were pooled and summarized to give the posterior distributions. In order to make all the parameter estimates directly comparable to those estimated with the ML phylogeny, each value was multiplied by the ratio of the depth of the Bayesian tree to the depth of the ML tree.

2. Results

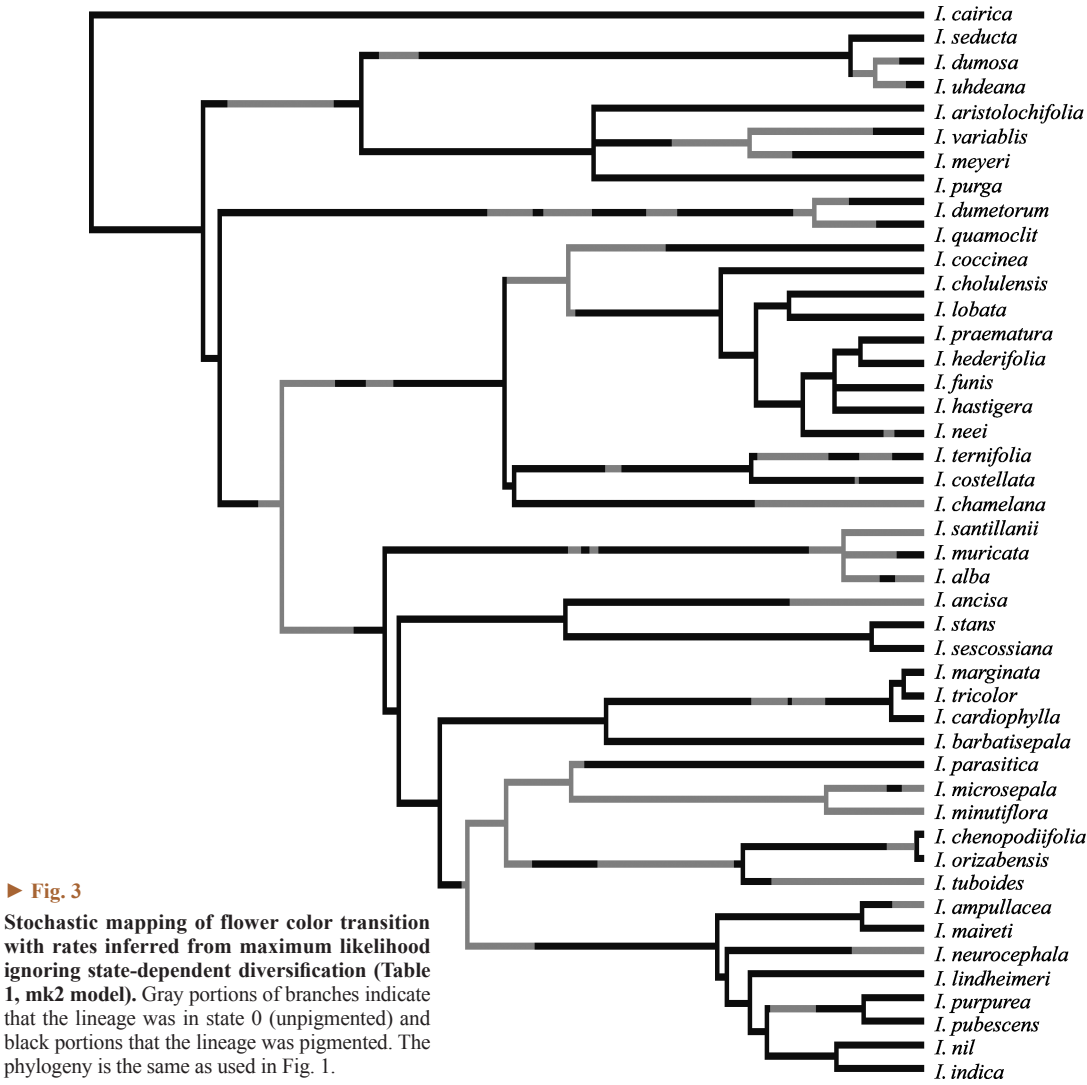### 2.1 Transition Rate Analysis Using Traditional ML Approach

Analysis of the maximum-likelihood tree for 45 Quamoclit species using the program Multistate within the BayesTraits software package indicated that the transition rates between flower colors are highly unequal, with greater gains of pigmentation than losses. The ML estimate of the reverse rate (from white to pigmented) is approximately four times that of the forward rate, and this difference is statistically significant as judged by a likelihood-ratio test (Table 1). By calculating the likelihood across a range of q values, we found that the 95% confidence interval for the ratio of q_{01}/q_{10} spans from 1.6 to 4.1, rejecting the possibility of equal rates when the model includes only transition rates but not state-dependent speciation and extinction rates.
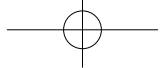
Simulations of character state evolution using the ML rate estimates indicate that multiple reversals of character state often occur along any one lineage from branch to tip. Across the 1,000 stochastic mappings of flower color evolution on the ML phylogeny using the asymmetric mk2 rates, we observed an average of 61.6(±9.0 SD) transitions in flower color. Of these transitions,

