



TEMPO AND MODE IN PLANT BREEDING SYSTEM EVOLUTION

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Classic questions about trait evolution—including the directionality of character change and its interactions with lineage diversification—intersect in the study of plant breeding systems. Transitions from self-incompatibility to self-compatibility are frequent, and they may proceed within a species (“anagenetic” mode of breeding system change) or in conjunction with speciation events (“cladogenetic” mode of change). We apply a recently developed phylogenetic model to the nightshade family Solanaceae, quantifying the relative contributions of these two modes of evolution along with the tempo of breeding system change, speciation, and extinction. We find that self-incompatibility, a genetic mechanism that prevents self-fertilization, is lost largely by the cladogenetic mode. Self-compatible species are thus more likely to arise from the isolation of a newly self-compatible population than from species-wide fixation of self-compatible mutants. Shared polymorphism at the locus that governs self-incompatibility shows it to be ancestral and not regained within this family. We demonstrate that failing to account for cladogenetic character change misleads phylogenetic tests of evolutionary irreversibility, both for breeding system in Solanaceae and on simulated trees.

KEY WORDS: Comparative methods, Dollo’s law, macroevolution, self-incompatibility, Solanaceae.

Use of simple stochastic processes to model macroevolution (Raup et al. 1973) revolutionized the study of tempo and mode in evolution, as framed by Simpson (1944). The resulting shift in perspective transformed paleontological studies (Sepkoski and Ruse 2009) and enabled the continuing development of methods to infer historical processes from neontological data, especially molecular phylogenies (Pagel 1999; Nee 2006; Stadler 2011). A prominent area of inquiry is how trait evolution interacts with lineage diversification. Breeding system traits are of particular interest in this regard because they strongly affect the spatial and temporal distribution of genetic variation (Barrett 2003), and so potentially influence processes that lead to speciation and extinction (Stebbins 1974; Holsinger 2000).

We focus here on self-incompatibility (SI), a genetic mechanism by which hermaphrodite plants recognize and reject their own pollen. The counterpart to this powerful means of enforced outcrossing is self-compatibility (SC), a commonly derived state that allows self-fertilization. We investigate three aspects of evolutionary tempo and mode in relation to this breeding system

trait within the nightshade family (Solanaceae): the relative contributions of trait evolution within lineages versus during lineage splitting (anagenetic vs. cladogenetic modes of character change), the influence of a trait on speciation and extinction rates (state-dependent diversification), and directionality of trait evolution (particularly irreversibility).

Transitions from SI to SC are frequent—as judged by independent lines of evidence from genetic, ecological, and phylogenetic analyses (Stebbins 1974; Igić et al. 2008; Busch et al. 2011)—but it is presently unknown whether they are more likely to proceed through the anagenetic or cladogenetic mode. Understanding the possible relationships between breeding system shifts and speciation events could inform the study of population genetic processes likely to be involved in each. The spread of mutations associated with SC is generally opposed by the expression of inbreeding depression following self-fertilization, but this disadvantage can be overcome by the reproductive assurance and automatic advantage of selfing held by SC individuals (Porcher and Lande 2005; Busch and Schoen 2008). In the anagenetic

mode of trait evolution, SI is lost from an entire species when SC mutants increase in frequency until the formerly SI parental species becomes SC. Alternatively, in the cladogenetic mode, the fixation of SC mutants may take place in only one or a few populations, allowing the progenitor SI lineage to persist alongside the newly established daughter SC species. In the latter case, rapid evolution of other characters to the “selfing syndrome” may drive the swift reproductive isolation of the SC population (Stebbins 1957; Fishman and Wyatt 1999; Foxe et al. 2009; Guo et al. 2009; Busch et al. 2011; Grossenbacher and Whittall 2011). If reproductive isolation within SI species instead proceeds first, perhaps following the formation of a geographic barrier, reduced population size may hasten the fixation of SC mutants and complete the breakdown of SI (Porcher and Lande 2005). This would also be interpreted as cladogenetic loss of SI if SC mutants fix rapidly relative to the timescale of genetic divergence.

Quantifying the separate contributions of anagenetic and cladogenetic modes of SI loss from comparative data requires careful consideration of related processes. In particular, the pitfalls of failing to account for interactions between anagenetic change and character state-dependent speciation and extinction (Maddison 2006) are now widely recognized. The expectation that breeding system affects lineage diversification rates arises from the higher levels of inbreeding, increased extinction risk, and greater differentiation among populations seen in self-compatible species (Schoen and Brown 1991; Hamrick and Godt 1996; Glémin et al. 2006; Schoen and Busch 2008). Within Solanaceae, phylogenetic analyses support these expectations, finding higher net diversification rates for SI than SC species (Goldberg et al. 2010).

The interaction between state-dependent diversification and anagenetic trait change is especially pronounced for traits in which transitions occur in only one direction (Goldberg and Igić 2008). When character change is cladogenetic, however, the reliability of phylogenetic tests of evolutionary irreversibility is presently untested. Issues involving strong directionality in trait evolution are particularly relevant for SI. In the history of angiosperms, SI systems of various forms have evolved independently dozens of times, but loss of SI is far more frequent. This asymmetry is unsurprising because the mechanisms underlying SI require considerable genetic complexity (de Nettancourt 1977; Steinbachs and Holsinger 2002; Igić et al. 2008). Within Solanaceae, the asymmetry in breeding system transitions takes the extreme form—irreversibility. In this family, all SI species exhibit the homologous form of gametophytic SI, and at the locus governing the SI response, distantly related species carry several closely related alleles (Ioerger et al. 1990; Igić et al. 2006). This shared ancestral polymorphism at the SI locus provides persuasive evidence that the SI mechanism is ancestral and has not re-evolved within the family (Ioerger et al. 1990; Igić et al. 2006). We use this direct

genetic evidence for the irreversibility of SI loss in Solanaceae as a basis against which the performance of statistical phylogenetic tests is compared. Breeding system evolution in this family is thus consistent with the modern interpretation of “Dollo’s law,” which holds that once lost, a complex trait is not regained in the same form (Gould 1970).

Here, we analyze breeding system evolution within Solanaceae, using a phylogenetic model that fully integrates anagenetic and cladogenetic loss of SI with the effect of breeding system on speciation and extinction. We demonstrate how simultaneously considering the tempo and mode of lineage diversification and evolutionary transitions widens the scope of inference of historical processes from neontological data. We also show that accounting for cladogenetic character change can improve the accuracy of phylogenetic tests of evolutionary irreversibility.

