

Plant sex and the evolution of plant defenses against herbivores

Marc T. J. Johnson^{a,1}, Stacey D. Smith^b, and Mark D. Rausher^b

^aDepartment of Plant Biology, North Carolina State University, Raleigh, NC 27695; and ^bDepartment of Biology, Duke University, Durham, NC 27612

Edited by Douglas J. Futuyma, Stony Brook University, Stony Brook, NY, and approved May 28, 2009 (received for review April 29, 2009)

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.

coevolution | herbivory | phylogenetics | plant–insect | tradeoff

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 Onagreae tribe within the family is comprised of 259 species, of which 85% exhibit “normal” sexual reproduction, which typically involves meiotic recombination between 7 pairs ($2 \times = 14$) 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 synapsis 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

Author contributions: M.T.J.J., S.D.S., and M.D.R. designed research; M.T.J.J. performed research; M.T.J.J., S.D.S., and M.D.R. contributed new reagents/analytic tools; M.T.J.J., S.D.S., and M.D.R. analyzed data; and M.T.J.J., S.D.S., and M.D.R. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: marc.johnson@ncsu.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0904695106/DCSupplemental.

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 *Oenothera* 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 individ-

ual 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 ($r_{\text{phylo}} = 0.73$, $P < 0.05$). 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: $r_{\text{phylo}} = 0.75$, $P < 0.05$; tannins: $r_{\text{phylo}} = 0.74$, $P < 0.05$; 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

