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Minireview

Ecological mechanisms for the coevolution of mating systems and defence

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Summary

The diversity of flowering plants is evident in two seemingly unrelated aspects of life history: sexual reproduction, exemplified by the stunning variation in flower form and function, and defence, often in the form of an impressive arsenal of secondary chemistry. Researchers are beginning to appreciate that plant defence and reproduction do not evolve independently, but, instead, may have reciprocal and interactive (coevolutionary) effects on each other. Understanding the mechanisms for mating–defence interactions promises to broaden our understanding of how ecological processes can generate these two rich sources of angiosperm diversity. Here, I review current research on the role of herbivory as a driver of mating system evolution, and the role of mating systems in the evolution of defence strategies. I outline different ecological mechanisms and processes that could generate these coevolutionary patterns, and summarize theoretical and empirical support for each. I provide a conceptual framework for linking plant defence with mating system theory to better integrate these two research fields.

Introduction

Plants are attacked by a diverse array of herbivores. As a result of variation in natural selection by herbivores, plants have evolved a vast array of chemical, physical and phenological traits to prevent damage, and a range of physiological (tolerance) traits which allow compensatory growth and reproduction after damage (Mithöfer & Boland, 2011). The evolution of these defence traits in plants can, in turn, drive the evolution of herbivore counter-defences, host range and the assembly of herbivore communities, and the predator and parasitoid communities that feed on them.

The diversity of plant defences is rivalled by the variety of colour, form and scent traits of flowers, and by the range of reproductive modes that are mediated by these traits. Most plants are hermaphroditic, and sexually reproducing species can have dramatically different mating systems depending on where they

fall on the continuum from predominant self-fertilization ('selfing') to mixed mating to obligate outcrossing (Barrett, 2002). Transitions between mating systems are common: the transition from outcrossing to selfing, for example, represents one of the most common evolutionary shifts in angiosperms, with important influences on the morphology, ecology, demography and genetics of populations (Barrett, 2002). Shifts to selfing also have important genomic consequences, and can influence adaptation, speciation and diversification (Charlesworth & Wright, 2001; Igić *et al.*, 2008; Wright *et al.*, 2013). The shift from outcrossing to selfing is commonly accompanied by reductions in the size of floral traits that mediate pollination and by evolution of the selfing syndrome (Goodwillie *et al.*, 2010). Research on the ecological consequences of mating system transitions has understandably focused on interactions with mutualistic pollinators directly involved with mating system phenotypes (e.g. floral traits), with less attention to other biotic interactions.

In over 100 yr of research on defence and reproduction separately, comparatively little research has examined the potential for their coevolution. Levin (1975) was the first to propose a connection between herbivory and the reproductive strategies of

plants, positing that asexual species should show lower defences as a result of increased mutation accumulation and decreased effective recombination. Consistent with Levin's hypothesis, asexual *Oenothera* species have lower constitutive resistance to herbivores relative to sexual species, although they have greater diversity of defence-related secondary metabolites (Johnson *et al.*, 2009, 2014). It has only been in the last 10–15 yr that researchers have begun to extensively explore how variation in the mode of sexual reproduction (i.e. mating system variation) interacts with defence.

The interactions of mating systems and defence can be dissected into complementary components, considering the following: how herbivory influences the evolution of mating systems; how mating system variation influences the evolution of defence; and the macroevolutionary consequences of feedbacks between mating and defence. Herbivory can impose significant fitness costs and, when these costs are correlated with mating system parameters (e.g. selfing rate), there is the potential for herbivores to influence mating system evolution (Campbell *et al.*, 2013). In turn, mating systems can have profound effects on defence expression, and on the evolution of defence traits (Steets *et al.*, 2007; Campbell & Kessler, 2013; Carr & Eubanks, 2014). There is also growing appreciation that pollinators are affected by herbivore-induced changes to floral traits, with implications for the evolution of both mating systems and defence (Adler *et al.*, 2001; Kessler *et al.*, 2011). Collectively, these studies suggest that defence and reproduction are jointly and interdependently evolving (i.e. co-evolving) in plants (Fig. 1).

