



Macroevolutionary synthesis of flowering plant sexual systems

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Sexual system is a key determinant of genetic variation and reproductive success, affecting evolution within populations and within clades. Much research in plants has focused on evolutionary transitions away from the most common state of hermaphroditism and toward the rare state of dioecy (separate sexes). Rather than transitions predominantly toward greater sexual differentiation, however, evolution may proceed in the direction of lesser sexual differentiation. We analyzed the macroevolutionary dynamics of sexual system in angiosperm genera that contain both dioecious and nondioecious species. Our phylogenetic analyses encompass a total of 2145 species from 40 genera. Overall, we found little evidence that rates of sexual system transitions are greater in any direction. Counting the number of inferred state changes revealed a mild prevalence of transitions away from hermaphroditism and away from dioecy, toward states of intermediate sexual differentiation. We identify genera in which future studies of sexual system evolution might be especially productive, and we discuss how integrating genetic or population-level studies of sexual system could improve the power of phylogenetic comparative analyses. Our work adds to the evidence that different selective pressures and constraints act in different groups, helping maintain the variety of sexual systems observed among plants.

KEY WORDS: Dioecy, hermaphroditism, phylogenetic comparative analysis, plant sexual system.

Flowering plants exhibit an unparalleled variety of sexual systems, from combined male and female function within a single flower (hermaphroditism) to entirely separate male and female individuals (dioecy), and every combination in between. Sexual system is a key determinant of genetic variation and reproductive success, affecting evolutionary processes within populations (Bawa 1980; Charlesworth and Wright 2001; Eppley and Pannell 2007; Queenborough et al. 2009) and potentially the origination and extinction of species (Vamosi and Vamosi 2005; Kay et al. 2006; Käfer et al. 2014; Sabath et al. 2016).

A full understanding of sexual system patterns and the processes behind them will draw on diverse functional perspectives and time scales (e.g., Renner and Ricklefs 1995; Heilbut 2000; Diggle et al. 2011; Käfer et al. 2014). Population-level studies provide insight into mechanistic drivers of sex ratios and sex allocation variation (Ashman 2006; Spigler and Ashman 2011; Dufay and Billard 2012), while broad comparative analyses assess correlations with ecological factors or diversification rate (Renner and Ricklefs 1995; Heilbut 2000; Vamosi et al. 2003; Kay et al. 2006; Käfer et al. 2014; Caruso et al. 2015; Sabath

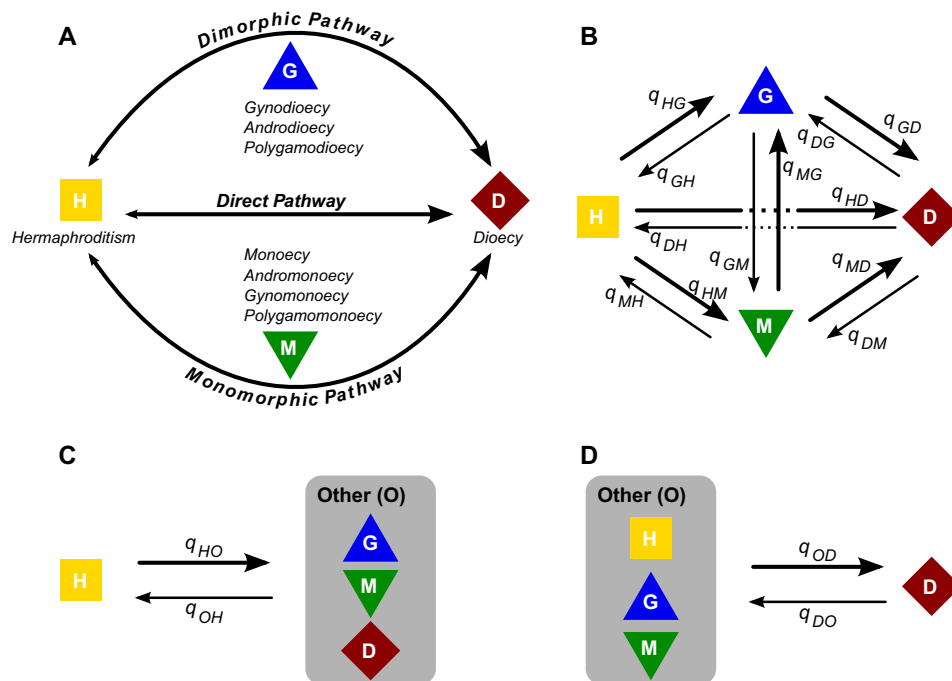


Figure 1. Sexual system states and transitions between them. (A) We consolidated the many plant sexual systems as indicated to four states (icons). Transitions are often expected to be toward greater sexual differentiation (\uparrow SD, larger arrowheads), but evolution may also proceed toward lesser sexual differentiation (\downarrow SD, smaller arrowheads). (B) Our full 4-state model allowed transitions between all pairs of states. Per-lineage rate parameters are shown by arrows: q_{XY} is the rate at which a species in state X evolves to state Y . Our 2-state analyses focused on (C) transitions away from hermaphroditism or (D) toward dioecy; the nonfocal states were grouped into a composite “other” state, reducing the number of rate parameters.

et al. 2016). Within-population processes translate into transitions among states, which can be investigated by phylogenetic studies of species-level shifts in sexual systems (e.g., Case et al. 2008; Volz and Renner 2008; Schaefer and Renner 2010; Njuguna et al. 2013; Rivkin et al. 2016). In this study, we seek generalities in transitions by synthesizing across multiple plant clades that are polymorphic for sexual system. As a complement to comparison of clades fixed for sexual system (e.g., all species dioecious; Renner 2014), a focus on clades with a mix of states provides a window into the flexibility of sexual system transitions.

Transitions between sexual systems may occur through several potential evolutionary pathways (Bachtrog et al. 2014). Because separate male and female individuals are relatively uncommon in flowering plants, these pathways are traditionally viewed as beginning in the common state of hermaphroditism and ending in the rare state of dioecy, with other states serving as intermediates. Broad categorizations are the “dimorphic” pathway, the “monomorphic” pathway, and the “direct” pathway (Fig. 1A). In the dimorphic pathway, dioecy evolves via an intermediate stage consisting of hermaphrodites and single-sexed individuals (females or males, for gynodioecy or androdioecy, respectively), followed by the replacement of hermaphrodites with the opposite-sexed type, although all three types may coexist

temporarily (polygamodioecy, sometimes called subdioecy or triecy; Lewis 1942; Lloyd 1976, 1980; Charlesworth 1989; Spigler and Ashman 2012). In the monomorphic pathway, the transition to dioecy is through a state where individual plants possess both unisexual male and female flowers (monoecy; Charlesworth and Charlesworth 1978b; Lloyd 1980). The dimorphic pathway requires that at least two mutations become linked to generate individuals with only male or female flowers, whereas in the monomorphic pathway both unisexual flowers already exist within an individual so selection acts on quantitative variation in sex allocation (Lewis 1942; Lloyd 1976, 1980; Charlesworth and Charlesworth 1978a; Webb 1999). The two pathways may intergrade, however (Barrett 1998; Renner and Won 2001; Dorken and Barrett 2004), with bisexual and one type of unisexual flower produced on the same individual (gynomonoecy and andromonoecy), or bisexual and both types of unisexual flowers on an individual (polygamomonoecy). Moreover, monoecy itself may arise from hermaphroditism via gynomonoecy or andromonoecy (Charlesworth and Charlesworth 1978b; de Jong et al. 2008; Torices et al. 2011). Finally, in the direct pathway, separate sexes evolve directly from hermaphrodite ancestors, which may be distylous or heterodichogamous, via reciprocal reductions in male and female function of the style morphs (Lloyd 1979). The

relative commonness of some of these transitions has been investigated with comparative analyses at high taxonomic levels (Weiblen et al. 2000; Torices et al. 2011), but the frequency of the various pathways in sexual system evolution is still not broadly understood.

