Plant Sex and the Evolution of Plant Defenses against Herbivores

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Plant sex and the evolution of plant defenses against herbivores

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Despite the importance of plant–herbivore interactions to the ecology and evolution of terrestrial ecosystems, the evolutionary factors contributing to variation in plant defenses against herbivores remain unresolved. We used a comparative phylogenetic approach to examine a previously untested hypothesis (Recombination-Mating System Hypothesis) that posits that reduced sexual reproduction limits adaptive evolution of plant defenses against arthropod herbivores. To test this hypothesis we focused on the evening primrose family (Onagraceae), which includes both sexual and functionally asexual species. Ancestral state reconstructions on a 5-gene phylogeny of the family revealed between 18 and 21 independent transitions between sexual and asexual reproduction. Based on these analyses, we examined susceptibility to herbivores on 32 plant species representing 15 independent transitions. Generalist caterpillars consumed 32% more leaf tissue, gained 13% greater mass, and experienced 21% higher survival on functionally asexual than on sexual plant species. Survival of a generalist feeding mite was 19% higher on asexual species. In a field experiment, generalist herbivores consumed 64% more leaf tissue on asexual species. By contrast, a specialist beetle fed more on sexual than asexual species, suggesting that a tradeoff exists between the evolution of defense to generalist and specialist herbivores. Measures of putative plant defense traits indicate that both secondary compounds and physical leaf characteristics may mediate this tradeoff. These results support the Recombination-Mating System Hypothesis and suggest that variation in sexual reproduction among plant species may play an important, yet overlooked, role in shaping the macroevolution of plant defenses against arthropod herbivores.

Plant species vary markedly in the expression and effectiveness of defenses against herbivores (1–3). Understanding the evolutionary processes that contribute to this variation is of interest to both basic and applied biologists because herbivory is an important feature of natural and managed ecosystems (2, 4, 5). Previous hypotheses on the evolution of plant defense successfully explain variation in the levels of defense and amount of herbivory incurred by plant species within particular ecosystems (6–8), or across broad phylogenetic scales (i.e., among plant families) (9, 10). However, these hypotheses are less successful at explaining patterns of defense among closely related plant species, where variation in defensive strategies originates (11–14), which suggests that there are additional explanations for variation in plant defense. Here, we report on a study that explores an unexamined explanation for this variation: a reduction in the amount of sexual reproduction decreases the ability of plants to evolve defenses in response to arthropod herbivores (15).

The importance of parasites for the maintenance of sexual reproduction has been well established (16–18), but the consequences of different plant reproductive systems for the evolution of defense has received little attention (15, 19, 20). Reproductive mode may be particularly relevant to flowering plants, which exhibit a near continuum in sexual systems, from self-incompatible species with high effective recombination rates to species that produce seeds asexually (21, 22). Levin (15) was the first to consider the dual significance of plant–parasite interactions for the evolution of sex and the evolution of plant defenses. He proposed the Recombination-Mating System Hypothesis, which predicts that species exhibiting higher rates of recombination and segregation of alleles (i.e., increased sexual reproduction) should display greater resistance to arthropod herbivores. This hypothesis is based on 2 ways in which sex influences the evolution of plant defenses (23, 24). First, while increased sexual reproduction allows populations to purge deleterious mutations, less sex (i.e., reduced recombination and segregation) allows for the accumulation of mildly deleterious mutations throughout the genome that might affect primary and secondary metabolism, a process called Muller’s Ratchet (25, 26). Second, sexual reproduction is expected to allow greater evolutionary responses by plants to selection imposed by herbivores. For example, sexually reproducing populations can create novel genotypes that vary in resistance every generation and maintain genetic variation over long periods of time (17, 27). By contrast, selection on host populations with reduced sex can quickly erode genetic variation, decreasing the ability of plant populations to respond to selection by parasites (17, 27).