Herbivory imposes natural selection on mating systems

Herbivore-mediated inbreeding depression

Inbreeding depression (ID), the average difference in fitness between inbred and outcross progeny within a population, is arguably the most relevant parameter in the maintenance of outcrossing. Under most basic models, ID must exceed 0.5 (outcrossed offspring at least twice as fit as selfed offspring) in order to prevent the evolution of selfing (Jarne & Charlesworth, 1993). However, many outcrossing species exhibit levels of ID considerably less than 0.5 (Goodwillie *et al.*, 2005). One solution to this paradox is that studies of ID have typically been conducted in benign glasshouse environments, sheltered from abiotic and biotic stresses that can exacerbate ID (Cheptou & Donohue, 2011). Indeed, any ecological factor (e.g. herbivory) which modulates the cost of inbreeding will influence the maintenance of outcrossing within populations.

Empirical tests of the hypothesis that herbivory modulates ID are still relatively scarce. Campbell *et al.* (2013) demonstrated that the magnitude of ID for survival, growth and reproduction exceeded 0.5 when *Solanum carolinense* plants (a normally outcrossing species) were exposed to herbivory for 3 yr, but was not significantly different from zero when herbivores were excluded with insecticide, indicating strong, ecologically mediated ID and a potentially critical role of herbivores in maintaining outcrossing. Several
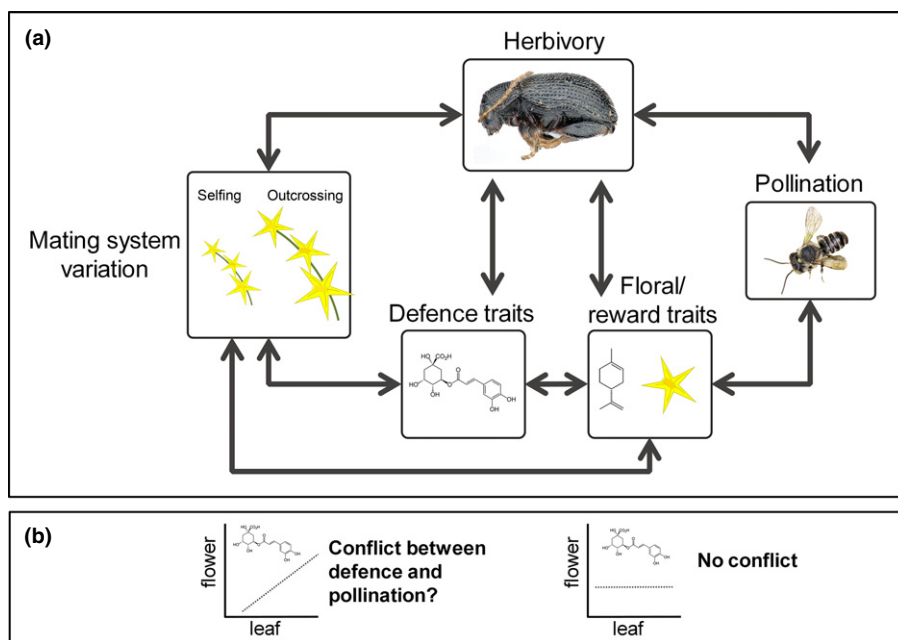
Fig. 1 (a) Conceptual framework for understanding the complex, reciprocal ecological interactions among mating systems, herbivory and pollination, as mediated through plant traits. Mating system variation (inbreeding vs outcrossing) can directly influence herbivory and defence traits, potentially leading to herbivore-mediated natural selection on mating systems. Although pollinators also affect the mating system directly, herbivore-induced changes to floral traits (e.g. floral chemistry) can impact pollinators and thereby indirectly affect the mating system. (b) The interaction of herbivory and pollination depends heavily on the quantitative relationship between leaf and flower traits. In situations in which there is a strong correlation between defensive leaf chemistry and the chemistry of flowers (specifically, reward tissues, such as pollen and nectar), a conflict may arise between successful defence and pollination. This could, in turn, lead to pollinator selection on leaf traits. However, decoupling of leaf and flower traits can allow these two ecological interactions to act more independently.