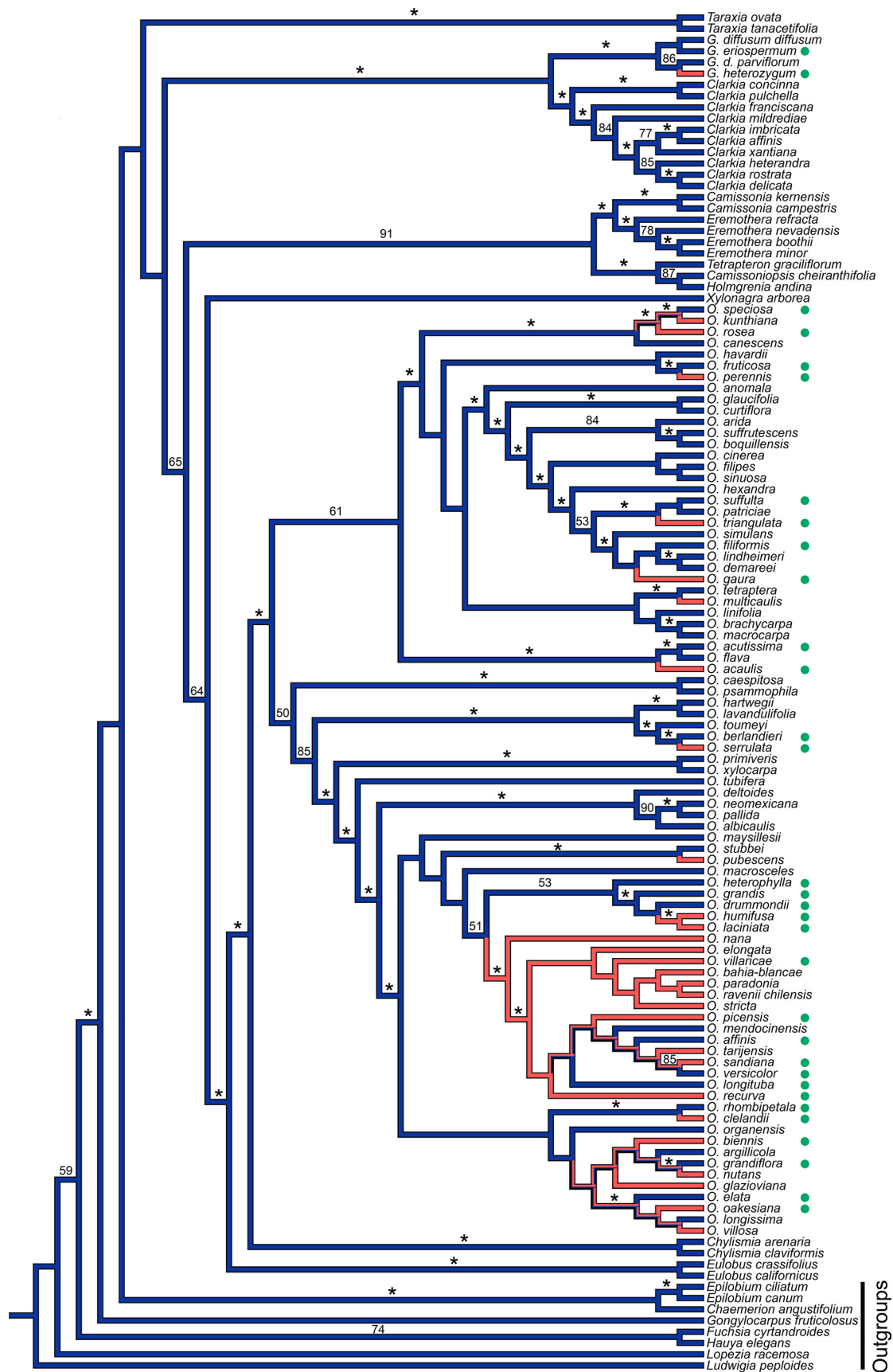


Fig. 1. Evolutionary history of PTH and sexual reproduction in the Onagraceae. Transitions between PTH (red) and sexual reproduction (blue) were estimated by maximum parsimony. A single maximum-likelihood tree from combined analysis of plastid and nuclear genes is shown. Bayesian posterior probabilities 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).

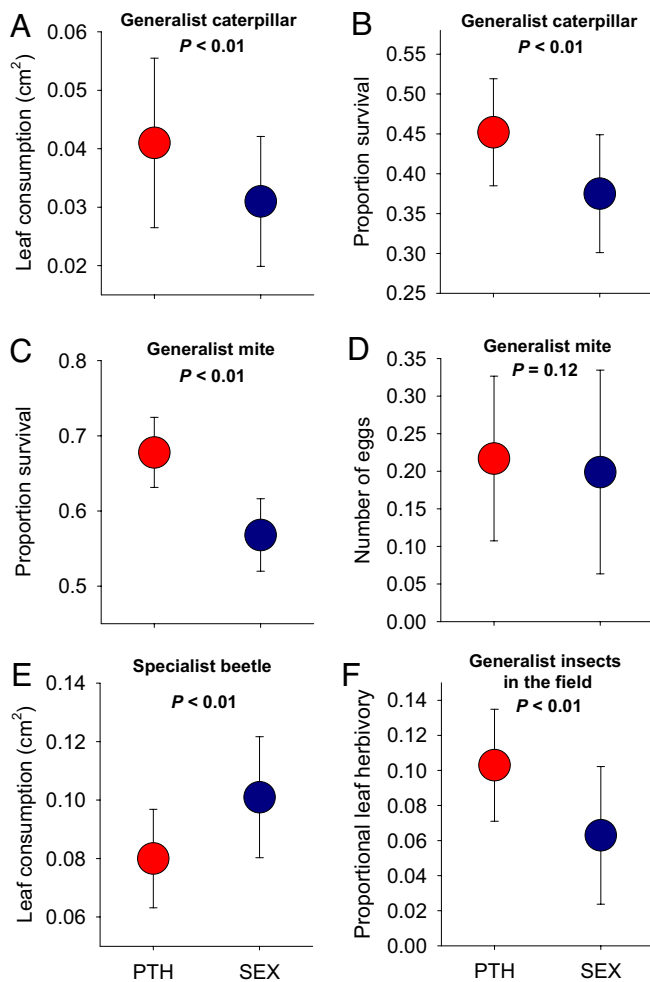


Fig. 2. Susceptibility of arthropod herbivores to PTH and sexual plant species. Susceptibility of PTH (red) and sexual (blue) plants measured according to consumption (A) and proportion survival (B) of generalist caterpillars, proportion survival (C) and the number of eggs laid (D) by generalist mites, the amount of leaf tissue consumed by specialist beetles (E), and the maximum herbivory incurred by plants in the field (F). Mean values were adjusted for phylogenetic relatedness among species after taking measurement error into account and modeling evolution as a Brownian motion process across the phylogeny (33). P values of mean differences were estimated by parametric bootstrapping (33). To portray the variation among species we show 1 SE among the raw means of plant species; these SE values were not calculated from the comparative analyses and do not provide information about the significance of mean differences between PTH and sexual species.

