Main Manuscript for

The effects of selfing on lineage diversification across multiple genera

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This PDF file includes:

Main Text
Figures 1 to 4
SI Appendix

Abstract (194 words)

The transition from outcrossing to self-fertilization is one of the most frequent evolutionary trends in flowering plants. Selfing has long been hypothesized as an evolutionary dead end, and the prevalence of intermediate selfing populations despite their evolutionary instability is considered an enigma Nevertheless, recent theories suggest that it is high selfing populations that elevate extinction, while an intermediate selfing rate may be a best-of-both-world strategy. By estimating the dependency of speciation and extinction rates on mating system states for 27 genera, we find that outcrossing lineages overall have significantly higher net diversification rates than selfing lineages. However, whether selfing promotes or reduces speciation rates vary greatly across genera, with no significant overall effects. Moreover, self-compatible annuals tend to have lower diversification rates than self-compatible perennials. Given that annuals are much more likely to be highly selfing than perennials, this finding indirectly supports the hypothesis that suppressed diversification rates may be restricted to highly selfing, instead of intermediate selfing, populations. Consistently, models incorporating hidden states are best fitted for most of the genera, suggesting that the effects of selfing on diversification and transitions in mating systems may often depend on other factors driving diversification variation.

Significance statement (120 words)

The consequences of mating system evolution have received great attention, and selfing has long been hypothesized as an evolutionary dead end. By analyzing 27 genera to mitigate clade dependency, we find that, overall, selfing lineages show lower diversification rates, but similar speciation rates, compared to outcrossing lineages. However, the dependency of diversification rates on mating system may be often caused by the association of mating system transitions with hidden factors driving diversification variation. Indeed, diversification rates for self-compatible annuals are much lower than self-compatible perennials, which also indirectly supports recent theories that suppressed diversification may be restricted to highly selfing, instead of intermediate selfing, populations. This study offers a definitive conclusion about the macroevolutionary consequences of self-fertilization with nuanced insights.

Main text (~4300 words excluding references)

1. Introduction

Transition from outcrossing to self-fertilization is one of the most frequent evolutionary trends in flowering plants (Barrett 2008), with selfing occurring in about 50% of species (Goodwillie et al. 2005). However, it has long been hypothesized that selfing is an evolutionary dead end (Igic & Busch 2013), as transitions back towards outcrossing are theoretically implausible (Igic et al. 2008) and rarely found in nature (Barrett, 2013, Fujii et al. 2016). Selfing lineages are thought to suffer high extinction risks due to multiple microevolutionary consequences of selfing (Hartfield et al. 2017).

Selfing increases homozygosity in the offspring and reduces the effective population size due to increased variance in the number of alleles an individual leaves to the next generation (Caballero & Hill 1992). Increased genome-wide homozygosity also reduces effective recombination rates, leading to a further reduction in the effective population size in the presence of deleterious mutations (background selection; Nordborg et al. 1996), and the accumulation of deleterious mutations (Charlesworth et al. 1993, Xu 2022). Moreover, theoretical work and meta-analyses suggest that selfing tends to have lower levels of additive genetic variance in quantitative traits compared to outcrossers (Clo et al. 2019), but selfing may reveal non-additive genetic variation and may increase adaptive potential at the short-term (Clo et al. 2020, Clo & Opedal 2021).

Due to relaxed selection pressure in selfing populations, selfers are expected to have a higher ratio of nonsynonymous to synonymous divergence (D_n/D_s) or polymorphism (π_n/π_s) than outcrossers (Glémin 2007, Wright et al. 2008). Some molecular studies comparing sister species with contrasting mating systems did not detect significant differences in these ratios (Escobar et al. 2010). This is often explained as selfing lineages are often of recent origin, so inbreeding may have not persisted for sufficient time to generate significant differences. More recent studies have found higher nonsynonymous versus synonymous ratios in selfers (Ness et al. 2012, Arunkumar et al. 2015, Wang et al. 2021).

Compared to the consequences on adaptation, the effects of selfing on speciation are less understood since selfing can have multiple effects on various speciation processes (Marie-Orleach et al. 2024). Selfing may promote speciation by reducing gene flow from other populations, but can also inhibit the accumulation of alleles leading to speciation by reducing the effective population size and effective recombination rate. Recent studies suggest that Dobzhansky-Muller incompatibilities may accumulate faster in selfing populations (Marie-Orleach et al. 2022, Marie-Orleach et al. 2024). Also, selfing may be selected as a mechanism to reduce gene flow between diverging populations (reinforcement; Castillo et al. 2016), and transitions to selfing are often accompanied by a series of morphological changes (selfing syndrome; Sicard & Lenhard 2011) which may contribute to reproductive isolation (Cutter 2019). In

addition, selfing may facilitate polyploid speciation by allowing polyploid species to avoid mating with diploid individuals during the early establishment (Rausch & Morgan 2005, Barringer 2007).

The macroevolutionary effects of selfing on lineage diversification have been estimated based on state-dependent speciation and extinction (SSE) models (Helmstetter et al., 2023), but the results vary across clades. Several studies found lineages with character states promoting selfing tend to have reduced rates of net diversification (speciation minus extinction) (Goldberg et al. 2010, de Vos et al. 2014, Gamisch et al. 2015, Castillo et al. 2016, Freyman & Höhna 2019), but opposite results were found in other clades (Castillo et al. 2016, Landis et al., 2018). Also, these studies found that selfing either enhances or inhibits speciation rates, depending on the focal clade.

There are several limitations in the previous diversification analyses. Most of these analyses were done at the family level, and the results tend to be biased towards well-studied genera that have richer character state data than other genera. For example, in the analyses of Solanaceae and Onagraceae, more than 2/3 and 1/3 of the included species are from the genus *Solanum* and *Oenothera*, respectively (Goldberg et al. 2010, Freyman & Höhna 2019). Furthermore, in many family-level analyses, mating system polymorphism is mainly contributed by variation between genera, while congeneric species have identical mating system states in many genera, which makes the estimation tend to be confounded by unknown genus-specific characters. In fact, SSE models are prone to inferring false positives: even it is other factors that impact diversification rates, significant dependency of diversification rates on the focal character may still be obtained when state transitions of the focal character happen to occur in a more/less diverse part of the tree (Rabosky & Goldberg 2015). For example, the breakdown of self-incompatibility may be often associated with unfavorable environmental changes such as pollen limitation (Busch & Schoen 2008, Eckert et al. 2010, Encinas-Viso et al. 2020), but it may be pollen limitation, rather than self-compatibility, that drives extinction.

The problem of coincidental associations and clade dependency may be alleviated by performing comparative analyses on multiple clades. For example, analyses of more than 30 genera revealed that the effects of dioecy on diversification rates varies across genera, with the overall effects being insignificant (Sabath et al., 2016). Also, the issue of false positives is explicitly addressed by recently developed methods such as the HiSSE model (Hidden State Speciation and Extinction; Beaulieu & O'Meara, 2016), which allows diversification rates to depend on both measured and unknown characters. HiSSE analyses in Polemoniaceae suggested that diversification rates appear to be independent of whether species are autogamous or cross-pollinated (Landis et al. 2018), while lower diversification rates of self-compatible lineages in Onagraceae were found for both unmeasured hidden states (Freyman & Höhna 2019).

Importantly, early theoretical and empirical investigations mainly focus on differences between predominantly outcrossing versus predominantly selfing populations. However, recent progress suggests

that the rate of selfing matters, especially when considering the fixation probabilities of adaptive alleles. A higher selfing rate reduces the fixation probability of adaptive alleles at a single locus only when alleles are dominant, but very high selfing can inhibit fixation even for recessive alleles (Glémin & Ronfort 2013). Also, an intermediate selfing rate tends to promote the fixation of adaptive alleles at multiple loci by preventing haplotypes with multiple adaptive alleles from being destroyed by recombination (Xu 2023a). Populations with intermediate selfing rates may also avoid the risk of rapid mutation accumulation in highly selfing populations since even a small amount of recombination is sufficient to greatly retard Muller's ratchet (Xu 2022). Compared to outcrossing populations, selfing also purges genetic load and can provide reproductive assurance when mates or resources are limited (Busch & Delph 2012, Xu 2023b).

