

Plant mating system transitions drive the macroevolution of defense strategies

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Understanding the factors that shape macroevolutionary patterns in functional traits is a central goal of evolutionary biology. Alternative strategies of sexual reproduction (inbreeding vs. outcrossing) have divergent effects on population genetic structure and could thereby broadly influence trait evolution. However, the broader evolutionary consequences of mating system transitions remain poorly understood, with the exception of traits related to reproduction itself (e.g., pollination). Across a phylogeny of 56 wild species of Solanaceae (nightshades), we show here that the repeated, unidirectional transition from ancestral self-incompatibility (obligate outcrossing) to self-compatibility (increased inbreeding) leads to the evolution of an inducible (vs. constitutive) strategy of plant resistance to herbivores. We demonstrate that inducible and constitutive defense strategies represent evolutionary alternatives and that the magnitude of the resulting macroevolutionary tradeoff is dependent on the mating system. Loss of self-incompatibility is also associated with the evolution of increased specificity in induced plant resistance. We conclude that the evolution of sexual reproductive variation may have profound effects on plant–herbivore interactions, suggesting a new hypothesis for the evolution of two primary strategies of plant defense.

herbivory | induced resistance | coevolution | plant defense theory | *Solanum*

Both plant and animal taxa exhibit remarkable variation in sexual reproduction, and this variation can have important consequences for population genetics and evolution (1). In general, outcrossing taxa exhibit larger, more genetically variable populations, whereas inbreeding taxa tend to exhibit smaller population sizes, lower genetic variability, and for highly self-fertilizing taxa, reduced recombination rates (1–4). Mating systems may thereby influence many aspects of a species' evolutionary ecology, including gene flow, extinction risk (5), local adaptation (6), and interactions with other species (7–11). Evolution of inbreeding may occur more often at species range edges, allowing the persistence of small, isolated populations in which pollinators and/or mates are limiting (12, 13). Such populations must overcome the costs of inbreeding (e.g., inbreeding depression) but can thereby fill new niches and potentially speciate (14). Ecological studies have shown that isolated populations, although lacking mutualists, may also escape herbivores and parasites (15–17), suggesting a role for mating system variation in the evolution of these antagonistic interactions. However, macroevolutionary studies of the broader effects of mating system variation have focused primarily on reproductive traits (18); for example, the evolution of self-fertilization in plants is associated with the loss or reduction of floral traits that mediate pollinator visitation (19). Thus, our understanding of whether mating systems broadly shape phenotypic evolution remains limited.

The role of mating systems for antagonistic interactions may be particularly relevant for plants, which are sessile and face the constant threat of attack by natural enemies, such as herbivores and pathogens. Plants have evolved complex syndromes of phenotypes (20) to mitigate attack and consumption by herbivores, including physical traits such as trichomes and leaf toughness, growth (tolerance) traits, and chemical traits such as toxins and

antinutritive compounds (21). There are numerous hypotheses for the evolution and maintenance of this variation, each emphasizing to varying degrees the relative importance of herbivore frequency, physiological constraints, resource limitation, and the costs of trait expression (reviewed in ref. 22). Fundamentally, however, plants may adopt two different primary strategies in how they deploy those traits: they may express defense at all times (i.e., constitutive strategy), or they may save the cost of trait expression in the absence of attackers by only inducing defenses after initial attack (inducible strategy). Theory predicts that plants that grow in environments in which the probability of attack is variable should exhibit the latter strategy and show a greater phenotypically plastic response to herbivory (inducibility) (23). However, considerable theoretical and empirical research on plant defense has yielded little consensus (22), indicating that important predictive factors may be missing from models of defense evolution, particularly in relation to macroevolutionary patterns (20). Prior theoretical work has predicted the evolution of greater plant defense in sexual vs. asexual taxa, potentially as a result of differential recombination rates (24) or reduced mutation accumulation (25). This prediction has been supported by a large study of constitutive plant resistance in the Onagraceae (evening primroses) (26) and complements research on the role of parasites in the evolution of sex (27). However, it remains unknown whether variation in sexual reproduction should similarly influence defense evolution, in part because recombination rates may be widely variable in all but extremely or obligately selfing taxa.