Materials and Methods

DATA

Phylogenetic relationships and breeding system of 356 species in the nightshade family Solanaceae have previously been published (Goldberg et al. 2010), representing approximately 16% of species in the family (Olmstead and Bohs 2007). Approximately 38% of these species are SI. Our analyses use these published trees and character states, which are summarized here and available from the Dryad database under accession no. 1888.

Sequence alignment and tree construction used the *Phyutility* pipeline developed by Smith and Dunn (2008). The initial sparse-matrix multiple alignment for the family consisted of 995 taxa by 16,405 bp from eight loci, six from the plastid (*atpB*, *matK*, *ndhF*, *rbcL*, *trnK*, *trnL-trnF*) and two from the nuclear genome (*GBSSI*, *ITS*). Bayesian phylogenetic inference was not computationally feasible, so the maximum likelihood tree was found in a heuristic search, and 100 bootstrap replicates were also obtained.

Trees were rooted with five closely related taxa from Convolvulaceae and then pruned to the best-sampled monophyletic group of Solanaceae, the “x=12” clade. This clade encompasses the subfamilies Nicotianoideae and Solanoideae and contains 85% of the species in the family. Trees were then made ultrametric with penalized likelihood (*r8s*, Sanderson 2002), and further pruned to remove cultivars and known hybrid varieties and retain only those species with character state data. The accuracy of absolute divergence dates is not crucial for our analyses, but we constrained the age of the “x=12” crown group to 36 Ma (Paape et al. 2008) to make the time units of our results meaningful for wider comparative study.

For macroevolutionary models of binary character evolution to be applied to breeding system, each species must be classified as either SI or SC. Of the 356 taxa in this clade included on the phylogeny and with known breeding system, 196 are SC and

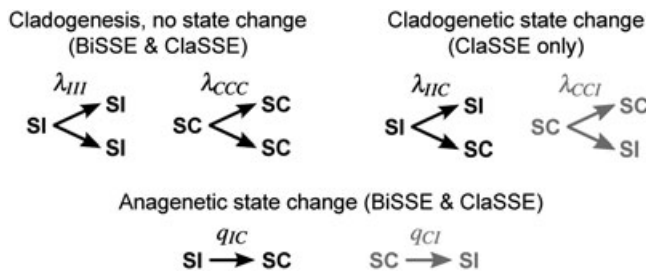


Figure 1. Processes and rates in the BiSSE and ClaSSE models. Four types of speciation (above) and two types of within-lineage state change (below) are shown. Processes in gray are constrained not to occur under the irreversible forms of the models. All models also include extinction of lineages in each state at rates μ_I and μ_C , not illustrated here.

116 are unambiguously SI. Breakdown of SI is so common that SI species studied in any detail reveal segregating SC mutants, SC populations, or sister species. Therefore, we additionally encoded as SI 17 species that have not entirely transitioned to SC. Of 27 species exhibiting dioecy, two that also show functional SI systems were encoded as SI. The other 25 were encoded as SC, following the expectation that loss of SI precedes gender dimorphism (Miller and Venable 2000). The final count of 135 SI species and 221 SC species did not significantly differ from the total available state data (Goldberg et al. 2010).

ANALYSIS

Models

To analyze these data, we extend the BiSSE model of anagenetic trait evolution and state-dependent diversification (Maddison et al. 2007) to include cladogenetic character change. The model we work with is equivalent to the BiSSE-ness model (Mayrose et al. 2011; Magnuson-Ford and Otto 2012) but framed to assign rate parameters directly to cladogenetic state change, de-

scribed below and in Figure 1. This formulation is available as the ClaSSE (Cladogenetic State change Speciation and Extinction) model in the R package *diversitree* (R Development Core Team 2009, FitzJohn 2010). The exact relationship between ClaSSE, BiSSE-ness, and related models is summarized in Appendix sA.

We fit six models to our data, each encapsulating a hypothesis about the macroevolutionary processes that shaped the history of the clade (Table 1). All models contain two character states, SI and SC, denoted by subscripts *I* and *C*, respectively. The most complex model we consider is the BiSSE-ness or ClaSSE model in which loss of SI is reversible (*classe-rev*), that is, transitions from SC to SI are allowed. There are four speciation rates in this model, denoted λ with subscripts indicating the state of the parent and the two daughter species. Thus, λ_{III} and λ_{CCC} are the per-lineage rates of speciation without state change, and λ_{IIC} and λ_{CCI} are the rates of cladogenetic state change. In the latter process, speciation produces one SI daughter and one SC daughter, for SI and SC parent lineages, respectively. We do not allow both daughter species to take states different than the parent because that is not biologically plausible for this system. Like BiSSE, this model also contains state-dependent extinction rates, μ_I and μ_C , and anagenetic transition rates, q_{IC} and q_{CI} (Fig. 1).

The parameters of this model can be constrained to prohibit regain of SI and/or to reduce the model to ones with simpler diversification processes (Fig. 1, Table 1). An irreversible model is formed by fixing $q_{CI} = 0$ and $\lambda_{CCI} = 0$, eliminating transitions from SI to SC (*classe-irr*). BiSSE, the model without cladogenetic state change, is recovered by setting $\lambda_{IIC} = \lambda_{CCI} = 0$ and can take both reversible (q_{CI} free, *bisse-rev*) and irreversible ($q_{CI} = 0$, *bisse-irr*) forms. Mk (Pagel 1994; Lewis 2001) is the model of discrete trait evolution that assumes anagenetic change only and no effect of the character states on speciation or extinction. This simpler model did not originally consider speciation or extinction, but its behavior can be recovered within this family of models by

Table 1. Comparison of model fits to the maximum likelihood tree. The “*classe*” and “*bisse*” models allow breeding system to affect speciation and extinction rates; the “*mk*” models do not. The “*classe*” models allow breeding system to change through both the anagenetic and cladogenetic mode; the “*bisse*” and “*mk*” models allow only the anagenetic mode. The “*irr*” models allow only irreversible loss of SI and not its regain; the “*rev*” models allow bidirectional transitions between SI and SC. Note that only the irreversible models are consistent with independent evidence showing that SI has not re-evolved in this clade. Median values from the posterior distributions are shown for each rate. Credibility intervals of relevant quantities are reported in the text. The best model is *classe-irr*; each other model is compared against it with a Bayes factor (*B*).