studies have similarly documented apparent increases in ID under herbivory (Ivey *et al.*, 2004; Stephenson *et al.*, 2004; Kariyat *et al.*, 2011). However, the primary goal of many studies to date has been to establish effects on herbivores, not plant fitness. Fewer have manipulated herbivory and mating system using replicate genetic families and populations (Carr & Eubanks, 2014). Classic ID studies entail controlled crossing of a large number of maternal plants so as not to generate biased estimates of the magnitude of ID by selectively sampling a small number of genotypes or single populations. Indeed, there appears to be substantial variation among species, populations and genetic families in the magnitude of inbreeding effects (Carr & Eubanks, 2014), and thus robust confirmation that herbivory increases ID in nature represents a research priority in this nascent field.

An intuitive explanation for variation among studies in components of herbivore-mediated ID (Carr & Eubanks, 2014) is that the strength of ID depends on the plant's mating system. In theory, outcrossing taxa should harbour a greater deleterious genetic load relative to inbreeding taxa that regularly expose this load to selection (Husband & Schemske, 1996) (Fig. 2a). Moreover, the strength of selection against such load should also depend on the magnitude and probability of herbivory (Fig. 2b). Regular, intense herbivory could effectively purge deleterious alleles from outcrossing populations, even if inbreeding was comparatively rare. Conversely, equivalent selective purging might occur in frequently selfing populations with relatively infrequent herbivory.
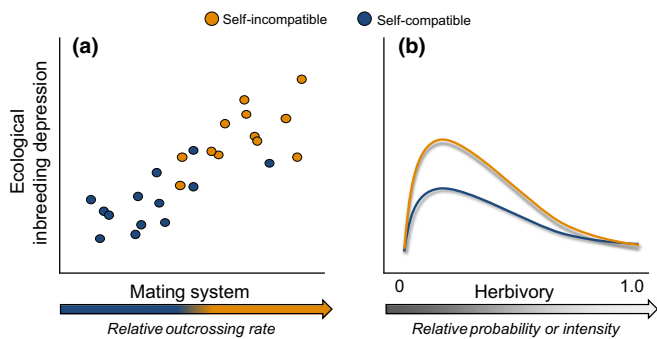
Fig. 2 The magnitude of ecological inbreeding depression in defence traits is a critical variable when predicting the effect of inbreeding on herbivory, and for predicting the reciprocal effect of herbivory on the fitness of inbred and outcrossed progeny. This conceptual model illustrates the relationship between herbivory stress and the mating system on the magnitude of inbreeding depression for defence. (a) Theory predicts that outcrossing species should harbour greater deleterious genetic load (i.e. recessive deleterious mutations) than inbreeders, which may have more opportunity to expose that load to selection over time. (b) Thus, the joint consideration of both the mating system and the intensity of herbivory could allow the prediction of when inbreeding depression for defence will be important. For example, under extremely high herbivore loads (towards the right of the abscissa), successful purging of deleterious mutations in both outcrossers and inbreeders may lead to a prediction of an equivalent effect of inbreeding on trait expression, all else being equal. At lower levels of herbivory, theory and data suggest a linear increase in inbreeding depression with increased stress (left of abscissa), presumably to some maximum. It should be noted that the shapes of the curves are arbitrary as they will depend on the particular genes underlying defence, their function and the interactions between them.

The consideration of both ID and natural variation in herbivory can lead to specific predictions about their role in mating system evolution (Table 1). Leimu *et al.* (2008), for example, used a comprehensive assessment of field patterns and genetic estimates of mating system and ID to show interactive effects of these factors on plant fitness. Theory would predict a more limited role for herbivores in shifts from mixed mating to outcrossing (a rarer transition among plants) given the greater opportunity for purging in selfing populations (Pujol *et al.*, 2009). Herbivory may instead play a greater role in the transition to selfing (Table 1). Interestingly, strong herbivore-mediated ID in outcrossing species (Campbell *et al.*, 2013) suggests that transitions to selfing may be more likely to occur in environments lacking herbivores, such as smaller populations or at range margins (Campbell & Kessler, 2013). These environments would probably favour selfing for reproductive assurance (Baker, 1955), and these confounding factors will present challenges to future studies seeking to disentangle the relative importance of herbivores for the evolution of selfing.