The above theoretical results imply that elevated extinction rates may be restricted to highly selfing, instead of intermediate selfing populations. This hypothesis may partly explain why intermediate selfing is common in nature despite being predicted to be evolutionarily unstable (Goodwillie et al. 2005, Whitehead et al. 2018). However, previous diversification analyses applied BiSSE (Binary-state SSE) by treating mating system as a binary-state character (e.g., self-incompatible/self-compatible, heterostyly/no heterostyly), which tells no information about the rate of selfing, as being self-compatible does not necessarily mean a high selfing rate (Raduski et al. 2012). Therefore, testing the proposed hypothesis requires diversification analyses that distinguish between predominantly outcrossing, mixed mating, and highly selfing lineages.

Regarding the limitations in previous diversification analyses, we revisit the question of the macroevolutionary consequences of the evolution of selfing rates, using replicated genus-level analyses across multiple genera to reduce clade dependency. We collected datasets on three selfing-related characters, including self-incompatibility, pollination systems and outcrossing rates, and carried out BiSSE analyses across 27 genera. In addition, to test whether transitions in mating systems tend to be associated with unmeasured factors, we applied HiSSE models and investigated whether selfing lineages consistently have lower or higher diversification rates than outcrossing lineages for different states of unmeasured characters.

To test the hypothesis that highly selfing lineages tend to lower diversification rates than lineages with intermediate selfing rates, it will be ideal to do diversification analyses based on outcrossing rates, but we did not find appropriate genera with sufficient data. Therefore, we instead performed diversification analyses on the combination of self-compatibility and life form states (annual/short-lived versus perennial). Regarding that annuals are much more likely to be highly selfing than perennials (Aarssen 2000, Ma et al. 2020), we expect that self-compatible annuals tend to have lower diversification rates and higher extinction rates than self-compatible perennials.

2. Results

2.1 *Data description*

We collected datasets of three characters related to selfing: self-compatibility, pollination system, and outcrossing rates. We filtered for genera with enough species that have character state data on the reconstructed phylogenies (see Materials and Methods). Our diversification analyses included 27 genera, summarized in Table S1. 21 herbaceous genera were from the self-incompatibility dataset, including 9 genera in Asteraceae, 4 genera in Brassicaceae, 4 genera in Solanaceae, 2 genera in Bromeliaceae, 1 genus in Onagraceae and 1 genus in Fabaceae. 4 genera in Polemoniaceae were from the pollination system dataset. The outcrossing rate dataset gave 2 gymnosperm genera (*Picea*, *Pinus*) that are suitable for analyses. In *Oenothera*, some self-compatible species are functionally asexual due to a special genetic system called permanent translocation heterozygosity (PTH) that nearly suppresses meiotic recombination and segregation (Holsinger & Ellstrand 1984), and thus were excluded from our diversification analyses.

We classified the mating-system-related characters into binary states 0 and 1, which indicate outcrossing and selfing, respectively. Specifically, for self-compatibility states 0 and 1 represent being self-incompatible (SI) and self-compatible (SC), respectively. For pollination system, states 0 and 1 stand for cross-pollination and autogamy, respectively. For outcrossing rates, states 0 and 1 represent species-level outcrossing rates above and below 0.8, respectively. In total, the diversification analyses of 27 genera included 360 species in state 0 and 453 species in state 1, with 19 genera having sampling fractions above than 10% (Fig. S1).

Two genera, *Solanum* and *Oenothera*, were suitable to perform diversification analyses on the combination of self-compatibility and life form states. Diversification analyses in *Solanum* included 73 SI perennial species, 25 SC annual/short-lived species, and 105 SC perennial species, with no SI annual/short-lived species found in our collected dataset. After excluding PTH species, analyses in *Oenothera* included 9 SI annual/short-lived species, 31 SI perennial species, 21 SC annual/short-lived species, and 11 SC perennial species.

2.2 *Diversification analysis on binary mating system states*

For each genus, we implemented BiSSE models using Markov chain Monte Carlo (MCMC) sampling (FitzJohn et al. 2009) across 100 bootstrap trees. Since estimations of extinction rates are suggested to be unreliable (Rabosky 2010), our analyses focused on the speciation and diversification rates (speciation minus extinction). We merged the results from 100 bootstrap trees and determined the proportion of the posterior probability distribution that supports higher diversification rates and speciation rates for state 0 than state 1, denoted by $P(r_0 > r_1)$ and $P(\lambda_0 > \lambda_1)$, respectively. The null expectation of these values should be 0.5 if the rate is state-independent. $P(r_0 > r_1)$ values of four genera from the pollination system and outcrossing rate dataset (*Gilia*, *Leptosiphon*, *Nicotiana*, *Picea*) exhibited sensitivity to the

162 choice of prior distributions or the BiSSE models (Table S2) and thus were excluded from the analyses
163 below. For details, see *Materials and Methods*.
164 Fig. 1 shows the distribution of $P(r_0 > r_1)$ and $P(\lambda_0 > \lambda_1)$ for 23 genera. In general,
165 diversification rates are significantly higher in outcrossing lineages than in selfing lineages (i.e., $P(r_0 >$
166 $r_1) > 0.5$; P=0.002). However, speciation rates do not show significant dependency on the mating system
167 state (P=0.286). These results are robust to different choices of prior distributions used in MCMC
168 sampling (Fig. S2). The higher diversification rates inferred for state 0 are not likely due to state 0 being
169 overrepresented in a clade, since no significant correlation was found between $P(r_0 > r_1)$ values and the
170 fraction of state-0 species in each genus (r=0.15; P=0.500), although genera with higher fractions of
171 state-0 species tended to have higher $P(\lambda_0 > \lambda_1)$ values (r=0.41; P=0.049).
172 The lower diversification rates inferred for selfing lineages may be contributed by both lower
173 rates of speciation and higher rates of extinction. Specifically, the $P(r_0 > r_1)$ value of each genus is
174 positively correlated with $P(\lambda_0 > \lambda_1)$ value (r=0.45; P=0.032), and negatively correlated with the
175 fraction of posterior probability distribution supporting higher extinction rates for state 0 than for state 1,
176 $P(\mu_0 > \mu_1)$ (r= -0.46; P=0.026). However, the extinction rate estimates may be unreliable (Rabosky
177 2010).
178 **2.3 *Diversification analyses on the combination of self-compatibility and life form***
179 BiSSE analyses in the previous subsection found lower diversification rates in SC lineages compared to
180 SI lineages for *Solanum* and *Oenothera*. Additional diversification analyses on the combination of self-
181 compatibility and life form states in both genera support the hypothesis that diversification rates in SC
182 annuals tend to be lower compared to SC perennials due to higher extinction rates. This suggests that low
183 diversification rates in SC lineages may be mainly contributed by highly selfing populations.
184 In *Solanum*, SC annuals have much lower diversification rates than the other two states (Fig. 2A)
185 due to very low speciation rates (Fig. 2B) and relatively high extinction rates (Fig. S3). In contrast, SC
186 perennials have slightly lower diversification rates than SI perennials (Fig. 2A), mainly due to higher
187 extinction rates (Fig. S3) rather than lower speciation rates (Fig. 2B). Moreover, Fig. 2C shows that the
188 transition rates from SI perennials to SC perennials (q_{13}), and from SC perennials to SC annuals (q_{32}),
189 are relatively high, while the transition rates from SC annuals to SC perennials are nearly 0 (q_{23}).
190 In *Oenothera*, SC lineages have lower diversification and speciation rates than SI lineages at both
191 annual and perennial states (Figs. 3A, 3B), but importantly, the differences in diversification rates
192 between SI and SC lineages are much higher at the annual state than at the perennial state (Fig. 3A).
193 Similar to the results found in *Solanum*, SC annuals have the lowest diversification rates (Fig. 3A),
194 mainly due to high extinction rates (Fig. S4). In contrast, in SI lineages, annuals have higher
195 diversification rates than perennials (Figs. 3A), mainly contributed by higher speciation rates (Fig. 3B,

196 S4). Transition rates from SI to SC were higher in annuals than in perennials (Fig. 3C). Contrary to the
197 results found in *Solanum*, transition rates from annuals to perennials are higher than in the reverse
198 direction, especially in SI lineages (Fig. 3D).