Much work has thus focused on evolution from hermaphroditism toward separate sexes (i.e., toward greater sexual differentiation, hereafter \uparrow SD; Lloyd 1980; Ross 1982; Charlesworth 1999; Webb 1999), and dioecy has even been called an evolutionary dead end (Bull and Charnov 1985). However, transitions in the other direction may also be possible (Barrett 2013; Käfer et al. 2017), from dioecy to gynodioecy, androdioecy, or monoecy, and from those states to hermaphroditism (i.e., toward lesser sexual differentiation, hereafter \downarrow SD). For transitions to hermaphroditism, a variety of mechanisms have been described. One is a population bottleneck in a gynodioecious or androdioecious species, depending on the genetics of sex determination (Bachtrog et al. 2014; VanBuren et al. 2015; Rivkin et al. 2016). Another is resolution of cyto-nuclear conflicts when gynodioecy is determined by cytoplasmic male-sterility mutations and matched nuclear fertility restorers (Bailey et al. 2003). A third is a combination of sex inconstancy and long-distance dispersal, allowing establishment in a new location (Baker 1955; Lloyd 1975b; Case et al. 2008). For transitions away from dioecy, theory has investigated the roles of pollen limitation (Maurice and Fleming 1995; Wolf and Takebayashi 2004; Ehlers and Bataillon 2007; Crossman and Charlesworth 2014), the need for reproductive assurance during colonization (Pannell 2000), and sex ratio evolution with hybridization (Barrett et al. 2010). Empirical evidence for loss of dioecy varies in strength and approach, ranging from observations of particular forms of sex inconstancy (Lloyd 1975a) to direct genetic studies (Wolf et al. 2001; Obbard et al. 2006) to phylogenetic reconstructions (Case et al. 2008; Volz and Renner 2008; Schaefer and Renner 2010; Leslie et al. 2013; Villarreal and Renner 2013; McDaniel et al. 2013; Njuguna et al. 2013).

Even with this great variety of microevolutionary processes, the macroevolutionary dynamics of sexual system could be more consistent, such as dioecy largely functioning as an evolutionary dead end (Bull and Charnov 1985). Here, we use phylogenetic analyses to gain a broad view of the various sexual system transitions. By fitting models of trait evolution to phylogenies resolved to the species level, we quantified the evolutionary lability of sexual systems within 40 genera that are highly variable for this trait. We evaluated whether some transitions are more common than others, and whether there is a predominant evolutionary direction toward greater or lesser sexual differentiation within each genus. We do not find consistent macroevolutionary dynamics, and we discuss the mixed directionality of transitions and the potential for integrating micro- and macroevolution in studies of sexual system evolution.

Materials and Methods

DATA

Sexual system

Data on plant sexual systems were taken from the Tree of Sex compilation, to which we contributed further updates (Tree of Sex Consortium et al. 2014; Sabath et al. 2016). This database includes genera containing both dioecious and nondioecious species, defined by morphology. Although functionally defined sexual system would perhaps be more appropriate, data on it are not as widely available. Cultivated species were excluded (based largely on FAOSTAT 2012), as were hybrids. It is inherently challenging to categorize the great variety of sexual systems into a manageable and meaningful number of states for summary and analysis. The original Tree of Sex dataset includes 10 sexual system states, plus an “other” category. That is far too many categories for an analysis such as ours, so we consolidated plant sexual systems into four states (Fig. 1A): species are denoted *H* if all individuals have bisexual flowers, *D* if each individual has only male or only female flowers, *G* if only some individuals have exclusively male or female flowers, or *M* if all individuals have floral organs of both sexes and not all flowers are bisexual. This consolidation allows us to compare transitions within the dimorphic pathway (where the intermediate state includes populations with hermaphrodite and single-sexed individuals) and the monomorphic pathway (where all individual plants in the intermediate stage possess both male and female function). Although retaining more finely resolved states would allow one to pose finer-scale questions, we found that there was insufficient power to answer such questions with these data.

We coded as polymorphic species that exhibit substantial intraspecific polymorphism. This applies if one subspecies, synonym, or major population exhibits a different sexual system than the rest of the species, but not if occasional individual plants or flowers deviated from the species’ norm. Figure S1 provides a visual summary of the extant sexual systems (tip states) in each genus, while Table S1 provides the precise numbers. Table S2 summarizes the diversity of life-history traits and geographic distributions encompassed by the genera.

Phylogenetic trees

We used recently constructed genus-level phylogenies based on sequence data from GenBank (Benson et al. 2012) and a relaxed-clock model in MrBayes (Ronquist and Huelsenbeck 2003); full methods are provided by Sabath et al. (2016). We incorporated phylogenetic uncertainty in topology and relative branching times within each genus by analyzing 100 trees drawn from its posterior set. We can compare transition rates within each genus, but because each tree was scaled to a root age of 1, our rate estimates do not include an absolute time scale or reflect uncertainty in root age.

Sampling

Most genera lack complete data, with unknown sexual system or phylogenetic placement for some species. We therefore assessed whether sampling is biased in a way that could be problematic for our analyses. First, we used the full Tree of Sex database as the best available estimate of true state frequencies in each genus. Comparing it against the representation of sexual system states in the species we were able to place on our phylogenies, in only 10% of genera was sampling significantly biased (Table S1). Our analyses nevertheless incorporate the probability of being included in the phylogenetic dataset, allowing it to depend on sexual system state but assuming that it is random with respect to other factors (FitzJohn et al. 2009). Second, we consider that data reported in the Tree of Sex database could be biased by state, with species descriptions perhaps less often noting hermaphroditism than the more unusual sexual systems. We therefore also applied sampling proportions based on the number of species in each genus, in total and with dioecy, reported by Renner (2014) (Table S1). Assessing the consequences of reporting bias was only possible for the model with dioecy as the focal state (Fig. 1D) because there is not an analogous tabulation of numbers of species with other sexual systems.