Within species and populations, feedbacks between herbivory and sexual reproduction could also contribute to observed variation in both defense and mating systems. Plants exhibit a remarkable diversity of traits that have ostensibly evolved to promote outcrossing and limit the costs of inbreeding depression, including aspects of floral morphology, the timing and location of sex expression, and gamete recognition (28, 29). At an intraspecific scale, antagonists such as herbivores can affect the expression of mating system and reproductive traits (30, 31) as well as the magnitude of inbreeding depression (32). Conversely, mating systems (inbreeding, outcrossing) and sexual or pollination systems (e.g., sex) can affect the expression of defense traits (32–35). Inbreeding effects on defense and genetic diversity (4) could thereby alter selection for defense strategies within populations.

Some of the most potent mechanisms for preventing inbreeding include the self-incompatibility systems found in more than 19 orders and 70 families of angiosperms, including the nightshades (Solanaceae) (36). In gametophytic self-incompatibility (SI), pollen from relatives is prevented from reaching the ovule in the case of a shared allele at the SI locus (*S*-locus), creating a predominantly outcrossing mating system. Some species exhibit

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plasticity in this system (37). SI is the ancestral condition in the Solanaceae; however, there have been more than 60 independent losses of SI in this plant family (38). These multiple independent transitions from SI to self-compatibility (SC) (i.e., from an outcrossing to a potentially more inbreeding reproductive strategy) seem to be irreversible (38) and provide a powerful framework for studying the evolutionary and ecological consequences of mating system transitions (39).

In this study we ask, what have been the consequences of SI loss for the evolution of plant defense? Our question arises from two complementary hypotheses discussed above: first, theory (24) and some data (26) suggest that outcrossing may favor higher levels of constitutive defense. Second, SI loss could alter natural selection on defense strategies by allowing plant colonization of novel habitats, thereby reducing the probability and intensity of herbivore attack (16). To address this question, we conducted manipulative experiments with 56 wild species (more than 900 plants) from 13 genera, including SI and SC petunia, tobacco, groundcherry, pepper, tomato, and potato taxa. We selected taxa a priori, on the basis of mating system status, to ensure that we assessed independent, replicate mating system transitions, and analyzed the evolution of mating and defense strategies (constitutive and inducible resistance) in a phylogenetic framework.

Results and Discussion

We estimated constitutive and inducible resistance to caterpillars of *Manduca sexta* L., a common specialist herbivore on Solanaceae. Using Bayesian analyses of trait evolution on a molecular phylogeny of our experimental taxa, we found a moderate decrease in constitutive resistance in SC compared with SI taxa, as indicated by a marginally significant likelihood ratio test and a Bayes Factor test (Fig. 1). Prior damage by *Manduca* larvae induced a 21% increase in average resistance across all species (Fig. 1); however, there were no consistent differences between mating systems in the overall magnitude of induced resistance. Thus, neither obligate outcrossing (SI) nor its loss confers a consistent evolutionary advantage in terms of the magnitude of overall plant resistance. Theoretical models (24) and some data (26) suggest an association between effective recombination and evolution of increased defense against antagonists. The marginally greater constitutive resistance in SI taxa is consistent with this hypothesis, although we note that our study was not designed to test the role of recombination per se. Moreover, prior theory (24) predicts higher rates of recombination in sexual, compared

with asexual and/or highly selfing taxa, and this prediction is likely blurred when comparing SI taxa [which may be more likely to be facultatively clonal (40)] and SC taxa (which may exhibit a wide range of recombination and outcrossing rates).

We next examined evolutionary patterns in how species deploy resistance traits. Estimates of the magnitude of inducibility of resistance reveal that the transition from outcrossing to inbreeding has been accompanied by a shift to a more inducible strategy of plant defense: despite considerable variation among species, resistance to *Manduca sexta* is on average 63% more inducible in self-compatible compared with self-incompatible taxa, indicating that phenotypic plasticity in response to herbivore attack has jointly evolved (i.e., coevolved in a broad sense) with plant mating strategies (Fig. 2). Because there is no known direct ecological interaction between mating and defense plasticity, we propose that this evolutionary relationship is an indirect result of the selective environment that accompanies a shift to increased inbreeding or self-fertilization. Self-compatible taxa often persist in marginal habitats and/or at range edges with a paucity of mates and pollen vectors (13). Theoretical models predict that these habitats should in turn favor the evolution of phenotypic plasticity in general (41), and plasticity in defense in particular if under variable herbivory (42), and our data support this hypothesis. Alternatively, there may exist indirect interactions among herbivores, plants, and pollinators that may collectively favor the evolution of greater inducibility in inbreeding taxa. Herbivory can alter floral phenotypes and influence pollinators (31, 43, 44), and the induction of deterrent or toxic metabolites in reward tissues such as pollen and nectar could be disproportionately costly to obligately outcrossing, compared with inbreeding, taxa (45). Because of the ancestral nature of SI in this family, a hypothesis of repeated herbivore-mediated shifts in both defense strategy and mating system seems less likely, although we note that our data cannot unequivocally exclude this possibility.