31.0±4.1 involved losses of pigmentation and 30.6±5.4 involved gains. An example of one of these stochastic mappings with the mk2 rates is shown in Fig. 3. Thus, traditional ML analyses suggest that white-flowered species are rare in Quamoclit because of high, asymmetric transition rates that rapidly yield an equilibrium ratio of pigmented to white species. This result is in

Table 1 Comparison of constrained and unconstrained maximum likelihood (ML) estimates of transition rates, ignoring state-dependent diversification. Two models were fit to the 45-taxon phylogeny shown in Fig. 1 using BayesTraits (see text). The mk1 model constrains the transition rates (q_{01} and q_{10}) to be equal, and the mk2 model allow the rates to vary freely. The two models were compared with a likelihood ratio test with 1 degree of freedom (df) and the *P*-value (*P*) is given.

Model	q_{01}	q_{10}	Ratio q_{01}/q_{10}	lnL	χ^2	df	*P*
mk1 (symmetrical rates)	0.0076	0.0076	1.00	−22.61			
mk2 (asymmetrical rates)	0.032	0.0087	3.71	−20.15	4.93	1	0.03

► **Fig. 3**

Stochastic mapping of flower color transition with rates inferred from maximum likelihood ignoring state-dependent diversification (Table 1, mk2 model). Gray portions of branches indicate that the lineage was in state 0 (unpigmented) and black portions that the lineage was pigmented. The phylogeny is the same as used in Fig. 1.

stark contrast with the parsimony reconstruction of ancestral character states, which shows 7 to 8 losses of pigmentation with no reverse transitions from white to pigmented (Fig. 1).

## 2.2 Joint Estimates of Diversification and Transition Rates: Parameter Estimation and Hypothesis Testing

The counterintuitive results from the traditional ML analysis could be due to its failure to account for possible effects of differential speciation or extinction rates between pigmented and white lineages. In order to examine this possibility, we used the ML BiSSE methods to estimate speciation, extinction and transition rates for each flower color for the single maximum likelihood tree (Table 2). The full BiSSE model (Model 1, all parameters unconstrained) suggests that pigmented lineages (state 1, 0.041) speciate at three times the rate of white lineages (state 0, 0.014) (Table 2). Extinction rates were estimated as zero for both states, although these may be more difficult to accurately estimate than speciation rates (Maddison *et al*. 2007), especially with the unresolved clades (Fig. 2). Finally, the reverse transition rate (from white to pigmented) was estimated to be 1.6 times higher than the forward rate (Table 2). Recall that the interpretation of these rate parameters depends on the depth of the tree (87 for the ML phylogeny) where the expected numbers of changes along a branch are equal to the branch length multiplied by the rate.

To determine whether these rate differences are statistically significant, we compared various models that differed in which parameters were constrained to be equal using likelihood criteria (Table 2). Models were compared in a hierarchical manner. We first compared the full model (model 1) to each model having one parameter equal for lineages with white and pigmented flowers (models 2, 3, and 4). None of these comparisons was statistically significant ($P > 0.1$

Table 2   Comparisons of full and constrained ML BiSSE analyses. The six BiSSE parameters are the speciation rates in white and pigmented lineages (λ_0 and λ_1), the extinction rates (μ_0 and μ_1), and forward and reverse transition rates (q_{10} and q_{01}). Nested models were compared with likelihood ratio tests with 1 degree of freedom; the comparison is given in the comparison column. Significant *P*-values (≤0.05) are bolded. For a given rate, the expected number of changes along a branch with the same length as the root-to-tip distance is the rate multiplied by 87 (the depth of the ultrametricized ML phylogeny).

Model	Contraints	λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}	lnL	Comparison	P
1	None	0.014	0.041	0.00	0.00	0.010	0.0062	−100.90		
2	$\lambda_0 = \lambda_1$	0.042		0.042	0.00	0.0070	0.0084	−101.96	vs. 1	0.14
3	$\mu_0 = \mu_1$	0.014	0.041	0.00		0.010	0.0062	−100.90	vs. 1	1.00
4	$q_{01} = q_{10}$	0.013	0.042	0.00	0.00	0.0059		−100.97	vs. 1	0.67
5	$\lambda_0 = \lambda_1, \mu_0 = \mu_1$	0.038		0.00		0.033	0.0067	−102.46	vs. 3	0.08
6	$\lambda_0 = \lambda_1, q_{01} = q_{10}$	0.042	3.46	0.00		0.00	0.0083	−101.97	vs. 3	0.16
7	$\mu_0 = \mu_1, q_{01} = q_{10}$	0.013	0.042	0.00		0.0059		−100.97	vs. 3	1.00
8	$\lambda_0 = \lambda_1, \mu_0 = \mu_1, q_{01} = q_{10}$	0.038		0.00		0.0049		−102.89	vs. 7	**0.05**
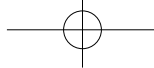
in each case). Because constraining extinction rates to be equal resulted in the smallest change in ln-likelihood, we then accepted the model with this constraint (model 3) as a better model than model 1. Next, we compared models 5 and 7 to model 3. These comparisons tested for significant differences for speciation rates and transition rates, respectively. The test for equal transition rates was not significant ($\chi^2 = 0.14$, df $= 1$, $P > 0.8$), while the test for equal speciation rates approached significance ($\chi^2 = 2.98$, df $= 1$, $P = 0.08$). We therefore accepted the model with the additional constraint of equal transition rates (model 7) as the best model of those compared so far. Finally, we compared model 8 (all three parameters constrained to be equal), with model 7, which tests whether speciation rates are equal. This test was statistically significant ($\chi^2 = 3.84$, df $= 1$, $P = 0.05$), indicating that model 7, with unequal speciation rates, is the best model. Conversely, if we started with the most constrained model (model 8) and compared this to models relaxing one constraint at a time (i.e., to model 5 allowing q_{01} to differ from q_{10}, model 6 allowing μ_0 to differ from μ_1, and model 7 allowing λ_0 to differ from λ_1), only model 7 with differential speciation resulted in a significant improvement in likelihood.