Conflicts between defence and pollination

A second mechanism for how herbivory could influence mating system evolution is indirect, and mediated through pollinators (Fig. 1). Both the presence and amounts of chemicals can be correlated in leaves and flowers (Kessler & Halitschke, 2009), and the expression of defensive compounds in floral and reward (pollen or nectar) tissue could drive indirect feedbacks from defence to mating system via pollination (Strauss *et al.*, 1996; Adler *et al.*, 2001; Gegear *et al.*, 2007) (Fig. 1). For example, in wild populations of the tomato *Solanum peruvianum*, herbivores induce changes to flower metabolites, which reduce pollinator visitation and plant fitness (Kessler & Halitschke, 2009; Kessler *et al.*, 2011).

The role of plant chemistry for mating system–herbivore–pollinator interactions is probably complex, and several hypotheses remain to be tested: Herbivory could result in pollinator repellence mediated by induced changes in volatile organic compound (VOC) emission (Schiestl *et al.*, 2014). Conversely, herbivore-induced VOCs could *attract* pollinators, or could indirectly benefit plants by promoting interplant movement and limiting geitonogamous self-pollination (Kessler *et al.*, 2012).

Antagonistically pleiotropic effects of defence traits on pollination should, in theory, influence the mating system, but there are few studies confirming these effects on, for example, outcrossing rates (Kessler *et al.*, 2008). In theory, such costs should be disproportionately greater for outcrossing species, leading to a prediction of greater selection against inducibility in outcrossing relative to selfing taxa (Campbell & Kessler, 2013) (Table 1). Alternatively, plants could resolve potential conflicts between defence and pollination by differentially expressing defence compounds in each tissue type, or by independently regulating the emission of compounds that signal toxic rewards to visitors (Fig. 1b). This hypothesis predicts greater independent regulation of emissions in outcrossing, relative to selfing, genotypes and species. In the only comparative analysis of leaf and floral chemistry of plants with different mating systems, Adler *et al.* (2012)

Table 1 Summary of how mating systems are predicted to influence defence evolution, and how herbivory is predicted to influence mating system evolution

(a) Predicted effects of mating system on natural selection for defence within populations

Mating system	Component of herbivory/defence						
	Herbivore abundance	Defence strategy	Reliance on compensatory growth (tolerance)	Defence trait diversity	Leaf/reward tissue integration	Role of pollinators in selection on defence	Role of inbreeding for defence variation
Outcrossing	High	Constitutive	High	High	Low	High	High
Mixed	Variable	Low inducibility	Mixed/high	Mixed/high	Mixed	Mixed/high	Mixed
Selfing	Low	High inducibility	Low	Low	Mixed/high	Low	Low/variable

(b) Predicted significance of aspects of herbivory for natural selection on mating system

Transition	Component of herbivory/defence		
	Role of herbivore-mediated inbreeding depression	Role of induced plant defence responses in reward tissue	Role of constitutive defence expression in reward tissue
Outcrossing → selfing	High		Low
Selfing → outcrossing	Low		High

demonstrated strong correlations in defence-related traits across these tissues in wild tobacco, *Nicotiana*, species. This suggests that ecological costs (pollination limitation) may be more common than independent trait regulation (Manson *et al.*, 2012). The association between leaf and reward chemistry (and hence between herbivory and pollination) could also constrain transitions between mating systems. For example, constitutive defence expression in reward tissue and upregulation of defence chemistry in rewards after damage (high inducibility) should both maintain selfing by limiting the recruitment of new pollinators. In other words, for mixed mating species, the defence strategy may play a greater role in limiting the evolution of greater outcrossing rates, relative to the evolution of greater selfing (Table 1). However, there are no data at present on this topic, which remains an exciting field of inquiry.

