

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

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Received March 4, 2011

Accepted March 27, 2011

Data Archived: Dryad doi:10.5061/dryad.dm690

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 hap-

loid 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 (Barracough 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 (Barracough 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

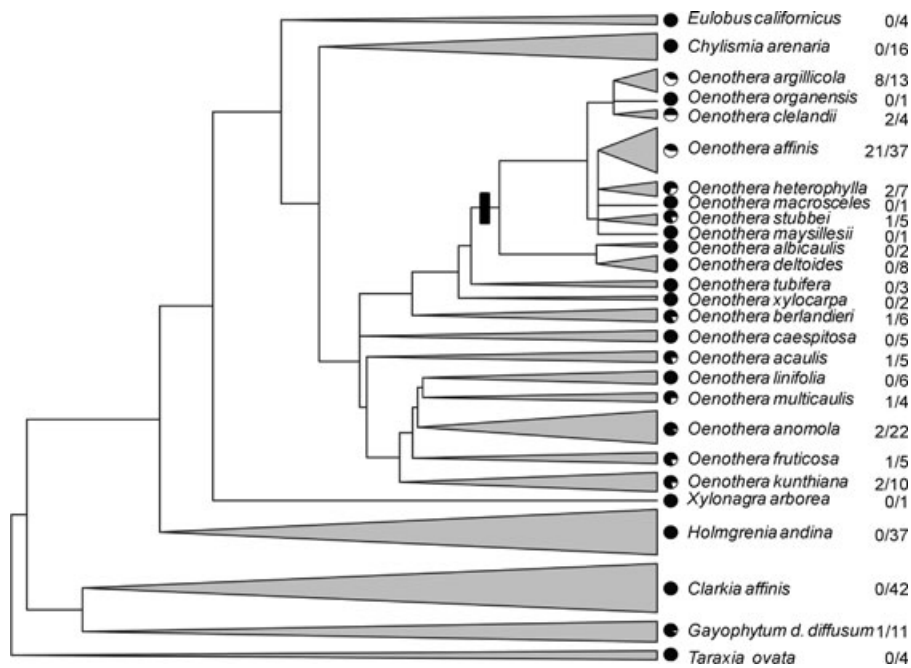


Figure 2. Phylogenetic distribution of sex and functionally asexual (PTH) evening primroses. A single ML tree is shown where gray triangles represent unresolved clades, with the width of triangles corresponding to the number of taxa. Although the ML tree contains several polytomies, the 100 MrBayes trees 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/phyloids/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 ($q_{P \rightarrow S} = q_{S \rightarrow P}$) 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: ($\lambda_{P,f}$, $\lambda_{S,f}$, $\mu_{P,f}$, $\mu_{S,f}$, $q_{P \rightarrow S,f}$, $q_{S \rightarrow P,f}$) in a particular clade of interest ("foreground," including the subtending branch) and ($\lambda_{P,b}$, $\lambda_{S,b}$, $\mu_{P,b}$, $\mu_{S,b}$, $q_{P \rightarrow S,b}$, $q_{S \rightarrow P,b}$) 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 diversification 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