Species in the evening primrose plant family (Onagraceae) offer an ideal system to examine the effects of sexual reproduction on the evolution of plant defense. The monophyletic Onagraceae tribe within the family is comprised of 259 species, of which 85% exhibit “normal” sexual reproduction, which typically involves meiotic recombination between 7 pairs (2 × 14) of homologous chromosomes and the segregation of heterozygous alleles during either self- or cross-fertilization (28, 29). By contrast, 15% of species from the genera Oenothera and Gayophytum experience a near-complete shutdown of meiotic recombination and segregation, and as such these species are functionally asexual (29, 30). This functional asexuality arises because of a well-studied genetic system called permanent translocation heterozygosity (PTH). PTH is characterized by 3 phenomena (29, 31). First, chromosomal translocations throughout the genome alter chromosomal homology in such a way that bivalent pairings do not occur during meiosis. Instead, the chromosomes form a complete ring with synopsis restricted to chromosome ends, effectively preventing recombination (29, 32). Second, segregation of alleles at heterozygous loci are prevented by sporophytic and gametophytic incompatibilities that cause a balanced lethal mortality of haploid gametes, such that one haploid set of chromosomes always segregates together and passes through the ovules, whereas the other haploid set always passes through the pollen (29, 32). And third, PTH species typically self-fertilize by dehiscing pollen onto receptive stigmas before flowers open. These characteristics lead to the production of seeds that are genetically identical to the parent plant. Although it has long been recognized

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that PTH reproduction evolved multiple times throughout the Onagraceae (28, 31), the number of independent transitions between sexual and asexual reproduction is unknown.

Here, we use the natural variation in sexual and functionally asexual PTH reproduction in *Ononisera* and *Gayophytum* spp., to perform a phylogenetically explicit test of the hypothesis that reduced sexual reproduction negatively influences the evolution of plant defenses against arthropod herbivores. We first created a phylogeny of the Onagraceae tribe and estimated the number and location of independent transitions between sexual and PTH reproduction. Using this information we selected multiple species that represented independent transitions between sexual and PTH reproduction and tested our hypothesis by measuring susceptibility of these species to arthropod herbivores in the lab and field.

Specifically, we asked: (i) do PTH plant species exhibit greater susceptibility to generalist and specialist herbivores compared with sexual plant species? (ii) Is resistance to different herbivore species correlated across plant species, indicative of correlated evolutionary mechanisms of defense? (iii) What plant traits differ between PTH and sexual plant species that might affect susceptibility to arthropod herbivores?

**Results**

**Phylogenetic Analyses and Ancestral State Reconstructions.** We used 5 genes to infer the molecular phylogeny of species in the Onagraceae tribe (Fig. 1). Our sampling included all putative independent occurrences of PTH reproduction across the phylogeny and closely related sexual species. Ancestral state reconstructions by maximum parsimony across 1,000 Bayesian trees identified 18–21 (median = 21) independent transitions between PTH and sexual reproduction (Fig. 1). Based on these results we selected 32 species that represented a minimum of 15 transitions in reproduction from across the phylogeny. Plants of these species were then used to assay susceptibility to generalist and specialist arthropod herbivores in lab and field experiments (Fig. S1 and Table S1).

**Herbivore Susceptibility.** Using recently developed phylogenetic comparative methods that incorporate measurement error (33), we found that PTH species exhibited increased susceptibility to generalist herbivores when compared with sexual plant species (Fig. 2, Fig. S1, and Table S2). As might be expected, there was considerable variation in susceptibility to herbivores among plant species across the phylogeny (Fig. S1). However, when we compared phylogenetically adjusted mean differences, we found that generalist feeding beet armyworm caterpillars (*Spodoptera exigua*) consumed 32% more leaf tissue (Fig. 2A), gained 13% more mass, and experienced 21% higher survival (Fig. 2B) on PTH plant species than sexual plant species in no-choice bioassays (Table S2). A second generalist herbivore, the two-spotted spider mite (*Tetranychus urticae*), experienced 19% higher survival on PTH plant species compared with sexual species (Fig. 2C) and exhibited 9% higher egg production on PTH species (Fig. 2D), although this last effect was not statistically significant (Table S2).

Contrary to our prediction, a breakdown in sexual reproduction was associated with decreased susceptibility to a specialist herbivore. The specialist flea beetle *Alticus foenae* consumed 21% less leaf tissue on PTH species compared with sexual plant species in no-choice bioassays (Fig. 2E). As we discuss below, this result suggests that a genetic tradeoff exists in the evolution of defense to generalist and specialist herbivores.

We conducted a common garden field experiment to understand whether the above results apply under more natural conditions, where arthropods were allowed to freely colonize plants. PTH plant species incurred 64% higher season-wide herbivory than sexual plant species, where most herbivory was imposed by 2 generalist-feeding caterpillars (*Spodoptera ornithogalli* and *Desmia funeralis*) and 1 generalist beetle (*Popillia japonica*). This difference was observed regardless of whether we performed analyses on individual sampling dates or over the entire season, with the exception of the first sampling date when plants had little damage (Table S2).