Model	λ_{III}	λ_{CCC}	λ_{IIC}	λ_{CCI}	μ_I	μ_C	q_{IC}	q_{CI}	$2 \ln B_{\text{best, other}}$
<i>classe-irr</i>	2.53	4.88	0.45	—	2.39	5.06	0.046	—	0
<i>classe-rev</i>	0.25	8.69	0.15	0.34	0.11	9.14	1.18	0.35	3.6
<i>bisse-irr</i>	2.98	5.12	—	—	2.40	5.34	0.52	—	19.0
<i>bisse-rev</i>	0.27	8.79	—	—	0.091	8.73	1.34	0.86	3.0
<i>mk-irr</i>	4.12	—	—	—	4.04	—	0.12	—	83.2
<i>mk-rev</i>	4.12	—	—	—	4.03	—	0.18	0.10	12.7

fixing $\lambda_{IIC} = \lambda_{CCI} = 0$, $\lambda_{III} = \lambda_{CCC}$, and $\mu_I = \mu_C$. It can also take both reversible (q_{CI} free, mk-rev) and irreversible ($q_{CI} = 0$, mk-irr) forms.

Fitting

We used Bayesian inference to obtain posterior distributions of the parameters under each model. We obtained 1,000,000 postburnin samples of slice-sampling Markov chain Monte Carlo (MCMC) on the maximum likelihood tree and 10,000 samples on each of the 100 bootstrap trees. Each tree was analyzed separately, and results are summarized for the maximum likelihood tree and across the set of bootstrap trees. Priors on each parameter were exponentially distributed and broad, with rate 0.3, intended to be uninformative. We included a correction for incomplete sampling (FitzJohn et al. 2009): assuming uniform sampling effort across the tree within each character state, the probability of a species being included in the analysis was 0.163 for SI species and 0.150 for SC species.

Models were compared with Bayes factors, B_{ij} , the ratio of the marginal likelihood of the better-performing model i to that of model j . These are reported as $2 \ln B_{ij}$, for which values between 2 and 6, 6 and 10, or greater than 10 indicate that evidence against model j is “positive,” “strong,” or “very strong,” respectively (Kass and Raftery 1995). The marginal likelihood of each model was computed from the MCMC samples by a non-parametric importance sampling algorithm, implemented in the R package *margLikArrogance* (R Development Core Team 2009, Escoto 2011).

SIMULATIONS

Extensive simulation testing has already confirmed substantial power and low bias when estimating rates of cladogenetic character change from phylogenies with related models (Goldberg et al. 2011; Magnuson-Ford and Otto 2012). Tests of unidirectional anagenetic change also show moderate power and good reliability, provided that state-dependent diversification is accounted for and the root state is handled appropriately (Goldberg and Igić 2008). In our analyses and tests, we use the conditional likelihood root state assumption (FitzJohn et al. 2009), which is appropriate for both reversible and irreversible models.

Model performance under unidirectional evolution, state-dependent diversification, and with the possibility of cladogenetic state change has not previously been examined. Because this scenario is central to our conclusions, we conducted two types of simulation tests in the region of parameter space relevant to our empirical analysis. First, we tested whether anagenetic and cladogenetic change can be reliably distinguished when trait evolution is irreversible. Second, we tested whether irreversible cladogenetic change is likely to be mistaken for reversible change, under either the BiSSE or ClaSSE models.

We used three batches of 1000 simulated trees for these tests. The first batch represented irreversible anagenetic change, with parameter values $\lambda_{III} = 3$, $\lambda_{CCC} = 5$, $\lambda_{IIC} = \lambda_{CCI} = 0$, $\mu_I = 2.41$, $\mu_C = 5.24$, $q_{IC} = 0.5$, $q_{CI} = 0$, and elapsed time 20. The second batch represented irreversible cladogenetic change, with the same parameter values except for $\lambda_{III} = 2.5$, $\lambda_{IIC} = 0.5$, $q_{IC} = 0$. These rates are based on our empirical results (Table 1). They yield an expected proportion of 40% SI tips, and only trees with both states represented at the tips were analyzed. The elapsed time was chosen to produce a median of approximately 300 tips, with tree sizes varying from 10 tips (the minimum allowed) to 2800. To further examine the effect of cladogenetic change, a third batch of trees elevated the rate of this process while keeping the tip state proportions and tree sizes similar: $\lambda_{III} = 2$, $\lambda_{CCC} = 5$, $\lambda_{IIC} = 1$, $\mu_I = 1.94$, $\mu_C = 5.6$, still with $\lambda_{CCI} = q_{IC} = q_{CI} = 0$.

Models were fit to each tree by likelihood maximization, taking the greatest likelihood value found in searches from several initial parameter sets. Models were compared with the Akaike information criterion (AIC), equal to twice the difference between the number of free parameters in a model and its maximum log-likelihood. The best model is the one with the lowest AIC score, but there is still “substantial” support for other models that score no more than two units higher (Burnham and Anderson 2002). Although we use maximum likelihood to expedite our simulation tests, we note that our empirical analysis shows that results from Bayesian inference need not agree. The discrepancies we observed, however, did not extend to parameter estimation under irreversible models or to significance in tests of irreversibility. We therefore proceed with maximum likelihood in our simulation tests, but we urge thorough statistical exploration of real datasets.

Results

CLADOGENETIC LOSS OF SI

To assess the importance of the cladogenetic mode in loss of SI, we fit the model allowing both anagenetic and cladogenetic state change and prohibiting regain of SI (classe-irr)—setting the regain rates to zero allows us to incorporate the independent observation of shared ancestral polymorphism at the SI locus (Igić et al. 2006). Posterior distributions of the six rates in this model are shown in Figure 2A, B.

