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LOSS OF SEXUAL RECOMBINATION AND SEGREGATION IS ASSOCIATED WITH INCREASED DIVERSIFICATION IN EVENING PRIMROSES

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The loss of sexual recombination and segregation in asexual organisms has been portrayed as an irreversible process that commits asexually reproducing lineages to reduced diversification. We test this hypothesis by estimating rates of speciation, extinction, and transition between sexuality and functional asexuality in the evening primroses. Specifically, we estimate these rates using the recently developed BiSSE (Binary State Speciation and Extinction) phylogenetic comparative method, which employs maximum likelihood and Bayesian techniques. We infer that net diversification rates (speciation minus extinction) in functionally asexual evening primrose lineages are roughly eight times faster than diversification rates in sexual lineages, largely due to higher speciation rates in asexual lineages. We further reject the hypothesis that a loss of recombination and segregation is irreversible because the transition rate from functional asexuality to sexuality is significantly greater than zero and in fact exceeded the reverse rate. These results provide the first empirical evidence in support of the alternative theoretical prediction that asexual populations should instead diversify more rapidly than sexual populations because they are free from the homogenizing effects of sexual recombination and segregation. Although asexual reproduction may often constrain adaptive evolution, our results show that the loss of recombination and segregation need not be an evolutionary dead end in terms of diversification of lineages.

KEY WORDS: **Apomixis, BiSSE, comparative biology, *Oenothera*, translocation heterozygote.**

Sex is the dominant mode of reproduction among eukaryotic organisms, but why sex prevails despite ecological and evolutionary advantages associated with asexual reproduction is a paradox that has fueled over a century of research and vigorous debate (Weismann 1889; Fisher 1930; Muller 1932; Otto 2009; Lively 2010; Becks and Agrawal 2011). Theory shows that a breakdown in the two most important components of sex—recombination and segregation—leads to an accumulation of deleterious mutations (Muller 1964; Lynch and Gabriel 1990) and slower rates of adaptive evolution (Fisher 1930; Muller 1932; Hill and Robertson 1966; Maynard Smith 1978; Barton 1995). These theoretical results are consistent with microbial microcosm experiments where asexual populations exhibited reduced rates of adaptive evolution compared to sexual populations (Colegrave 2002; Cooper 2007). The theoretical predictions are also supported by some phylogenetic analyses

in which asexual lineages accumulate deleterious mutations at faster rates than related sexual lineages (Paland and Lynch 2006; Johnson and Howard 2007; Neiman et al. 2010), but other studies show no clear genetic load in asexual lineages (Mark Welch and Meselson 2001; Birky et al. 2005; Swanstrom et al. 2011).

As a consequence, it is commonly believed that the loss of recombination and segregation associated with asexual reproduction dramatically reduces diversification. In particular, it has been argued that the reduced genetic variance and limited adaptive potential associated with many asexual populations might impede speciation (Stebbins 1950; Bell 1982). Additionally, higher mutational loads are expected to increase rates of extinction in asexual lineages (Lynch and Gabriel 1990). Together, the decrease in speciation rate and increase in extinction rate are expected to severely limit diversification of asexual lineages, such that asexuality has been characterized as an evolutionary "dead end" (Stebbins 1950; Maynard Smith 1978; Bell 1982). Despite these predictions and an abundance of theoretical investigations, we are not aware of any study that has quantified the effects of sexual recombination and segregation on diversification in a natural system.

The long-term persistence and observed distribution of asexuality across the tree of life also depends on the transition rate between sexual and asexual reproduction (Williams 1975; Schwander and Crespi 2009). In many forms of asexual reproduction, the loss of sex has been characterized as an irreversible or nearly irreversible process (Bull and Charnov 1985). Irreversibility may, however, reflect the inability of asexuals to recover the machinery necessary for fertilization (e.g., functional flowers, pollen, and genes essential for forming zygotes), rather than an absence of selection to regain recombination and segregation. Distinguishing between these explanations is not possible unless an organism maintains fertilization while experiencing suppressed recombination and segregation. Moreover, obtaining accurate estimates of transition rates between sexual and asexual reproduction requires the simultaneous estimation of speciation and extinction rates as these processes jointly shape the occurrence of a trait among extant species (Schwander and Crespi 2009).

The evening primrose plant family (Onagraceae) offers an ideal system to test predictions relating to the role of sexual recombination and segregation on diversification (Fig. 1). In many evening primrose species, multiple chromosomal translocations throughout the genome have resulted in the loss of homologous chromosome pairing during meiosis, causing a virtually complete suppression of recombination and segregation (Stebbins 1950; Cleland 1972). The result is a functionally asexual mode of reproduction, called Permanent Translocation Heterozygosity (PTH), which occurs in at least eight plant families and has independently arisen many times in the Onagraceae (Holsinger and Ellstrand 1984; Johnson et al. 2009; Johnson 2011). PTH is caused in part by extensive chromosomal translocations, which are a ubiquitous

Figure 1. An example of closely related sexual and functionally asexual (PTH) *Oenothera* species. (A) The sexually reproducing *Oenothera drummondii* is closely related to the (B) functionally asexual PTH *O. humifusa*. Flowers were always closed (i.e., cleistogamous) on the *O. humifusa* individual shown, although this trait is highly variable within this and other functionally asexual PTH species.

phenomenon among eukaryotes and frequently result in reduced recombination over extended genomic regions (Holsinger and Ellstrand 1984). Thus, the functional asexuality of PTH represents the extreme end of a larger continuum whereby chromosomal translocations completely eliminate recombination and segregation (Cleland 1972; Ranganath 2008). An advantage of studying PTH reproduction over other forms of asexuality is that PTH species typically exhibit the same ploidy as sexual relatives. By contrast, plants employing other forms of asexual seed production

(e.g., apomixis) often have higher ploidy levels, such that variation in sex and ploidy are confounded (Stebbins 1950; see Whitton et al. 2008 for a review of other forms of asexuality in plants). Moreover, PTH species continue to form zygotes via the fusion of male and female gametes, so that the loss of recombination and segregation occur while the machinery associated with fertilization remains intact. Thus, the evening primrose system allows us to specifically study the consequences of recombination and segregation (Ranganath 2008). This form of asexual reproduction is most similar to other asexual systems that maintain components of meiosis and fertilization to form endosperm in plant seeds (e.g., apospory), or to stimulate development in gynogenetic and hybridogenetic animals (for overviews of the various forms of asexuality, see Simon et al. 2003 and Whitton et al. 2008).

Here, we examine how sexual and asexual reproduction influence rates of diversification to shape extant patterns of plant diversity. Specifically, we tested whether a breakdown in recombination and segregation affects rates of speciation, extinction, and net diversification in the Onagraceae, and whether a loss of recombination and segregation is irreversible. We explored these effects using BiSSE (Binary State Speciation and Extinction), a recently developed comparative phylogenetic method that allows the simultaneous estimation of rates of speciation and extinction, as well as rates of transition between two states using maximum likelihood (Maddison et al. 2007) or Bayesian (FitzJohn et al. 2009) techniques.

Materials and Methods

STUDY SYSTEM AND DATA COLLECTION

We focused our sampling on the Onagreae clade within the Onagraceae (Levin et al. 2004), as it is only within this clade that we see variation in sexual reproduction and the functionally asexual permanent translocation heterozygote genetic system (PTH). The Onagreae is a New World monophyletic clade (Levin et al. 2004), whose species are common in open habitats of temperate and dry environments found in North America, Central America, and parts of South America. In total, there were 43 (16.4%) PTH species and 219 (83.6%) sexual species (Figs. 1 and 2, Table S1). The functional asexuality of PTH species arises because of three phenomena (Cleland 1972; Harte 1994; Rauwolf et al. 2008). First, meiotic recombination is completely suppressed throughout most of the genome in PTH individuals because of a loss of bivalent chromosome homology caused by reciprocal chromosome translocations. Instead of forming bivalents, a complete ring of 14 chromosomes ($x = n = 7$) is formed during meiosis, which synapse only at their distal ends; distal ends recombine but they are typically homozygous (Cleland 1972). Second, the production of homozygotes via segregation of heterozygous loci is prevented by a balanced lethal mortality of haploid gametes, such that one haploid set of chromosomes always segregates together and passes through ovules, while the other haploid set passes through the pollen. And third, PTH species self-fertilize by dehiscing pollen onto receptive stigmas before flowers open (Fig. 1B). In essence, seeds are formed by pulling apart the parental genome and putting it back together without recombination or segregation, resulting in functionally asexual seed production. This genetic system is most similar to apomictic genetic systems that require fertilization (e.g., apospory, gynogenesis and hybridogenesis), and it is distinct from self-fertilizing sexual systems where recombination and segregation are maintained and quickly lead to high levels of homozygosity within populations.

Chromosome pairing behavior has been sampled throughout the family and often for numerous replicate populations within species; we used these data to determine whether species employed sexual (S) or PTH (P) reproduction (Table S1) (Lewis and Szweykowski 1964; Towner 1970; Cleland 1972; Raven and Gregory 1972; Dietrich 1977; Straley 1977; Dietrich and Wagner 1988; Harte 1994; Dietrich et al. 1997; Wagner 2005). These studies show that a ring of 14 chromosomes is a diagnostic feature of PTH reproduction, and when a species exhibits PTH, typically all individuals within that species are fixed for meiotic rings of 14 chromosomes. Sexual species often have seven bivalent pairs or are polymorphic, where some individuals exhibit seven bivalent pairs and other individuals have small rings of 4, 6, 8, or 10 chromosomes, plus five to two bivalent pairs. Although one genetic system was always dominant, there were 10 cases in which a species was polymorphic for sexual and PTH genetic systems. Even in these ambiguous cases, either the sexual or PTH genetic system was dominant (average frequency of dominant genetic system = 90%; 39 individuals sampled, on average, per species). We therefore designated these polymorphic species as either sexual or PTH according to the dominant reproductive method (>50%) found across sampled individuals.