**Correlations in Susceptibility.** If differences in defense between PTH and sexual species are caused by the same genetic mechanisms for different herbivores, then we expect to observe correlations in performance among different herbivore species. Consistent with this prediction, we found that consumption by the generalist caterpillar was positively correlated with the proportion of mites that survived on plants (Fig. 3A) and herbivory in the field (Fig. 3B). Other measures of susceptibility to the generalist caterpillar (i.e., weight gain and caterpillar survival) were also significantly positively correlated with susceptibility to both mite survival and herbivory in the field (Table S3). Similarly, survival of mites was positively correlated with herbivory in the field (*r* _phylo_ = 0.73, _P_ < 0.05). By contrast, susceptibility to the specialist beetle was negatively associated with susceptibility to the generalist caterpillar (Fig. 3B), mite survival, and field herbivory. Some caution is needed in interpreting this latter result because these correlations were significant only under a model of Brownian motion evolution, which was marginally better statistically at explaining variation among species than models that assumed trait evolution was independent of phylogeny.

**Plant Traits.** Our final objective was to identify plant traits that vary between PTH and sexual plant species and predict susceptibility to herbivores. We measured 5 traits that were previously shown to be correlated with resistance against herbivores in the Onagraceae and other plant families (34–37); these traits included plant secondary chemistry (protein precipitation capacity of hydrolysable and condensed tannins), physical leaf characteristics (leaf toughness, trichome density), and physiological traits (percentage of leaf water content, specific leaf area). Using the same phylogenetic methods as described above (33), we found that all plant traits except leaf water content significantly differed between PTH and sexual plant species (Table S2). The largest differences were observed for leaf toughness (40% lower in PTH species than sexual species; Fig. 4A), the protein binding capacity of tannins in leaves (10% lower in PTH species; Fig. 4B), and trichome density (32% higher on PTH species) (Table S2). Of these traits, leaf toughness and tannins were the most consistent predictors of herbivore susceptibility (Table S4). For example, herbivory by the generalist caterpillar and survival rate of the generalist mite both significantly decreased with increasing leaf toughness and tannins (Fig. 4 C–F), whereas herbivory by the specialist beetle positively correlated with these same variables (leaf toughness: _r_ _phylo_ = 0.75, _P_ < 0.05; tannins: _r_ _phylo_ = 0.74, _P_ < 0.05; Table S4). Variation in other plant traits (trichome density, percentage of leaf water content, and specific leaf area) also correlated with susceptibility to herbivores, although the magnitude and statistical significance of these correlations was less consistent (Table S4).

**Discussion**

This study tested the hypothesis that reduced recombination and segregation negatively affects the evolution of plant defenses against herbivores (15). Our results demonstrate that a breakdown in sexual reproduction within species of the Onagraceae has large effects on the evolution of plant defense, yet the consequences of this evolution for generalists are opposite to that for specialist arthropod herbivores.

In this study, reduced sexual reproduction was associated with increased susceptibility to generalist herbivores and decreased susceptibility to specialist herbivores (Fig. 2 and Table S2). One explanation for this pattern assumes a genetic tradeoff in defense against generalist and specialist herbivores, where defense against generalists is negatively correlated genetically with defense against the specialist. This correlation could arise, for example, when a particular defensive trait that is effective at deterring generalists is...
Fig. 1. Evolutionary history of PTH and sexual reproduction in the Onagraceae. Transitions between PTH (red) and sexual reproduction (blue) were estimated by maximum parsimony. A single maximum-likelihood tree from combined analysis of plastid and nuclear genes is shown. Bayesian posterior probabilities for each branch are indicated when >50%; * denotes posterior probability >95%. Green circles show *Oenothera* and *Gayophytum* species used in experiments. Taxonomy follows Wagner et al. (43).
also attractive to specialists (38, 39). The results presented here are consistent with this expectation: all generalist herbivores were positively correlated in their performance (Table S3), and correlations between generalist herbivores and the specialist beetle were all negative (Fig. 3C and Table S3). The traits that provided the strongest predictors of this pattern were leaf toughness and tannins (Fig. 4C–F), which also differed significantly between PTH and sexual plant species (Fig. 4A and B).