Our results reveal that cladogenetic state change is relatively common in this system. More than one in seven speciation events among SI lineages yield one SC daughter species. (The median value of the posterior distribution of $\lambda_{IIC}/(\lambda_{III} + \lambda_{IIC})$ is 0.15. See also Table 1 and Fig. 2A. On each of the bootstrap trees, the median of $\lambda_{IIC}/(\lambda_{III} + \lambda_{IIC}) > 0.10$.) Furthermore, transitions from SI to SC are 10 times more likely to proceed through the cladogenetic than the anagenetic mode. (The median of q_{IC}/λ_{IIC} is 0.10. See

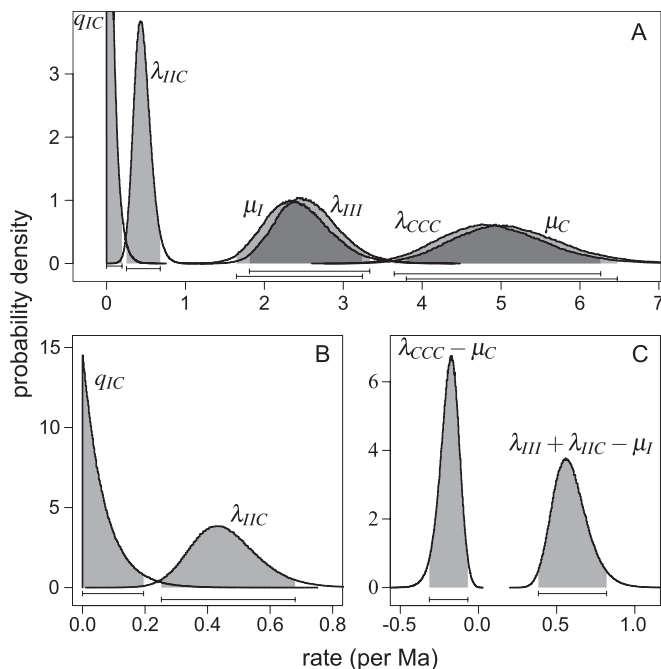


Figure 2. Posterior distributions of macroevolutionary rates under the best-fitting model (Table 1), allowing cladogenetic state change and no regain of SI (classe-irr). (A) All six rates estimated. (B) The cladogenetic rate of SI-to-SC state change (λ_{IIIC}) is substantially larger than the anagenetic rate (q_{IC}). This is a magnification of the left side of panel A. (C) The net diversification rate of SI lineages ($\lambda_{III} + \lambda_{IIIC} - \mu_I$) is significantly greater than the net diversification rate of SC lineages ($\lambda_{CCC} - \mu_C$).

also Table 1 and Fig. 2B. On each of the bootstrap trees, the median of $q_{IC}/\lambda_{IIIC} < 0.2$.) We also find that self-incompatible lineages undergo a much greater rate of net diversification than do self-compatible lineages (Fig. 2C; on each of the bootstrap trees, the difference in median net diversification rates is at least 0.5), in agreement with a previous analysis using BiSSE (Goldberg et al. 2010).

Among the models in which loss of SI is irreversible, there is very strong evidence against the two that do not allow cladogenetic state change ($2 \ln B_{\text{classe-irr, bisse-irr}} = 19.0$, $2 \ln B_{\text{classe-irr, mk-irr}} = 83.2$; Table 1). Two informal model comparisons are also instructive, focusing on the results from the two irreversible models that allow state-dependent diversification. First, the ClaSSE model decomposes the speciation and state change events that an SI lineage may undergo into separate anagenetic and cladogenetic pieces. Putting those pieces back together allows a direct comparison with the analogous processes under BiSSE. The total speciation rate for an SI lineage is $\lambda_{III} + \lambda_{IIIC}$, and the total rate of change from SI to SC is $q_{IC} + \lambda_{IIIC}$. We find that the median estimates of these quantities are the same under classe-irr and bisse-irr (in which $\lambda_{IIIC} = 0$), but that the confidence intervals from ClaSSE are narrower (Table 2). Second, we find

Table 2. Comparison of rate estimate precision on the maximum likelihood tree, with and without cladogenetic state change. Both models allow state-dependent diversification and prohibit regain of SI. ClaSSE includes the cladogenetic state change parameter λ_{IIIC} , whereas $\lambda_{IIIC} = 0$ in BiSSE. Median and 90% credibility intervals are shown for the total rate of SI speciation and the total rate of SI-to-SC state change. Averaged over the bootstrap trees, the width of the 90% CI is greater for bisse-irr than for classe-irr by 0.24 and 0.12 for $\lambda_{III} + \lambda_{IIIC}$ and $q_{IC} + \lambda_{IIIC}$, respectively.

Model	SI speciation $\lambda_{III} + \lambda_{IIIC}$	SI loss $q_{IC} + \lambda_{IIIC}$
classe-irr	2.99 (2.45, 3.66)	0.51 (0.36, 0.72)
bisse-irr	2.98 (2.31, 3.78)	0.52 (0.36, 0.78)

that the correlations between parameters are all lower when cladogenetic state change is allowed. In particular, correlation between each pair of parameters is less than 35% with classe-irr, whereas correlations between q_{IC} and the other parameters range from 40% up to 66% with bisse-irr. The exceptions are very strong correlations in both models between λ_{III} and μ_I , and between λ_{CCC} and μ_C , which are reflected in the much narrower credibility intervals of the net diversification rates (in Fig. 2, compare panels C and A; see also Fig. 1A, B in Goldberg et al. 2010). Unlike the Bayes factors reported above, these comparisons of uncertainties and correlations are not formal statistical model comparisons. They do, however, illustrate that incorporating the cladogenetic mode of character change improves the description of the processes underlying SI loss and diversification.

IRREVERSIBILITY OF SI LOSS

Independent genetic evidence shows that once SI has been lost from a lineage in Solanaceae, it has not been regained (Igić et al. 2006), as discussed in the introduction. We incorporated this knowledge into our analyses above by prohibiting transitions from SC to SI, but it can instead be withheld to assess the accuracy of phylogenetic tests of irreversibility. We therefore tested whether failing to include state-dependent diversification and cladogenetic state change causes regain of SI to be erroneously inferred. We performed statistical comparisons among the six models described above: the irreversible and reversible forms of ClaSSE, BiSSE, and Mk.