A consistent species definition is critical when comparing diversification among sexual and asexual taxa (Barraclough et al. 2003). In such cases the biological species definition is inappropriate because each asexual individual is reproductively isolated. Instead, the most appropriate method for delimiting species involves applying a consistent and objective species concept that identifies evolutionary independent lineages (Barraclough et al. 2003). The taxonomy and systematics of the Onagraceae is among the most comprehensively studied of any plant group (Munz 1965; Raven 1979; Wagner et al. 2007). More importantly, the same criteria have been used to identify evolutionarily independent lineages of PTH and sexual taxa in Onagreae based on using a combination of morphology, population genetic markers, and molecular systematics (Munz 1965; Cleland 1972; Stubbe and Raven 1979; Harte 1994; Dietrich et al. 1997; Wagner et al. 2007). The resolution of taxonomic boundaries within clades

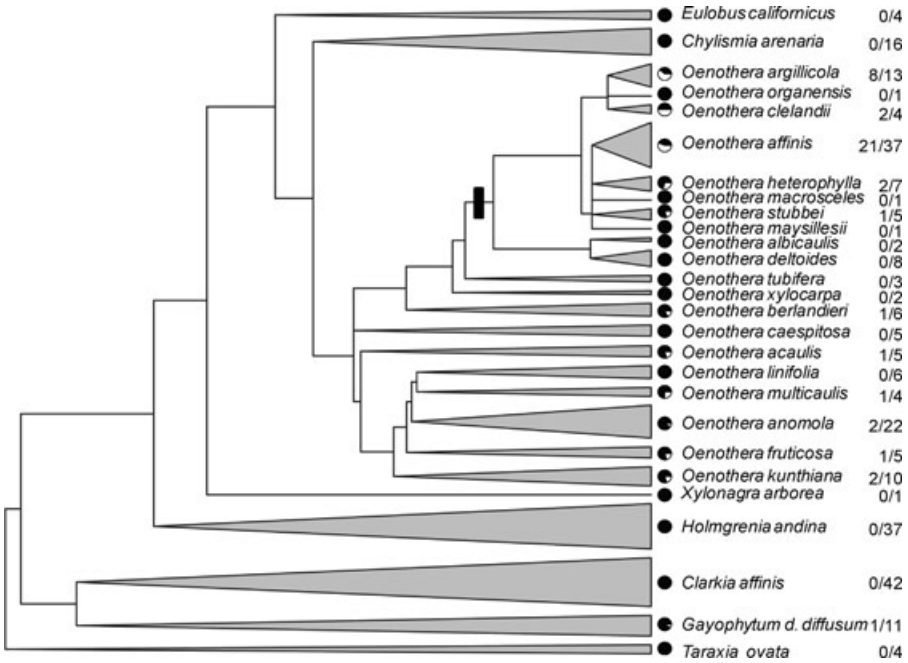

Figure 2. Phylogenetic distribution of sex and functionally asexual (PTH) evening primroses. A single ML tree is shown where gray triangles represent unresolved clades, with the width of triangles corresponding to the number of taxa. Although the ML tree contains several polytomies, the 100 MrBayes trees are fully resolved. The thick vertical hash mark indicates the branch leading to a shift in diversification rates associated with PTH and sexual reproduction (Fig. S1). A single representative species is shown for each clade; all sexual and PTH species within clades are provided in Table S1. Pie diagrams show the proportion of species that are sexual (black) and PTH (white). The fraction refers to the number of PTH species over the total number of species in the clade.

containing many PTH species has also involved literally thousands of experimental crosses within and between species to assess hybrid viability, meiotic behavior, the segregation of traits and chromosomes, and the homology of plastid and nuclear genomes (Cleland 1972; Harte 1994; Rauwolf et al. 2008); although PTH species are functionally asexual with respect to recombination and segregation, the sexual machinery required for fertilization are intact and therefore species can be forced to cross via hand pollinations. The data from these crosses have been used by taxonomists to differentiate morphologically similar PTH and sexual species based on the homology of plastid and nuclear genomes. A thorough description of the methods and philosophy taken toward the taxonomy of the Onagraceae is provided by Dietrich and colleagues (1977, 1997) and Wagner et al. (2007). Although biases in taxonomic rules applied to asexual versus sexual taxa can influence results, such biases should be relatively minor in the Onagraceae.

PHYLOGENETIC INFERENCE

The implementation of BiSSE requires a phylogenetic tree or a sample of trees. We inferred the phylogeny of the Onagraceae using the molecular dataset of Johnson et al. (2009), which is based on two plastid (*trnL-trnF* and *rps16*) and three nuclear gene regions (*PgiC*, ITS, and ETS) from 121 species, with 113 species within the Onagreae clade and eight species outside the clade. We obtained a single maximum likelihood (ML) tree using RAxML 7.0.4 (Stamatakis 2006) and a sample of 100 post-burnin trees from a Monte carlo Markov Chain Bayesian analysis using MrBayes 3.1.2 (Ronquist et al. 2005). BiSSE requires the use of ultrametric trees, which we performed in r8s (Sanderson 2003) using penalized likelihood with the truncated Newton (TN) algorithm and an optimal smoothing parameter selected using cross-validation. To assess whether our results were robust to our method for creating ultrametric trees, we compared BiSSE results from the r8s trees (see below) with results based on trees inferred using the relaxed clock methods implemented in a pre-release version of MrBayes 3.2. For the relaxed clock analyses, we assigned *Fuchsia cyrtandroides* to the outgroup and used 15 million generations with a Brownian motion model of clock rate evolution and a temperature of 0.02. The BiSSE results from both sets of trees (r8s and relaxed clock) resulted in qualitatively identical conclusions and so we used the former trees in all subsequent analyses. The molecular dataset and the trees inferred from it are available through Treebase (http://purl.org/phylo/treebase/phylows/study/TB2:S10384). Although outgroups were included when building trees, we excluded the outgroup taxa prior to BiSSE analyses because outgroup taxa were poorly sampled. All trees were then standardized to a

root-to-tip length of 1, so that parameter estimates from different trees could be directly compared.

To obtain unbiased estimates of speciation and extinction rates using BiSSE, either we must include all extant species in the phylogeny, or we must account for the sampling of taxa in the statistical analysis (FitzJohn et al. 2009). Because Onagreae contains 262 species, we included the remaining 149 unsampled species using two methods: (1) an "unresolved clades" approach and, (2) a "skeletal tree" approach, both of which account for uncertainty in species placement (FitzJohn et al. 2009). Using the first approach, we grouped the 149 species not included in the molecular phylogenetic analysis, as well as the 113 species on the tree, into 27 putatively monophyletic clades (Table S1), based on the most recent taxonomic revision of Onagreae (Wagner et al. 2007), as well as additional recent molecular systematics of the group (Ford and Gottlieb 2003; Hoggard et al. 2004; Levin et al. 2004; Evans et al. 2005; Ford and Gottlieb 2007). This required dropping some of the phylogenetic information in the 113 species tree (Fig. 2). The majority of these clades (22 of 27) appear in the maximum likelihood phylogeny and in Bayesian trees with 100% posterior probability support. Of the remaining five clades, one clade had 95% support, two clades were subdivided differently using morphological versus molecular methods but consist only of sexual taxa and so the exact composition of these two clades does not influence the results, and the remaining two clades had 54% and 46% support (detailed in Table S1). Phylogenetic relationships within these 27 clades were considered to be unknown, and the statistical analysis took into account all possible phylogenetic resolutions within each clade (FitzJohn et al. 2009). Analyses were carried out both on a single maximum likelihood (ML) tree and on a sample of 100 trees obtained from Bayesian analysis. We focus here on results from the sample of 100 trees because conclusions based on the single ML tree were equivalent (Tables S2 and S3).

We also applied an alternative "skeletal tree" approach (FitzJohn et al. 2009), which keeps the full phylogeny of 113 species but assumes that these species represent a random sample from the 262 extant taxa (see methods below). Because there are 43 asexual species, only 29 of which are represented on the tree, the skeletal tree method weights each PTH tip species by its probability of being sampled, given its state, set to the sampling fraction 29/43. Similarly, the sampling fraction for sexual species is 84 of 219. As reported in the Supporting information, qualitatively similar results to the unresolved clade approach were obtained using this skeletal tree approach (FitzJohn et al. 2009) (Figs. S2 and S3).

BiSSE ANALYSES

BiSSE (Maddison et al. 2007) was used to estimate six parameters: the speciation rates of lineages in states P (PTH) and S (sex) (λ_P and λ_S, respectively); the extinction rates of lineages in states P and S (μ_P and μ_S, respectively); and the transition rates from PTH to sex ($q_{P\rightarrow S}$) and sex to PTH ($q_{S\rightarrow P}$), applying either the unresolved tip clade approach or the skeletal tree approach (FitzJohn et al. 2009), implemented in the R package Diversitree (version 0.4–5).