Overall, we hypothesize that mating system evolution could have widespread impacts on a range of species interactions, including parasitism, competition, and predation, as well as mutualism, provided that mating system transitions were accompanied by changes in population genetic and/or ecological factors relevant to the interaction. Our study suggests a unique mechanism for the evolution of a primary strategy of plant defense. Induced plant responses to herbivory are considered to have evolved as a result of variable herbivory and the costs of trait

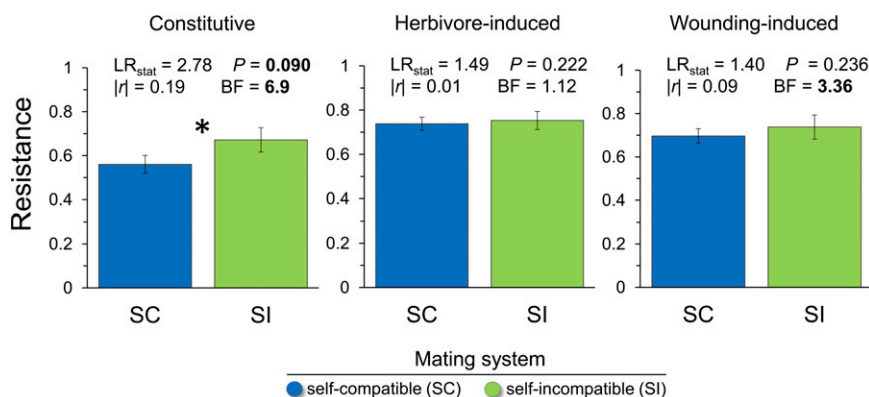


Fig. 1. Absolute magnitude of constitutive and induced resistance based on larval *Manduca sexta* performance across self-incompatible (green, SI) and self-compatible (blue, SC) Solanaceae. Data are raw phenotypic means (± 1 SE), whereas statistics account for phylogenetic relationships among taxa. Above each panel are the results of likelihood ratio (LR) tests of the hypothesis that resistance has been shaped by mating system (LR_{stat} and associated *P* value), and corresponding Bayes factor (BF) tests: BF ≥ 2 indicates positive support, BF ≥ 5 indicates strong support, and BF ≥ 10 indicates very strong support in favor of a model of correlated evolution. |*r*| is the phylogenetically corrected correlation coefficient. *Marginally significant difference in constitutive resistance between mating systems (*P* = 0.080) before correcting for phylogeny.

natural populations, including comparing the probability of herbivore attack in SI and SC populations, remains a goal for future research.

The observed increase in plasticity under the loss of SI could also be interpreted as the evolution of developmental instability under an inbreeding mating system (46), rather than adaptive correlated evolution of mating and defense. Developmental instability can increase plasticity to variable environments and is considered maladaptive (47). If this hypothesis were true, we would predict SC taxa to show equally strong induced responses to herbivore attack and mechanical wounding; that is, SC taxa would show a lower degree of specificity to herbivore attack than SI taxa. Many plant species are capable of inducing responses that are specific to herbivore consumption (vs. mechanical wounding), allowing plants with multiple attackers and plants at risk for wounding from stochastic abiotic damage to fine-tune their defenses in a putatively adaptive manner (48). Thus, an instability hypothesis would not be supported by the putatively adaptive evolution of greater specificity in SC taxa. We tested the instability hypothesis using the significance of the correlation between responses to *Manduca* caterpillar damage vs. mechanical wounding as a unique test of specificity evolution. Specifically, we postulated that under broad selection for increased specificity, the responses to mechanical wounding and real herbivory would be evolving independently, appearing as a nonsignificant correlation across replicate species; conversely, a significant correlation in the responses to each type of damage would indicate a lack of independence in the evolution of responses to each wounding type, or low specificity. We find that SI taxa show a pattern consistent with low specificity (a strong and significant relationship between responses, $r = 0.67$; $P = 0.02$), whereas SC taxa show a pattern of independent evolution of responses to each wounding type, consistent with greater specificity ($r = 0.24$; $P = 0.32$) (Fig. S1). This specificity is inconsistent with an instability hypothesis for SC taxa and incidentally suggests a role for mating systems in the evolution of specificity. Additional research is needed to examine the role of mating systems in the macroevolution of specificity in plant responses to different herbivores.