Median rate estimates and statistical model comparisons based on Bayes factors are shown in Table 1. (Results in a maximum likelihood framework are similar, except for parameter estimates under classe-rev and bisse-rev. Further discussion is in Appendix sB.) The best-fitting model allows cladogenetic state change but not regain of SI—this is the model on which our main results are based (classe-irr; e.g., Fig. 2). Using ClaSSE as the basis for a phylogenetic test the irreversibility

of SI loss produces positive evidence against the regain of SI ($2 \ln B_{\text{classe-irr, classe-rev}} = 3.6$). The opposite conclusion about SI regain is reached under the other models, however. Using BiSSE as the basis for the irreversibility test, the model allowing transitions from SC to SI is very strongly preferred over the model prohibiting them ($2 \ln B_{\text{bisse-rev, bisse-irr}} = 16.0$). Under Mk, the conclusion of SI regain is even stronger ($2 \ln B_{\text{mk-rev, mk-irr}} = 70.5$). Failing to incorporate the cladogenetic mode of state changes from SI to SC therefore leads to confident but incorrect conclusions regarding the irreversibility of SI loss.

SIMULATION TESTS OF CLADOGENETIC CHARACTER CHANGE

Distinguishing anagenetic from cladogenetic character change

Both anagenetic and cladogenetic modes of unidirectional character change were successfully identified on simulated trees. When fitting the classe-irr model to trees simulated with only anagenetic change, $q_{IC} > \lambda_{IIC}$ was correctly found on 94% of the trees, and $q_{IC}/\lambda_{IIC} > 10$ on 77% of trees. When fitting the same model to trees simulated with only cladogenetic change, $\lambda_{IIC} > q_{IC}$ was correctly found on 79% of the trees, and $\lambda_{IIC}/q_{IC} > 10$ on 52% of trees. Distributions of maximum likelihood parameter estimates across all simulated trees are shown in Figure s1.

For parameter values comparable to those we estimate in Solanaceae, we thus find that anagenetic change is more reliably identified than is cladogenetic change. Therefore, our finding that cladogenetic character change is the dominant mode in this clade is likely not an artifact of bias or power in parameter estimation.

Inferring irreversible cladogenetic character change

Irreversible cladogenetic change is generally correctly inferred through ClaSSE-based model comparisons. For parameter values comparable to our empirical estimates, the classe-rev model provided a negligible improvement in the maximum likelihood value over classe-irr for 70% of the simulated trees, yielding a preference for classe-irr with $\Delta AIC = 4$. Only 1% of trees showed any preference for the reversible model, and for only 0.3% was classe-rev preferred with $\Delta AIC > 2$. Performance was slightly worse, although still quite good, for trees simulated with greater cladogenetic character change, consistent with other findings that more rapid trait evolution makes irreversibility tests more difficult (Goldberg and Igić 2008). On 61% of these trees classe-irr was preferred with $\Delta AIC = 4$, on 3% classe-rev was preferred at all, and on 0.8% classe-rev was preferred with $\Delta AIC > 2$. When irreversibility was tested on the same trees through BiSSE-based model comparisons, performance was somewhat worse. Again, on 70% of trees simulated with the Solanaceae-like parameter values, bisse-rev provided no improvement in maximum likelihood value, yielding a preference for bisse-irr with $\Delta AIC = 2$.

On 6.6% of trees there was some preference for the reversible model, and on 3.1% bisse-rev was preferred with $\Delta AIC > 2$. For trees simulated with greater cladogenetic character change, on 41% bisse-irr was preferred with $\Delta AIC = 2$, on 24% bisse-rev was preferred at all, and on 13% bisse-rev was preferred with $\Delta AIC > 2$.

In our data, the mild preference for classe-irr over classe-rev is consistent with these simulation results. It appears unlikely, however, that the strong preference we find for bisse-rev over bisse-irr is due solely to failing to account for cladogenetic character change. For greater rates of cladogenetic state change, BiSSE-based tests of irreversibility are more likely to confidently but incorrectly infer regain of the character in question.

We also compared the fits of all four models, the irreversible and reversible forms of ClaSSE and BiSSE, on trees simulated with parameter values similar to our empirical estimates. On 68% of the simulated trees, there is not substantial support for any model other than classe-irr ($\Delta AIC > 2$). Moreover, classe-irr is preferred over bisse-irr by $\Delta AIC > 4$ on 63% of trees. The preference for classe-irr in our data is thus consistent with the power indicated by these simulation results.

Discussion

We applied a macroevolutionary model that decomposes transitions from SI to SC into anagenetic and cladogenetic modes. This yields more precise estimates of the effect of breeding system on the tempo of lineage diversification, and it demonstrates that transitions to SC occur predominantly in conjunction with speciation events. Our findings also have broader implications for phylogenetic tests of species selection and evolutionary irreversibility.

TEMPO AND MODE OF BREEDING SYSTEM EVOLUTION

The transition from SI to SC and selfing is widely held to be one of the most common evolutionary pathways in flowering plants (Stebbins 1974). Comparative studies conducted for over a century reveal that the shift can occur quickly, and that it is often accompanied by changes in floral morphology and life-history traits (Müller 1873; Darwin 1876; Clausen et al. 1940; Fryxell 1957; Stebbins 1974; Barrett et al. 2009). Theoretical studies predict potentially rapid fixation of the selfing syndrome in subpopulations through positive feedbacks between breeding system, demography, genetics, and environment that create increasingly favorable conditions for SC mutations (Porcher and Lande 2005). Population genomic data also reveal that this shift can be startlingly fast, especially when it involves severe bottlenecks (Foxe et al. 2009; Guo et al. 2009; Busch et al. 2011; Pettengill and Moeller 2012). Here, we report a slower rate of transition from SI to SC in Solanaceae, approximately the same as the net rate of

diversification of SI lineages ($\sim 0.5 \text{ Ma}^{-1}$, Table 1, Fig. 2). The time scale resolved by our study is coarser, however, encompassing the waiting time for appropriate SC mutations to arise as well as the rate of SC fixation. We therefore cannot use our phylogenetic analysis to infer the mechanisms or rates of fixation of SC mutants. Additionally, the rate we report for SI loss is an average for the entire clade, likely including lineages in which shifts are either faster or slower. In contrast, studies of mating system transitions in closely related species or subspecies (e.g., Foxe et al. 2009; Pettengill and Moeller 2012) focus on the rapid end of this spectrum. Such rate variation would add to the uncertainty in our parameter estimates, and the credibility interval of q_{IC} does extend to small values (Fig. 2B).