We used Bayesian methods to estimate posterior probability distributions for each of these six parameters using Markov chain Monte Carlo (MCMC) as described by FitzJohn et al. (2009). This Bayesian approach provides a measure of parameter uncertainty and, when repeated across a sample of plausible trees, can account for uncertainty in both the phylogeny and parameter estimates.

Posterior distributions were estimated separately using the single ML tree (Fig. 2) and for each of the 100 post-burnin trees sampled by MrBayes. We used an exponential prior distribution for each parameter with a mean set to two times r, where $r = \ln(262)$ is the deterministic growth rate required to account for growth from one species to 262 across a tree standardized to a length of one. Our choice of prior thus uses only information about the size of the clade and not the data on character states or branch lengths and is based on the assumption that the rate of character change is not much faster (or slower) than the overall rate of diversification in this group. Setting the prior to an exponential distribution was motivated by previous phylogenetic studies (Churchill 2000; Drummond et al. 2006) and by the advantage of using a proper (integrable) distribution (Gelman et al. 2004). As noted by Churchill (2000), the exponential distribution places more weight on small rates of change, in the absence of evidence to the contrary, which is a reasonable assumption for evolutionary processes where we do not expect instantaneous change to be possible. The mean of the exponential distribution was multiplied by 2 (i.e., $2r$ rather than r) to flatten the distribution and allow the model to explore the parameter space more freely. The factor of "2" implies that values of the parameters more than six times r occur in less than 5% of the prior distribution. We performed additional analyses where the mean prior for each parameter was set to $4r$, implying that parameter values must be more than 12 times r before occurring in less than 5% of the prior distribution, but the results were qualitatively the same. Similarly, we performed an MCMC analysis where the mean of a parameter (e.g., $\bar{\lambda} = (\lambda_0 + \lambda_1)/2$) was assigned an exponential prior with mean $2r$, but the parameter specific to PTH lineages was assigned a uniform prior between 0 and twice the mean (e.g., $\lambda_0 \sim U[0,\ 2\bar{\lambda}]$); again the results were qualitatively the same. Finally, we performed an ML analysis using log-likelihood ratio tests, which make no assumptions regarding priors (Table S3). Again, the results were qualitatively the same, indicating that our main results are robust to the assumptions made about the priors.

For the six-parameter BiSSE model, MCMC chains were started at the mean of the prior distribution and run for

10,000 steps. Initial graphical analyses indicated rapid convergence (within a few dozen steps); to be conservative, we discarded the first 2500 steps. The effective sample size of the remaining 7500 MCMC steps was calculated for each parameter and each tree separately using the CODA package (Plummer et al. 2006). The effective sample size was greater than 460 for each parameter, either for the ML tree or when averaged across the 100 MrBayes trees, indicating that we have obtained good samples from the posterior probability distribution. For the ML tree, the posterior probability distribution for each parameter was based on the last 7500 steps. For the 100 trees sampled by MrBayes, we concatenated the last 7500 steps for each tree together to form the posterior probability distributions. To test a hypothesis involving a compound parameter, a posterior probability distribution was constructed for that parameter. For example, to test whether extinction rates differ, we calculated the compound parameter $\mu_P - \mu_S$, and determined whether this parameter differed significantly from zero. To assess significance, we calculated 95% credibility intervals, defined as the smallest interval containing 95% of the MCMC samples, for a parameter or compound parameter and examined whether the credibility interval contained the value under the null hypothesis (e.g., zero). Likelihood ratio tests were also applied using BiSSE to confirm our major conclusions; for all 100 trees, a constrained model with equal extinction rates ($\mu_P = \mu_S$) could not be rejected, but a further constrained model with equal speciation rates ($\lambda_P = \lambda_S$) or transition rates ($q_{P\to S} = q_{S\to P}$) could be rejected (Table S3).

The MCMC BiSSE analyses required an average of 18.4 h of computation time per tree on an Intel Xeon E5450 3.0 GHz processor, with multiple trees analyzed in parallel on western Canada's WestGrid cluster (www.westgrid.ca).

SPLIT BISSE ANALYSES

It is possible that patterns of diversification associated with sexual and asexual reproduction are concentrated on particular branches of the phylogeny. To explore this, we expanded BiSSE to a 12-parameter model: ($\lambda_{P,f}$, $\lambda_{S,f}$, $\mu_{P,f}$, $\mu_{S,f}$, $q_{P\to S,f}$, $q_{S\to P,f}$) in a particular clade of interest ("foreground," including the subtending branch) and ($\lambda_{P,b}$, $\lambda_{S,b}$, $\mu_{P,b}$, $\mu_{S,b}$, $q_{P\to S,b}$, $q_{S\to P,b}$) for the remainder of the tree (background). MCMC BiSSE analyses were run as before, using Diversitree 0.5–2. To identify a clade of interest, we used MEDUSA (Alfaro et al. 2009), an algorithm that searches for branches on a tree that show evidence that diversification rates have changed using a birth–death model, ignoring transition events. We averaged the Akaike information criterion (AIC) across the 100 trees to identify the single branch with the strongest evidence of a shift in diversification, which was then used to define the foreground clade.

Although the position of the shift varied from tree to tree, it always included the unresolved tip clades represented by *O. affinis* and *O. heterophylla* (Fig. 2). For 80 of the 100 MrBayes trees, the split occurred on the branch leading to the same clade as shown in Figure 1. In the remaining 20, the split occurred closer to the present. In 99 of the 100 MrBayes trees, the split occurred in such a way that it captured 34 of the 43 PTH species (as in Fig. 2). In one tree (#77), the split only included the two unresolved tip clades represented by *O. affinis* (21 PTH and 16 sexual species) and *O. heterophylla* (two PTH and five sexual species). The posterior probability distribution for each of the 12 parameters was obtained by concatenating the results from the last 7500 steps of a 10,000 step MCMC BiSSE analysis across the 100 trees (Fig. S1).

Results and Discussion

EFFECTS OF SEXUAL RECOMBINATION AND SEGREGATION ON SPECIES DIVERSIFICATION

Variation in sexual reproduction is predicted to influence speciation and extinction dynamics and shape the contribution of lineages to contemporary patterns of species diversity. Specifically, a reduction or complete loss of sexual recombination and segregation is commonly believed to limit the ability of asexual populations to diverge into new species because asexual populations frequently have lower genetic variation than sexual populations (Stebbins 1950; Bell 1982). In contrast to this prediction, we found that speciation rates (λ) were on average 4.1 times higher in functionally asexual PTH lineages than in sexual evening primrose lineages (Fig. 3A), a difference that was significant (95% credibility interval: $9.1 < \lambda_P - \lambda_S < 38.3$) and consistent across trees (Table 1; Table S3). By contrast, extinction rates (μ) were on average 2.1 times higher in PTH than sexual lineages (Fig. 3B), consistent with the predicted pattern. Nevertheless, the posterior probability distributions for the extinction rate in PTH lineages (μ_P) and in sexual lineages (μ_S) were largely overlapping (Fig. 3B, Table 1), and statistically indistinguishable (95% credibility interval for the difference was $-10.4 < \mu_P - \mu_S < 26.5$; Table S3). Thus, if PTH species experience higher extinction rates the trend is weak compared to differences in speciation. These patterns of speciation and extinction resulted in the net diversification ($r = \lambda - \mu$) being 8.7 times greater in PTH than sexual lineages (95% credibility interval: $4.8 < r_P - r_S < 29.3$). These results strongly suggest that the loss of recombination and segregation in PTH lineages is correlated with higher speciation and net diverisifcation rates than sexual lineages.

Given that extinction rates are difficult to estimate with great precision (Maddison et al. 2007; Rabosky 2010), we sought to determine whether our conclusions were robust to different assumptions regarding μ_P and μ_S. To do this, we implemented maximum likelihood (ML) BiSSE estimation methods and compared a series of nested models fit to our 100 Bayesian trees as