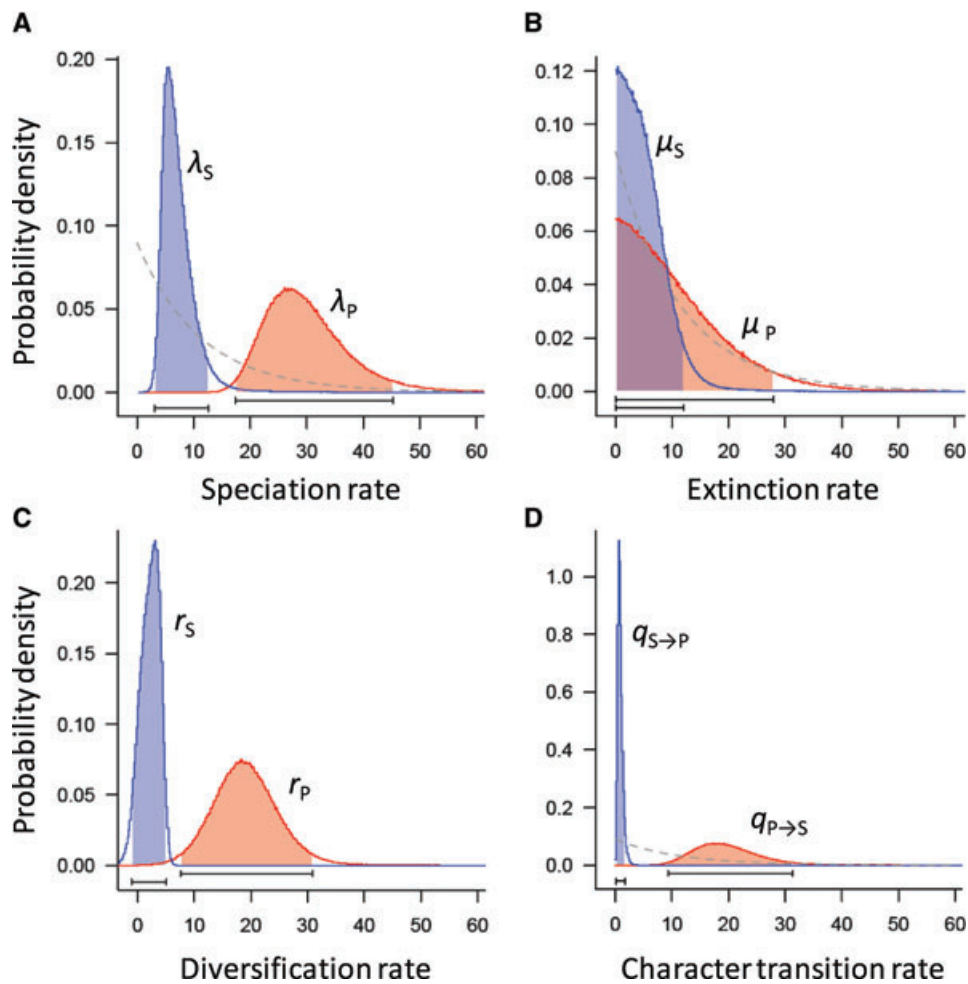


Figure 3. Diversification and transition rates in sexual and functionally asexual (PTH) lineages. Posterior probability distributions are shown for rates of (A) speciation (λ), (B) extinction (μ), and (C) net diversification ($r = \lambda - \mu$) in PTH (state P—red) and sexual (state S—blue) lineages. (D) The rates of evolutionary transitions from PTH to sexual reproduction ($q_{P \rightarrow S}$) and from sexual to PTH reproduction ($q_{S \rightarrow P}$) 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 ($\Delta AIC = 7.94$) or the standard MEDUSA model where there is a shift in overall diversification rate but no difference in diversification between PTH and sexual lineages ($\Delta AIC = 3.84$; in contrast with MEDUSA, $q_{P \rightarrow S}$ and $q_{S \rightarrow P}$ 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 ($\Delta AIC = 11.66$ 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; Gavrillets 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; Gavrillets 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 disproportionately 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 ($q_{P \rightarrow S} = 0$), 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: $8.5 < q_{P \rightarrow S} - q_{S \rightarrow P} < 30.3$), 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 ASEQUAL 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.

ACKNOWLEDGMENTS

This research was enabled in part through the use of advanced computing resources provided by WestGrid, Compute/Calcul Canada, and the Zoology Computing Unit of the University of British Columbia (UBC). We thank F. Ronquist for advice in using MrBayes 3.2, W. Birky and anonymous reviewers who provided constructive comments on earlier versions of the article. SDS received support from a National Institute of Health Ruth L. Kirschstein NRSA Fellowship and Duke University's Center for Evolutionary Genomics. RGF was supported by a University Graduate Fellowship from UBC, a Capability Fund Grant from Manaaki Whenua/Landcare Research, and a Vanier Commonwealth Graduate Scholarship from Natural Sciences and Engineering Research Council of Canada (NSERC). Funding was provided by NC State University and National Science Foundation (NSF) grants DEB-0919869 and DEB-0950486 to MTJJ, DEB-0448889 and DEB-0841521 to MDR, and a Natural Sciences and Engineering Research Council (Canada) Discovery Grants to MTJJ and SPO.

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Associate Editor: M. Johnston

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.

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