The relative contributions of cladogenetic and anagenetic shifts in plant breeding system evolution have not previously been quantified, despite being of considerable importance in understanding the underlying processes. We find that transitions from SI to SC are an order of magnitude more likely to occur in conjunction with speciation events than within single lineages (Table 1). Although our study does not reveal the forces responsible for the predominance of the cladogenetic mode of change, there are at least two likely explanations. First, SC and the associated morphological and life-history traits may fix in small populations with a favorable environmental and genetic context. In particular, transitions from SI to SC are commonly thought to take place in marginal habitats or after dispersal events (Baker 1955; Busch et al. 2011). Such SC populations may quickly acquire reproductive isolation, preventing fusion with the parent SI lineage upon subsequent contact (Fishman and Wyatt 1999; Grossenbacher and Whittall 2011). Second, polyploidization instantly and almost invariably breaks down SI in Solanaceae (Robertson et al. 2011) and yields strong reproductive isolation (Ramsey and Schemske 1998). A history of genome duplication can affect diversification rate (Mayrose et al. 2011), so the interacting effects of ploidy and breeding system on speciation and extinction may be a fruitful avenue of future study.

Our results also point to the importance of considering mating system shifts in the study of speciation. It has been argued that increase in the amount of self-fertilization should not be considered a contributor to speciation because it reduces both inter- and intrapopulation mating (Coyne and Orr 2004, ch. 6). Although this view is logically consistent with the biological species concept, it does not admit the potentially strong influence of mating system on the formation of other barriers. Empirical data (e.g., Fishman and Wyatt 1999; Grossenbacher and Whittall 2011) and our finding of frequent cladogenetic loss of SI indicate that we need to make a place for mating systems in the ongoing philosophical debates on the nature of species and study of isolation mechanisms.

FURTHER IMPLICATIONS OF CLADOGENETIC CHARACTER CHANGE: SPECIES SELECTION AND IRREVERSIBILITY TESTS

Including cladogenetic SI loss in our analysis does not affect our earlier finding that species selection maintains SI (Goldberg et al. 2010). It does, however, reduce the inferred strength of species selection by reducing the heritability of breeding system across speciation events. The total rate at which an SI lineage speciates is the same whether or not cladogenetic change is allowed ($\lambda_{III} + \lambda_{IC}$, Table 2). The rate at which new SI daughter lineages are produced, however, is reduced with cladogenetic change because speciation of an SI parent lineage sometimes produces an SC daughter instead. Because the rates of SI extinction and SC net diversification are little changed by the addition of the cladogenetic mode (Table 1), the advantage afforded by species selection, $\Delta r = (\lambda_{III} - \mu_I) - (\lambda_{CCC} - \mu_C)$, is lower under ClaSSE than BiSSE. Even with cladogenetic change, however, species selection is sufficient to maintain SI at a positive equilibrium frequency because anagenetic losses are reduced. (The equilibrium proportion of SI is $(\Delta r - q_{IC})/(\Delta r + \lambda_{IC})$, which has median and 90% CI of 0.33 [0.26, 0.40] under *classe-irr.*) Thus, cladogenetic character change may generally reduce the strength of species selection by lowering the heritability of species-level traits, but a balance between selection acting at different levels may still be preserved.

Species selection plays a prominent role in the theory of punctuated equilibrium, which explains macroevolutionary trends in character evolution by a combination of low anagenetic change, rapid but undirected cladogenetic trait change, and state-dependent extinction (Stanley 1975; Gould and Eldredge 1977). This mechanism is not directly relevant in our study, where species selection opposes rather than drives the trend toward SC. The components needed for testing aspects of punctuated equilibrium in other systems, however, are present in the BiSSE-ness/ClaSSE model (Magnuson-Ford and Otto 2012). Phylogenetic analysis of a discrete character with several ordered states, perhaps in lieu of using a continuously valued character, opens the possibility of new tests of punctuated equilibrium from neontological data. The same framework can be used to examine trends that may arise through other interactions between species selection and directionality in either mode of character change.

Our results raise both hopes and concerns regarding phylogenetic tests of strong directionality in trait evolution. We find that models that omit important processes are vulnerable to yielding strong but erroneous support for the re-evolution of SI within Solanaceae. Incorporating state-dependent diversification and cladogenetic state change shows those processes to contribute substantially to the macroevolutionary dynamics and weakens statistical support for the regain of SI (Table 1). Furthermore,

simulation tests show that failing to account for the cladogenetic mode of state change—at least when it is very strong—can lead to incorrect inference of trait regain. Our finding that more detailed models allow more powerful and accurate inferences is encouraging for the continuing quest to extract historical processes from phylogenetic data.

On the other hand, it is concerning that tests of irreversibility can be so susceptible to incompleteness in the underlying model. Models necessarily represent simplifications of reality, but for this test at least, two commonly made assumptions—the character state does not affect diversification, and state changes are only anagenetic—can lead not merely to reduced power, but to confident, incorrect conclusions. Furthermore, our simulation results suggest that omission of cladogenetic character change is not the sole cause for failure of a BiSSE-based test of irreversibility in our data. We do not yet know why, but additional rate heterogeneity is one possible culprit: it remains to be investigated whether rates that vary explicitly over time, among clades, or in conjunction with the evolution of other traits can mislead this test. The lingering concern, therefore, is that the extent to which model mis-specification endangers phylogenetic tests of irreversibility, in our system and others, is not yet fully appreciated.

The already prominent role of phylogenetic approaches in macroevolutionary inference continues to expand. This is driven in part by the advent of more powerful models of macroevolutionary processes, but these models rely on character state and phylogenetic data of high quality. Ongoing improvement of phylogeny estimation includes not only the acquisition of more extensive sequence data, but also more sophisticated methods for dealing with divergence time estimation and signal from multiple genes (Heled and Drummond 2010; Sibon Li and Drummond 2012). We again urge caution when phylogenetic data yield counterintuitive conclusions about lineage diversification and trait evolution, particularly with regard to irreversibility. Continuing communication between theoretical and empirical studies is essential to identify additional common assumptions that affect irreversibility tests, develop solutions when possible, and reveal the limits on what processes can be reliably inferred from phylogenies.

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Supporting Information

The following supporting information is available for this article:

Appendix sA. Model Relationships and Formulation.

Appendix sB. Alternative Model Fitting.

Figure s1. Maximum likelihood estimates of character change rates on simulated trees.

Supporting Information may be found in the online version of this article.