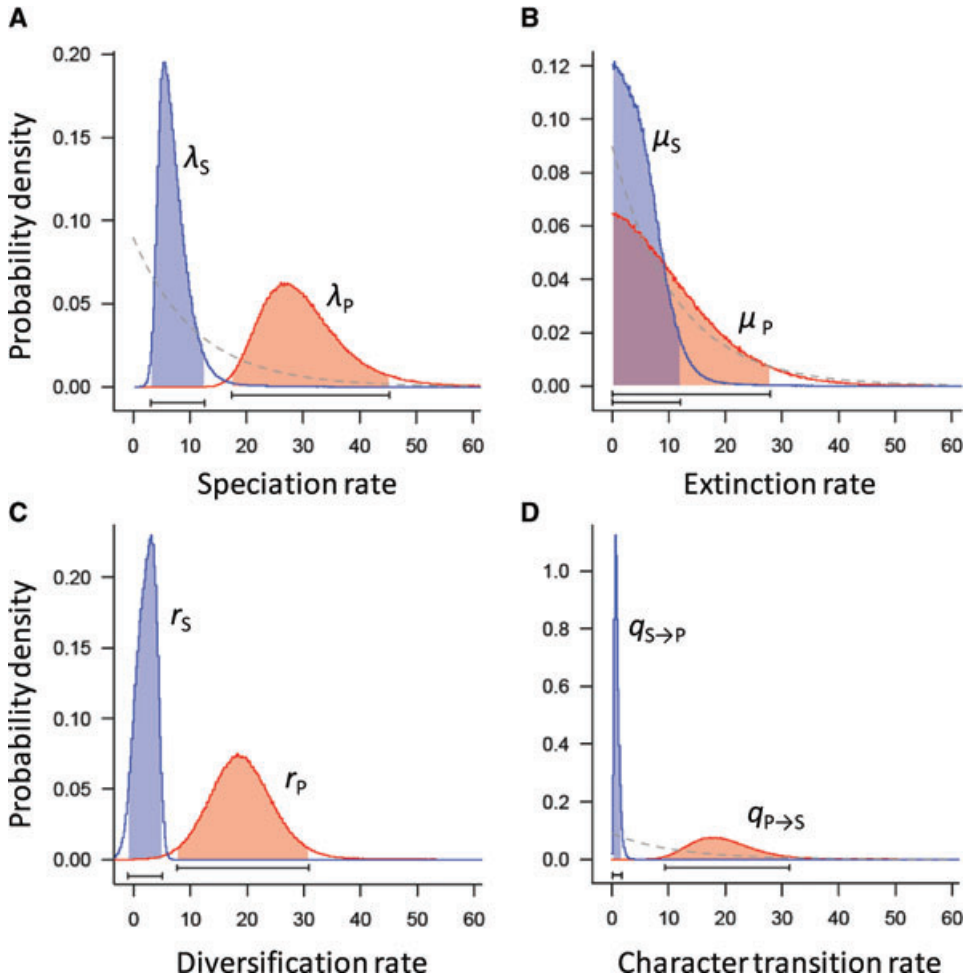

Figure 3. Diversification and transition rates in sexual and functionally asexual (PTH) lineages. Posterior probability distributions are shown for rates of (A) speciation (λ), (B) extinction (μ), and (C) net diversification ($r = \lambda - \mu$) in PTH (state P—red) and sexual (state S—blue) lineages. (D) The rates of evolutionary transitions from PTH to sexual reproduction ($q_{P\to S}$) and from sexual to PTH reproduction ($q_{S\to P}$) are also shown. Each distribution represents the posterior distribution obtained from the final 7500 steps in an MCMC BiSSE analysis combined together from each of the 100 phylogenetic trees sampled by MrBayes. Dashed gray lines represent the exponential priors used in analyses; three different starting conditions were used for the MCMC analyses, but there were no qualitative differences in our results (see Methods). Ninety-five percent credibility intervals are shown as whiskered lines beneath each curve for distributions associated with sexual reproduction (lower interval) and PTH (upper interval). All rates represent the number of transitions expected along a branch equal in length to the depth of the tree.

Table 1. Means of posterior distributions for diversification and transition rates in PTH and sexual lineages. Estimates were obtained from MCMC BiSSE analyses on 100 MrBayes trees. Estimates for the single ML phylogeny shown in Fig. 2 are provided in Table S2. Maximum likelihood analyses and log-likelihood ratio tests support the results reported here and are provided in Table S3A.

Parameter	PTH	Sex	95% CI of the difference	No. trees PTH > Sex
Extinction	10.8	5.1	(−10.4, 26.5)	98/100
Speciation	30.0	7.3	(9.1, 38.3)	100/100
Diversification	19.2	2.2	(4.8, 29.3)	100/100
	PTH to Sex	**Sex to PTH**		**PTH to Sex > Sex to PTH**
Transition	19.73	0.86	(8.5, 30.3)	100/100

well as our single best ML tree (Table S3). Once again, speciation rates were substantially greater for PTH lineages than sexual lineages when we constrained extinction rates to be the same ($\mu_P = \mu_S$) or forced both to equal zero ($\mu_P = \mu_S = 0$). Additional MCMC analyses also showed that the difference in diversification rates between PTH and sexual lineages was even stronger when we constrained extinction rates to be equal between modes of reproduction (95% credibility interval for $r_P - r_S$: 10.4 – 29.1). Therefore our results for speciation and diversification rates are robust to assumptions regarding the occurrence of extinction and differences in extinction between PTH and sexual taxa.

An alternative explanation for the above results is that diversification rate increased within a predominantly PTH clade of the tree for some reason unrelated to the loss of recombination and segregation within PTH lineages. If this explanation were true, then we would not expect to see a difference in diversification rate between PTH and non-PTH species within that clade. To assess this possibility, we identified the clade within each tree that exhibited the strongest evidence for a shift in diversification rate and applied a 12-parameter version of BiSSE, with different rate parameters in this "foreground" clade versus the background rates (see Methods). The 12-parameter model fit substantially better than either the original six-parameter BiSSE model (ΔAIC = 7.94) or the standard MEDUSA model where there is a shift in overall diversification rate but no difference in diversification between PTH and sexual lineages (ΔAIC = 3.84; in contrast with MEDUSA, $q_{P\to S}$ and $q_{S\to P}$ were allowed to differ). Furthermore, a Bayesian MCMC analysis showed that the same qualitative pattern of higher speciation and extinction associated with PTH remained, at least within the foreground clade (Bayesian MCMC analysis; Fig. S1). PTH was rare in the background clades, which resulted in insufficient power to support or reject an association between PTH and increased diversification (Fig. S1). Consequently, the best model was one where diversification rates were allowed to differ between PTH and sexual lineages in the foreground clade without these additional parameters in the background clade (ΔAIC = 11.66 and 7.56 improvement over the original six-parameter BiSSE and standard MEDUSA models, respectively). A simple shift in diversification parameters independent of PTH and sexual reproduction provided a worse fit to these data. We therefore conclude that the alternative explanation is not supported by the data.

The seemingly unexpected result that asexual (PTH) taxa diversify at a higher rate than sexual species lends support to an alternative hypothesis: recombination and segregation hinders speciation by remixing divergent genotypes each generation (Felsenstein 1981; Barraclough et al. 2003; Gavrilets 2004). If speciation in sexual lineages requires allopatry or strong assortative mating to occur, while asexuality allows divergence in both allopatry and sympatry, we would expect higher speciation rates in asexuals, as we observe (Felsenstein 1981; Dieckmann and Doebeli 1999). Thus our findings provide the first empirical support for the theoretically predicted negative effect of recombination on diversification (Felsenstein 1981; Gavrilets 2004). Interestingly, a recent study that examined rates of diversification in self-incompatible (SI) and self-compatible (SC) Solanaceous plant species, found that SC lineages exhibited approximately two times higher speciation rates than SI lineages, although lower net diversification rates due to disprortionately higher extinction rates in SC lineages (Goldberg et al. 2010). This finding of higher species rates complements our results because SC mating systems tend to decrease the effective rate of recombination compared to SI systems.

REVERSIBILITY OF LOSING SEXUAL RECOMBINATION AND SEGREGATION

Our results clearly show that the loss of recombination and segregation is reversible in evening primroses. We first tested the hypothesis that the transition rate from PTH to sex was zero ($q_{P\to S} = 0$), which was rejected for each of the 100 phylogenetic trees (log likelihood ratio test: average $\Delta \ln L = 13.3$, $P < 0.001$). When we allowed the parameters to vary freely in an MCMC BiSSE analysis, we found that the rate of transition from PTH to sexual reproduction was 23 times higher than the reverse transition rate (Fig. 3D), a result that was significant (95% credibility interval: $8.5 < q_{P\to S} - q_{S\to P} < 30.3$), consistent across all trees (Table 1; Table S3), and robust to assumptions regarding extinction rates ($\mu_P = \mu_S$ or $\mu_P = \mu_S = 0$, Table S3). Therefore it appears that restoring recombination and segregation is not only possible in functionally asexual PTH lineages, but was in fact common during the diversification of PTH clades.

This finding contradicts the hypothesis that a loss of sex is always irreversible and requires us to consider the mechanisms by which evening primroses might regain recombination and segregation. Because the machinery for fertilization remains intact within PTH species (as it does in some apomictic species), it is possible to experimentally cross PTH species with closely related species (Cleland 1972). Controlled mating experiments show that some combinations of crosses between species can partially or wholly restore recombination in PTH species when the parents' genomes share chromosomal homology (Cleland 1972; Rauwolf et al. 2011), suggesting that hybridization in natural populations might play a role in restoring sexual reproduction. This result suggests that it is not the loss of recombination and segregation per se that renders transitions to asexuality irreversible in other organisms, but instead it is the loss of the machinery involved in fertilization (Bull and Charnov 1985; Domes et al. 2007).

There is mounting evidence from both plant and animal systems that gains and losses of sex can be evolutionarily labile in some systems, involving multiple transitions from sexual to

asexual reproduction (Simon et al. 2003), as well as repeated transitions from asexual back to sexual reproduction, even within species thought to be obligately asexual (Menken et al. 1995; Domes et al. 2007). In both plants and animals, many asexual species require fertilization to produce viable offspring and others continue to produce some viable male and female haploid gametes, which could lead to sexual descendant lineages through either the reevolution of the machinery required for meiosis and fertilization, or through hybridization with related sexual individuals (Domes et al. 2007; Whitton et al. 2008). Even when reversion to sexuality is an extremely rare event, if sex provides an advantage in allowing individuals to purge deleterious mutations or combine beneficial alleles in their progeny (Fisher 1930; Muller 1932), revertants might become established and outcompete their parental asexual strain, leading to a higher reverse transition rate than expected from the rarity of mutations back to sexuality.