To examine the sensitivity of this conclusion to variation in tree topology, we performed a similar hierarchical analysis for 100 Bayesian trees. We considered the full model and the seven constrained models (Table 2), and in all cases, model 7 proved to be the best model. In the test for equal speciation rates (model 7 vs. model 8), the average χ^2 value was 3.94, corresponding to $P < 0.05$. For seven of the trees, $P < 0.025$, for an additional 47 trees $P < 0.05$, for 43 additional trees $P < 0.1$, and only for three trees was $P > 0.1$. Thus, the majority of the Bayesian trees support the conclusion from the BiSSE analysis of the single ML tree, namely that speciation rates differ between pigmented and white-flowered lineages, while extinction rates and transition rates do not.

Under model 7, the speciation rate for lineages with pigmented flowers is 3.12 times greater than that for lineages with white flowers (mean

ML estimate for 100 Bayesian trees) while transition rates and extinction rates do not differ for the two types of lineages. Moreover, the estimated transition rates under model 7, averaged over the 100 Bayesian trees ($q_{01} = q_{10} = 0.00580$), are only slightly smaller than the forward transition rate (0.00874) estimated by the Multistate analysis. These results suggest two predictions: (1) without differences in speciation rates, transition rates are high enough to yield an equilibrium of equal proportion of species with white and pigmented flowers within the time represented by the diversification of Quamoclit; and (2) when unequal speciation rates are added to the observed transition rates, they prevent this equilibrium from being reached and keep the proportion of white-flowered species small.

To examine these predictions, we employed the stochastic simulation functions within the diversitree package to estimate the proportion of white-flowered species resulting from the ML transition rates, allowing for differences in speciation. Our first set of simulations used the transition, speciation and extinction rates estimated by model 8 (Table 2). Starting from an ancestral state with pigmented flower and growing to a tree of 87 taxa, this all-rates-equal model resulted in an average proportion of 0.35 white species (± 0.20 SD) across 1000 simulations. Even though this average is below the expected equilibrium of 50% white species, in 22% percent of the trials the proportion of white species exceeded 50%, indicating that transition rates are large enough to frequently allow the equilibrium to be reached. Also, only 13% of the simulations with the equal-rates model gave proportions of white-flowered species below the 0.15 observed in Quamoclit. In contrast, if we incorporate differential speciation by simulating with the model 7 parameters (Table 2), we obtain significantly fewer white species ($t = 27.6$, df $= 1201$, $P < 2.2 \times 10^{-16}$), with the proportion across 1,000 simulations equal to 0.17 ± 0.04 on average (Fig. 4). These values compare well to the observed proportion of whites in Quamoclit (15%). Thus, while low transition rates may in part explain the observation that there are fewer white species than would be expected at equilibrium with equal transition rates, the observed
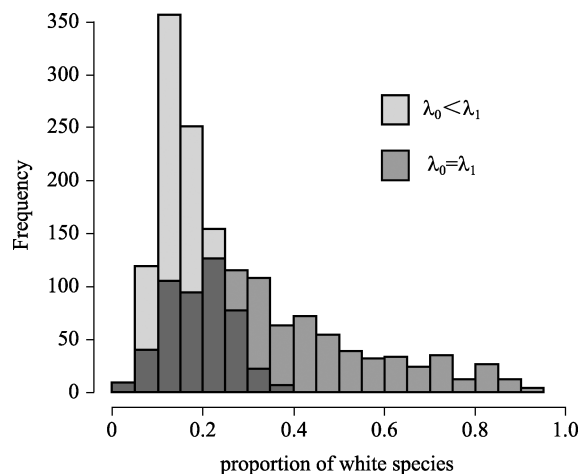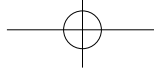
difference in speciation rates is entirely consistent with the low proportion of white-flowered species.