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

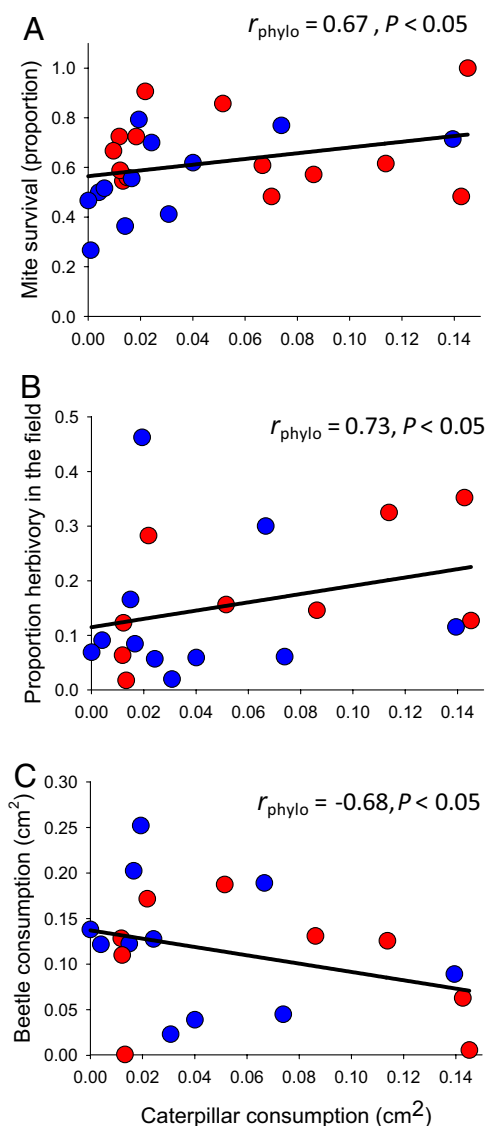


Fig. 3. Correlations in susceptibility to generalist and specialist herbivores among PTH (red) and sexual (blue) plant species. Raw mean values for each species for susceptibility to the generalist caterpillar are plotted against mite survival (A), maximum herbivory in the field (B), and consumption by the specialist beetle (C). We show the correlation coefficients for the relationship between each pair of variables after accounting for phylogeny. P values were estimated as described in Fig. 2 (33).

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

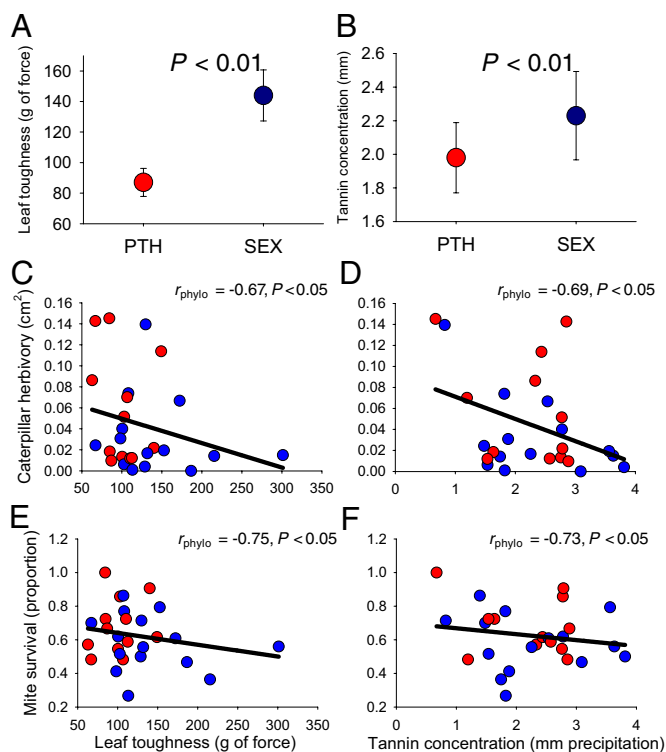


Fig. 4. The association between plant sexual reproduction, plant traits, and herbivore susceptibility. (A and B) Mean differences in leaf toughness (A) and tannin concentration (B) between PTH (red) and sexual (blue) plant species are shown. P values were estimated as described in Fig. 2. (C–F) Species 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 tolerance 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 generalists 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 phylogeny 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 phosphoglucose 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 dataset 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 independent origins of PTH reproduction. Sequences were aligned by using ClustalW followed by manual manipulations in BioEdit (61).