This association between plant sex, resistance traits, and the tradeoff in susceptibility could be explained by 2 nonexclusive mechanisms. First, asexual species are expected to accumulate deleterious mutations (25, 40) that can cause deterioration in the production of defensive traits. Second, asexual species are predicted to be slower in their response to selection than sexual species because of reduced genetic variance within populations of the former (41–42). Given the observed tradeoff, the disparity in evolutionary potential between PTH and sexual species should lead to a greater increase in leaf toughness and tannins in sexual species when generalist herbivores impose stronger selection, and the opposite pattern when specialists impose stronger selection (41, 42). Previous experimental evidence indicates that selection by generalist herbivores on *Oenothera* is weak (35), whereas specialist herbivores can be abundant and often have direct impacts on plant fitness (44, 45). Because our results indicate that specialist herbivores have not caused the evolution of decreased leaf toughness and tannins in sexual species compared with PTH species (in fact we observed the opposite pattern) our data are most consistent with Muller’s ratchet process, where an accumulation of mutations in PTH species impaired the expression of defensive traits relative to sexual species.

Although the evolution of asexuality by PTH is restricted to Onagraceae and a few other plant families (30), a variety of plant mating systems (e.g., extreme inbreeding) and demographic conditions (e.g., small population size) commonly cause decreases in
coefficients and Pcaterpillar consumption (P tannin concentration (herbivore susceptibility. (Johnson et al. PNAS

Materials and Methods

persistence times of asexual lineages (53). asexuality, which is hypothesized to contribute to the relatively short
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of the Red Queen Hypothesis, i.e., sexual reproduction causes
Hypothesis (18, 50–52). Our result of higher herbivory by gener-
tion of PTH itself. We therefore predict that our results portray a
PTH plants was likely caused by reduced sexual reproduction,
be more complex. These macroevolutionary predictions comple-
whereas the effects of plant sex on specialist herbivores will likely
of increased susceptibility to generalist herbivores (but see ref. 20),
and 3 nuclear regions

Fig. 4. The association between plant sexual reproduction, plant traits, and herbivore susceptibility. (A and B) Mean differences in leaf toughness (A) and tannin concentration (b) between PTH (red) and sexual (blue) plant species are shown. P values were estimated as described in Fig. 2. (C–F) Species raw mean values for leaf toughness and tannin concentration were plotted against
caterpillar consumption (C and E) and mite survival (D and F). Correlation coefficients and P values were calculated as described in Fig. 3.

effective rates of recombination and segregation (22, 42, 46). The observed pattern of increased herbivory by generalist herbivores on PTH plants was likely caused by reduced sexual reproduction, rather than any cytological mechanism associated with the evolution
of PTH itself. We therefore predict that our results portray a
general phenomenon, and we expect that plant mating systems associated with reduced sex will frequently result in the evolution
of increased susceptibility to generalist herbivores (but see ref. 20),
whereas the effects of plant sex on specialist herbivores will likely
be more complex. These macroevolutionary predictions complement
microevolutionary studies that show that plants derived from
self-fertilized seeds can experience decreased resistance and toler-
ance to herbivores, presumably because of increased homozygosity
of deleterious mutations (47–49).

Our results also have important implications for the ongoing
debate concerning the role of host–parasite coevolution for the
maintenance of sexual reproduction, the so-called Red Queen Hypothesis (18, 50–52). Our result of higher herbivory by gener-
alisers on PTH plant species is consistent with a necessary condition
of the Red Queen Hypothesis, i.e., sexual reproduction causes
reduced fitness impacts by parasites on hosts (18). Thus, increased
damage by generalist herbivores may render asexual lineages more
susceptible to extinction, resulting in lineage selection against
asexuality, which is hypothesized to contribute to the relatively short
Persistence times of asexual lineages (53).

Materials and Methods

Molecular Sequence Data and Phylogenetic Inference. To estimate the phylog-
ey of Onagraceae, we combined existing datasets from GenBank for 2 plastid
regions [trnL-trnF spacer (54) and rps16 intron (55–57)] and 3 nuclear regions
[introns 1–6 (plus exons) of the cytosolic isozyme phosphoglucone isomerase
(PgiC) (58, 59), nuclear ribosomal internal transcribed spacer region (ITS) and the
external transcribed spacer (ETS) (55, 56)] (Table S1). We expanded the existing
data set to include additional species in clades exhibiting variation in sexual
 reproduction by sequencing trnL-trnF and ITS from 33 species and PgiC from 43
species by using standard PCR conditions (Table S1 and Table S5) (55, 56, 60). In
total, our taxon sampling encompassed 113 species of Onagraceae (44% of
described species) plus 8 outgroup taxa (Fig. 1). Although this sampling does not
include all taxa in the family, it does include species from all clades containing
PTH reproduction and therefore our methods allowed us to accurately detect inde-
pendent origins of PTH reproduction. Sequences were aligned by using ClustalW
followed by manual manipulations in BioEdit (61).