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Tempo and Mode in Plant Breeding System Evolution

Emma E. Goldberg & Boris Igić

APPENDIX A: MODEL RELATIONSHIPS AND FORMULATION

The central technical difficulty in phylogenetic models of cladogenetic character change is accounting for “hidden” speciation events—lineage bifurcations that do not produce a node in the reconstructed phylogeny because one or both daughter clades go extinct before the present or are otherwise not sampled. Previous treatments either ignored hidden nodes by relying on branch length transformations (Mooers et al. 1999; Pagel 1999), or they summarized the effects of hidden nodes by estimating speciation and extinction rates before modeling state changes (Bokma 2002, 2008). The best existing model of character state-dependent diversification for discrete traits (BiSSE; Maddison et al. 2007) does, however, incorporate hidden speciation events directly when computing the likelihood of the observed tip states and tree shape. In this framework, cladogenetic character change can be treated as a Poisson process—just as anagenetic change, speciation, and extinction are—without further assumptions about how its occurrence should scale with phylogenetic branch lengths or nodes.

The model we use to analyze anagenetic and cladogenetic breeding system shifts, ClaSSE with two character states, is equivalent to the BiSSE-ness model (Magnuson-Ford and Otto 2012), a recent extension of BiSSE. We find the ClaSSE parameterization more convenient for our purposes, but the two models produce identical likelihood values for corresponding sets of rates. It is also straightforward to generalize ClaSSE to more than two states. The formulation is given below, and the implementation is available in the *R* package *diversitree* (FitzJohn 2010). With three character states, ClaSSE can then be viewed as a generalization of GeoSSE (Goldberg et al. 2011), a model for the evolution of a character representing geographic range. The relationships among these models are summarized in Table A1.

ClaSSE formulation

We define λ_{ijk} to be the rate of speciation for which a parent lineage in state i produces one daughter lineage in each of states j and k , μ_i to be the rate of extinction from state i , and q_{ij} to

be the rate of transition within a lineage from state i to j , or 0 when $i = j$. For $j \neq k$, one of the parameters λ_{ijk} and λ_{ikj} is set equal to zero because they describe the same process and so can not be estimated separately.

As in BiSSE (Maddison et al. 2007) and all its model relatives, let $D_{Ni}(t)$ be the likelihood that a lineage in state i at time t gives rise to the clade N that is observed, and let $E_i(t)$ be the likelihood that a lineage in state i at time t goes extinct by the present time. The time-evolution of these quantities in the ClaSSE model is described by

$$\frac{dD_{Ni}}{dt} = - \left(\sum_{j,k} \lambda_{ijk} + \sum_j q_{ij} + \mu_i \right) D_{Ni} + \sum_j q_{ij} D_{Nj} + \sum_{j,k} \lambda_{ijk} (D_{Nj} E_k + D_{Nk} E_j) \quad (\text{A1})$$

$$\frac{dE_i}{dt} = - \left(\sum_{j,k} \lambda_{ijk} + \sum_j q_{ij} + \mu_i \right) E_i + \sum_j q_{ij} E_j + \mu_i + \sum_{j,k} \lambda_{ijk} E_j E_k, \quad (\text{A2})$$

where summations over j and k cover all character states. The initial conditions come from the tips of the tree, at $t = 0$: $D_{Ni}(0) = 1$ if the tip is in state i and 0 otherwise, and $E_i(0) = 0$. Computations proceed backwards from the tips towards the root, integrating Eq. A1–A2 along the branches of the tree. At each node C , the likelihoods for its two descendant clades N and M are merged with

$$D_{Ci}(t_C) = \frac{1}{2} \sum_{j,k} \lambda_{ijk} (D_{Nj} D_{Mk} + D_{Nk} D_{Mj}). \quad (\text{A3})$$

At the root, the overall likelihood of the tree and tip states, given the model parameters, is obtained as the sum of the likelihoods for each state weighted by the conditional probability of the root being in the corresponding state (FitzJohn et al. 2009).

Table A1: Relationships among parameters in ClaSSE and other models of the same family. For the parameters of each model indicated (first column), the equivalent parameters in ClaSSE are given (second column), along with a brief description of their meaning (third column). ClaSSE parameters not specified for a model are removed, i.e., fixed equal to zero.

BiSSE ^a or MuSSE ^b		
λ_i	λ_{iii}	speciation for state i
μ_i	μ_i	extinction for state i
q_{ij}	q_{ij}	anagenetic transition from state i to j
BiSSE-ness ^c		
λ_0	$\lambda_{000} + \lambda_{001} + \lambda_{011}$	speciation for state 0
λ_1	$\lambda_{111} + \lambda_{101} + \lambda_{100}$	speciation for state 1
p_{0c}	$(\lambda_{001} + \lambda_{011})/(\lambda_{000} + \lambda_{001} + \lambda_{011})$	cladogenetic state change from state 0
p_{1c}	$(\lambda_{101} + \lambda_{100})/(\lambda_{111} + \lambda_{101} + \lambda_{100})$	cladogenetic state change from state 1
p_{0a}	$\lambda_{001}/(\lambda_{001} + \lambda_{011})$	asymmetric cladogenetic change from state 0
p_{1a}	$\lambda_{101}/(\lambda_{101} + \lambda_{100})$	asymmetric cladogenetic change from state 1
μ_0	μ_0	extinction for state 0
μ_1	μ_1	extinction for state 1
q_{01}	q_{01}	anagenetic transition from state 0 to 1
q_{10}	q_{10}	anagenetic transition from state 1 to 0
GeoSSE ^d		
s_A	$\lambda_{112}, \lambda_{222}$	speciation within region A
s_B	$\lambda_{113}, \lambda_{333}$	speciation within region B
s_{AB}	λ_{123}	between-region speciation
x_A	μ_2, q_{13}	extinction/extirpation from region A
x_B	μ_3, q_{12}	extinction/extirpation from region B
d_A	q_{21}	dispersal/range expansion from region A
d_B	q_{31}	dispersal/range expansion from region B

^aBinary State Speciation and Extinction, Maddison et al. 2007

^bMulti-State Speciation and Extinction, FitzJohn in press

^cBiSSE-node enhanced state shift, Magnuson-Ford and Otto 2012

^dGeographic State Speciation and Extinction, Goldberg et al. 2011

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APPENDIX B: ALTERNATIVE MODEL FITTING

We also conducted a maximum likelihood, rather than Bayesian, analysis of breeding system evolution and lineage diversification in Solanaceae. Results are summarized in Table B1, for comparison with Table 1. Relative support for the six models is similar when judged with AIC rather than Bayes factors. Maximum likelihood parameter estimates are also similar to those from Bayesian inference, except for the reversible models allowing state-dependent diversification (*classe-rev* and *bisse-rev*).