We conducted statistical analyses only on genera with phylogenies that include at least 10 species with known sexual system, at least two different sexual systems, and at least two *H* or two *D* species. Of the 77 clades in the Tree of Sex database, 40 genera met these criteria, spanning 34 families. All character state and phylogenetic data used in this study are archived at <http://dx.doi.org/10.5061/dryad.7n82c>. Model-fitting (described next) was conducted with the R package *diversitree* (R Core Team 2015; FitzJohn 2012), and the analysis scripts and results summaries are also included in the Dryad archive.

MODEL FITTING

Phylogenetic models

We fit to each genus a model describing sexual system evolution with a continuous time Markov chain (“Mkn”; Pagel 1994; Lewis 2001). The most general, 4-state model allows transitions among any of the four sexual system states present in the genus (Fig. 1B). The simplified 2-state models contain *H* or *D* as the focal state, with all other states combined into an “Other” state, *O*. Thus, the *HO* model considers evolution toward or away from any degree of sexual differentiation (Fig. 1C), while the *OD* model considers the loss or gain of some plants with both male and female function (Fig. 1D).

The Mkn models assume that the character does not affect rates of speciation or extinction. Sexual system has, however, been investigated as a possible correlate of lineage diversification rate (Heilbut 2000; Kay et al. 2006; Käfer et al. 2014; Sabath

et al. 2016). We recently analyzed this same dataset using the BiSSE model (Maddison et al. 2007), which allows for character-dependent speciation and extinction rates. We found that diversification rates do not depend consistently on sexual system in these genera and, furthermore, that the range of correlations could be explained simply by chance associations (Sabath et al. 2016). Nevertheless, it is reasonable to assess whether our conclusions about sexual system transitions from the Mkn models could potentially be misled by failing to account for the influence of sexual system on speciation or extinction. We therefore also fit BiSSE models for each of our 2-state analyses. Because comparison of the Mkn and BiSSE results did not reveal qualitative differences in our overall conclusions (described below), we refrained from fitting MuSSE (FitzJohn 2012) for our 4-state analysis, which would have involved an unwieldy number of parameters.

Transition rates

We fit the phylogenetic models to estimate the transition rate parameters for each genus. The rates are denoted q_{XY} for transitions from *X* to *Y*, which are each one of *H*, *G*, *M*, *D*, or *O* (Fig. 1B–D). We used Bayesian inference and assembled the posterior distribution of rates as the concatenation of 1000 Markov chain Monte Carlo (MCMC) samples (following a burn-in of 250 steps) on each of the 100 phylogenies in the posterior set. The total of 100,000 samples per genus per model captured uncertainty in the rate estimates both within and among trees.

We found that using priors so broad as to be uninformative led to inference of unrealistically large transition rate estimates in cases where there was little signal in the data (especially when a state was rare), and this in turn confounded our estimates of transition rate directionality. We consequently took an empirical Bayesian approach and, for each model, estimated a single transition rate prior from the dataset as a whole. First, we conducted each analysis with the constraint of equal transition rates in both or all directions, using an exponential with rate 1 as the prior. (The time unit is the root age of the tree, which was always scaled to 1.) Second, we computed the median value of those rate estimates across all the MCMC samples for all genera. Those rate values were 0.6 for the *HO* analysis, 0.4 for the *OD* analysis, and 0.35 for the 4-state analysis. Third, we used exponential distributions with those rates as the prior in each final analysis. This empirical Bayesian approach approximates the posterior distributions that would be obtained from a fully Bayesian hierarchical analysis (Gelman et al. 2013, Section 5.1).

Model selection is an alternative means to test for transition rate asymmetry. We prefer not to rely on it here, however, because our interpretations would not be enhanced by rejecting constrained models that represent symmetric transition rates, only \uparrow SD transitions, or only \downarrow SD transitions. Model selection results do, however, provide an indication of power. In likelihood ratio

tests for the 2-state analyses (not shown), we found that there was power to reject one or more of those three constrained models in roughly two-thirds of genera; the other one-third are genera for which our main analyses did not recover confident results.

Species with multiple sexual systems were treated as equally likely to be in any of their observed states. For the 2-state models, this is equivalent to dropping the polymorphic species from the analysis as uninformative. For the 4-state model, however, polymorphism provides information when it excludes some states for a species.

Numbers of transitions

With stochastic character mapping (Huelsenbeck et al. 2003), we used the transition rate estimates to simulate many possible realizations of trait evolution on each phylogeny, inferring how many times transitions occurred in our genera. The number of transitions from sexual system state X to Y is denoted n_{XY} . We obtained one stochastic mapping realization for each sample from the posterior distribution of rates, on its corresponding tree, yielding a total of 100,000 simulated histories per genus per model. For polymorphic species, a single character state was selected randomly for each mapping. (We conducted stochastic mapping under the Mkn model also in the case where the transition rates were estimated by fitting BiSSE. This is not a fully consistent procedure, but stochastic mapping methods have not been developed for BiSSE.)

We did not analyze node-based ancestral state reconstructions (Pagel 1999) because they are not as well suited to questions of which transitions have occurred. They can also be sensitive to tree topology, biased sampling, and model inadequacy (Wright et al. 2015), although we have taken measures to address each of those issues in our analyses. We did, however, reconstruct the root state for each genus. We compiled the posterior probabilities as the proportion of stochastic character mappings in each state at the root under the 4-state model.

SUMMARY STATISTICS

Summaries of transition rates and stochastic mappings allow us to assess the overall prevalences and asymmetries of transitions between sexual systems and their consistency across genera. Each of the following summaries is computed for each pair of sexual system states (X and Y) under each model (4-state, or 2-state *HO* or *OD*).

To assess the overall prevalence of sexual system transitions, we sum the total number of each kind of transition, n_{XY} , across all stochastic mappings for all genera. Dividing those sums by the total number of mappings (4,000,000 across the 40 genera) yields the average number of transitions per mapping.

To assess the overall signal of asymmetry in transitions between each pair of states, we pool results for all genera in

two ways. First, for transition rates, we compute $r_{XY} = (q_{XY} - q_{YX}) / (q_{XY} + q_{YX})$ for each step in the MCMC chain. The resulting distribution describes the relative asymmetry in \uparrow SD and \downarrow SD rates, normalized by the overall rate for a genus because the phylogenies are not time calibrated. The range of r_{XY} is from -1 to 1 ; if its 95% highest posterior density region does not include zero, the transition rate asymmetry is judged significant. We use the same criterion when assessing asymmetry of the unscaled rate difference, $q_{XY} - q_{YX}$, for each genus. Second, we quantify overall net asymmetry in the number of transitions between each pair of states. We compute, for each stochastic mapping, the proportion of transitions that are in the \uparrow SD direction: $a_{XY} = n_{XY} / (n_{XY} + n_{YX})$, which ranges from 0 to 1. (We exclude mappings that lack the transition entirely, $n_{XY} = n_{YX} = 0$.) We then examine the distribution of a_{XY} across all the mappings for all genera. Justification for obtaining posterior distributions of r_{XY} and a_{XY} by combining MCMC samples is provided by Gelman et al. (2013, Section 1.9).