199 **2.4 *Diversification analyses with hidden states***

200 Results in the previous subsection imply that the effects of selfing on lineage diversification may depend
201 on states of other characters. Therefore, for each genus, we fitted six HiSSE models from the R package
202 "hisse" (Beaulieu & O'Meara 2016). Three models assumed no hidden states (BiSSE), and three models
203 that incorporated two hidden states, A and B (HiSSE), resulting in a total of four states: 0A, 1A, 0B, and
204 1B. For both the BiSSE and HiSSE models, we considered three types of model set-up: state-independent
205 diversification model (BiSSE null, HiSSE CID2), state-dependent diversification with irreversible
206 transitions from state 0 (outcrossing) to state 1 (selfing) (BiSSE irr, HiSSE irr), and state-dependent
207 diversification with reversible transitions between states 0 and 1 allowed (BiSSE rev, HiSSE rev). For
208 each genus, we found that the ML tree and the majority of 100 bootstrap trees consistently supported one
209 of the six models as best fitted (for details, see *Materials and Methods*).

210 Out of 27 genera, models assuming hidden states were better fitted than models assuming no
211 hidden states in 19 genera (Fig. 4A), suggesting that state transitions in mating systems may often be
212 associated with other unmeasured factors driving diversification variation. The model assuming no
213 backward transition from selfing to outcrossing (HiSSE irr) was best fitted in 12 genera (Fig. 4A).
214 However, models that allow reversible transitions (BiSSE rev, HiSSE rev) were best fitted in 7 genera
215 from the self-compatibility dataset. This may not reflect reality since most of these genera belong to
216 Asteraceae and Brassicaceae, where SI systems are maintained by self-recognition mechanisms that
217 should rarely allow transitions from SC back to SI (Fujii et al. 2016). Among the 17 genera for which
218 state-dependent models with hidden states were best fitted, we found no significant pattern in terms of
219 whether outcrossing lineages consistently have higher or lower diversification rates than selfing lineages
220 at both hidden states or not (Fig. 4B).

221 **3. Discussion**

222 Our analyses show that although the impacts of mating system states on diversification and speciation
223 rates vary across genera, outcrossing lineages overall have significantly higher net diversification rates
224 than selfing lineages, which is suggested to be attributed to both higher speciation rates and lower
225 extinction rates. However, we did not detect significant overall effects of mating systems on speciation
226 rates. This may be because speciation involves various mechanisms (Coyne & Orr 2004) and selfing can
227 have complicated effects on them, as reviewed in the introduction. Compared to previous analyses
228 performed at the family level, opposing effects of selfing on diversification were found for different
229 genera within the same family. For example, analyses of Solanaceae found lower diversification rates in

230 self-compatible lineages than self-incompatible lineages (Goldberg et al. 2010). This result is found for
231 *Solanum*, but self-compatibility is found to have no effects on diversification for *Nicotiana*.
232 We propose that highly selfing lineages tend to have much higher extinction rates than mixed-
233 mating lineages. Given that annuals are more likely to be highly selfing than perennials (Aarssen 2000,
234 Ma et al. 2020), results from diversification analyses on the combination of self-compatibility and life
235 forms (annual vs. perennial) in *Solanum* and *Oenothera* indirectly support this hypothesis. In *Solanum*,
236 SC annual lineages were found to have much lower diversification rates than SC perennial lineages. In
237 *Oenothera*, diversification rate differences between SI and SC lineages are much larger in perennials than
238 in annuals. This hypothesis may be also indirectly tested by diversification analyses on characters
239 associated with selfing rates, including anther-stigma distance and flower size (Thomann et al. 2013).
240 However, a better test of this hypothesis may require diversification analyses on a full range of selfing
241 rates once outcrossing rates in more species are measured. Moreover, although it is expected that selfing
242 may promote evolution towards shorter lifespan (Lesaffre & Billiard 2020), we found mixed results.
243 Nearly unidirectional transitions from SC perennials to SC annuals were detected in *Solanum*, but the
244 opposite trends were found in *Oenothera*.
245 Analyses incorporating life form states suggest that diversification rates may be often affected by
246 other factors associated with mating system transitions, which is also supported by the fact that models
247 incorporating hidden states are better fitted than models without hidden states for a majority of the studied
248 genera. This may be partly because transitions from outcrossing to selfing may often triggered by
249 environmental changes, such as colonization and pollen limitation (Busch & Schoen 2008, Eckert et al.
250 2010, Pannell 2016, Encinas-Viso et al. 2020). Nevertheless, how the effects of selfing on diversification
251 rates depend on the hidden states vary across genera. In some genera, selfing consistently promotes or
252 inhibits diversification for both hidden states, but in other genera, selfing has opposite effects on
253 diversification in different hidden states.
254 Briefly, our analyses support that highly selfing is an evolutionary dead end. However, our
255 analyses only reveal a correlation, instead of causation, between selfing and low diversification rates, and
256 suggest that mating system transitions may be often associated with other factors affecting lineage
257 diversification.

258 **4. Materials and methods**

259 **4.1 *Data collection***

260 We collected three types of mating system datasets: self-compatibility, pollination system, and
261 outcrossing rate. The genera for which species belong to were updated based on the accepted names in
262 WFO Plant List (https://wfoplantlist.org). The self-compatibility dataset was mainly from previously
263 publication (Wagner et al. 2017, Grossenbacher et al. 2017, Freyman & Höhna 2019, Delaney and Igić

2022, Krakos et al. 2022, Cascante-Marín et al. 2023), with additional searching for self-compatibility data of a few species. For data reported in Delaney & Igić (2022), we only included self-compatibility data based on hand-pollination experiments, while data based on verbal statements without pollination experiments were considered unreliable and excluded. A species is considered as self-incompatible (SI) when the index of self-compatibility is smaller than 0.2, and otherwise considered as self-compatible (SC). The pollination system dataset is from Landis et al. (2018).

For the outcrossing rate dataset, we assembled datasets from three studies (Dumini et al. 2009, Goodwillie et al. 2012, Whitehead et al. 2018), and added estimates from more recent publications. We focused exclusively on multi-locus estimates of outcrossing rates, since these estimates are less influenced by selection compared to other estimates (Ritland 2002, Bürkli et al. 2017). Therefore, we searched for studies reported in Web of Science through Nov. 12, 2023 that cited at least one of the five papers that developed multi-locus outcrossing rate estimations (Ritland 1990, Ritland 2002, Ritland & Jain 1981, David et al. 2007, Jones & Wang 2010). When outcrossing rate estimates for multiple populations or/and across multiple flowering seasons were reported in the study, we averaged across populations and/or flowering seasons to obtain the mean outcrossing rate at the species level.