EXPLAINING EXTANT PATTERNS OF SEXUAL AND ASEXUAL SPECIES DIVERSITY

Previous ancestral state reconstructions showed a "twiggy" distribution of PTH reproduction across the Onagraceae phylogeny, where there is an absence of deep, exclusively PTH clades and an abundance of apparently repeated recent derivations of functional asexuality (Johnson et al. 2009). Twiggy distributions of asexual reproduction are common across the tree of life, and they have been interpreted as the hallmark signature of an elevated extinction risk associated with asexuality (Maynard Smith 1978; Bell 1982). We find no evidence in support of this explanation in *Oenothera*. The twiggy distribution of PTH in evening primroses is best explained by frequent reversions to sexuality from PTH lineages (Fig. 3D), coupled with increased diversification rates while in the PTH state (Fig. 3C). We thus caution that the twiggy distribution of asexual taxa in other groups should not automatically be taken as evidence for increased extinction or reduced speciation in asexual relative to sexual taxa (Schwander and Crespi 2009).

IMPLICATIONS FOR PERSISTENCE OF SEXUALITY

One explanation for the continued persistence of sexual reproduction among species is that sexual lineages have higher rates of speciation or lower rates of extinction than asexual species (Fisher 1930; Stebbins 1950; Stanley 1975; Bell 1982; Nunney 1989). According to this argument, greater diversification rates of sexual lineages balance the short-term ecological advantages of asexual reproduction and maintain sexuality. Our results lend no support to this view. Although extinction rates were somewhat higher in PTH evening primrose lineages, the differences were not significant. By contrast, speciation rates, as well as net diversification rates, were higher for PTH lineages and these results were robust to assumptions made about extinction rates. In this system, lineage selection operates in the same direction as the presumed short-term advantage of asexuality and thus cannot explain the maintenance of sexually reproducing species. We caution, however, that we are unable to say, based on the current analysis, whether the extinction rate might rise over time within asexual lineages due to genetic deterioration in the absence of segregation and recombination, a possibility that warrants further study.

CONCLUSIONS

We find little support in the evening primroses for the prevailing view that the loss of sexual recombination and segregation associated with asexual reproduction is an evolutionarily dead end caused by decreased net species diversification. In the evening primroses, functional asexuality is accompanied by higher rates of speciation and no clear differences in extinction rates compared to sexual reproduction. Therefore, in this evolutionary context, there is life after sex.

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Supporting Information

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

Table S1. Species included in the study and their placement within unresolved clades.

Table S2. Means of posterior distributions for diversification and transition parameters estimated for the single maximum likelihood tree.

Table S3. Maximum likelihood estimates of parameters and summary of likelihood-ratio tests of alternative BiSSE models.

Figure S1. Diversification and transition rates in sexual and functionally asexual lineages allowing for a shift in diversification rates.

Figure S2. First of the 100 MrBayes trees showing character states.

Figure S3. Diversification and transition rates in sexual and functionally asexual lineages using the skeletal tree method.

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

SUPPORTING INFORMATION

Table S1 Species, phylogenetic placement, reproductive type and method for placing species within the phylogeny. Species shown in bold are also used in Fig. 2 to show the placement of each of the 27 clades. All clades have 100% posterior probability support unless otherwise indicated.

Species*	Clade†	Reproduction‡	Phylogenetic placement§
Eulobus angelorum	1	Sex	Taxonomy
Eulobus californicus	1	Sex	MrBayes
Eulobus crassifolius	1	Sex	MrBayes
Eulobus sceptrostigma	1	Sex	Taxonomy
Chylismia arenaria	2	Sex	MrBayes
Chylismia atwoodii	2	Sex	Taxonomy
Chylismia brevipes	2	Sex	Taxonomy
Chylismia cardiophylla	2	Sex	Taxonomy
Chylismia claviformis	2	Sex	MrBayes
Chylismia confertiflora	2	Sex	Taxonomy
Chylismia eastwoodiae	2	Sex	Taxonomy
Chylismia exilis	2	Sex	Taxonomy
Chylismia heterochroma	2	Sex	Taxonomy
Chylismia megalantha	2	Sex	Taxonomy
Chylismia multijuga	2	Sex	Taxonomy
Chylismia munzii	2	Sex	Taxonomy
Chylismia parryi	2	Sex	Taxonomy
Chylismia scapoidea	2	Sex	Taxonomy
Chylismia specicola	2	Sex	Taxonomy
Chylismia walkeri	2	Sex	Taxonomy
Oenothera argillicola	3	Sex	MrBayes
Oenothera biennis	3	PTH	MrBayes
Oenothera elata	3	Sex	MrBayes
Oenothera glazioviana	3	PTH	MrBayes
Oenothera grandiflora	3	Sex	MrBayes
Oenothera jamesii	3	Sex	Taxonomy
Oenothera longissima	3	Sex	MrBayes
Oenothera nutans	3	PTH	MrBayes
Oenothera oakesiana	3	PTH	MrBayes
Oenothera parviflora	3	PTH	Taxonomy
Oenothera stuchii	3	PTH	Taxonomy
Oenothera villosa	3	PTH	MrBayes
Oenothera wolfii	3	PTH	Taxonomy
Oenothera organensis	4	Sex	MrBayes
Oenothera clelandii	5	PTH	MrBayes
Oenothera cordata	5	Sex	Taxonomy

Oenothera curtisii	5	PTH	Taxonomy
Oenothera rhombipetala	5	Sex	MrBayes
Oenothera affinis	6	Sex	MrBayes
Oenothera arequipensis	6	PTH	Taxonomy
Oenothera bahia-blancae	6	PTH	MrBayes
Oenothera catharinensis	6	Sex	Taxonomy
Oenothera coquimbensis	6	Sex	Taxonomy
Oenothera elongata	6	PTH	MrBayes
Oenothera featherstonei	6	Sex	Taxonomy
Oenothera grisea	6	PTH	Taxonomy
Oenothera indecora	6	Sex	Taxonomy
Oenothera lasiocarpa	6	Sex	Taxonomy
Oenothera longiflora	6	PTH	Taxonomy
Oenothera longituba	6	Sex	MrBayes
Oenothera magellanica	6	PTH	Taxonomy
Oenothera mendocinensis	6	Sex	MrBayes
Oenothera mollissima	6	PTH	Taxonomy
Oenothera montevidensis	6	PTH	Taxonomy
Oenothera nana	6	PTH	MrBayes
Oenothera nocturna	6	PTH	Taxonomy
Oenothera odorata	6	Sex	Taxonomy
Oenothera paradonia	6	PTH	MrBayes
Oenothera pedunculifolia	6	Sex	Taxonomy
Oenothera peruana	6	Sex	Taxonomy
Oenothera picensis ssp picensis	6	PTH	MrBayes
Oenothera punae	6	PTH	Taxonomy
Oenothera ravenii ssp chilensis	6	PTH	MrBayes
Oenothera recurva	6	PTH	MrBayes
Oenothera rivadaviae	6	PTH	Taxonomy
Oenothera sandiana	6	PTH	MrBayes
Oenothera santarii	6	Sex	Taxonomy
Oenothera scabra	6	Sex	Taxonomy
Oenothera siambonensis	6	PTH	Taxonomy
Oenothera stricta stricta	6	PTH	MrBayes
Oenothera tafiensis	6	Sex	Taxonomy
Oenothera tarijensis	6	PTH	MrBayes
Oenothera verrucosa	6	Sex	Taxonomy
Oenothera versicolor	6	Sex	MrBayes
Oenothera villaricae	6	PTH	MrBayes
Oenothera drummondii	7	Sex	MrBayes
Oenothera falfurriae	7	Sex	Taxonomy
Oenothera grandis	7	Sex	MrBayes
Oenothera heterophylla¶	7	Sex	MrBayes

Oenothera humifusa	7	PTH	MrBayes
Oenothera laciniata	7	PTH	MrBayes
Oenothera mexicana	7	Sex	Taxonomy
Oenothera macrosceles	8	Sex	MrBayes
Oenothera breedlovei	9	Sex	Taxonomy
Oenothera pennellii	9	Sex	Taxonomy
Oenothera pubescens	9	PTH	MrBayes
Oenothera stubbei	9	Sex	MrBayes
Oenothera tamrae	9	Sex	Taxonomy
Oenothera maysillesii	10	Sex	MrBayes
Oenothera albicaulis[#]	11	Sex	MrBayes
Oenothera coronopifolia	11	Sex	Taxonomy
Oenothera arizonica	12	Sex	Taxonomy
Oenothera californica	12	Sex	Taxonomy
Oenothera deltoides	12	Sex	MrBayes
Oenothera engelmannii	12	Sex	Taxonomy
Oenothera neomexicana	12	Sex	MrBayes
Oenothera nuttallii	12	Sex	Taxonomy
Oenothera pallida	12	Sex	MrBayes
Oenothera wigginsii	12	Sex	Taxonomy
Oenothera muelleri	13	Sex	Taxonomy
Oenothera rivadaviae	13	Sex	Taxonomy
Oenothera tubifera	13	Sex	MrBayes
Oenothera primiveris	14	Sex	MrBayes
Oenothera xylocarpa	14	Sex	MrBayes
Oenothera berlandieri	15	Sex	MrBayes
Oenothera hartwegii	15	Sex	MrBayes
Oenothera lavandulifolia	15	Sex	MrBayes
Oenothera serrulata	15	PTH	MrBayes
Oenothera toumeyi	15	Sex	MrBayes
Oenothera tubicula	15	Sex	Taxonomy
Oenothera brandegeei	16	Sex	Taxonomy
Oenothera caespitosa	16	Sex	MrBayes
Oenothera cavernae	16	Sex	Taxonomy
Oenothera harringtonii	16	Sex	Taxonomy
Oenothera psammophila	16	Sex	MrBayes
Oenothera acaulis	17	PTH	MrBayes
Oenothera acutissima	17	Sex	MrBayes
Oenothera centauriifolia	17	Sex	Taxonomy
Oenothera flava	17	Sex	MrBayes
Oenothera triloba	17	Sex	Taxonomy
Oenothera brachycarpa	18	Sex	MrBayes
Oenothera coryi	18	Sex	Taxonomy