To assess whether the signal of asymmetry in sexual system transitions is consistent among genera, we summarize results for each genus separately and then synthesize the results. For transition rates and numbers of transitions, respectively, we compute the median of r_{XY} and mean of a_{XY} for each genus, which we call r_{XY}^i and a_{XY}^i for genus i . Averaging across genera, we denote the mean value of the r_{XY}^i as \bar{r}_{XY} , and the mean value of the a_{XY}^i as \bar{a}_{XY} . We then use t -tests with the Holm–Bonferroni correction for multiple testing to test whether any of the \bar{r}_{XY} differ from 0 or from one another, and whether any of the \bar{a}_{XY} differ from 0.5 or from one another. Thus, we use genus as the unit of replication to test whether any sexual system transitions are significantly biased toward greater or lesser sexual differentiation.

Finally, to identify individual genera with substantial power, we examine the uncertainties of each r_{XY}^i and a_{XY}^i . We highlight genera in which the 90% credibility interval of these quantities spans no more than 25% of the possible range of values (width of 0.5 for r_{XY}^i or 0.25 for a_{XY}^i). Compared with other methods for power assessment, such as repeating the inference procedure on simulated traits or comparing prior and posterior rate distributions, this procedure has the advantage of directly probing the summary statistics on which we focus our interpretation.

Results

PREVALENCE OF TRANSITIONS

Simply counting the numbers of transitions inferred across all stochastic mappings reveals broad patterns of the relative prevalence of sexual system transitions (Fig. 2). In the 4-state model (Fig. 2A), transitions between H and the other three states are similarly common, whereas transitions involving D are most commonly with M and least commonly with G . Asymmetry is greatest for the HM pair of states (in the \downarrow SD direction) and the HD pair

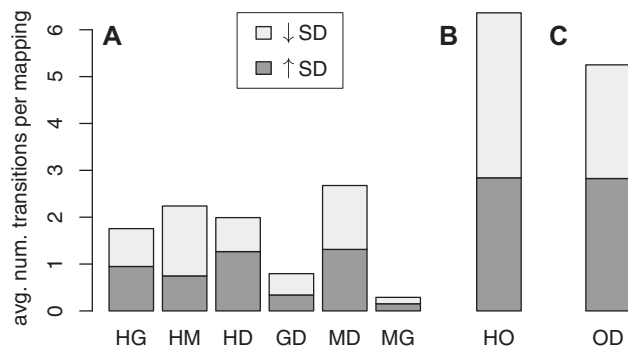


Figure 2. The average inferred number of transitions between each pair of states, across all genera, from the stochastic mappings. Results from the 4-state model are in (A), while (B) and (C) show each of the 2-state models.

(in the \uparrow SD direction). Transitions for the *MG* pair of states are more often from *M* to *G*; they occur in 11 genera but are relatively rare, and we do not consider them further. In the 2-state models, there are more transitions involving hermaphroditism, *HO*, than involving dioecy, *OD*, despite our dataset including only genera that contain some dioecious species. The summary presented in Fig. 2 tends to weight most heavily large genera with many transitions. For example, *Solanum* and *Valeriana* account for most of the \downarrow SD transitions between the *HM* and *HG* pairs of states, respectively. In contrast, *MD* transitions are highly represented because they are common in both directions in many genera.

OVERALL DIRECTION OF ASYMMETRY

The overall transition rate asymmetry, r_{XY} pooled across genera, shows essentially no directionality for any pair of sexual system states (Fig. 3). There is thus no statistical support in this dataset as a whole for the idea that lineages tend to be more susceptible to evolve toward either greater or lesser sexual differentiation. This conclusion could be due to lack of power, lack of directionality in each genus, or opposing directionality among genera. We investigate these alternatives below when we consider the consistency of asymmetry among genera.

Stochastic mapping of sexual system transitions reveals striking bimodality in the proportion of transitions that are \uparrow SD (Fig. 4). For each stochastic mapping on a particular tree with a particular set of rate values, transitions tended to be either all in one direction or all in the other direction, that is either $a_{XY} = 1$ (all \uparrow SD) or $a_{XY} = 0$ (all \downarrow SD). Across multiple stochastic mappings under the posterior of transition rates, however, the orientation is mixed, with both extreme values of a_{XY} common for each pair of states. The inferred root state explains much of this directionality, as is apparent when the distributions of a_{XY} are annotated with the root state of each stochastic mapping (Fig. S2). When transitions are not frequent, the state at the root of a genus strongly

influences the overall direction of evolution within it, with transitions being predominantly away from the root state. Nevertheless, within the bimodality of a_{XY} , different sexual systems show different strengths and directions of asymmetry. Roughly speaking, transitions are predominantly \uparrow SD for *HG*, *HM*, *HD*, and *OD*; transitions are about equally likely in either direction for *MD* and *HO*; and transitions are predominantly \downarrow SD for *GD*.

In Figure 2, each transition within a mapping is counted separately, so large genera contribute more. In Figure 4, each full mapping of transitions on a tree counts equally, allowing larger proportional contributions from smaller genera. For example, *GD* shows similar \uparrow SD and \downarrow SD in Figure 2 because two opposing genera with many transitions dominate the result, *Fraxinus* in the \uparrow SD direction and *Bursera* in the \downarrow SD direction. The Figure 4 results, however, reflect more equitable contributions of the 15 (out of 19) genera with predominantly \downarrow SD transitions for *GD*. Similarly, *HM* is predominantly \downarrow SD in Figure 2 because of *Solanum*, whereas many other genera point instead to \uparrow SD in Figure 4.

CONSISTENCY OF ASYMMETRY AMONG GENERA

We next separate the results by genus to allow statistical tests of taxonomic consistency in asymmetry. Considering the transition rate differences, we find that the lack of asymmetry in the pooled r_{XY} (Fig. 3) is underlain by a lack of significant asymmetry within most genera, rather than by opposing conclusions in different genera. The posterior distributions of the rate differences, $q_{XY} - q_{YX}$ for each pair of states *X* and *Y*, often exhibit some asymmetry, but we do not find strong statistical support for a higher rate of transition in either direction (Fig. S3–S7; with the exception of transitions to *H* from *O* and from *M* in *Solanum*). This lack of significant asymmetry largely reflects a lack of power, although the rate estimates do in general differ substantially from the prior (which is also shown in Fig. S3–S7).

Examining the proportional rate asymmetry values for each genus separately, r_{XY}^i , reveals little power in determining whether transition rates are consistently toward either greater or lesser sexual differentiation (Fig. 5A). Averaging across genera, none of the \bar{r}_{XY} differ significantly from each other, and most do not differ significantly from zero. The exceptions are \bar{r}_{HG} and \bar{r}_{OD} , which are significantly negative ($p = 0.01$ and 0.05 , respectively); however, this finding is further weakened by the additional uncertainty inherent in each r_{XY}^i (apparent in Fig. S3–S7). Of all the states in all the genera, we find that the estimate of r_{XY} has a notable amount of confidence only for transitions away from hermaphroditism in *Solanum*.