Our diversification analyses only included genera for which the resulting phylogeny contains at least 9 species and at least three species in each state, and genera with excessively low sampling fraction were also not dropped. Two genera with rich character state data, *Solanum* and *Oenothera*, were chosen for diversification analyses on the combination of self-compatibility and life form states. Life forms of *Solanum* species were determined mainly based on the dataset from Hilgenhof et al. (2023). Species marked as shrub or tree were considered as perennials, and for species marked as herb, we determined whether they are short-lived or perennial based on descriptions from Solanaceae Source (http://solanaceaesource.org) with additional literature searching. Life forms of *Oenothera* species were determined based on descriptions in previous studies (Dietrich & Wagner 1988, Wagner et al. 2007, Evans et al. 2011).

4.2 *Phylogeny reconstruction*

For genera chosen from the pollination system dataset, we directly used the ML trees and 100 bootstrap trees from Landis et al. (2018). For other genera, phylogenies were constructed by following the procedure described below.

1). Outgroup species were chosen based on previous phylogenetic studies (see references in Table S1). Sequence data for all species in the genus and the outgroup species were downloaded from GenBank and clustered using the R package “phylotaR” (Bennett et al. 2018).

2). Within each cluster, sequences two times longer or shorter than the median sequence length were discarded. The best-represented sequence per species was chosen using the drop_by_rank() function

in the package "phylotaR". Sequences within each cluster were then aligned using MAFFT version 7 (Katho & Standley 2013), and unreliable sites were removed using Gblocks (Castresana 2000). Clusters that contain sequences from only few species were dropped, but since previous studies suggested that filtering genes based on missing data may reduce the y accuracy of phylogenies (Molloy & Warnow 2018), most clusters were retained. The remaining clusters were concatenated into a partitioned supermatrix for phylogeny constructions.

3). Phylogenetic trees were reconstructed using raxmlGUI 2.0 (Edler et al. 2021) with 100 bootstraps and an initial model test to choose the best model. To ultrametricize trees, we used the function "chronos" from the R package "ape" (Paradis & Schliep 2019). The smoothing parameter λ in "chronos" function, which weighs the penalty for rate variation across branches (Sanderson 2002), was chosen by performing cross-validation on the maximum-likelihood (ML) tree using the "CV" function from the R package "chronos" (https://github.com/josephwb/chronos), by varying λ from 10^{-2} to 10^{3} with an interval of 0.5 in the log10 scale. Outgroups and species with missing character state information were then pruned from ultrametricized trees for diversification analyses.

One genus, *Eucalyptus*, was excluded from diversification analyses due to unreliable phylogeny reconstruction results. Mating system character states, supermatrices and phylogenies of each genus are available at https://doi.org/10.5281/zenodo.13265323. For each genus, diversification analyses were performed on 100 bootstrap trees to account for phylogenetic uncertainty.

4.3 *Diversification analyses*

We assessed the impacts of mating system states on lineage diversification in several ways. We used the R package "diversitree" (Fitzjohn 2012) and implemented BiSSE models for each genus with the Markov chain Monte Carlo (MCMC) sampling approach to estimate the posterior probability distributions of parameters (FitzJohn et al. 2009). We assessed the influence of the combination of self-compatibility and life form states on diversification by implementing Multiple State Speciation and Extinction (MuSSE) models with MCMC sampling using the "diversitree" package. We also fitted a series of HiSSE models using the R package "hisse" for each genus (Beaulieu & O'Meara 2016). The sampling fraction used in these SSE models was calculated based on the number of accepted species from the WFO Plant List (https://wfoplantlist.org), by assuming that there is state-dependent sampling bias, since we lacked complete character state information of all taxa in each genus.

In a full BiSSE model, six parameters were estimated: the speciation rates for states 0 and 1 (λ_0, λ_1), the extinction rates for states 0 and 1 (μ_0, μ_1), and the transition rates between states 0 and 1 (q_{01}, q_{10}). However, for genera with character states being self-compatibility, to account for the theoretical prediction and observation that breakdown of self-incompatibility is hardly reversible (Igic et al. 2008, Barrett 2013), we constraint the transition rates from state SI to SC to be 0 (i.e., $q_{10} = 0$). For genera

from the pollination system and outcrossing rate datasets, we implemented both the full BiSSE model and the BiSSE model with no transition from state 1 to 0, and genera were excluded from the analyses in Fig. 1 if the two models give qualitatively different results (Table S2).

For MuSSE analyses on the combination of self-compatibility and life form state in *Solanum* and *Oenothera*, we assumed no simultaneous state transitions at self-compatibility and life form (e.g., transitions between SI annual and SC perennial). Also, following previous studies (Goldberg et al. 2010, Freyman & Höhna 2019), we assumed no transitions from SC to SI.

In MCMC sampling, an exponential prior distribution was used for each parameter, with the mean being fp_0. Here p_0 is the initial rate estimate from the function starting.point.bisse() in the "diversitree" package, and f is a parameter that we varied (see below). For each tree, MCMC chains were started at the maximum likelihood estimates of the model parameters. MCMC chains were run for 2500 steps with the initial 20% steps discarded as burn-in for BiSSE models, and were run for 10000 steps with the initial 25% steps discarded for MuSSE models (Soltis et al. 2013). For each genus, results from 100 bootstrap trees were combined to calculate the proportion of the posterior probability in which the parameter rate was higher for state 0 lineages than for state 1 lineages. Specifically, we use $P(r_0 > r_1)$ and $P(\lambda_0 > \lambda_1)$ to denote the proportion of MCMC steps in which outcrossing lineages were inferred to have higher diversification rates and speciation rates than selfing lineages, respectively.

To examine whether the results were sensitive to the prior distributions used in our MCMC simulations, we used three values of f=1, 2, 4 in the prior distributions for each parameter (Sabath et al. 2016). Genera were considered not robust and were excluded from the analyses in Fig. 1 if $P(r_0 > r_1)$ values differed by > 30% across the different values of f (see Table S2).

For each genus, we fitted six models from the R package "hisse" on the ML tree and bootstrap trees as described in Section 2.4 (BiSSE null, BiSSE rev, BiSSE irr, HiSEE CID2, HiSSE rev, HiSSE irr). It should be noted that the BiSSE models from the package "hisse" differ from the BiSSE model from the package "diversitree" used in our MCMC analyses due to different model set up. In all models, we assumed no simultaneous transition in more than one state. For each tree, the best fitted model was chosen based on the lowest AICc values instead of AIC values to account for small sample size (Table S2). The best fitted model for each genus was determined based on the best-fitted model from the 100 bootstrap trees and the ML tree.

When a state-dependent model with hidden states (HiSSE rev or HiSSE irr) was best fitted for a genus, we classified the rate estimations from a bootstrap tree into two scenarios: 1) diversification rates for state 0 are higher or lower than for state 1 in both hidden states ($r_{0A} > r_{1A}$ and $r_{0B} > r_{0B}$, or $r_{0A} < r_{1A}$ and $r_{0B} < r_{0B}$); 2) diversification rates for state 0 are higher than for state 1 in one hidden state but lower in the other hidden state ($r_{0A} > r_{1A}$ and $r_{0B} < r_{1B}$, or $r_{0A} < r_{1A}$ and $r_{0B} > r_{1B}$). We recorded the

366 occurrence of the two scenarios in 100 bootstrap trees to assess whether outcrossing consistently gives
367 higher or lower diversification rates than selfing at both hidden states or not.