2.3 Bayesian Analysis of Diversification and Transition Rates

The ML implementation of BiSSE provides the best point estimates of the transition, speciation, and extinction rates for a given phylogeny and allows formal hypothesis tests on these rates. It is difficult, however, to perform hypothesis tests on combinations of these parameters (e.g., diversification rate) using ML, as it requires that a new search procedure be implemented with each new set of parameters. In addition, the likelihood ratio tests described above rely on a chi-squared distribution to assess significance, which holds only when there is sufficient data. Bayesian BiSSE methods using MCMC circumvent these problems. The distribution of any parameter combination can be obtained from previous MCMC runs by calculating the value of that combination at each step. Furthermore, Bayesian BiSSE methods approximate the full posterior distribution and allow credibility inter-
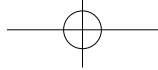
vals to be estimated directly without reliance on asymptotic properties.

Qualitatively, the Bayesian analysis revealed patterns similar to those found in the ML analyses. In particular, speciation rates appear to be about 2-3 times higher for lineages with pigmented flowers than for lineages with white flowers (Table 3, Fig. 5a), while extinction and transition rates are very similar for the two types of lineages (Table 3, Fig. 5b,c). The diversification of white species ($\lambda_0 - \mu_0$) is centered around zero while the most probable rate for pigmented lineages is 0.04 (Table 3, Fig. 5d). However, the speciation-rate and diversification-rate credibility intervals overlap broadly (Fig. 5a,d), implying that there is no significant difference in either rate between white and pigmented lineages.

This comparison is potentially conservative, though, because it does not constrain extinction and transition rates to be equal. A more powerful test compares speciation rates when extinction and transition rates are constrained to be approximately

Table 3   Summary statistics for posterior distributions of BiSSE parameters. The MCMC results were pooled across runs with 100 Bayesian trees. For each parameter, the mean, mode and the width of the 95% credibility interval ("low" and "high") is given. The credibility interval is the smallest interval that contains 95% of the MCMC samples. To estimate the mode, we took the midpoint between the high and low values of the smallest interval containing 1% of the samples. Diversification rates (r_0 and r_1) are the difference between speciation and extinction rates ($\lambda_0 - \mu_0$, $\lambda_1 - \mu_1$).

| Parameter	mean	mode	low	high
Speciation in state 0 (λ_0)	0.037	0.017	0.00	0.098
Speciation in state 1 (λ_1)	0.062	0.052	0.032	0.10
Extinction in state 0 (μ_0)	0.057	0.0071	0.00	0.17
Extinction in state 1 (μ_1)	0.022	0.0001	0.00	0.064
Diversification in state 0 (r_0)	–0.020	0.00023	–0.14	0.070
Diversification in state 1 (r_1)	0.039	0.039	0.017	0.062
Transition rate from state 0 to 1 (q_{01})	0.080	0.0078	0.00	0.27
Transition rate from state 1 to 0 (q_{10})	0.024	0.0094	0.0022	0.063
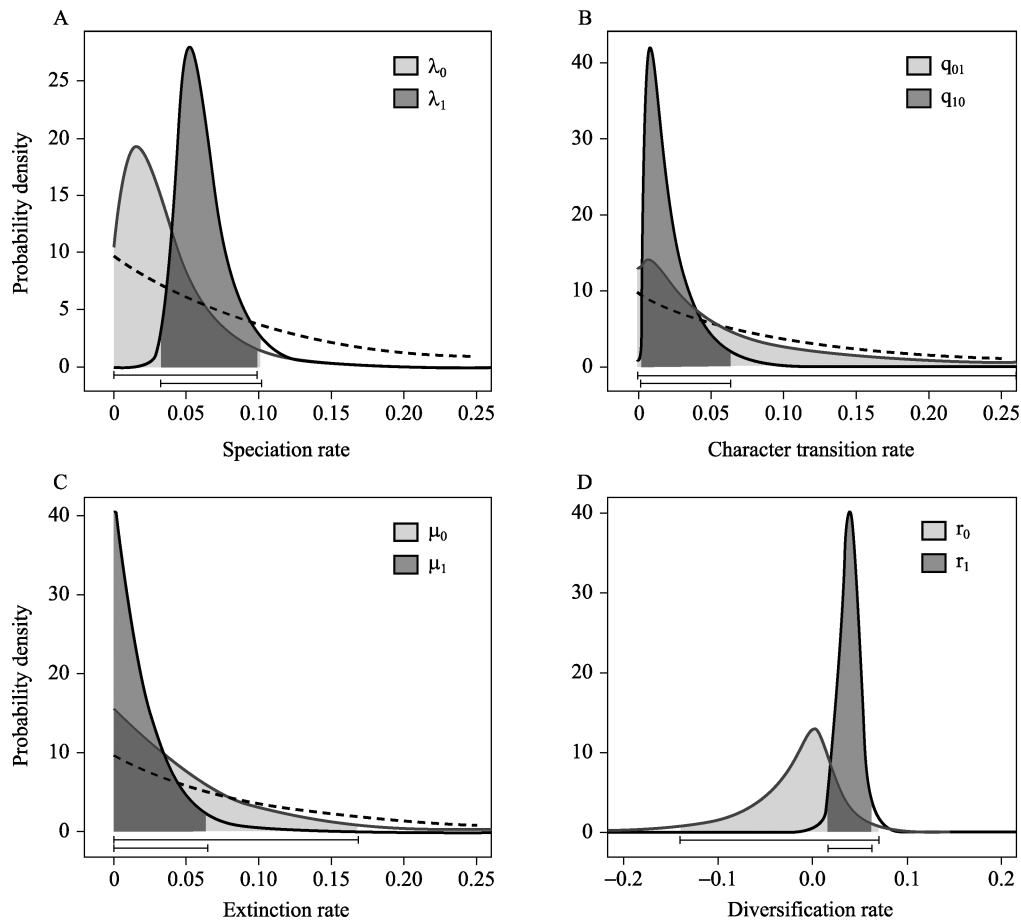
equal. To perform this test, we filtered the Bayesian BiSSE data to retain only those samples in which transition and extinction rates are roughly equal (i.e. samples in which the absolute difference between pigmented and white lineages was less than 0.01 for both extinction rates and transition rates). With this reduced data set, which contained 41,113 sample estimates from the 100 Bayesian trees, the overlap in the posterior frequency distributions of speciation rates for white and pigmented species is greatly reduced