Oenothera havardii	18	Sex	MrBayes
Oenothera howardii	18	Sex	Taxonomy
***Oenothera linifolia*[1]**	18	Sex	MrBayes
Oenothera macrocarpa	18	Sex	MrBayes
Oenothera epilobiifolia	19	Sex	Taxonomy
Oenothera multicaulis	19	PTH	MrBayes
Oenothera seifrizii	19	Sex	Taxonomy
Oenothera tetraptera	19	Sex	MrBayes
Oenothera anomala	20	Sex	MrBayes
Oenothera arida	20	Sex	MrBayes
Oenothera boquillensis	20	Sex	MrBayes
Oenothera calcicola	20	Sex	Taxonomy
Oenothera cinerea	20	Sex	MrBayes
Oenothera coloradensis	20	Sex	Taxonomy
Oenothera curtiflora	20	Sex	MrBayes
Oenothera demareei	20	Sex	MrBayes
Oenothera filiformis	20	Sex	MrBayes
Oenothera filipes	20	Sex	MrBayes
Oenothera gaura	20	PTH	MrBayes
Oenothera glaucifolia	20	Sex	MrBayes
Oenothera hexandra	20	Sex	MrBayes
Oenothera lindeimeri	20	Sex	MrBayes
Oenothera mckelveyae	20	Sex	Taxonomy
Oenothera patriciae	20	Sex	MrBayes
Oenothera simulans	20	Sex	MrBayes
Oenothera sinuosa	20	Sex	MrBayes
Oenothera suffulta	20	Sex	MrBayes
Oenothera sufrutescens	20	Sex	MrBayes
Oenothera triangulata	20	PTH	MrBayes
Oenothera xenogaura	20	Sex	Taxonomy
Oenothera fruticosa	21	Sex	MrBayes
Oenothera perennis	21	PTH	MrBayes
Oenothera pilosella	21	Sex	Taxonomy
Oenothera riparia	21	Sex	Taxonomy
Oenothera spachiana	21	Sex	Taxonomy
Oenothera canescens	22	Sex	MrBayes
Oenothera deserticola	22	Sex	Taxonomy
Oenothera dissecta	22	Sex	Taxonomy
Oenothera kunthiana	22	PTH	MrBayes
Oenothera luciae-julianiae	22	Sex	Taxonomy
Oenothera orizabae	22	Sex	Taxonomy
Oenothera platanorum	22	Sex	Taxonomy
Oenothera rosea	22	PTH	MrBayes

Oenothera speciosa	22	Sex	MrBayes
Oenothera texensis	22	Sex	Taxonomy
Xylonagra arborea	23	Sex	MrBayes
Camissonia benitensis	24	Sex	Taxonomy
Camissonia campestris	24	Sex	MrBayes
Camissonia contorta	24	Sex	Taxonomy
Camissonia dentata	24	Sex	Taxonomy
Camissonia integrifolia	24	Sex	Taxonomy
Camissonia kernensis	24	Sex	MrBayes
Camissonia lacustris	24	Sex	Taxonomy
Camissonia parvula	24	Sex	Taxonomy
Camissonia pubens	24	Sex	Taxonomy
Camissonia pusilla	24	Sex	Taxonomy
Camissonia sierrae	24	Sex	Taxonomy
Camissonia strigulosa	24	Sex	Taxonomy
Camissoniopsis bistorta	24	Sex	Taxonomy
Camissoniopsis cheiranthifolia	24	Sex	MrBayes
Camissoniopsis confusa	24	Sex	Taxonomy
Camissoniopsis hardhamiae	24	Sex	Taxonomy
Camissoniopsis hirtella	24	Sex	Taxonomy
Camissoniopsis ignota	24	Sex	Taxonomy
Camissoniopsis intermedia	24	Sex	Taxonomy
Camissoniopsis lewisii	24	Sex	Taxonomy
Camissoniopsis luciae	24	Sex	Taxonomy
Camissoniopsis micrantha	24	Sex	Taxonomy
Camissoniopsis proavita	24	Sex	Taxonomy
Camissoniopsis quadalupensis	24	Sex	Taxonomy
Camissoniopsis robusta	24	Sex	Taxonomy
Camissoniospsis pallida	24	Sex	Taxonomy
Eremothera boothii	24	Sex	MrBayes
Eremothera chamaenerioides	24	Sex	Taxonomy
Eremothera gouldii	24	Sex	Taxonomy
Eremothera minor	24	Sex	MrBayes
Eremothera nevadensis	24	Sex	MrBayes
Eremothera pygmaea	24	Sex	Taxonomy
Eremothera refracta	24	Sex	MrBayes
Holmgrenia andina[2]	24	Sex	MrBayes
Holmgrenia hilgardii	24	Sex	Taxonomy
Tetrapteron graciliflorum	24	Sex	MrBayes
Tetrapteron palmeri	24	Sex	Taxonomy
Clarkia affinis	25	Sex	MrBayes
Clarkia amoena	25	Sex	Taxonomy
Clarkia arcuata	25	Sex	Taxonomy

Clarkia australis	25	Sex	Taxonomy
Clarkia biloba	25	Sex	Taxonomy
Clarkia borealis	25	Sex	Taxonomy
Clarkia bottae	25	Sex	Taxonomy
Clarkia breweri	25	Sex	Taxonomy
Clarkia concinna	25	Sex	MrBayes
Clarkia cylindrica	25	Sex	Taxonomy
Clarkia davyi	25	Sex	Taxonomy
Clarkia delicata	25	Sex	MrBayes
Clarkia dudleyana	25	Sex	Taxonomy
Clarkia epilobioides	25	Sex	Taxonomy
Clarkia exilis	25	Sex	Taxonomy
Clarkia franciscana	25	Sex	MrBayes
Clarkia gracilis	25	Sex	Taxonomy
Clarkia heterandra	25	Sex	MrBayes
Clarkia imbricata	25	Sex	MrBayes
Clarkia jolonensis	25	Sex	Taxonomy
Clarkia lassenensis	25	Sex	Taxonomy
Clarkia lewisii	25	Sex	Taxonomy
Clarkia lingulata	25	Sex	Taxonomy
Clarkia mildrediae	25	Sex	MrBayes
Clarkia modesta	25	Sex	Taxonomy
Clarkia mosquinii	25	Sex	Taxonomy
Clarkia prostata	25	Sex	Taxonomy
Clarkia pulchella	25	Sex	MrBayes
Clarkia purpurea	25	Sex	Taxonomy
Clarkia rhomboidea	25	Sex	Taxonomy
Clarkia rostrata	25	Sex	MrBayes
Clarkia rubicunda	25	Sex	Taxonomy
Clarkia similis	25	Sex	Taxonomy
Clarkia speciosa	25	Sex	Taxonomy
Clarkia springvillensis	25	Sex	Taxonomy
Clarkia stellata	25	Sex	Taxonomy
Clarkia tembloriensis	25	Sex	Taxonomy
Clarkia tenella	25	Sex	Taxonomy
Clarkia unguiculata	25	Sex	Taxonomy
Clarkia virgata	25	Sex	Taxonomy
Clarkia williamsonii	25	Sex	Taxonomy
Clarkia xantiana	25	Sex	MrBayes
Chylismiella pterosperma	26	Sex	Taxonomy
Gayophytum decipiens	26	Sex	Taxonomy
Gayophytum diffusum diffusum	26	Sex	MrBayes

Gayophytum d. parviflorum	26	Sex	MrBayes
Gayophytum eriospermum	26	Sex	MrBayes
Gayophytum heterozygum	26	PTH	MrBayes
Gayophytum humile	26	Sex	Taxonomy
Gayophytum micranthum	26	Sex	Taxonomy
Gayophytum oligospermum	26	Sex	Taxonomy
Gayophytum racemosum	26	Sex	Taxonomy
Gayophytum ramosissimum	26	Sex	Taxonomy
Taraxia breviflora	27	Sex	Taxonomy
Taraxia ovata	27	Sex	MrBayes
Taraxia subacaulis	27	Sex	Taxonomy
Taraxia tanacetifolia	27	Sex	MrBayes

[*]Nomenclature follows Wagner *et al.* (2007)

[†]Clades refer to those shown in Fig. 2 and are numbered from top (1) to bottom (27).