Mating systems and the evolution of defence

Mating systems alter defence trait expression

Biologists since Darwin have quantified the effects of inbreeding on offspring vigour and fitness (Husband & Schemske, 1996). However, there is growing evidence that inbreeding can also significantly influence the expression of defence against herbivores (Chautá-Mellizo *et al.*, 2012; Campbell *et al.*, 2013; Carr & Eubanks, 2014) and pathogens (Carr *et al.*, 2003). The effect of inbreeding on defence expression is thought to arise from the expression of deleterious, recessive mutations at defence genes; mutations that accumulate during a period of outcrossing become exposed in the more homozygous genomes of inbred individuals. The effects of inbreeding on resistance to herbivores has been reviewed recently by Carr & Eubanks (2014), who also provided the first compelling evidence for this phenomenon (Carr & Eubanks, 2002).

Plant resistance is robustly measured as the performance (growth) of a herbivore or as the percentage of tissue removed, and thus most studies have focused on these operational measures of resistance (Carr & Eubanks, 2014). The mechanisms in terms of defence chemistry or physical traits remain poorly understood; however, several model systems are emerging to address this deficit. Leimu and colleagues, working with *Vincetoxicum hirundinaria* (Asclepiadaceae) (Kalske *et al.*, 2014), have shown reductions in phenolic expression in inbred relative to outcrossed progeny. Horsenettle, *S. carolinense* (Solanaceae), has been the subject of numerous studies on how inbreeding reduces constitutive and induced expression of functional leaf chemistry (Campbell *et al.*, 2013), spine density (Kariyat *et al.*, 2013), volatile emissions (Kariyat *et al.*, 2012) and plant tolerance traits (Campbell *et al.*, 2014). These two study systems have revealed that most, if not all, of a plant's defensive arsenal has the potential to be strongly affected by inbreeding.

One area deserving greater attention in the study of inbreeding effects is the role of herbivore feeding mode and degree of specialization. Different herbivore species (e.g. specialists vs generalists) are differentially affected by defence traits of the same host species, and this variation is likely to be important in determining the cost of herbivory. Indeed, studies with numerous

herbivores of *Mimulus guttatus* have even revealed opposing effects of inbreeding on resistance, with inbred progeny occasionally being more resistant (Hull-Sanders & Eubanks, 2005; Carr & Eubanks, 2014). Further study is needed in order to establish whether any pattern exists across feeding guilds or degree of adaptation.

The regulation of defence traits by plant hormones is also negatively affected by inbreeding (Campbell *et al.*, 2014). Phyto-hormones regulate adaptive responses to a range of abiotic and biotic stresses, suggesting that inbreeding effects on hormone production may be linked to heightened ID under stress (Cheptou & Donohue, 2011; Campbell *et al.*, 2014). These results also predict that selfing plants may be less likely to utilize tolerance (compensatory regrowth) as a defence, given the negative effects of inbreeding on plant growth traits (Table 1).

Overall, such mechanistic studies, when coupled with estimates of plant fitness, could broaden our understanding of how inbreeding and herbivore defence might interact at the scales of both gene expression and regulation (Campbell *et al.*, 2013).

Genetic consequences of selfing

In addition to the immediate effects of inbreeding on trait expression, population genetics theory predicts reduced efficacy of selection and reduced effective recombination rates across the genome in selfers (Wright *et al.*, 2002). These genome-wide predictions may influence the evolution of phenotypes, such as defence. For example, in highly selfing populations experiencing high levels of herbivory, adaptive evolution of defence may be constrained by a relaxation in natural selection and reduced genetic diversity. Opposing these effects would be countervailing selection by the environments occupied by selfers, in particular by parasites (Gos *et al.*, 2012, and see next section). As there are few data or models at present to indicate a role for genome-wide effects of selfing on specific defence phenotypes, the interaction of genomic and ecological forces remains speculative.