(Fig. 6a). Moreover, in more than 98 percent of the samples (40,350) the speciation rate for pigmented lineages exceeded that for white lineages (Fig. 6b). Finally, for 99 of the 100 trees examined, speciation rate for pigmented lineages exceeded that for white lineages in 95 percent or more of the samples. We interpret these Bayesian results as further support for the conclusion that the paucity of white flowers in Quamoclit is due largely to the difference in speciation rates between white and pigmented lineages.
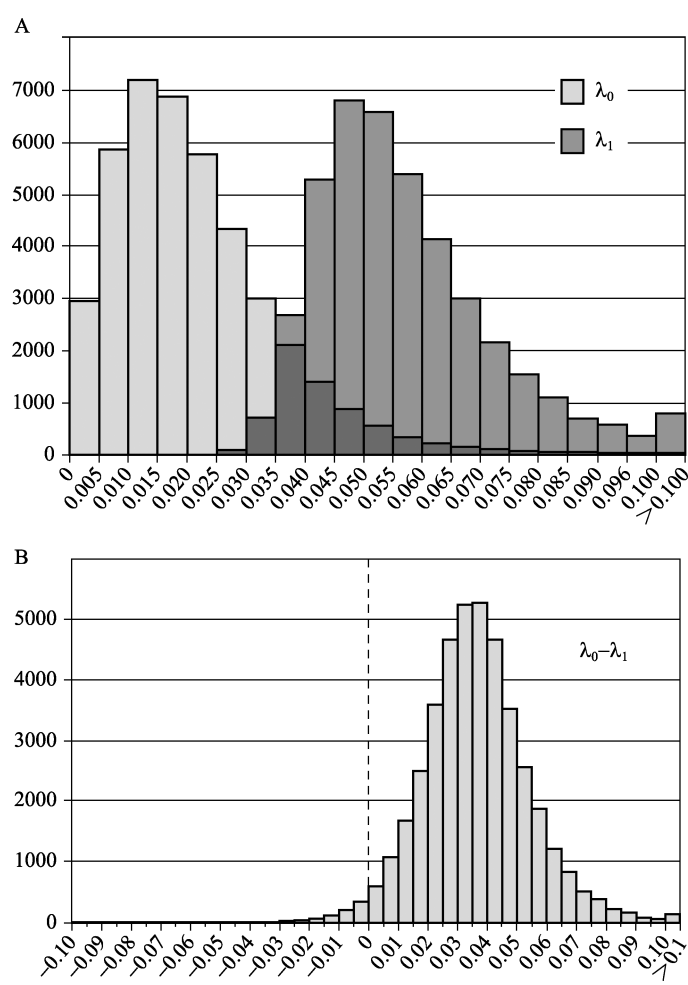
▲ **Fig. 6**

Distribution of speciation rates in white and pigmented lineages in constrained Bayesian BiSSE samples. The full Bayesian BiSSE sample (100 trees × 7500 generations) was filtered to include only those samples for which $\mu_0 \sim \mu_1$ and $q_{01} \sim q_{10}$ (difference < 0.01). (A) The distribution of speciation rates in this constrained subset. (B) The difference in speciation rates between white and pigmented lineages. The dashed line marks zero.
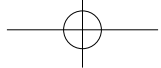
3. Discussion

In this investigation, we have sought to understand the causes of the disparity between the number of species with pigmented and unpigmented flowers in the Quamoclit clade of morning glories. The relative rarity of unpigmented species is a pattern observed across many floral radiations (Perret *et al*. 2003; Wilson *et al*. 2004; Smith and Baum 2007; Tripp and Manos 2008; but see Kay *et al*. 2005 for counter-example) and raises the question of what evolutionary factors could account for this disparity. We considered three explanations, which are not mutually exclusive. First, asymmetric transition rates may bias the distribution in favor of pigmented species. Second, transition rates may simply be too low for the number of white-flowered species to increase substantially within the period of time represented by the diversification of Quamoclit. Finally, excluding asymmetric or low transition rates, the diversification rate may be higher for lineages with pigmented flowers. The series of analyses reported here allow us to distinguish among these explanations.