[‡]Reproduction was determined according to the meiotic behavior, patterns of gamete viability and crossing results reported in multiple publications (Lewis & Szweykowski 1964; Towner 1970; Cleland 1972; Raven & Gregory 1972; Dietrich 1977; Straley 1977; Dietrich & Wagner 1988; Dietrich *et al.* 1997; Wagner 2005)

[§]Method used to place species within unresolved clades. "MrBayes" refers to the 113 species included in a 5-gene phylogeny (Johnson *et al.* 2009) (Fig. S1). "Taxonomy" refers to placement of species based on the most recent taxonomic treatment (Wagner *et al.* 2007), which incorporates information from molecular systematics, crossing experiments that assess compatibility of crosses, cytogenetics, morphology and biogeography.

[¶] Clade 7 has 54% posterior probability support

[#]The species assigned to clades 11 and 12 are supported by taxonomy but not by a recent molecular phylogeny (Evans *et al.* 2009). Evans et al. (2009) infer an identical topology to the one shown (i.e. two well supported exclusively sexual clades containing two and eight species, respectively), but with different species assigned to each clade than shown here. This disparity has no effect on our results because both the taxonomy and Evans *et al.'s* (2009) molecular phylogeny have equal numbers of species in two well supported and exclusively sexual clades.

[1]46% posterior probability support

[2]95% posterior probability support

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Table S2 Means of posterior distributions for diversification and transition parameters estimated for the single ML tree. Estimates were obtained from MCMC BiSSE analyses using the single ML phylogeny shown in Fig. 2. Maximum likelihood analyses and log-likelihood ratio tests support the results reported here and are provided in Table S3B.

Parameter	PTH	Sex	95% CI of the Difference
Extinction	12.6	3.8	(–8.3, 32.8)
Speciation	35.8	6.3	(15.7, 47.3)
Diversification	23.2	2.5	(7.1, 33.6)
	PTH to Sexual	**Sex to PTH**	
Transition	25.2	1.1	(12.4, 36.2)

Table S3 Maximum likelihood estimates of parameters and summary of likelihood-ratio tests of alternative BiSSE models. (**A**) Parameters estimated by ML BiSSE, averaged across 100 MrBayes trees. (**B**) Parameters estimated by ML BiSSE using single ML tree.

(A)

Model	λ_P	λ_S	μ_p	μ_S	$q_{P\rightarrow S}$	$q_{S\rightarrow P}$	% [*] trees that reject H_0
1) Full	41.27	8.25	27.00	6.45	16.67	0.96	
2) $\lambda_P=\lambda_S$	20.47	20.47	0.43	21.04	21.49	0.44	100%
3) $\mu_p=\mu_S$	29.07	9.03	8.13	8.13	21.42	0.65	0%
4) $q_{P\rightarrow S}=q_{S\rightarrow P}$	166.51	8.05	176.04	0.43	5.35	5.35	100%
5) $\lambda_P=\lambda_S$, $\mu_p=\mu_S$	23.94	23.94	21.42	21.42	11.50	1.09	100%[†]
6) $\mu_p=\mu_S$, $q_{P\rightarrow S}=q_{S\rightarrow P}$	24.56	30.67	27.97	27.97	1.11	1.11	100%[‡]

(B)

Model	λ_P	λ_S	μ_p	μ_S	$q_{P\rightarrow S}$	$q_{S\rightarrow P}$	ΔlnL
1) Full	59.57	6.09	46.24	2.87	18.84	1.49	
2) $\lambda_P=\lambda_S$	23.38	23.38	0.00	24.29	24.82	0.45	8.03[***]
3) $\mu_p=\mu_S$	32.41	6.87	4.77	4.77	28.26	0.8	1.20
4) $q_{P\rightarrow S}=q_{S\rightarrow P}$	211.09	8.17	222.22	0	6.40	6.40	6.75[***]
5) $\lambda_P=\lambda_S$, $\mu_p=\mu_S$	17.29	17.29	14.57	14.57	58.29	5.80	32.22[†***]
6) $\mu_p=\mu_S$, $q_{P\rightarrow S}=q_{S\rightarrow P}$	27.18	33.95	31.21	31.21	1.22	1.22	27.74[‡***]

[*] The final column shows the percentage of trees where the more complex model provided a significant improvement (P <0.05) over the simpler constrained model (H_0) according to a likelihood ratio test, where P-values were based on a χ^2 distribution with 1 degree of freedom, corresponding to a model difference of one free parameter (for more details see Maddison *et al.* 2007).

[†] Model 5 compared to model 3.

[‡] Model 6 compared to model 3.

[***] p<0.001; likelihood ratio test comparing -2ΔlnL to a $\chi^2_{[1]}$-distribution.

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Figure S1 Diversification and transition rates in sexual and functionally asexual lineages allowing for a shift in rates. Here we show the posterior distributions marginalized over all 100 trees from MCMC BiSSE analyses based on a 12-parameter model that allows a single shift in rates to occur. The six background rates for (**A**) speciation, (**B**) extinction, (**C**) diversification (speciation minus extinction) and (**D**) character transitions between sexual and PTH reproduction are illustrated in the main boxes, and the six foreground rates (after the shift) in the inset boxes. In the background, there are few PTH clades and the posterior distributions are very similar to the prior distributions (dashed grey curves), indicating little power to detect trends associated with PTH in the background. In the foreground, we observe similar patterns to the 6-parameter BiSSE where there is no shift in rates (Fig. 3). Specifically, foreground speciation rates were inferred to be significantly higher for PTH than for sexuals for 70 of the trees, as were diversification rates for 54 of the trees, while no trees displayed significant results in the opposite direction. Again, the foreground transition rate from PTH to sex was inferred to be significantly higher than transitions from sex to PTH (for 64 of the trees). The posterior distributions are slightly wider, however, again indicating lower power. The shift was localized using the MEDUSA comparative method, as the most likely position of a shift in diversification rates (Alfaro *et al.* 2009). Even though the trees differed slightly in the placement of the split, the posterior distributions are similar for the majority of trees considered separately.

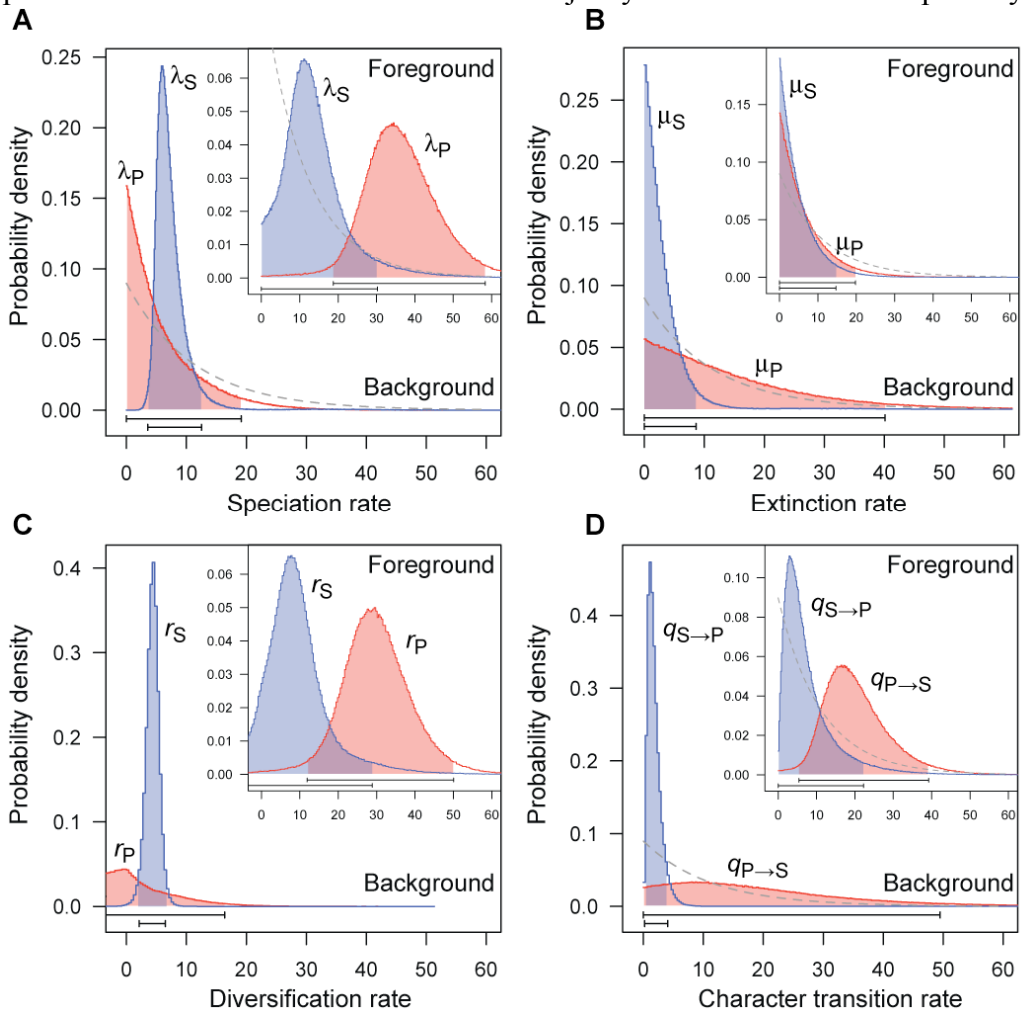


Figure S2 First of the 100 MrBayes trees, showing the character state (PTH: red; sexual: blue) of each of the 113 taxa in the Onagreae clade.