After assessing the combinability of nuclear and plastid datasets, we analyzed the combined dataset of all genes [5,228 characters; 1,992 variable sites (965 parsimony-informative)] by using Bayesian and likelihood methods (SI Text). Bayesian analyses were performed by using MrBayes 3.1.2 (<http://mrbayes.csit.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+G+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 randomly sampled from the Bayesian trees using Mesquite (<http://mesquiteproject.org>). The reproductive strategy of each species (Fig. 1 and Table S1) was determined 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 (SI 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 experiment, we grew 2–38 replicate plants (mean = 24) from each of 27 *Oenothera* species (Fig. S1 and Table S6). We used no-choice Petri dish assays to measure susceptibility to newly hatched beet armyworm caterpillars (*Spodoptera exigua*, Noctuidae, Lepidoptera) supplied by Benzoin 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 measured 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. S1 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 (SI Text).

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

Phylogenetic Regression Analyses. The associations between plant sexual reproduction, susceptibility to herbivores, and plant traits were assessed statistically by using phylogenetic regression methods that incorporate measurement error within species (33) (SI Text). We used these methods to estimate the phylogenetic signal of traits according to K^* ($K^* = 1$ corresponds to Brownian motion evolution; $K^* = 0$ corresponds to evolution independent of phylogeny) (63), the correlation coefficient (r_{phylo}) between continuously varying traits, and the association 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).

ACKNOWLEDGMENTS. We thank V. Bender, B. Calhoun, T. Chappell, R. Hopkins, D. Des Marais, R. Johnson, T. Kossler, A. Manzaneda, L. Rausher, M. Streisfeld, K. Wright, and workers at Fort Macon State Park (Atlantic Beach, NC) for logistical support; the U.S. Department of Agriculture, A. Agrawal (Cornell University, Ithaca, NY), S. Barrett (University of Toronto, Toronto), S. Blaney (Atlantic Canada Conservation Data Centre, Sackville, Canada), M. Gibbons (Birmingham-Southern College, Birmingham, AL), G. and R. Hoggard (University of Oklahoma, Oklahoma City), K. Krakos (Washington University,

St. Louis), J. Meurer (Ludwig-Maximilians University, Munich), R. Raguso (Cornell University), and P. Van Zandt (Birmingham-Southern College) for plant material; B. Husband (University of Guelph, Guelph, Canada) for *Chamerion* DNA; and T. Garland (University of California, Riverside) and T. Ives (University of Wisconsin, Madison) for software and helpful advice. M.T.J.J. was supported by the Natural Sciences and Engineering Research Council of Canada and North Carolina State University. S.D.S. received support from a National Institutes of Health Ruth L. Kirschstein National Research Service Award Fellowship and Duke University's Center for Evolutionary Genomics. This work was supported by National Science Foundation Grant DEB-0448889 and a Duke Research Council grant (to M.D.R.).