3.1 Rarity of White-Flowered Species due to Lineage Selection

Two features characterize the distribution of flower colors among species in the Quamoclit clade. The first feature is the rarity of white-flowered species, which make up only 15% of species in the clade. The second is that white-flowered species appear to be confined to the "tips" of the tree. In other words, the white-flowered species are typically sister to pigmented species as opposed to other white species (Fig. 1). This so-called "tippy" or "twiggy" pattern is similar to that exhibited by traits such as asexuality (Schwander and Crespi 2009) and specialization (Kelley and Farrell 1998). A common explanation for this pattern is that one state (e.g., asexuality, specialization) has a lower diversification rate than the other, either through decreased speciation or increased extinction. In few cases, however, have attempts been made to rigorously evaluate this explanation (but see Igic *et al*. 2008 and Takebayashi and Morrell 2001).
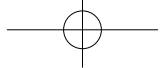
Here, we have used several phylogenetic approaches to evaluate the evolutionary processes that generate this pattern for flower color in the Quamoclit clade. These analyses suggest that the rarity of white species in Quamoclit is not well-explained by the first hypothesis, that is, asymmetrical transition rates with higher gains than losses of floral pigmentation. Comparing BiSSE models is an ML framework (Table 2), we found that allowing for rate asymmetry did not significantly improve the likelihood and the best fitting model (model 7) had equal transition rates. This inference is also supported by the Bayesian analysis, which found little difference between transition rates.

A second explanation, that transition rates are sufficiently low that they have not allowed proportions of white and pigmented species to reach an equilibrium within the time required for diversification of the Quamoclit clade, may in part explain the low proportion of white flowers. In our simulations involving no differences in speciation rates, the average proportion of white-flowered species was 0.35, somewhat less than the equilibrium of 0.5, but substantially higher than the observed proportion of white species (0.15).

By contrast, our analyses indicate that a third explanation is sufficient to account for the observed flower-color distribution within Quamoclit: higher speciation rates, and thus higher diversification rates, for lineages with pigmented flowers prevent white-flowered lineages from increasing substantially in frequency. Both the ML analysis and the Bayesian analysis supported a correlation between flower color and speciation rates. Moreover, the stochastic simulations showed that the estimated difference in speciation rates was large enough to shift the proportion of white-flowered species from that projected by equal transition rates (white and pigmented species equally abundant) to a proportion of whites similar to that seen in the clade. Our results thus demonstrate the potential importance of lineage selection (Jablonski 2008; Rabosky and Mc-Cune 2009) in shaping the distribution of flower colors within Quamoclit.

Although we have little information about why lineages with pigmented flowers have higher

speciation rates, one possibility is that they differ in degree of pollinator specialization. In general, *Ipomoea* species with pigmented flowers tend to be pollinated by numerous species of generalist bees and Lepidoptera, as well as hummingbirds (e.g. McDonald 1991; Austin 1997; Galetto and Bernardello 2004; Maimoni-Rodella and Yanagizawa 2007), while many (but not all) white-flowered species are pollinated either by moths or bats (e.g. McDonald 1991; Galetto and Bernardello 2004; McMullen 2009). This pollinator specialization may limit the spread of white-flowered species into new geographic regions that lack their pollinators and may thus reduce the rate of allopatric speciation.

The different speciation rates between lineages with pigmented and white flowers that we have demonstrated here does not necessarily indicate that flower color directly affects speciation rates. We have demonstrated only a correlation between speciation rate and flower color. It is possible that other characters that evolve in a co-ordinated manner with flower color are causally responsible. For example, flower color is often correlated with other characters that make up a pollinator syndrome (Wilson *et al.* 2004; but see Smith *et al.* 2008). In particular, white-flowered species pollinated by moths and bats often have increased nectar content, produce tubular flowers, emit strong scents, and flower at night (Faegri and van der Pijl 1971). Any of these, or possibly other, factors correlated with white flowers could be responsible for differential speciation rates. In this situation, the low frequency of white-flowered species in Quamoclit would be a correlated macroevolutionary response to lineage selection on some other character. We can thus infer that lineage selection affects the distribution of flower colors within Quamoclit, but that this selection may be indirect.

3.2 Effects of Differential Diversification on Estimation of Transition Rates

A number of previous studies have examined flower-color transitions using phylogenetic methods (Armbruster 2002; Beardsley *et al.* 2003; Perrett *et al.* 2003; Kay *et al.* 2005; Whittall *et al.* 2006; Wilson *et al.* 2007), and many have concluded that transition rates have been asymmetrical. For example, Whitall *et al.* (2006) estimated that in *Aquilegia*, the rate of loss of floral pigmentation is roughly 500 times greater than the rate of gain, and accordingly, a model incorporating rate asymmetry is a significantly better fit than one assuming equal rates. However, our results suggest that it may be premature to conclude from these studies that there is overwhelming evidence for asymmetry in flower color transition rates if, as in Quamoclit, diversification rates vary among lineages with different flower colors. Both differences in diversification rates and asymmetric transition rates can produce a similar pattern of unequal numbers of species with different color states, and only methods that simultaneously estimate both sets of rates can distinguish their effects.