Macroevolutionary patterns

The consequences of the reciprocal effects of herbivory on mating systems and mating on defence could lead to long-term, macro-evolutionary trends across plant lineages and the coordinated evolution of defence and mating systems. Although not explicitly looking at mating system transitions, a few studies have attempted to link pollination and floral herbivory (e.g. Armbruster, 1997), highlighting the importance of the correlation between defence and floral traits (Adler *et al.*, 2012) (Fig. 1b). The transition to clonality

Fig. 3 (a) Schematic illustration of predictions arising from mating system theory and plant defence theory when plants disperse to novel, distant habitats. Baker's law posits that long-distance dispersal events are accompanied by the evolution of self-compatibility, as a form of reproductive assurance in the absence of mates or pollinators. Plant defence theory posits that, in such habitats, variable or unpredictable herbivory should select for defence only expressed after initial attack (i.e. inducible defences), in contrast with the constitutive defence expression expected to be adaptive when herbivores are more abundant and predictable. Together, these two bodies of theory provide predictions for the correlated evolution of mating systems and defence. (b) This coevolution is borne out in studies considering patterns of defence among inbreeding and outcrossing taxa. For example, in the Solanaceae, analysis of defence-related secondary metabolites and resistance reveals convergent strategies of defence across repeated independent transitions from outcrossing to inbreeding. Shifts to increased selfing are accompanied by a shift to greater inducibility and reductions in the diversity of defensive phenolics, illustrated here by high-performance liquid chromatograms. The bar chart shows the mean (± 1SE) inducibility in 24 outcrossing and 32 inbreeding species of Solanaceae (Campbell & Kessler, 2013; S. A. Campbell *et al.* unpublished). SC, self-compatible; SI, self-incompatible.
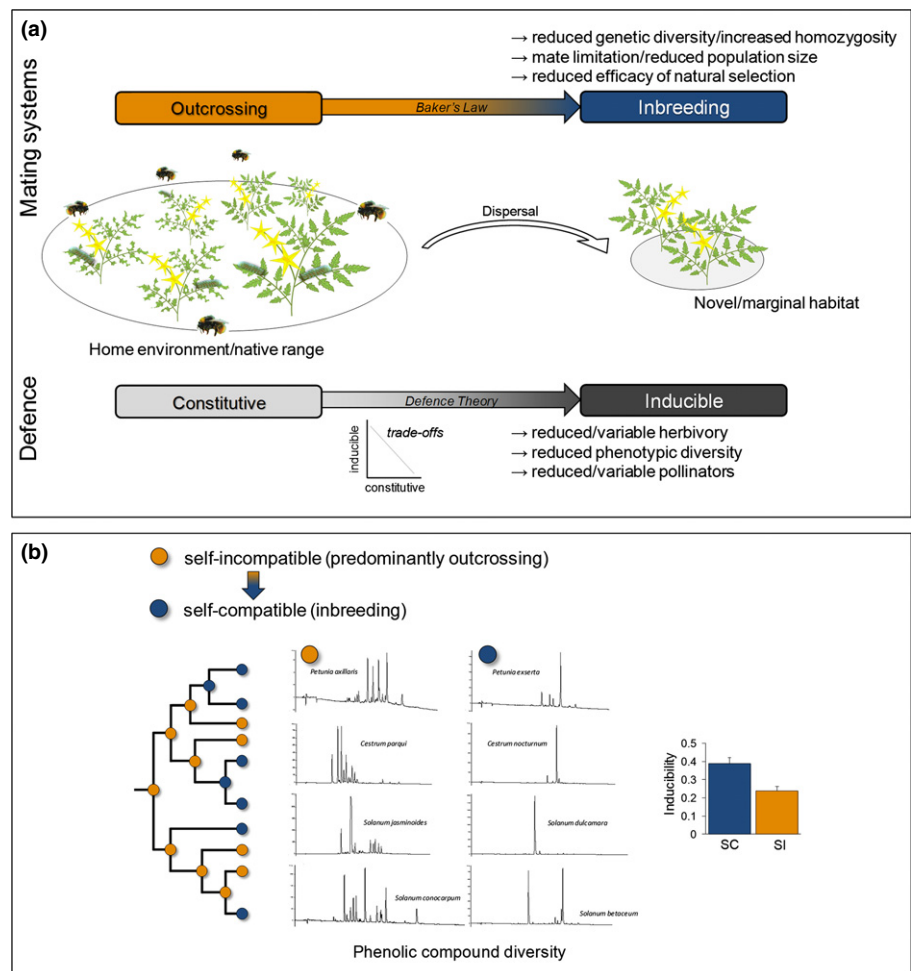
(asexuality) has led to the evolution of decreased resistance traits (Johnson *et al.*, 2009, 2014), indicating important consequences of a broader range of reproductive modes beyond the mating system. Although few, these studies make a strong case for novel coevolutionary interactions between mating and defence over macroevolutionary scales. However, there remains considerable ambiguity as to the ecological or population genetic mechanisms for these patterns. In particular, it remains unclear how these are linked with the comparatively robust effects of inbreeding on trait expression.