1. Agrawal AA (2007) Macroevolution of plant defense strategies. *Trends Ecol Evol* 22:103–109.
2. Berenbaum MR, Zangerl AR (2008) Facing the future of plant–insect interaction research: Le retour à la "Raison d'Être." *Plant Phys* 146:804–811.
3. Stamp N (2003) Out of the quagmire of plant defense hypotheses. *Q Rev Biol* 78:23–55.
4. Fraenkel GS (1959) The raison d'être of secondary plant substances. *Science* 129:1466–1470.
5. Cyr H, Pace ML (1993) Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* 361:148–150.
6. Coley PD, Bryant JP, Chapin FS (1985) Resource availability and plant antiherbivore defense. *Science* 230:895–899.
7. Fine PVA, Mesones I, Coley PD (2004) Herbivores promote habitat specialization by trees in Amazonian forests. *Science* 305:663–665.
8. Van Zandt PA (2007) Plant defense, growth, and habitat: A comparative assessment of constitutive and induced resistance. *Ecology* 88:1984–1993.
9. Feeny PP (1976) in *Biochemical Interaction Between Plants and Insects*, eds Wallace JW, Mansell RL (Plenum, New York), pp 1–40.
10. Silvertown J, Dodd M (1996) Comparing plants and connecting traits. *Philos Trans R Soc London Ser B* 351:1233–1239.
11. Berenbaum MR (1995) The chemistry of defense: Theory and practice. *Proc Natl Acad Sci USA* 92:2–8.
12. Agrawal AA, Fishbein M (2008) Phylogenetic escalation and decline of plant defense strategies. *Proc Natl Acad Sci USA* 105:10057–10060.
13. Ehrlich PR, Raven PH (1964) Butterflies and plants: A study in coevolution. *Evolution (Lawrence, Kans)* 18:586–608.
14. Becerra JX (1997) Insect on plants: Macroevolutionary chemical trends in host use. *Science* 276:253–256.
15. Levin DA (1975) Pest pressure and recombination systems in plants. *Am Nat* 109:437–451.
16. Lively CM (1996) Host-parasite coevolution and sex: Do interactions between biological enemies maintain genetic variation and cross-fertilization. *Bioscience* 46:107–114.
17. Hamilton WD, Axelrod R, Tanese R (1990) Sexual reproduction as an adaptation to resist parasites (A review). *Proc Natl Acad Sci USA* 87:3566–3573.
18. Salathé M, Kouyos RD, Regoes RR, Bonhoeffer S (2008) Rapid parasite adaptation drives selection for high recombination rates. *Evolution (Lawrence, Kans)* 62:295–300.
19. Busch JW, Neiman M, Koslow JM (2004) Evidence for maintenance of sex by pathogens in plants. *Evolution (Lawrence, Kans)* 58:2584–2590.
20. Koslow JM, DeAngelis DL (2006) Host mating system and the prevalence of disease in a plant population. *Proc R Soc London Ser B* 273:1825–1831.
21. Stebbins GL (1950) *Variation and Evolution in Plants* (Columbia Univ Press, New York).
22. Barrett SCH (2002) The evolution of plant sexual diversity. *Nat Rev Genet* 3:274–284.
23. Otto SP, Lenormand T (2002) Resolving the paradox of sex and recombination. *Nat Rev Genet* 3:252–261.
24. Howard RS, Lively CM (1994) Parasitism, mutation accumulation, and the maintenance of sex. *Nature* 367:554–557.
25. Muller HJ (1964) The relation of recombination to mutational advance. *Mut Res* 1:2–9.
26. Paland S, Lynch M (2006) Transitions to asexuality result in excess amino acid substitutions. *Science* 311:990–992.
27. Seger J, Hamilton WD (1988) in *The Evolution of Sex: An Examination of Current Ideas*, eds Michod RE, Levin BR (Sinauer, Sunderland, MA), pp 176–193.
28. Raven PH (1979) A survey of reproductive biology in Onagraceae. *New Zeal J Bot* 17:575–593.
29. Cleland RE (1972) *Oenothera: Cytogenetics and Evolution* (Academic, New York).
30. Holsinger KE, Ellstrand NC (1984) The evolution and ecology of permanent translocation heterozygotes. *Am Nat* 124:48–71.
31. Harte C (1994) *Oenothera: Contributions of a Plant to Biology* (Springer, New York)
32. Rauwolf U, Golczyk H, Meurer J, Herrmann RG, Greiner S (2008) Molecular marker systems for *Oenothera* genetics. *Genetics* 180:1289–1306.
33. Ives AR, Midford PE, Garland T, Jr (2007) Within-species variation and measurement error in phylogenetic comparative methods. *Syst Biol* 56:252–270.