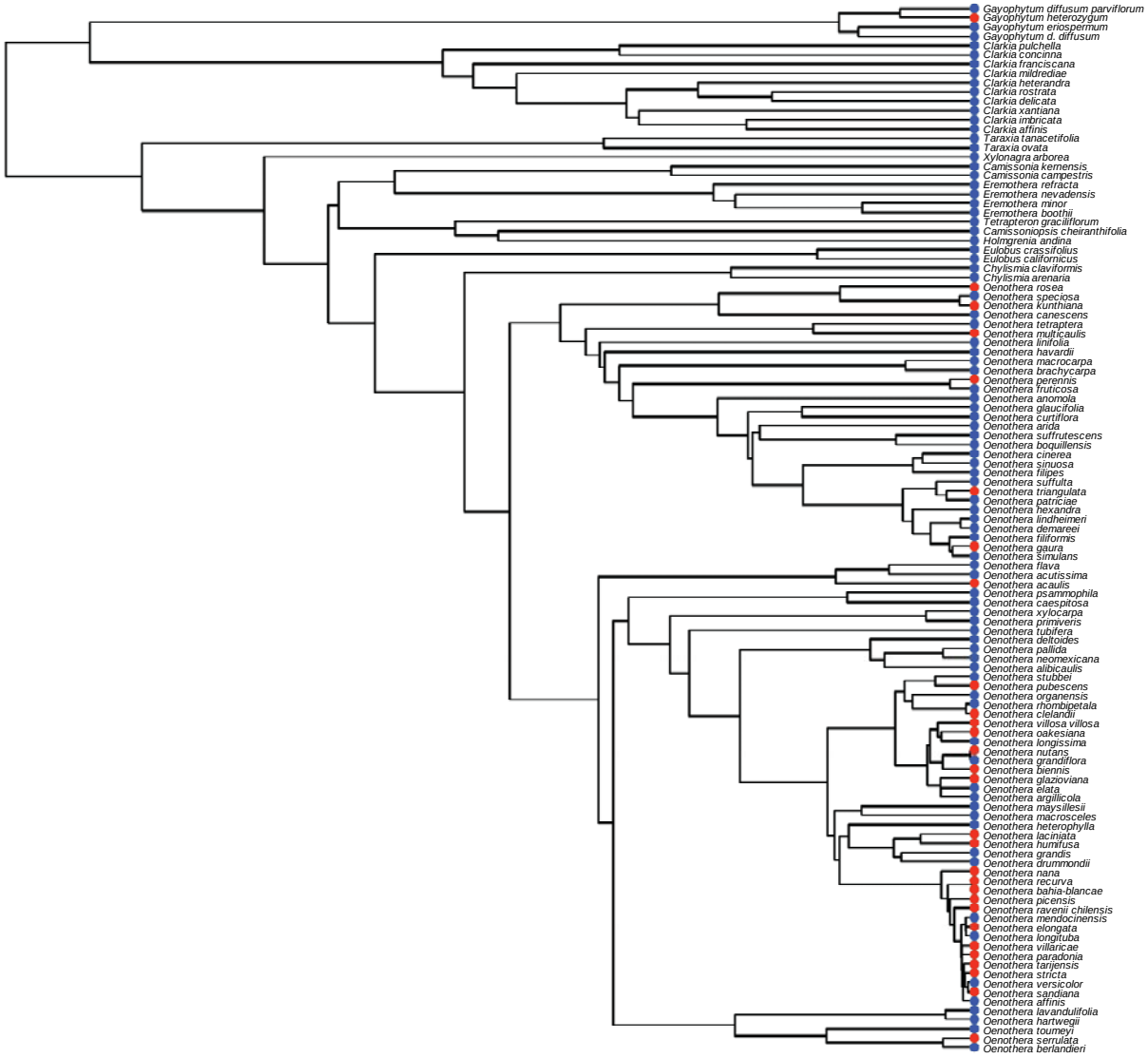
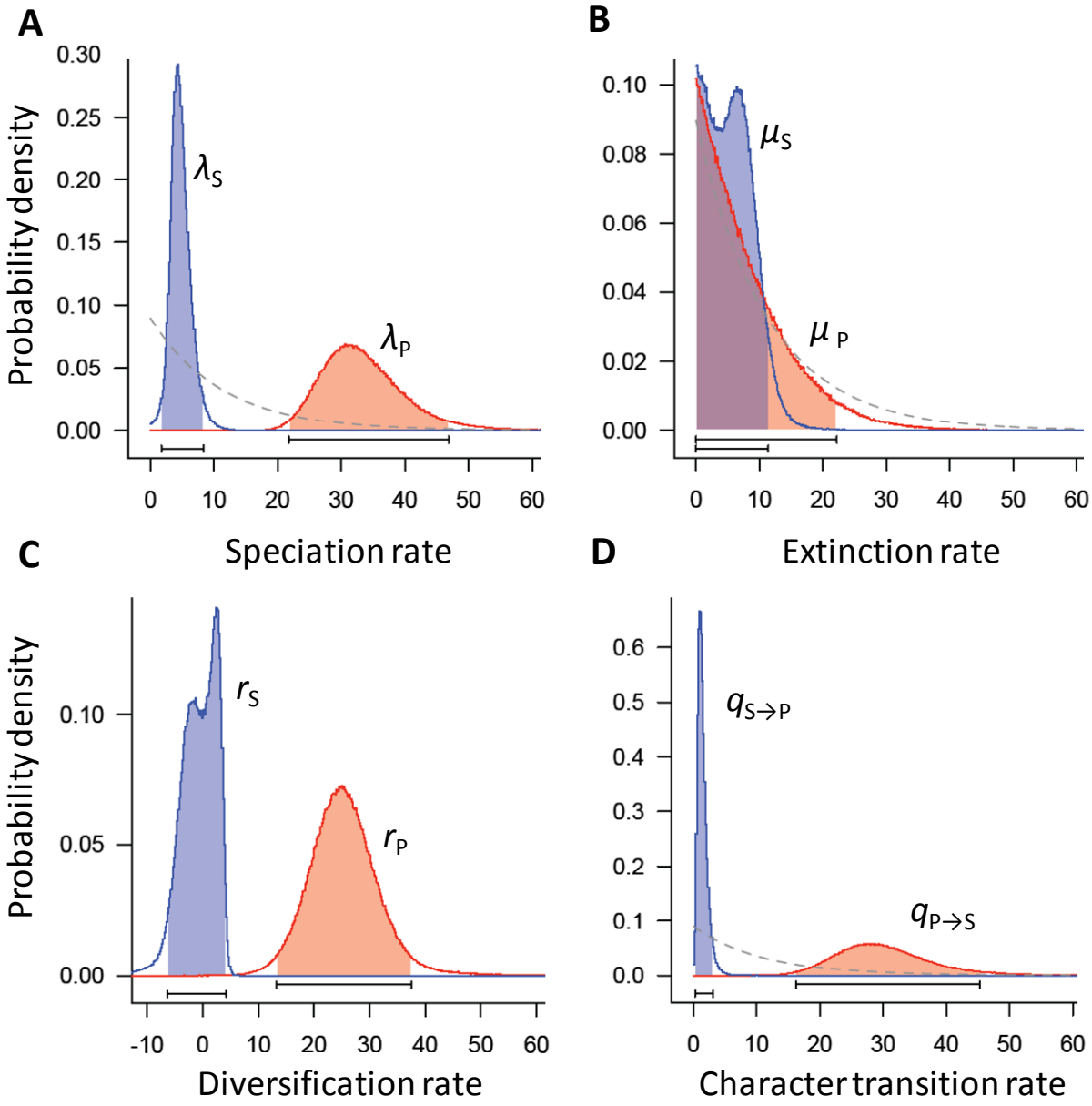


Figure S3 Diversification and transition rates in sexual and functionally asexual lineages using the skeletal tree method. Each distribution showing the rate of (**A**) speciation, (**B**) extinction, (**C**) diversification (speciation minus extinction) and (**D**) character transitions between sexual and PTH reproduction represents the posterior distribution from an MCMC BiSSE analysis, as in Fig. 3 but with the skeletal tree method (FitzJohn *et al.* 2009). This method assumes that the taxa included in the phylogeny are randomly sampled from the extant taxa. Specifically, the 29 PTH species and 84 sexual taxa included in the phylogeny are assumed to be random samples from the 43 extant PTH taxa and 219 sexual taxa in the Onagreae (sampling fraction set to 29/43 for PTH and 84/219 for sexuals). The skeletal tree method typically has less power than the unresolved clade method, because it does not use information about the likely phylogenetic location of the unsampled taxa (FitzJohn *et al.* 2009). Because we had to collapse the 113 taxon tree down to a tree with only 27 unresolved clades (main text), however, the power and results for the skeletal tree method and the unresolved clade method are very similar in this case. Fig. S2 represents the first of 100 trees that were used in this analysis.

Fig. S3


A
Probability density
0.30
0.25
0.20
0.15
0.10
0.05
0.00
λ_S
λ_P
0
10
20
30
40
50
60
Speciation rate
B
0.10
0.08
0.06
0.04
0.02
0.00
μ_S
μ_P
0
10
20
30
40
50
60
Extinction rate
C
Probability density
0.10
0.05
0.00
r_S
r_P
-10
0
10
20
30
40
50
60
Diversification rate
D
0.6
0.5
0.4
0.3
0.2
0.1
0.0
$q_{S\rightarrow P}$
$q_{P\rightarrow S}$
0
10
20
30
40
50
60
Character transition rate