The contrasting effects of mating systems on constitutive resistance and inducibility prompted us to investigate how these traits covaried across the phylogeny. A longstanding tenet of plant defense theories (and trait evolution in general) is that a tradeoff should exist between these two strategies, primarily because plants should benefit from being inducible when defenses are costly and herbivory uncertain, because this saves the cost of defense expression in the absence of herbivores (22). This hypothesis has been supported by comparisons within species (49); conversely, prior phylogenetic studies have demonstrated positive correlations in the expression of individual defense-related secondary compounds (50) but have not examined actual plant resistance among wild species when the amount of damage was controlled (cf. ref. 51). The measurement of induced plant resistance (i.e., based on herbivore performance) is necessary for fully understanding the evolution of defense strategies, because it integrates all of the individual physical and metabolic traits that are relevant to the herbivore, but that may be highly divergent even between closely related species. Across the Solanaceae we find a highly significant, negative relationship between direct, constitutive resistance and inducibility of resistance (Fig. 3). This is robust comparative evidence that these represent evolutionary alternative strategies in plant defense. It is significant because it demonstrates a potentially strong correspondence between classic microevolutionary processes (defense tradeoffs) and constraints on the evolution of species-level variation in strategy, regardless of the particular defensive tactics (e.g., chemicals) used by each species. Mating systems differ in the magnitude of the constraint based on a comparison of slope estimates (Fig. 3), indicating that this fundamental relationship

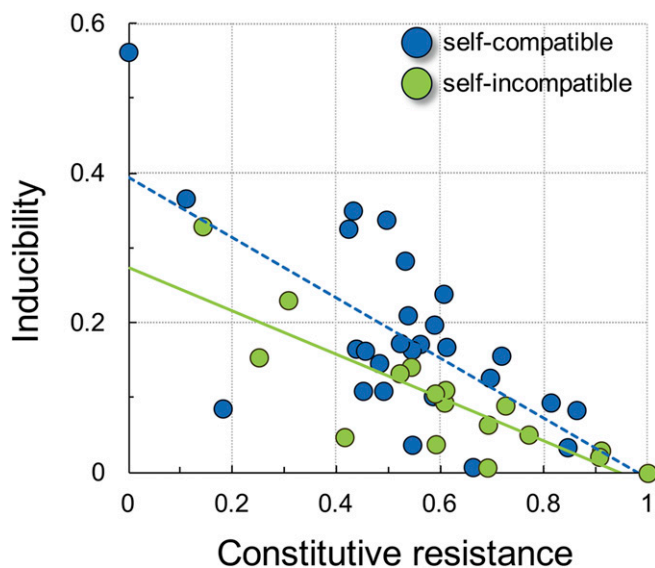


Fig. 3. Inducible and constitutive resistance strategies represent evolutionary alternatives. Shown is the relationship between constitutive plant resistance and inducibility in resistance for self-incompatible (solid green line, $R^2 = 0.822$; $\beta_{SI} = -0.275 \pm 0.030$) and self-compatible (dashed blue line, $R^2 = 0.499$; $\beta_{SC} = -0.390 \pm 0.075$) taxa. Data are raw phenotypic mean values, whereas statistics are corrected for phylogenetic relationships among taxa. LR tests for both SI ($LR_{stat} = 25.22$; $P < 0.0001$) and SC taxa ($LR_{stat} = 21.56$; $P < 0.0001$) strongly support a model of a negative phylogenetic relationship between resistance strategies, as do BF tests ($BF_{SI} = 25.84$; $BF_{SC} = 23.78$, where $BF > 10$ indicates extremely strong support for a model of correlated evolution). Mating systems differ in the steepness of the relationship based on an LR test that $\beta_{SI} < \beta_{SC}$ ($LR_{stat} = 3.72$; $P = 0.05$).

in plant defense theory is shaped in part by the joint evolution of SC and inducibility.