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6. References

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7. Figure Legends

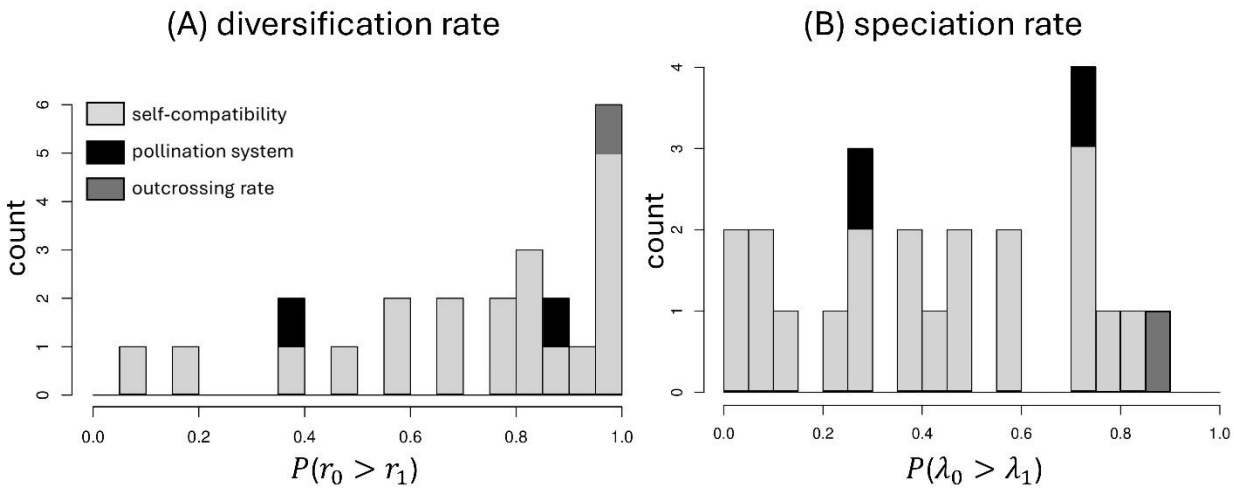

Figure 1. The proportion of the posterior probability distribution from BiSSE MCMC sampling that supports higher diversification rates and speciation rates for state 0 than state 1, assuming no transition from state 1 to state 0. Figures show the distribution for 23 genera. Colors represent genera from different datasets. The p-value from one-sample Wilcoxon rank-sum test is 0.002 for panel (A), and 0.111 for panel (B).

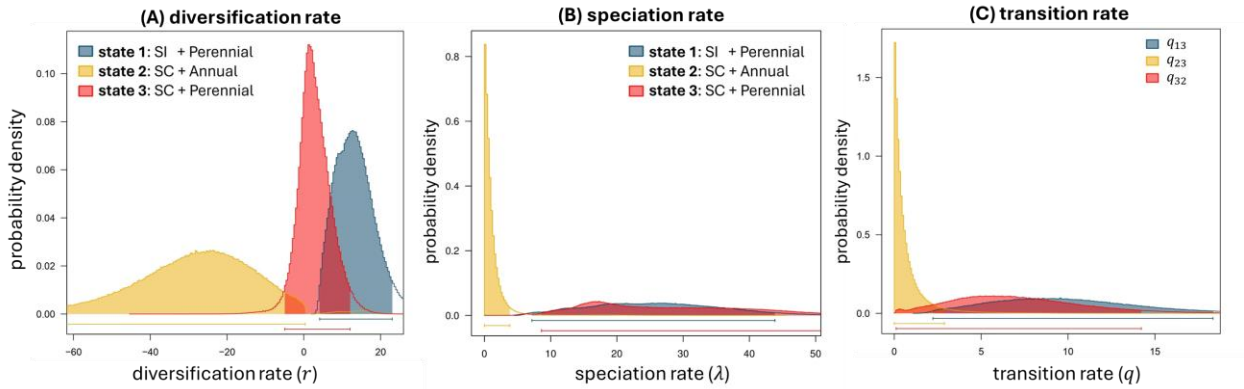

Figure 2. Posterior distribution of the diversification rates, speciation rates and transition rates for the combination of self-compatibility and life form states in *Solanum*. In panel (C), q_{ij} is the rate of transition from state i to j. Results were obtained from MuSSE MCMC simulations on 100 bootstrap trees, with $f = 2$ used in the prior distribution. Results are robust to different values of f used in the prior distribution (Fig. S3).

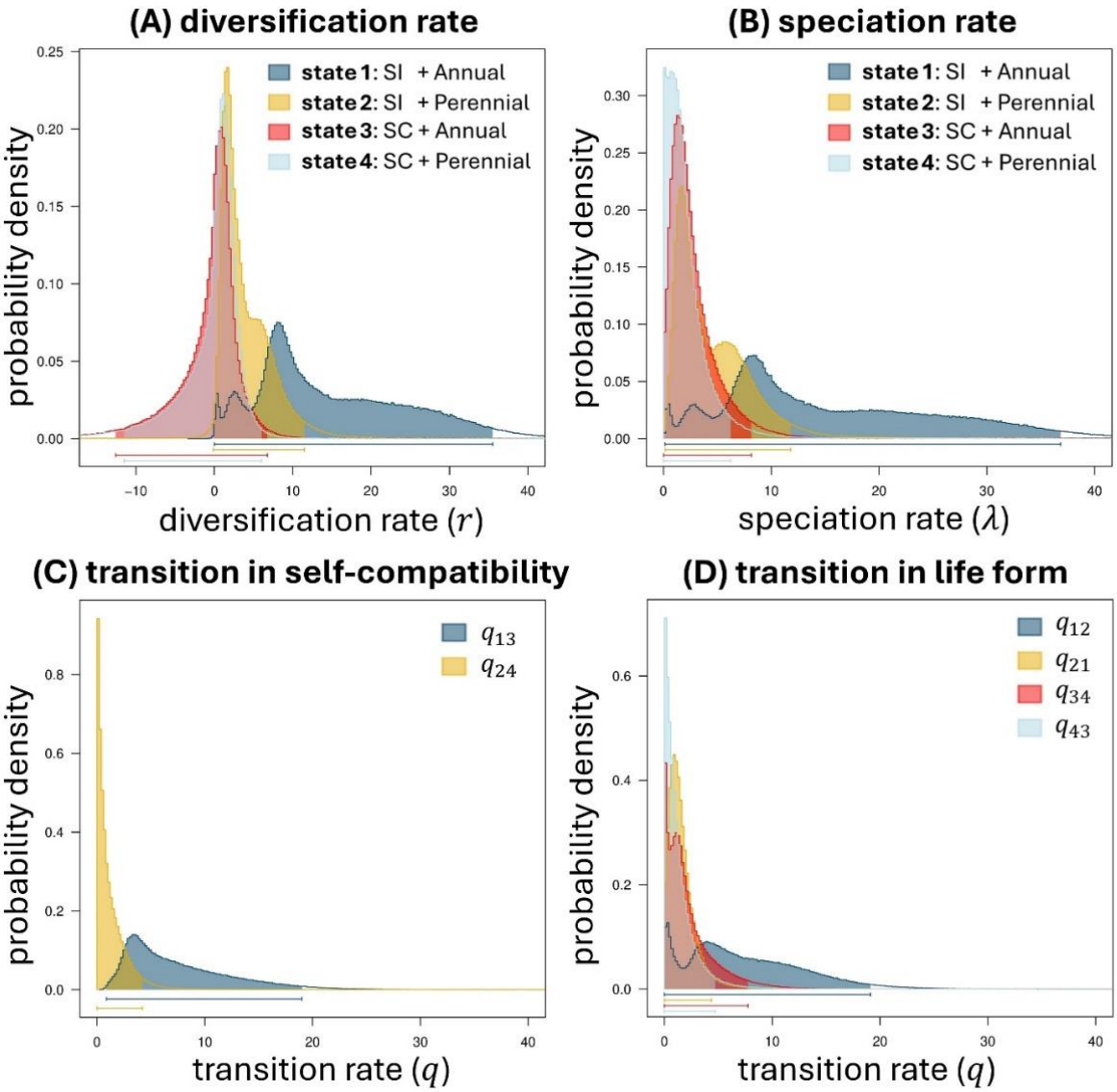

Figure 3. Posterior distribution of the diversification rates, speciation rates and transition rates for the combination of self-compatibility and life form states in *Oenothera*. Results were obtained from MuSSE MCMC simulations on 100 bootstrap trees, with $f = 2$ used in the prior distribution. Results are robust to different values of f used in the prior distribution (Fig. S4).