Mating strategies could drive defence macroevolution if mating system transitions were accompanied by changes to ecological (e.g. habitat) features relevant to herbivory (Fig. 3). For example, transitions to selfing tend to occur at range edges and/or in isolated populations where mates and pollen vectors may be limiting (Griffin & Willi, 2014). Populations in such conditions can also escape herbivores and parasites (Torchin *et al.*, 2003), suggesting a potential connection between mating systems and the evolution of antagonistic species interactions. Defence theory (Stamp, 2003) suggests that plants in environments with variable or unpredictable herbivory should evolve an inducible defence strategy (thereby saving the costs of defence expression in the absence of herbivores). These two theories jointly predict the correlated evolution of selfing and inducibility after dispersal to habitats lacking mates and enemies (Fig. 3a; Table 1). This led Campbell & Kessler (2013) to propose that the coevolution of mating and defence could occur as an indirect consequence of convergence as a result of habitat and ecology: dispersal into habitats lacking pollen vectors would select for selfing, where reductions in herbivores would reduce selection against inbred progeny and then drive selection for an inducible defence strategy. In support of this prediction, the repeated transition to selfing in the Solanaceae was associated with the convergent evolution of an inducible, rather than constitutive, defence strategy (Campbell & Kessler, 2013). This convergence was remarkable given the wide ecological scope encompassed by the 56 species in that study. Moreover, these results indicate a potent role of ecology in driving adaptation of selfers, despite any genomic constraints (e.g. reduced effective recombination) imposed by selfing (Wright *et al.*, 2013). Changes in strategy were coupled with reductions in secondary metabolite diversity (Fig. 3b; S. A. Campbell *et al.*, unpublished), although whether these reductions arose from population genetic processes (e.g. bottlenecks) or adaptive evolution remains unclear. In the future, the examination of range margins may reveal whether reduced herbivory and reduced pollination (Moeller *et al.*, 2011) jointly facilitate transitions to selfing (Table 1).

Conclusions

Merging the fields of mating system biology and plant–herbivore–pollinator interactions promises to broaden our understanding of both plant defensive and reproductive diversity. Progress towards this goal will hinge on testing genetic and ecological predictions (Table 1) that arise from a hypothesis of reciprocal natural selection between mating and defence. Theory has emphasized that defence evolution is driven by the response to two intersecting axes of variation: the intensity of herbivory and the relative benefits of producing defences in different abiotic environments (Stamp, 2003). The effects of mating systems on a range of variables relevant to defence theory (Table 1) suggest that mating systems may constitute a third axis of variation in defence theory. These include the probability of colonizing novel habitats, ID for defence traits, reliance on animal pollinators, phenotypic variation and the population genetics of defence genes. Moreover, data and theory predict (Table 1) that herbivory could influence mating system evolution. Herbivore-mediated ID may play a critical role in the transition from outcrossing to selfing, particularly when coupled with shifts in habitat that reduce the availability of both herbivores and pollen vectors. Conversely, the evolution of herbivore-induced responses in pollen and nectar may play a greater role in limiting the evolutionary transition from mixed mating to outcrossing. Field experiments, coupled with comparative molecular evolution studies, are likely to reveal complex, reciprocal ecological and evolutionary interactions between defence trait variation and mating systems in plants.

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