Investigating the directionality of the stochastic mapping results, a_{XY} , the bimodality seen in Figure 4 persists within many genera (Fig. S8–S12). Some genera support only \uparrow SD transitions and some only \downarrow SD transitions, but many are uncertain and

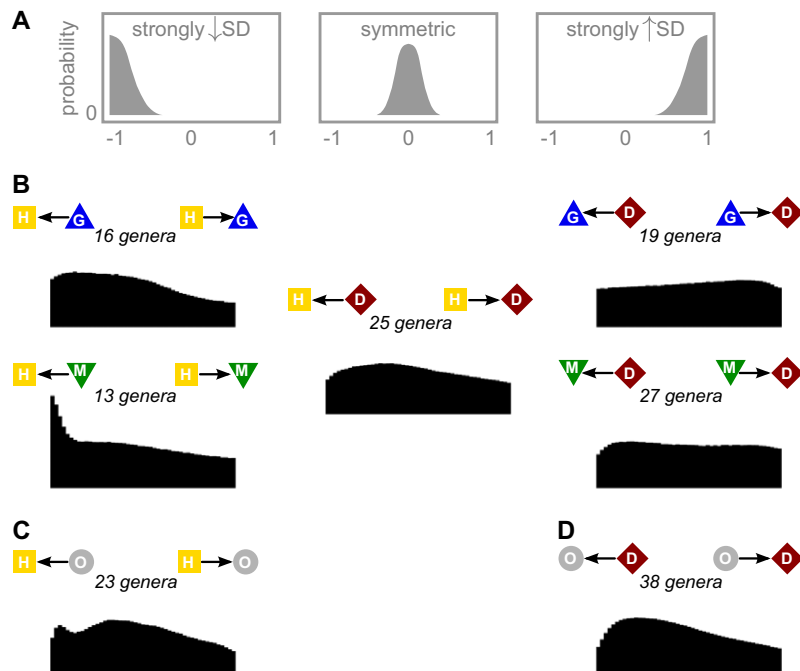


Figure 3. Distributions of the inferred asymmetry in transition rates, $r_{XY} = (q_{XY} - q_{YX}) / (q_{XY} + q_{YX})$. Hypothetical outcomes are shown in (A) as a guide to reading the figures. Real results are shown for the 4-state model in (B) and the 2-state models in (C) and (D), corresponding to Figure 1B, C, D, respectively. Icons at the extreme values of each plot show the pair of states and direction of transitions. All subpanels have the same horizontal axis scale, ranging from -1 to 1 . All subpanels in (B–D) also have the same vertical axis scale and hence the same area of each shaded distribution. The number of genera containing each pair of states is indicated.

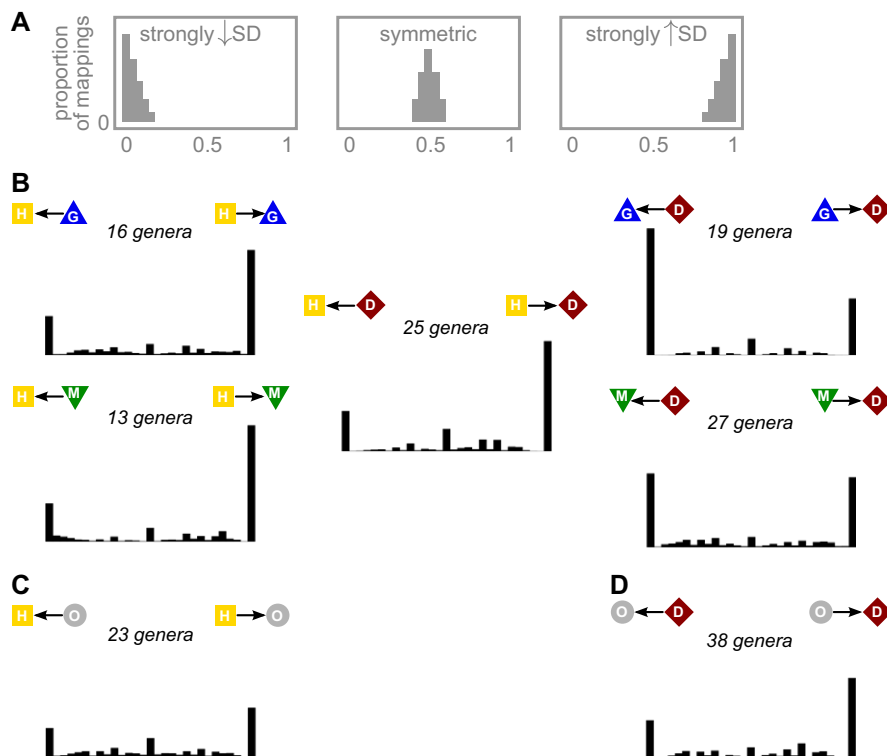


Figure 4. Distributions of the inferred proportion of transitions that are toward greater sexual differentiation for each stochastic mapping, $a_{XY} = n_{XY} / (n_{XY} + n_{YX})$. The guide (A), 4-state model results (B), and 2-state model results (C, D) are arranged as in Figure 3. All subpanels have the same horizontal axis scale, ranging from 0 to 1 . All subpanels in (B–D) also have the same vertical axis scale and hence the same area of each shaded distribution.