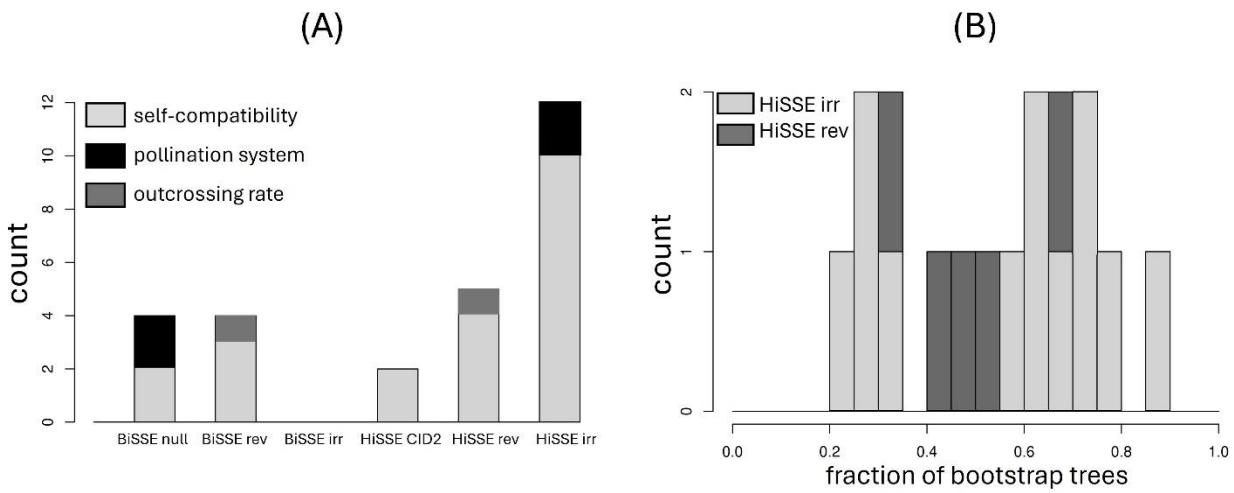

Figure 4. (A) Distribution of the best fitted model across 27 genera. (B) The fraction of 100 bootstrap trees of each genus for which the estimated diversification rates from the package "hisse" for state 0 are consistently higher or lower than state 1 in both hidden states A and B. The distribution of the fractions did not deviate from 50% by Wilcoxon test, no matter whether the two HiSSE models were analyzed separately (P=0.478 for HiSSE irr; P=1.000 for HiSSE rev) or merged (P=0.505).

Supporting Information for

The effects of selfing on lineage diversification across multiple genera

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This PDF file includes:

Figures S1 to S4

Tables S1 to S3

Supplementary Figures

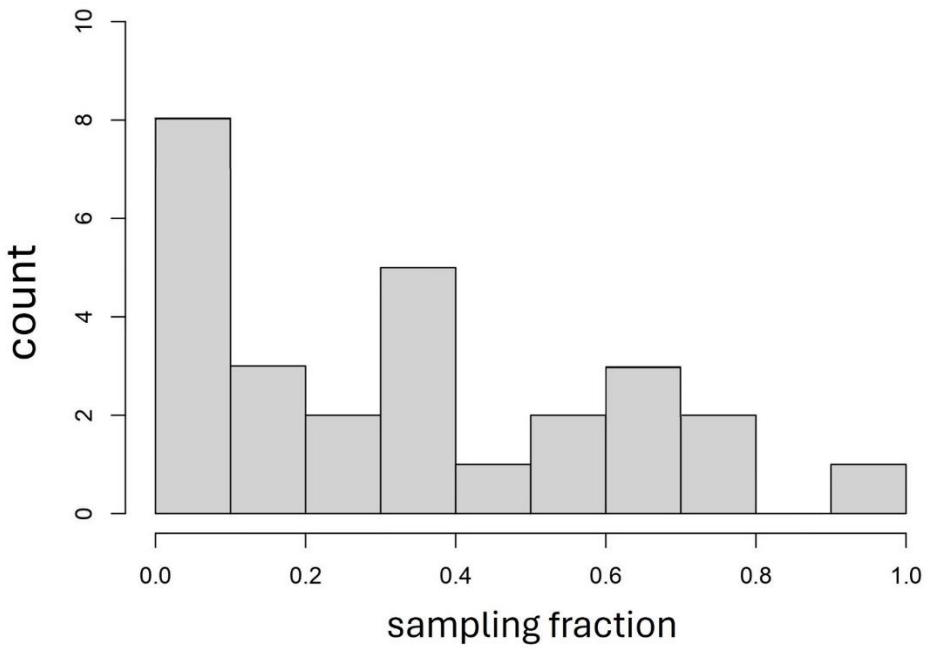

Figure S1. Distribution of the fraction of species used in diversification analyses out of the number of accepted species in the genus, calculated based on Table S1.