Conclusions

Our study indicates that variation in plant sexual reproduction has broadly shaped the macroevolution of defense strategies across the Solanaceae, including the evolution of tradeoffs between constitutive and inducible resistance. Our results should be robust, first, because our analyses are based on repeated, independent evolutionary transitions in a key mating system trait (SI); second, our conclusions are based on plant resistance to a common herbivore, rather than secondary metabolite production, and thus are robust to taxon-specific differences in which traits constitute the resistance phenotype; finally, our comparative study is among the few to consider plant defense in terms of both inducible and constitutive phenotypes (50). Future work could compare these results with responses to herbivores with different feeding modes or host ranges. Although sex per se (vs. asexuality) is long thought to have been favored in part by coevolution of antagonistic interactions (26, 27, 52), we have demonstrated that such interactions may also coevolve with variation in the mode and expression of sexual reproduction. These findings also complement prior research showing that intraspecific variation in mating systems (32) and associated traits such as sex (33) can alter plant defense trait expression, with the potential for herbivores to influence the evolution and maintenance of plant reproduction within populations (30, 32). Thus, interactions between herbivory and mating systems could have consequences at both micro- and macroevolutionary scales. Finally, we propose that host strategies of sexual reproduction may represent a previously unappreciated factor that may explain the occurrence of particular defense strategies in nature.

Materials and Methods

We sampled 56 species of Solanaceae from 13 genera, including *Petunia*, *Nicotiana*, *Datura*, *Brugmansia*, *Cestrum*, *Capsicum*, *Physalis*, and *Solanum* taxa, selecting SI species for which DNA sequence data and seeds were available, and then selecting closely related SC species (Table S1). We preferentially selected diploid taxa to avoid confounding polyploidization with mating system transition (full details in *SI Materials and Methods*). Our taxon sampling took advantage of the repeated, independent losses of SI in the family (38, 53) to specifically test the a priori hypothesis that shifts from obligate outcrossing to potentially increased inbreeding have been independently and repeatedly associated with defense evolution. Plants from each species were grown in a rooftop greenhouse from botanical seed until they had four to eight true leaves. Before bolting/flowering (with the exception of *Petunia* species, which bolted extremely rapidly), plants were used in a manipulative experiment in which plants were exposed to the following: damage by *Manduca sexta* (Lepidoptera: Sphingidae) larvae [a highly damaging Solanaceous specialist found on most of the experimental taxa (54)]; damage by manual tissue removal (hole punch) and puncture wounding (fabric wheel punctures to the distal third of the leaf); or nothing (control). Both biological and mechanical tissue removal were standardized (20% leaf area) to control for covariation between damage level and induction. A few species only produced sufficient seedlings for the control treatment and thus were only used to estimate constitutive resistance; these appear as missing data in Fig. 2. Performance bioassays with fresh *M. sexta* larvae on treatment and control plants were used to generate estimates of induced and constitutive resistance, respectively. Such resistance estimates integrate all of the individual traits (e.g., specific metabolites) that are relevant to the plant's defense and thereby address the issue of different taxa using different suites of secondary metabolites. Leaf discs from two leaves on each plant were taken using a cork borer and mounted on a pin over moist filter paper in a Petri dish. For plants with compound/divided leaves, we used the terminal leaflet as the bioassay unit. This technique minimizes the confounding effect of induction during the bioassay (as can happen with the use of whole plants). Larvae initiated feeding on all species in our study, but larvae that fell of the leaf disk and died without feeding were excluded. Treatment averages for each species were relativized to the most resistant species to allow comparison among species and mating systems on a common scale. Performance was converted to a measure of resistance ($1 - [\text{relativized treatment average}]$) to facilitate interpretation. Inducibility in resistance was estimated as the contrast between the resistance in control

and damaged plants, thereby addressing known biases in testing for relationships between, for example, constitutive resistance and proportional inducibility (55). *Petunia* species were excluded from measurements of inducibility because they were extremely constitutively resistant to *Manduca*, and it would have been inappropriate to conclude low inducibility per se when feeding rates were so low (experimental details in *SI Materials and Methods*).

Relationships among traits were analyzed in *BayesTraits* v2.0. We constructed a molecular phylogeny of our experimental taxa (*SI Materials and Methods*) using published sequence data from three chloroplast and two nuclear regions, and analyzing a gene-partitioned data matrix in *MrBayes* v.3.1.2 (56) for 180 million postburn-in generations under a GTR + I + G model of evolution. This analysis yielded a well-resolved phylogeny that was broadly congruent with published hypotheses for the Solanaceae (57–61). From the posterior distribution of this analysis, 1,000 trees were randomly selected to incorporate phylogenetic uncertainty into our trait analyses. The need for phylogenetic correction was confirmed by estimating the phylogenetic signal parameter, λ , for the resistance traits (Table S2) (62). Pairwise relationships between mating system and the measures of resistance and inducibility of resistance, as well as between constitutive resistance and inducibilities, were tested as phylogenetic correlations (63–65) using standard likelihood ratio tests comparing nested models in which the correlation between the two traits was constrained to be zero or allowed to take the maximum likelihood value (details in *SI Materials and Methods*).

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