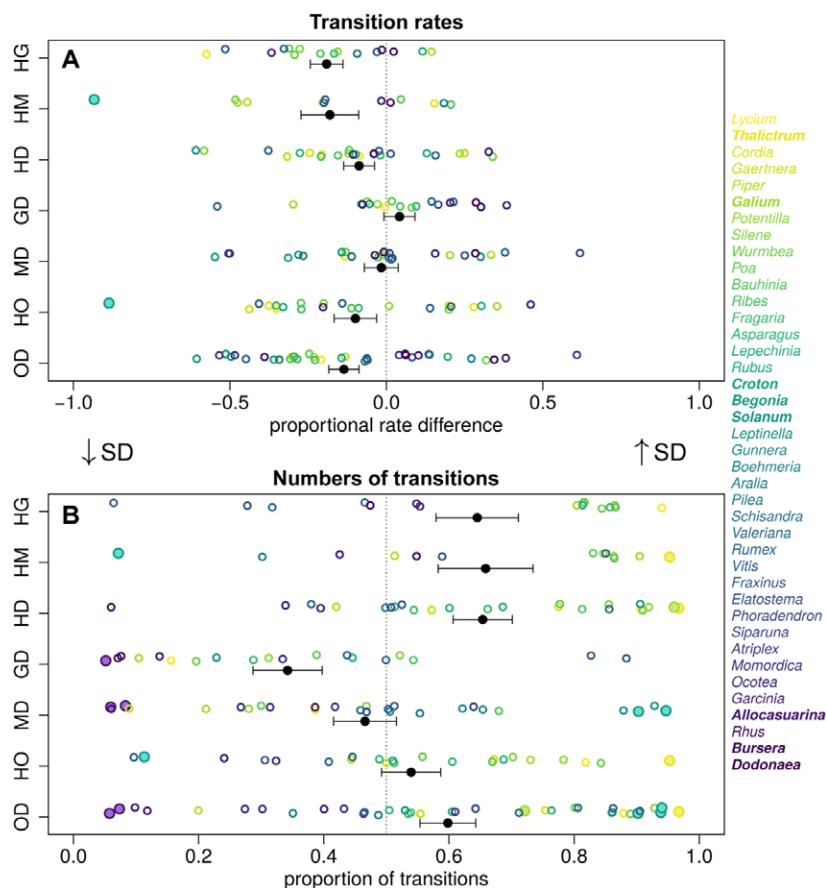


Figure 5. Proportional asymmetry in transition rates and numbers of transitions, computed separately for each genus. Each open circle is a value of (A) $r_{XY}^i = (q_{XY} - q_{YX}) / (q_{XY} + q_{YX})$ or (B) $a_{XY}^i = n_{XY} / (n_{XY} + n_{YX})$. The vertical axis shows the seven categories for pairs of sexual system states, with jitter in the points to reduce overlap. The larger, filled circles mark findings with substantial confidence (90% CI spans no more than 25% of the possible range); these genera are also named in bold. The ordering and shading of the genera roughly reflect the inferred root state (Fig. S1). Below the points for each pair of states, black circles with whiskers show the mean (\bar{r}_{XY} or \bar{a}_{XY}) and standard error.

support both or neither. For example, consider the *OD* analysis (Fig. S9). Nearly all transitions are \uparrow SD in *Begonia*, and nearly all are \downarrow SD in *Bursera*. In *Asparagus* directionality is uncertain, but most mappings include only \uparrow SD transitions or only \downarrow SD transitions, leading to both extreme values of a_{XY} . In contrast, in *Fragaria* directionality is also uncertain, but most stochastic mappings include both \uparrow SD and \downarrow SD transitions, leading to intermediate values of a_{XY} .

Finally, we consider the proportion of transitions that are toward greater sexual differentiation for each genus separately, a_{XY}^i . This summary of separate genera (Fig. 5B) agrees qualitatively with the previous description that combined genera (Fig. 4). In general terms, transitions involving hermaphroditism (*HG*, *HM*, and *HD*) are \uparrow SD, while transitions involving dioecy (*GD* and to some extent *MD*) are \downarrow SD. The 2-state *HO* and *OD* analyses exhibit less asymmetry than the 4-state analyses. However, when averaged across genera, only \bar{a}_{HD} differs significantly from 0.5 (\uparrow SD, $p = 0.02$), with marginal significance

for \bar{a}_{GD} (\downarrow SD, $p = 0.06$). Comparing the \bar{a}_{XY} with one another, \bar{a}_{GD} is significantly less than all but \bar{a}_{MD} and \bar{a}_{HO} ($p = 0.03, 0.05, 0.003, 0.02$ for *HG*, *HM*, *HD*, *OD*, respectively). In some of the genera with notably confident estimates of a_{XY} , transitions are predominantly \downarrow SD (*Allocasuarina*, *MD*; *Bursera*, *GD*, *OD*; *Dodonaea*, *MD*, *OD*; *Solanum*, *HM*, *HO*) while in others transitions are predominantly \uparrow SD (*Begonia*, *MD*, *OD*; *Croton*, *MD*, *OD*; *Galium*, *HD*, *OD*; *Solanum*, *OD*; *Thalictrum*, *HM*, *HD*, *HO*, *OD*).

The transition rate results (Fig. 5A) and stochastic mapping results (Fig. 5B) show an apparent discrepancy: when \bar{r}_{XY} is \downarrow SD, \bar{a}_{XY} is \uparrow SD (for state pairs *GD*, *HM*, *HD*, *HO*, *OD*), and vice versa (for state pair *GD*). This is explained by the rarity of some transitions in many genera. Our results about transition rate asymmetry are strongly influenced by the uncertainty associated with inferring a probabilistic process from few events, which is larger when the process is rare. For example, with few *Y*-to-*X* transitions, little information exists about q_{YX} , causing the

posterior distribution for q_{YX} to reflect the broad prior distribution and thus making r_{XY}^i seem artificially negative. Our results about numbers of transitions, however, are much less subject to being influenced by the prior because they are better anchored by the observed tip states. Our conclusions about whether transition rates are more frequently \uparrow SD or \downarrow SD are thus primarily influenced by differences in the number of lineages available to change.

INFLUENCE OF SAMPLING INCOMPLETENESS

Re-running our *OD* analysis with sampling proportions from Renner (2014) did not greatly change the overall picture. The rate estimates for most genera were little affected, though *Galium* gained significant \downarrow SD asymmetry (Fig. S13). There was almost no effect on transition asymmetries inferred by stochastic mapping (Fig. S14).

INFLUENCE OF STATE-DEPENDENT DIVERSIFICATION

Allowing for the possible effects of sexual system on rates of speciation and extinction does not qualitatively change any of the findings reported above. In only three genera was there statistical support for net diversification rate depending on sexual system (*Galium* and *Garcinia* for *HO*, and *Lycium* for *OD*). The overall transition rate asymmetry still shows essentially no directionality (Fig. S15 A, B). The overall proportion of transitions that are \uparrow SD remains strongly bimodal across genera (Fig. S15 C, D) and within them (Figs. S16 and S17). The distribution of transitions inferred for each genus separately is largely unchanged, relative to the state-independent results, with the exception of a_{HO}^i for *Galium*, where the inference shifts toward more transitions to hermaphroditism (\downarrow SD).

Considering the distribution of r_{XY}^i across genera, the pattern remains broadly similar (compare Fig. S15 E to Fig. 5A), although the values change noticeably for a few genera (especially for *HO* for *Galium* and *Garcinia*, and for *OD* for *Lycium*). Only for the *HO* transition rate asymmetry in *Galium* does the conclusion become notably confident. Considering the distribution of a_{XY}^i across genera, the proportion of transitions that are \uparrow SD also does not change much (compare Fig. S15 F to Fig. 5B), although by allowing for state-dependent diversification we lose confidence in our conclusions for some genera (*Begonia* and *Dodonaea* for *OD*).

Discussion

We investigated patterns of sexual system evolution by applying phylogenetic comparative methods to 40 genera of flowering plants. We did not find a clear and consistent signal of evolution toward greater sexual differentiation (\uparrow SD), predominantly away from hermaphroditism and toward dioecy. This was in part due to low statistical power, as our analyses were thorough in incorporating various forms of uncertainty. Here, we highlight the specific

conclusions from this study, how they relate to previous work, and how they can be built upon in future work. We also discuss more broadly the potential and limits of learning about sexual system evolution from statistical phylogenetic methods, including the promise of integrating other types of data into studies like ours.