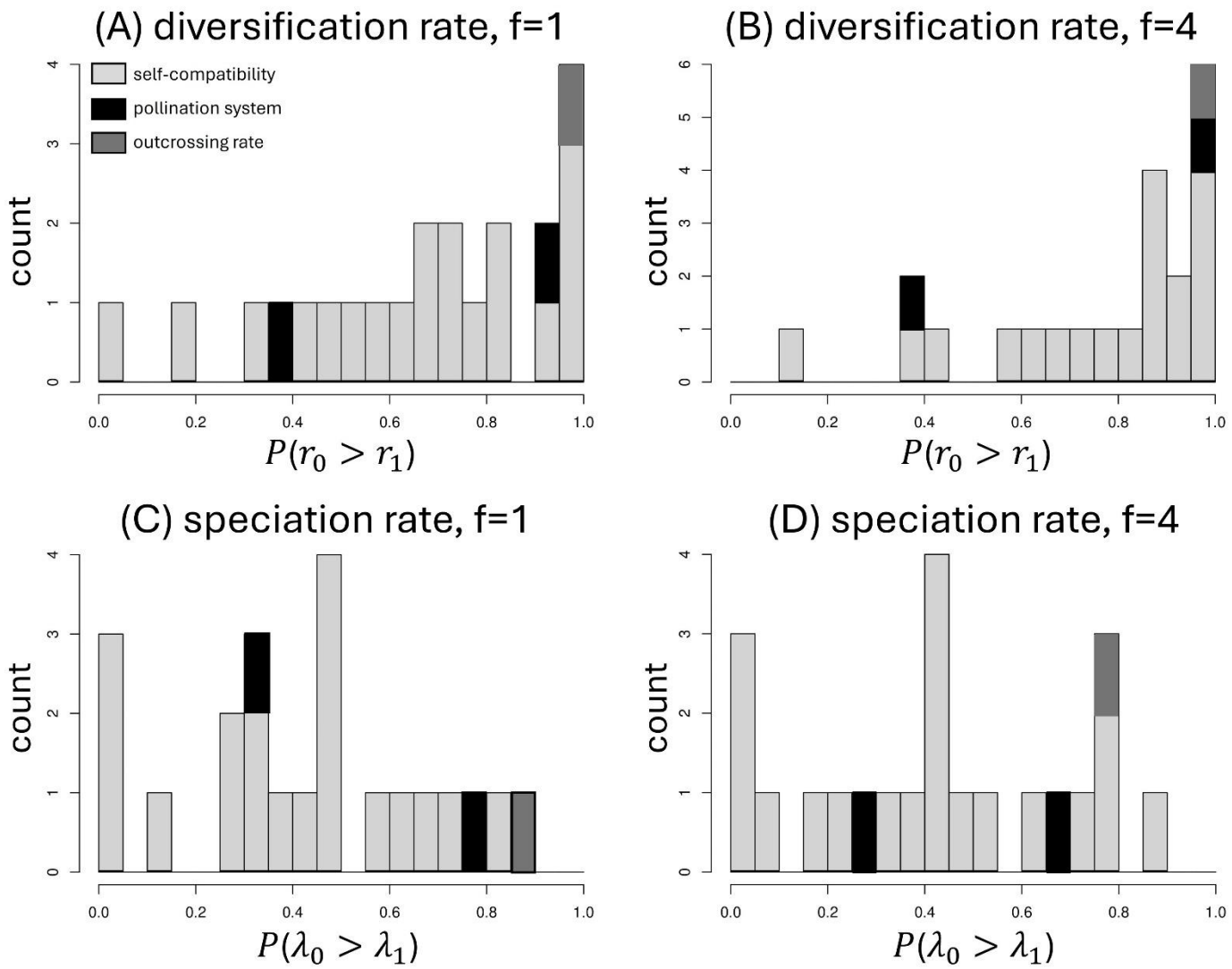

Figure S2. Proportion of the posterior probability distribution from BiSSE MCMC simulations that supports higher diversification rates and speciation rates for state 0 than state 1, assuming no transition from state 1 to state 0. Figures show the distribution for 22 genera. The figures show that results are robust to different choices of the prior distribution. The p-value based on one-sample Wilcoxon rank-sum test is 0.019 for panel (A), 0.00015 for panel (B), 0.222 for panel (C), and 0.276 for panel (D).

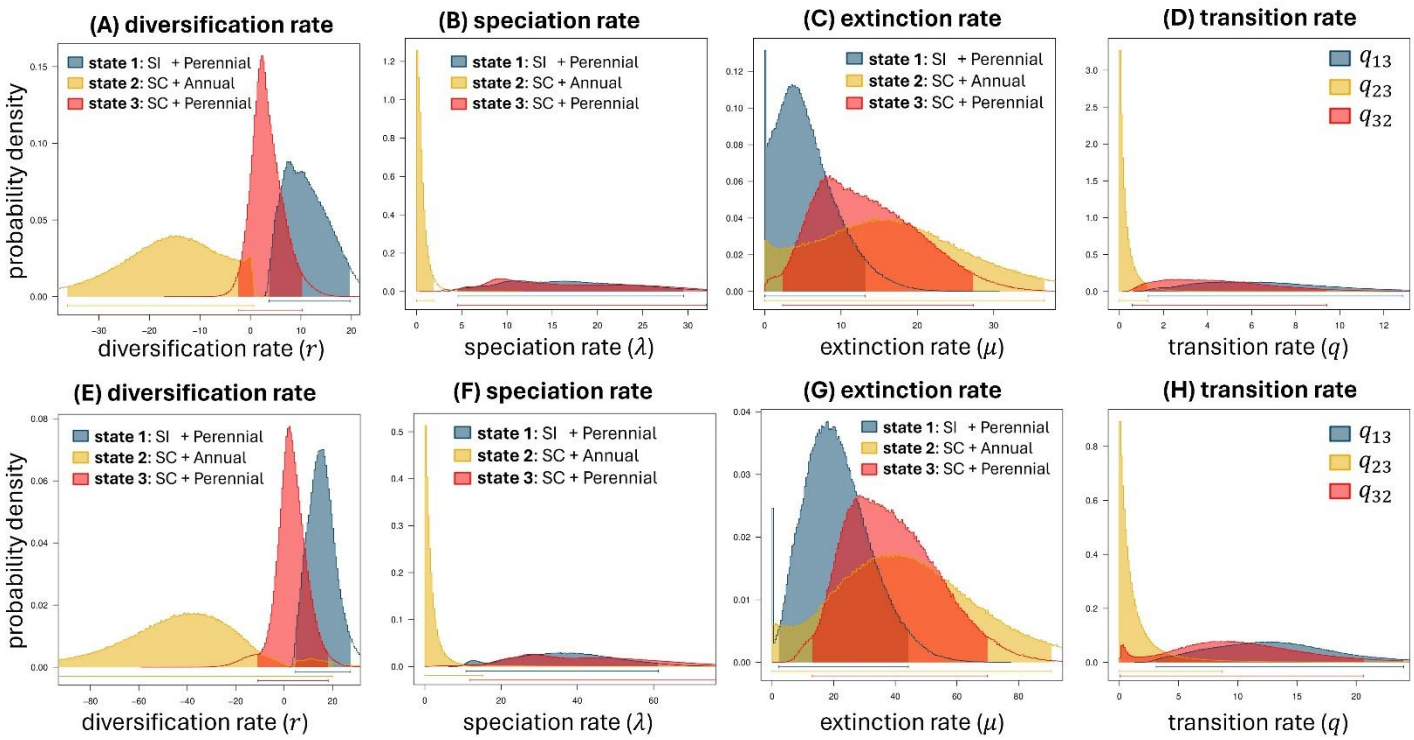

Figure S3. Posterior distribution of the net diversification rates, speciation rates, extinction rates, and transition rates obtained from MuSSE MCMC simulations on 100 bootstrap trees of *Solanum*. The parameter f used in the prior distribution is $f = 1$ for upper panels and $f = 4$ for bottom panels.

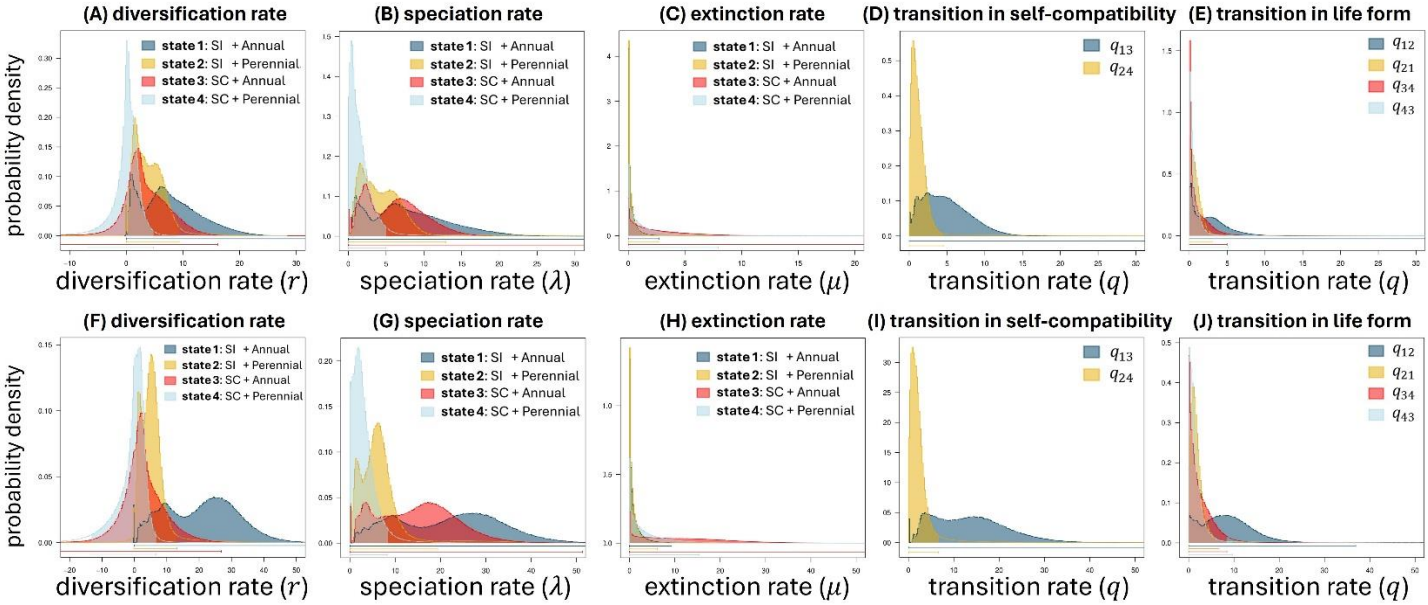

Figure S4. Posterior distribution of the net diversification rates, speciation rates, and transition rates obtained from MuSSE MCMC simulations on 100 bootstrap trees in the genus *Oenothera*. The parameter f used in the prior distribution is $f = 1$ for upper panels and $f = 4$ for bottom panels.