Why do estimates of transition rates change so much when diversification rates are included in the analysis? We believe that this effect arises because of the "tippy-ness" of white species in Quamoclit (Fig. 1). White species form clades of only one or two species, which are smaller than clades of pigmented species. The only way to get this pattern in a model that allows only transition rate differences is to have high, asymmetric transition rates, with the rate of transitions from white to pigmented being much higher than the rate from pigmented to white. In this situation, an equilibrium proportion of white-flowered species is quickly reached, and the maximum likelihood estimates of the q's are such as to make $q_{10}/(q_{10}+q_{01})$ approximately equal to the observed proportion (Nosil and Mooers 2005). Although these values lead to high inferred number of character state changes (Fig. 3), they are the only ones that give a reasonable fit to the observed distribution of white species. However, when speciation and extinction rates are added to the model, higher extinction or lower speciation rates for white-flowered species can yield a "tippy" tree without necessitating asymmetric transition rates.

Incorporating the knowledge that traits not only evolve along the tree but also shape the tree may allow us to finally address long-standing questions about a wide range of traits of adaptive significance. In the extensive phylogenetic studies of the evolution of host specialization,

for example, several authors have cited potential pitfalls of parsimony and traditional ML transition-rate methods, and noted that the two can yield directly contradictory results (Stireman 2005; Nosil and Mooers 2005; Goldberg and Igic 2008). Our analysis of Quamoclit yielded similar apparently contradictory results: the parsimony results indicated a small number of losses of pigmentation with no gains, while the traditional ML analysis focusing solely on character transitions suggested high rates of transition with gains occurring at a much higher rate than losses. The joint analyses of transition rates and diversification rates, however, resolved this contradiction by demonstrating that there is no strong asymmetry in transition rates and that the rarity of white species is instead due primarily to differences in speciation. By extending the application of these methods to other characters and clades, we can begin to understand the relative importance of transition rates and differential diversification in determining the distribution of traits across the tree of life.

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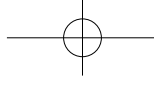
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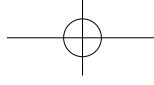
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Appendix 1. Species belonging to the Quamoclit clade and their flower colors. Section names are in all caps and those in bold are well-established taxa. Those not in bold represent our current systematic hypotheses for these species. Species in bold were sampled for phylogenetic analysis; genbank numbers for the ITS sequences and voucher information are available upon request. **I. hederacea* treated as synonymous with *I. nil*.

Section and associated species	Color code	Description (reference)
EXOGONIUM (Choisy) Griseb.		
I. aristolochiifolia G. Don	1	Limb light sky-blue or pink, throat white, tube base white or yellow (1)
I. bracteata Cav.	1	Magenta or rarely lavender or greenish (2)
I. caudata Fernald	1	Red-purple (2)
I. dumetorum Roem & Schult.	1	Pink to dark lavender limb, tube paler (2)
I. dumosa Benth. L. O. Williams	1	Mauve (2)
I. elongata Choisy	1	Red-purple limb (2)
I. eximia House	1	Purple (2)
I. expansa J. A. McDonald	1	Pale lavender-blue (2)
I. ignava House	1	Rose or purple (2)
I. jicama Brandegee	0	White tube, pale lavender or white limb (2)
I. mcvaughii McPherson	1	White tube, pink limb (2)
I. meyeri (Spreng.) G. Don	1	Limb sky-blue, tube whitish in the exterior, yellow in the interior (3)
I. miquihuanensis J. A. McDonald	1	Purple (2)
I. monticola (Meisn.) O'Donell	1	Rose (4)
I. noctulifolia McPherson	1	Red-purple limb, white tube (2)
I. piurensis O'Donell	1	White or with a rose limb and darker tube (5)
I. puncticulata Benth.	0	White or pale pink along interplicae (2)
I. purga (Wender.) Hayne	1	Magenta (2)
I. schaffneri Watson	1	Rose (2)
I. seducta House	1	Mauve (2)
I. simulans Harb.	1	Magenta (2)
I. suffulta (Kunth) G. Don	1	Red-purple or white limb, white tube (2)
I. tastensis Brandegee	0	White or pale pink along interplicae (2)
I. thurberi A. Gray	1	Purple flowers opening in the evening (6)