CONCLUSIONS FROM THE PRESENT STUDY

Our work continues a long tradition of relying on phylogenetic data to investigate sexual system transitions. Previous conclusions in these genera include transitions away from hermaphroditism and toward dioecy (\uparrow SD; Miller and Venable 2000; Venter 2000; Malcomber 2002; Weigend et al. 2002; Senter and Soltis 2003; Navajas-Pérez et al. 2005; Miller et al. 2008; Wallander 2008; Martine et al. 2009; Soza and Olmstead 2010; Himmelreich et al. 2012; Soza et al. 2012, 2013) and away from dioecy and toward hermaphroditism (\downarrow SD; Bécerra and Venable 1999; Sweeney 2008; Soza and Olmstead 2010). In particular, the confident outcomes from our statistical analysis provide renewed support for transitions from hermaphroditism to monoecy and dioecy in *Thalictrum*, transitions both to and from dioecy in *Galium*, and transitions away from dioecy in *Bursera* and *Dodonaea* (compare, respectively, with Soza et al. 2012; Soza and Olmstead 2010; Bécerra and Venable 1999; Harrington and Gadek 2010). Our broad dataset additionally yielded confident conclusions in a few genera in which sex system evolution has apparently not previously been extensively investigated. Specifically, we found strong support for transitions only from monoecy to dioecy in *Croton* and *Begonia*, and for transitions primarily from dioecy to monoecy in *Allocasuarina*. In *Rhus*, although data are more limited and the directionality of transitions is somewhat less confident, the root state was confidently reconstructed as dioecious, implying loss of dioecy in multiple lineages.

Our analyses point to specific lineages that are particularly good candidates for more in-depth studies of possible loss of dioecy. Our stochastic mapping results yielded 20 species in which loss of dioecy was inferred with at least 85% confidence to occur on the external branch leading to them (Table S3). Some are from genera in which loss of dioecy has been inferred by other studies (*Atriplex*, *Bursera*, *Galium*, *Garcinia*; see respectively Freeman and McArthur 1984; Crossman and Charlesworth 2014; Soza and Olmstead 2010; Sweeney 2008). However, others are from genera in which sexual system evolution has not been heavily investigated (*Allocasuarina*, *Rhus*) or in which previous results have been equivocal (*Dodonaea*, *Piper*, *Ribes*; see respectively Harrington and Gadek 2010; Jaramillo and Manos 2001; Senter and Soltis 2003).

One surprising outcome was strong statistical support in *Solanum* for an *M* root (where *M* is andromonoecy in this genus) and for transitions primarily from *M* to *H*. This could be due to

sampling bias toward documenting andromonoecy in this genus, which has been a trait of particular interest (e.g., Whalen and Costich 1986; Anderson and Symon 1989). Almost half of *Solanum* species have unknown states in the Tree of Sex database, and our inferences could be affected if these are mostly hermaphrodites. It is also possible that transitions between andromonoecy and hermaphroditism are especially easy in this genus, necessitating only activation or suppression of female function in the flower at the tip of the cyme, and that the environment frequently favors this transition (e.g., shifting resource allocation between male and female function; Miller and Diggle 2007).

The overall difference in our results based on transition rates versus numbers of transitions raises a subtle but important distinction in discussing which evolutionary transitions are likely. For example, the statement that transitions are more likely from *H* to *G* than the reverse is true (according to our stochastic mapping results; Fig. 4B) in the sense that more lineages are inferred to have undergone that evolutionary change. It is not true, however (according to our transition rate results; Fig. 3B), that a single *H* lineage is more likely to evolve to *G* than a single *G* lineage is to evolve to *H*. The latter perspective is taken by population-level studies, both theoretical and empirical, that focus on the conditions under which a given sexual system evolves to another. The former perspective includes not only the susceptibility of a given sexual system to change, but also how common that initial state is, providing a picture of which evolutionary transitions have occurred. Both perspectives are valid, but they may disagree in their conclusions of the relative commonness of the various sexual system transitions. Phylogenetic comparative analyses provide a means to quantify both of these measures of trait evolution.

POTENTIAL AND LIMITS OF PHYLOGENETIC COMPARATIVE METHODS

Future phylogenetic comparative analyses of sexual system evolution can be greatly improved in several ways. Incompleteness in our dataset, including missing species and uncertain phylogenetic relationships, will gradually be remedied by continuing data collection. Even with complete sampling, though, using genus as the unit of taxonomic focus inherently restricts some clades to small size. Rather than analyze genera separately, as we did here, a different style of synthetic study could combine them into a hierarchical multiclade analysis, which would not necessitate estimating a separate set of rates in each genus (e.g., Sanmartín et al. 2008). Alternatively, working with deeper, larger clades could provide the power to fit more complex models of trait evolution (e.g., Torices et al. 2011). For example, one might hope to test the relative prevalence of the monomorphic versus dimorphic pathways to dioecy (Lloyd 1980), or whether androdioecy typically evolves by the breakdown of dioecy (Charlesworth and Charlesworth 1978a; Pannell 2002). (Androdioecy is extremely

rare in our dataset, as in nature, so the prevalence of transitions we found from *D* to *G* is not due to the inclusion of androdioecy in *G*.) Allowing for heterogeneous evolutionary processes within the tree, such as different sets of transition rates in different subclades, may be especially important for studies of older clades. We found some evidence of transition rate variation among genera, at least in their relative directions though we could not compare rate magnitudes across clades. We also found a hint of higher level structure in that the strongest evidence for transitions away from dioecy comes from three genera within Sapindales (*Rhus*, *Bursera*, *Dodonaea*), and other work has indicated particularly labile sexual systems in some large clades (e.g., Cucurbitales; Zhang et al. 2006).

Comparative methods also provide the possibility of investigating not only whether other traits are correlated with dioecy (Renner and Ricklefs 1995; Vamosi et al. 2003), but whether particular changes in sexual system occur more often on the background of specific traits. This could be modeled either for proposed traits, or for unknown traits or attributes yet to be identified (Beaulieu et al. 2013). Although we did not undertake such an analysis, we did investigate whether any driving processes or life-history traits clearly differentiate the genera showing evolution toward versus away from dioecy in our study. However, we found no significant association between the proportion of transitions in a genus that are toward dioecy and the prevalence of woody versus herbaceous species or perennials versus annuals, nor with whether the genus has primarily tropical versus temperate distributions, has abiotic versus biotic dispersal of seeds or pollen, or belongs to monocots versus nonmonocots (Table S2; see also analysis in Sabath et al. 2016). In short, it does not appear that an obvious trait affects either the directionality of sexual system evolution or its influence on diversification (Sabath et al. 2016), although a study explicitly focused on trait combinations (e.g., O'Meara et al. 2016) could reveal more nuanced effects. Given the diverse processes proposed to drive sexual system evolution, this is perhaps not surprising. Reproductive assurance, pollinator interactions, polyploidization, and sex inconstancy or stress-driven plasticity have all been suggested as forces driving transitions away from dioecy within genera for which we inferred \downarrow SD (Lloyd 1975a; Freeman and McArthur 1984; Miller and Venable 2000; Schaefer and Renner 2010; Soza and Olmstead 2010). Additionally, loss of self-incompatibility, selective pressure on resource allocation to male and female function, and selection to avoid inbreeding have been suggested as driving the evolution of dioecy within genera for which we inferred \uparrow SD (Heithaus et al. 1974; Opler et al. 1975; Anderson and Stebbins 1984; Anton and Connor 1995; Case and Barrett 2004; Miller and Diggle 2007; Wallander 2008; Himmelreich et al. 2012; Soza et al. 2012). Overall, the lack of clear and consistent directionality in sexual system evolution suggests that no single mechanism underlies the transitions.