34. Johnson MTJ (2008) Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology* 89:145–154.
35. Johnson MTJ, Agrawal AA, Maron JL, Salminen J-P (2009) Heritability, covariation, and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. *J Evol Biol* 22:1295–1307.
36. Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. *Phytochemistry* 55:481–504.
37. Appel HM (1993) Phenolics in ecological interactions: The importance of oxidation. *J Chem Ecol* 19:1521–1552.
38. Da Costa CP, Jones CM (1971) Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in *Cucumis sativus* L. *Science* 182:1145–1146.
39. Agrawal AA, Gorski PM, Tallamy DW (1999) Polymorphism in plant defense against herbivory: Constitutive and induced resistance in *Cucumis sativus*. *J Chem Ecol* 25:2285–2304.
40. Felsenstein J (1974) The evolutionary advantage of recombination. *Genetics* 78:737–756.
41. Hill WG, Robertson A (1966) The effect of linkage on limits to artificial selection. *Genet Res* 8:269–294.
42. Barton NH, Otto SP (2005) Evolution of recombination due to random drift. *Genetics* 169:2353–2370.
43. Wagner WL, Hoch PC, Raven PH (2007) Revised classification of the Onagraceae. *Syst Bot Monogr* 83:1–222.
44. Johnson MTJ, Agrawal AA (2005) Plant genotype and environment interact to shape a diverse arthropod community on Evening Primrose (*Oenothera biennis*). *Ecology* 86:874–885.
45. Johnson MTJ, Agrawal AA (2007) Covariation and composition of arthropod species across plant genotypes of evening primrose (*Oenothera biennis*). *Oikos* 116:941–956.
46. Wright SI, Ness RW, Foxe JP, Barrett SCH (2008) Genomic consequences of outcrossing and selfing in plants. *Int J Plant Sci* 169:105–118.
47. Carr DE, Eubanks MD (2002) Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). *Evolution (Lawrence, Kans)* 56:22–30.
48. Strauss SY, Karban R (1994) The significance of outcrossing in an intimate plant-herbivore relationship. I. Does outcrossing provide an escape from herbivores adapted to the parent plant? *Evolution (Lawrence, Kans)* 48:454–464.
49. Núñez-Farfán J, Fornoni J, Valverde PL (2007) The evolution of resistance and tolerance to herbivores. *Annu Rev Ecol Syst* 38:541–566.
50. Bell G (1984) *The Masterpiece of Nature* (Univ California Press, Berkeley)
51. Otto SP, Nuismer SL (2004) Species interactions and the evolution of sex. *Science* 304:1018–1020.
52. Agrawal AF (2009) Differences between selection on sex versus recombination in red queen models with diploid hosts. *Evolution (Lawrence, Kans)*, in press.
53. Rice WR (2002) Experimental tests of the adaptive significance of sexual recombination. *Nat Rev Genet* 3:241–251.
54. Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of 3 noncoding regions of chloroplast DNA. *Plant Mol Biol* 17:1105–1109.
55. Levin RA, et al. (2004) Paraphyly in tribe Onagreae: Insights into phylogenetic relationships of Onagraceae based on nuclear and chloroplast sequence data. *Syst Bot* 29:147–164.
56. Hoggard GD, Kores PJ, Molvray M, Hoggard RK (2004) The phylogeny of *Gaura* (Onagraceae) based on ITS, ETS, and *trnL-F* sequence data. *Am J Bot* 91:139–148.
57. Evans MEK, Hearn DJ, Hahn WJ, Spangle JM, Venable DL (2005) Climate and life-history evolution in evening primroses (*Oenothera*, Onagraceae): A phylogenetic comparative analysis. *Evolution (Lawrence, Kans)* 59:1914–1927.
58. Ford VS, Gottlieb LD (2007) Tribal relationships within Onagraceae inferred from *PgiC* sequences. *Syst Bot* 32:348–356.
59. Gottlieb LD, Ford VS (1996) Phylogenetic relationships among the sections of *Clarkia* (Onagraceae) inferred from the nucleotide sequences of *PgiC*. *Syst Bot* 21:45–62.
60. Ford VS, Gottlieb LD (2003) Reassessment of phylogenetic relationships in *Clarkia* sect. *Sympherica*. *Am J Bot* 90:284–292.
61. Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98.
62. Stamatakis A (2006) RAXML-VI-HPC: Maximum-likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
63. Blomberg SP, Garland T, Jr, Ives AR (2003) Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution (Lawrence, Kans)* 57:717–745.