Stacey D. Smith *et al.*   **223**

Section and associated species	Color code	Description (reference)
I. uhdeana **(Hallier f.) D. F. Austin**	1	Red (2)
I. variabilis **(Schltdl & Cham.) Choisy**	1	Blue or purple, tube white (7)
LEPTOCALLIS **(G. Don) J. A. McDonald**		
I. capillacea (Kunth) G. Don	1	Limb purple, throat pink, basal tube white (8)
I. chamelana **J. A. McDonald**	0	Yellow (8)
I. costellata **Torr.**	1	Limb blue, tube white (3)
I. madrensis S. Watson	1	Limb blue-purple, tube pink (8)
I. perpartita McPherson	1	Purple inside, limb white (8)
I. plummerae A. Gray	1	Limb purple, tube pink (8)
I. tenuiloba Torr.	1	White, pink, or purple (8)
I. ternifolia **Cav.**	1	Limb bluish or lavender or rarely cream, tube white, throat white or yellow inside (8)
I. subrevoluta Choisy	1	Lavender or purple (9)
MINA **(Cerv.) Griseb.**		
I. cholulensis **Kunth**	1	Orange-red (3)
I. coccinea **L.**	1	Orange-red or red with yellow tube (10)
I. cristulata Hallier f.	1	Orange-red (10)
I. decemcornuta O'Donell	1	Violet (11)
I. dubia Roem. & Schult.	1	Red (5)
I. fissifolia (McPherson) Eckenw.	1	Dark bronzy red or green with faint red tinge (12)
I. funis **Schltdl & Cham.**	1	Limb orange-red (3)
I. gloverae J. A. McDonald	1	Distal portion striate pigmentation, maroon (13)
I. hastigera **Kunth.**	1	Red or orange (3)
I. hederifolia **L.**	1	Red or yellow-red (9)
I. indivisa Hallier f.	1	Red or orange-red (14)
I. lobata **(Cerv.) Thell.**	1	Red, later becoming whitish or pale yellow (15)
I. lutea Hemsl.	1	Purple (7)
I. neei **(Spreng.) O'Donell**	1	Yellow or violet or yellow with purple or violet markings (16)
I. praematura **Eckenw.**	1	Tube greenish pink, limb alternating pink and orange (17)
I. quamoclit **L.**	1	Crimson or white (18)
I. rubriflora **O'Donell**	1	Red (14)
I. spectata J. A. McDonald	1	Red or orange (13)
PHARBITIS (Choisy) Griseb.		
I. ampullacea **Fernald**	0	White, pink-colored when dry (7)
I. emetica Choisy	1	Scarlet (19)
I. hederacea Jacq.*	1	Light blue (20)
I. indica **(Burm. F.) Merr.**	1	Limb blue, tube whitish (3)
I. jamaicensis G. Don.	1	Bright crimson to magenta (21)
I. laeta A. Gray	1	Purple (7)

Section and associated species	Color code	Description (reference)
I. lindheimeri **A. Gray**	1	Blue-lavender, sometimes with pale or white center (10)
I. mairetii **Choisy**	1	Pink limb, white tube (3)
I. neurocephala **Hallier f.**	0	Whitish, densamente cubierta con pelos amarillentos en todo tubo (7)
I. nil **(L.) Roth**	1	Blue, purple or almost scarlet, throat often white (5)
I. pubescens **Lam.**	1	Limb blue, tube white (3)
I. purpurea **(L.) Roth**	1	Limb blue and purple, tube white or rose (3)
I. temascaltepecensis P. Wilkin	1	Limb purple, tube white or pale pink (22)
I. villifera House	1	Purple (23)
TYRIANTHINAE (House) D. F. Austin		
I. chenopodiifolia **(M. Matens & Galeotti) Hemsl.**	1	Magenta (2)
I. collina House	1	Purple (11)
I. jacalana Matuda	1	Pink purple (7)
I. orizabensis **(Pelletan) Steud.**	1	Limb magenta-purple, tube white or rose (3)
I. sawyeri D. F. Austin	1	Limb lavender, tube white (24)
I. tuboides **O. Deg. & Ooststr.**	0	White, lavender when wilted (25)
"SESCOSSIANAE"		
I. ancisa **House**	0	White (11)
I. sescossiana **Baill.**	1	Purple (7)
I. stans **Cav.**	1	Purple (7)
CALONYCTION **(Choisy) Griseb.**		
I. alba **L.**	0	White, greenish banded (26)
I. magniflora O'Donell	0	White (4)
I. muricata **(L.) Jacq.**	1	Limb lilac, interior violet (7)
I. santillanii **O'Donell**	0	White (7)
TRICOLORES **D. F. Austin**		
I. cardiophylla **A. Gray**	1	Dark blue, throat white, tube interior yellow (1)
I. marginisepala **O'Donell**	1	Limb sky blue, throat white, interior tube yellow (1)
I. tricolor **Cav.**	1	Limb sky blue, throat white, interior tube yellow (1)
I. velardei O'Donell	1	Violet-blue, greenish within (26)
"BARBATISEPALAE"		
I. barbatisepala **A. Gray**	1	Light-rosy-purple (10)
"PARASITICAE"		
I. parasitica G. Don	1	Limb blue-purple, tube white (3)

Section and associated species	Color code	Description (reference)
***MICROSEPALAE* (House) D. F. Austin**		
I. microsepala **Benth.**	0	Yellow (3)
I. minutiflora **(M. Martens & Galeotti) House**	0	Yellow (3)
OUTGROUP		
I. cairica **(L.) Sweet**	1	Purple, red or white with purple center & purple tinge on outside of limb, rarely entirely white (15)

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