The source of this difference in parameter estimates is bimodality in the likelihood surface. A single clear peak exists under the irreversible forms of ClaSSE and BiSSE. This same peak also appears in their reversible forms, although it is very narrow along the additional axes of λ_{CCI} (for *classe-rev*) and especially q_{CI} . The reversible models also show a second peak, corresponding to a set of parameter values that allows substantial regain of SI, and correspondingly higher rates of (anagenetic) SI loss, SC speciation, and SC extinction, and lower rates of SI speciation and SI extinction. This second peak is lower but broader than the first, particularly along the λ_{CCI} and q_{CI} axes. The two sets of parameter values corresponding to the two peaks collapse, however, to unimodal posterior distributions of net diversification for each state ($\lambda_{III} + \lambda_{IIC} - \mu_I$ and $\lambda_{CCC} + \lambda_{CCI} - \mu_C$) and of net state change ($q_{IC} + \lambda_{IIC} - q_{CI} - \lambda_{CCI}$).

The relative support for each of these two peaks in the reversible models depends on the model-fitting methodology. Maximum likelihood identifies the first, taller peak, and so the rate estimates under *classe-rev* and *bisse-rev* are similar to those under the irreversible forms of those models (Table B1). Bayesian inference gives more weight to the peak with the greater area under it, which in this case is the second peak. Rate estimates under *classe-rev* and *bisse-rev* thus include a moderate amount of SI regain (Table 1). Even so, the *classe-irr* model is preferred overall, consistent with the independent evidence that SI has not been regained in this family, and with the important role of cladogenesis in the loss of SI.

Table B1: Model fitting results in a maximum likelihood framework. Compare with Table 1, which shows conclusions under Bayesian inference. Relative model supports are similar under the two statistical frameworks, with classe-irr being preferred over all. Parameter estimates are also similar, except for classe-rev and bisse-rev.

Model	λ_{III}	λ_{CCC}	λ_{IIC}	λ_{CCI}	μ_I	μ_C	q_{IC}	q_{CI}	$\ln L$	ΔAIC
classe-irr	2.6	4.9	0.46	—	2.6	5.1	1e-7	—	-937	0
classe-rev	2.6	4.9	0.28	0.04	2.9	4.9	1e-6	1e-7	-936	3.4
bisse-irr	3.1	5.2	—	—	2.6	5.4	0.48	—	-949	23.4
bisse-rev	3.0	5.3	—	—	2.7	5.3	0.31	0.05	-942	7.7
mk-irr	4.2	—	—	—	4.1	—	0.12	—	-985	91.4
mk-rev	4.2	—	—	—	4.1	—	0.17	0.09	-947	16.3

Maximum Likelihood Rate Estimates on Simulated Trees

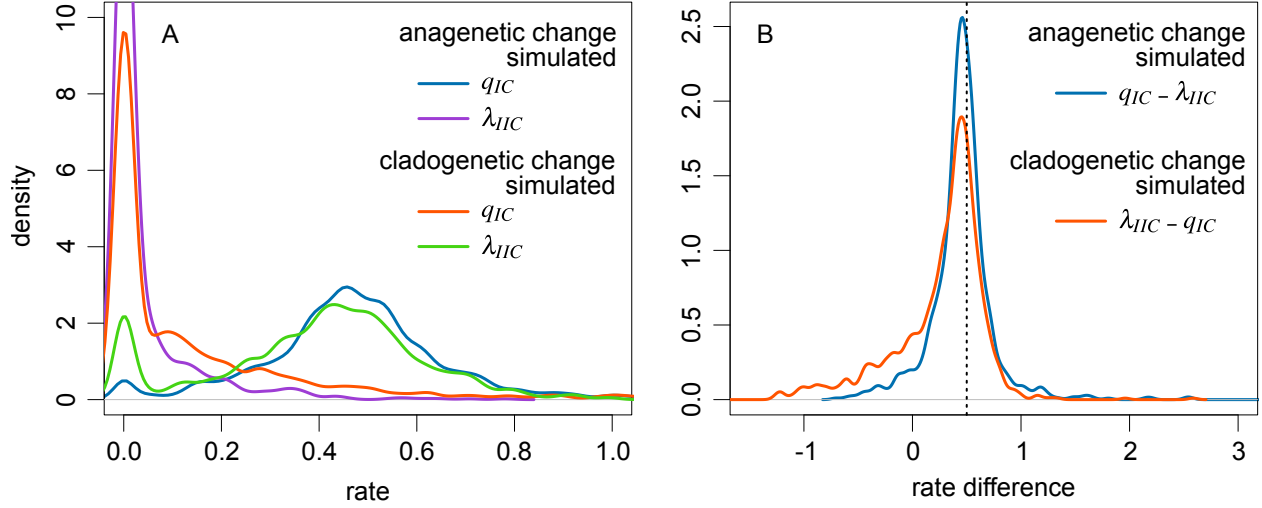


Figure S1: Maximum likelihood estimates of character change rates on simulated trees. Trees were simulated with unidirectional character change that was either purely anagenetic (blue, purple; $\lambda_{III} = 3$, $\lambda_{CCC} = 5$, $\lambda_{IIC} = \lambda_{CCI} = 0$, $\mu_I = 2.41$, $\mu_C = 5.24$, $q_{IC} = 0.5$, $q_{CI} = 0$) or purely cladogenetic (orange, green; same parameter values except for $\lambda_{III} = 2.5$, $\lambda_{IIC} = 0.5$, $q_{IC} = 0$). For 1000 trees under each of these two simulation scenarios, smoothed histograms are shown for rate estimates obtained by fitting the classe-irr model. (A) Estimates of anagenetic (q_{IC}) and cladogenetic (λ_{IIC}) character change rates. Values displayed to the left of zero are merely an artifact of smoothing; rate estimates are constrained to be non-negative. (B) Estimates of the difference between anagenetic and cladogenetic character change rates. The vertical dotted line marks the true value of this difference.