Although phylogenetic comparative methods will continue to be a valuable tool for learning about sexual system evolution, they do have important limitations. Power to reconstruct transitions is inherently lacking when the evolution of the focal trait is extremely rapid or extremely slow. Even statistically confident results from phylogenetic analyses may not reflect what happened in nature, especially if the model used for trait evolution is inadequate. For sexual system in particular, there is often a continuum of sexual states (e.g., morphologically hermaphroditic plants that function largely as males or as females), and intraspecific polymorphism is common. Such complexities may prevent simplistic macroevolutionary models from capturing interesting context-dependent dynamics. As with any trait, interpreting results from analyses such as ours is best done in light of other evidence from the study system.

INCORPORATING OTHER LINES OF EVIDENCE IN PHYLOGENETIC ANALYSES

Sexual system in particular has great promise as a trait whose macroevolution can be better understood by incorporating additional types of evidence into phylogenetic comparative analyses. One general approach is using informative priors. If an understanding of the genetic and ecological changes involved in loss of dioecy suggests that this process should be rare within a group, a prior with a lower rate could be applied to this transition.

Another technique would be to refine the coding of states. For example, consider two closely related dioecious species. In a traditional analysis, they would each be coded as the same state (e.g., *D* in our study), and the trait evolution model could allow that state to have been inherited from a common ancestor. However, if it were known that dioecy had been gained separately in each lineage, the two species could be coded as different states (e.g., *D*₁ and *D*₂, to reflect distinct genetic changes), and this could increase the inferred rate of transitions to dioecy. One potential example comes from *Silene*, in which sex chromosomes in different species have independent genetic origins (Mrackova et al. 2008). Similarly, gynodioecy in *Fragaria* has different sex-determiners in different species (Ashman et al. 2015).

When there is knowledge of past states within specific lineages, phylogenetic analyses could be constrained to prohibit or enforce particular transitions along particular branches. For example, in *Cotula*, Lloyd (1975a) inferred transitions from monoecy to dioecy, and the reverse, based on morphological patterns of polymorphism. Theory further indicates that different forms of inconstancy are expected in a dioecious species if it evolved from monoecy versus from gynodioecy (Charlesworth and Charlesworth 1978a, 1978b). Other traits can also yield information on the direction of state changes. For example, in *Lycium*, knowledge about self-incompatibility and ploidy indicates that hermaphrodites are not derived from dioecious species

(Miller and Venable 2000; Yeung et al. 2005). Ploidy can also implicate sexual system transitions from dioecy to monoecy or androdioecy, as in *Mercurialis* (Obbard et al. 2006). The genetic basis of sex determination can be another means to identify the transition direction within a pair of species, as for the transition from dioecy to androdioecy in *Datisca* (Wolf et al. 2001).

More detailed knowledge of sexual systems may provide information on how long a state has been maintained within a lineage, which could also be included as a constraint in a phylogenetic model of trait evolution. One common idea is that the extremely low incidence of heteromorphic sex chromosomes in flowering plants (Charlesworth 2002; Ming et al. 2011; Renner 2014) reflects low residence times in a dioecious state. As long as transitions away from dioecy are frequent, there may not be sufficient time for forces that favor sex chromosome development (e.g., fusions bringing together loci that determine sex with those experiencing sexually antagonistic selection; Charlesworth 2015). Once sex chromosomes have evolved and become critical to proper development, transition rates away from dioecy may slow substantially. We did not use this reasoning to constrain transitions of dioecious species with and without sex chromosomes, but our results are consistent with it. For the genera in our dataset that are known to have heteromorphic sex chromosomes (*Rumex*, *Silene*), we obtained strong support for transitions only to dioecy. For the genera with species known to have homomorphic or younger sex chromosomes, we infer transitions in both directions for *Fragaria*, transitions mainly toward dioecy in *Vitis*, and equivocal results in *Asparagus*. A different approach to non-phylogenetic estimation of the duration of a sexual system state is taken by Barrett et al. (2010), who combine sex ratio data and theory to investigate the origin and stability of polygamodioecious populations in *Sagittaria*.

Conclusion

A consistent macroevolutionary picture of flowering plant sexual system evolution has proven elusive. The appearance of dioecy has been cited as one of the major evolutionary transitions (Maynard Smith and Szathmary 1997, p. 12). However, dioecy is rare (Renner 2014), and broad comparative studies have so far not demonstrated that its frequency is suppressed by either common loss of dioecy within lineages or lower net diversification of dioecious lineages. More extensive compilations of plant sexual system data and phylogenetic relationships will aid future efforts, but it may well be that more sophisticated macroevolutionary analysis approaches are also required, including incorporating findings from population-level empirical studies. Our results overall suggest that different selective forces act in different clades, helping to explain the evolution and maintenance of the myriad sexual systems observed among plants.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1: Tip state frequencies, inferred root state frequencies, and inferred duration in each state.

Figure S2: Histograms of numbers of transitions, colored by root state.

Figure S3: Transition rate estimates for the model with hermaphroditic and other states (Fig. 1C).

Figure S4: Transition rate estimates for the model with dioecious and other states (Fig. 1D).

Figure S5: Transition rate estimates for the two steps of the dimorphic pathway (Fig. 1B).

Figure S6: Transition rate estimates for the two steps of the monomorphic pathway (Fig. 1B).

Figure S7: Transition rate estimates for the direct pathway (Fig. 1B).

Figure S8: Histograms of a_{HO} , separated by genus.

Figure S9: Histograms of a_{OD} , separated by genus.

Figure S10: Histograms of a_{HG} and a_{GD} , separated by genus.

Figure S11: Histograms of a_{HM} and a_{MD} , separated by genus.

Figure S12: Histograms of a_{HD} , separated by genus.

Figure S13: Transition rate estimates for the model with dioecious and other states (Fig. 1D), using sampling proportions from Renner (2014) (Table S1).

Figure S14: Histograms of a_{OD} (left) and $n_{OD} - n_{DO}$ (right), using rates estimated with sampling proportions from Renner (2014) (Table S1).

Figure S15: Results when the transition rates are estimated while allowing for state-dependent diversification.

Figure S16: Histograms of a_{HO} , separated by genus, when the transition rates are estimated while allowing for state-dependent diversification.

Figure S17: Histograms of a_{OD} , separated by genus, when the transition rates are estimated while allowing for state-dependent diversification.

Table S1: Sexual system representation in our dataset.

Table S2: Other traits, and transitions to or from dioecy.

Table S3: Species inferred to have recently lost dioecy.