After assessing the combinatorial of nuclear and plastid datasets, we analyzed
the combined dataset of all genes [5,228 characters; 1,992 variables sites (965
parasimony-informative)] by using Bayesian and likelihood methods (St Text). Bayesian
analyses were performed by using MrBayes 3.1.2 (http://mrbayes.cit.fsu.edu) with
partitioning by gene and further by coding versus noncoding regions
within genes, and analyses used a general time-reversible model with
gamma-distributed rates and invariant sites (GTR + I) with model parameters
unlinked across partitions. We retained 40,000 post burn-in trees for subsequent
ancestral state reconstruction analyses. The single best tree was inferred by using
likelihood analyses of the combined data in RAxML 7.0.4 (62) with the same
partitioning and models as in the Bayesian analyses. The ultrametricized
RAxML tree was subsequently used in phylogenetic comparative analyses.

Ancestral State Reconstructions. The evolutionary history of PTH and sexual
reproduction was inferred by maximum parsimony on each of 1,000 trees ran-
domly sampled from the Bayesian trees using Mesquite (http://mesquiteproject.
org). The reproductive strategy of each species (Fig. 1 and Table S1) was deter-
mined from the original publications that performed chromosome squashes of
meiotic pollen cells and controlled crosses, which have been reviewed elsewhere
(refs. 29–31 and http://botany.si.edu/onagraceae/index.cfm).

Experimental Details. We assayed susceptibility of plant species to generalist and
specialist herbivores by using lab and field experiments (St Text). Seeds were
obtained from natural populations by M.T.J.J., colleagues, and the U.S.
Department of Agriculture’s Ornamental Plant Germplasm Center. In the lab experi-
nment, we grew 2–38 replicate plants (mean = 24) from each of 27 Oenothera
species (Fig. 1 and Table S6). We used no-choice Petri dish assays to measure
susceptibility to newly hatched beet armyworm caterpillars (Spodoptera exigua,
Neohelicidae, Lepidoptera) supplied by Benzon Research and 2-spotted spider
mites (Tetranychus urticae, Tetranychidae, Prostigmata) collected from Duke’s
greenhouse and maintained on soybean. We measured susceptibility by excising 2
leaves from each plant that were placed individually onto moistened filter paper
in 60-mm Petri dishes sealed with parafilm. Caterpillar and adult parthenogenetic
female mites were placed individually onto each leaf and allowed to feed at
23 °C. The survival and wet mass of caterpillars were measured after 6 days of
feeding; wet mass and dry mass of caterpillars were highly correlated (r = 0.92,
P < 0.001, n = 197). The total area consumed by caterpillars was estimated from
leaves by using fine grid paper printed onto transparent cellular acetate. The
survival and number of eggs laid by mites was measured after 5 days.

We conducted a field experiment in the summer of 2008 to assay susceptibility
to naturally colonizing herbivores. We grew 5–42 replicate plants (mean = 36)
from each of 25 Oenothera and Gayophytum species (Fig. 1 and Table S6) and
measured insect herbivory on each plant 4 times through the season by visually
estimating the percentage of leaf tissue removed on each of 10 haphazardly
selected leaves; herbivory was averaged among leaves to derive a single estimate
per plant. Seasonwide maximum herbivory was determined as the maximum
herbivory recorded from a plant across all sampling dates. We also used leaves
from plants in the field to measure susceptibility to the specialist beetle Alticus
foenae (Chrysomelidae, Coleoptera) by using no-choice assays. Specialist beetles
were collected along the North Carolina coast, and susceptibility was determined by
using the protocols described for caterpillars and mites (St Text).

All plant traits were measured from plants grown in the first lab experiment (Table S7 and St Text).

Phylogenetic Regression Analyses. The associations between plant sexual repro-
duction, susceptibility to herbivores, and plant traits were assessed statistically
by using phylogenetic regression methods that incorporate measurement error
within species (33) (St Text). We used these methods to estimate the phylogenetic
signal of traits according to K* (K* = 1 corresponds to Brownian motion evolu-
tion; K* = 0 corresponds to evolution independent of phylogeny) (63), the
correlation coefficient (rphylo) between continuously varying traits, and the asso-
ciation between plant sex (PTH/sex) and herbivore susceptibility, using restricted
maximum likelihood under a model of Brownian motion trait evolution across
the phylogeny. Statistical significance of estimates was determined by parametric bootstrapping (SI Text).

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