Table S1. Genera included in this study.

Genus	Family	Species richness[a]	No. sp. tree[b]	State 0	State 1	No. clusters	Alignment Length	Missing site (%)[c]	Outgroup reference
Self-compatibility dataset									
Aechmea	Bromeliaceae	257	158	7	10	38	33323	78	
Bidens	Asteraceae	246	88	5	30	25	20530	64	
Brassica	Brassicaceae	46	27	13	3	75	54952	81	
Cardamine	Brassicaceae	253	180	6	10	20	16044	82	
Carthamus	Asteraceae	26	28	2	8	46	33372	67	
Cirsium	Asteraceae	497	153	3	7	16	10869	55	
Diplotaxis	Brassicaceae	40	29	13	3	26	17729	82	
Flaveria	Asteraceae	21	20	16	3	43	40264	54	
Layia	Asteraceae	16	10	7	3	18	14642	71	
Lepidium	Brassicaceae	249	140	5	9	52	29997	86	
Microseris	Asteraceae	44	15	4	5	12	8040	61	
Nicotiana	Solanaceae	101	72	7	41	73	71676	81	
Oenothera	Onagraceae	165	112	40	32	23	24604	76	
Petunia	Solanaceae	16	17	8	3	38	30449	59	
Physalis	Solanaceae	85	61	6	8	40	28310	72	
Senecio	Asteraceae	1681	485	21	17	15	10944	70	
Simsia	Asteraceae	30	22	14	3	7	3759	52	
Solanum[d]	Solanaceae	1238	741	73	133	9	10908		
Symphyotrichum	Asteraceae	104	64	5	4	39	22174	80	
Tillandsia	Bromeliaceae	678	291	6	16	20	18552	71	
Trifolium	Fabaceae	369	225	5	11	9	6382	53	
Pollination system dataset									
Cobaea[e]	Polemoniaceae	17		8	2				
Gilia[e]	Polemoniaceae	39		10	21				
Ipomopsis[e]	Polemoniaceae	27		15	4				
Leptosiphon[e]	Polemoniaceae	33		7	4				
Outcrossing rate dataset									
Picea	Pinaceae	43	34	7	5	197	144673	75	
Pinus	Pinaceae	134	116	34	7	362	174017	80	

a. The number of accepted species per genus in WFO Plant List.
b. The number of species on the reconstructed phylogenetic tree.
c. Percentage of sites with missing data in the concatenated supermatrix.
d. Supermatrix from Gagnon et al. (2022) was directly used.
e. Phylogenetics from Landis (2018) were directly used.

Table S2. Comparison of models with and without transition from state 1 to state 0 in genera with mating system characters being pollination systems and outcrossing rates. "$q_{10} = 0$ model" stands for the BiSSE model in the R package "diversitree" with the transition rate from state 1 to 0 set to 0, and the "full model" represents the model without this constraint. The table shows the proportion of posterior distribution supporting higher diversification rates for state 0 than state 1, $P(r_0 > r_1)$, obtained for each model under different values of parameter f used in the prior distribution of MCMC sampling.

Genus	**Family**	$\boldsymbol{q_{10} = 0}$ **model**			**full model**		
		$\boldsymbol{f = 1}$	$\boldsymbol{f = 2}$	$\boldsymbol{f = 4}$	$\boldsymbol{f = 1}$	$\boldsymbol{f = 2}$	$\boldsymbol{f = 4}$
Pollination system dataset							
Cobaea		0.40	0.38	0.36	0.51	0.38	0.27
Gilia		1.00	1.00	1.00	0.06	0.06	0.06
Ipomopsis		0.91	0.93	0.95	0.88	0.84	0.82
Leptosiphon		0.51	0.54	0.58	0.54	0.36	0.27
Outcrossing rate dataset							
Picea		0.96	0.97	0.98	0.45	0.39	0.38
Pinus		0.71	0.78	0.86	0.97	0.96	0.90

Table S3. Best fitted model for each genus inferred from R package "hisse" based on the lowest AIC values or AICc values. The last three columns are the percentage of 100 bootstrap trees of each genus with rate estimation falling in three scenarios: diversification rates for state 0 are higher than for sate 1 at both hidden states ($r_{0A} > r_{1B}, r_{0B} > r_{1B}$), diversification rates for state 0 are lower than for sate 1 at both hidden states ($r_{0A} < r_{1B}, r_{0B} < r_{1B}$), and diversification rates for state 0 are higher than state1 at one hidden state while lower at the other hidden state ($r_{0A} < r_{1B}, r_{0B} > r_{1B}$ or $r_{0A} > r_{1B}, r_{0B} < r_{1B}$).

Genus	**Family**	**Best fitted model based on AIC**	**Best fitted model based on AICc**	$\boldsymbol{r_{0A} > r_{1B}}$ $\boldsymbol{r_{0B} > r_{1B}}$	$\boldsymbol{r_{0A} < r_{1B}}$ $\boldsymbol{r_{0B} < r_{1B}}$	$\boldsymbol{r_{0A} < r_{1B}}$ $\boldsymbol{r_{0B} > r_{1B}}$
Self-compatibility						
Aechmea	Bromeliaceae	BiSSE null	BiSSE null			
Bidens	Asteraceae	BiSSE rev	BiSSE rev			
Brassica	Brassicaceae	HiSSE irr	HiSSE rev	25	17	58
Cardamine	Brassicaceae	BiSSE null	HiSSE rev	5	50	31
Carthamus	Asteraceae	BiSSE null	HiSSE irr	76	1	23
Cirsium	Asteraceae	BiSSE null	HiSSE irr	55	3	41
Diplotaxis	Brassicaceae	BiSSE rev	HiSSE rev	47	6	47
Flaveria	Asteraceae	BiSSE rev	BiSSE rev			
Layia	Asteraceae	BiSSE null	HiSSE irr	60	0	38
Lepidium	Brassicaceae	HiSSE CID2	HiSSE irr	84	2	12
Microseris	Asteraceae	BiSSE null	HiSSE irr	5	20	75
Nicotiana	Solanaceae	HiSSE CID2	HiSSE CID2			
Oenothera	Onagraceae	HiSSE irr	HiSSE irr	71	1	28
Petunia	Solanaceae	BiSSE null	HiSSE irr	19	7	72
Physalis	Solanaceae	BiSSE rev	HiSSE irr	62	1	36
Senecio	Asteraceae	HiSSE CID2	HiSSE CID2			
Simsia	Asteraceae	BiSSE rev	BiSSE rev			
Solanum	Solanaceae	HiSSE rev	HiSSE rev	11	22	67
Symphyotrichum	Asteraceae	BiSSE null	HiSSE irr	25	4	71
Tillandsia	Bromeliaceae	BiSSE null	BiSSE null			
Trifolium	Fabaceae	BiSSE irr	HiSSE rev	9	39	52
Pollination system dataset						
Cobaea	Polemoniaceae	BiSSE null	HiSSE irr	16	17	67
Gilia	Polemoniaceae	BiSSE null	BiSSE null			
Ipomopsis	Polemoniaceae	BiSSE null	BiSSE null			
Leptosiphon	Polemoniaceae	BiSSE null	HiSSE irr	66	0	34
Outcrossing rate dataset						
Pinus	Pinaceae	BiSSE null	BiSSE rev			
Picea	Pinaceae	BiSSE irr	HiSSE irr	71	0	29