the plant journal

SEB Society for Experimental Biology

The Plant Journal (2014) **78,** 481–490 doi: 10.1111/tpj.12492

Plant mating systems affect adaptive plasticity in response to herbivory

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Received 25 November 2013; revised 12 February 2014; accepted 17 February 2014; published online 1 March 2014.

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SUMMARY

The fitness consequences of mating system variation (e.g. inbreeding) have been studied for at least 200 years, yet the ecological consequences of this variation remain poorly understood. Most plants are capable of inbreeding, and also exhibit a remarkable suite of adaptive phenotypic responses to ecological stresses such as herbivory. We tested the consequences of experimental inbreeding on phenotypic plasticity in resistance and growth (tolerance) traits in *Solanum carolinense* (Solanaceae). Inbreeding reduced the ability of plants to up-regulate resistance traits following damage. Moreover, inbreeding disrupted growth trait responses to damage, indicating the presence of deleterious mutations at loci regulating growth under stress. Production of the phytohormones abscisic and indole acetic acid, and wounding-induced up-regulation of the defence signalling phytohormone jasmonic acid were all significantly reduced under inbreeding, indicating a phytohormonal basis for inbreeding effects on growth and defence trait regulation. We conclude that the plasticity of induced responses is negatively affected by inbreeding, with implications for fragmented populations facing mate limitation and stress as a consequence of environmental change.

Keywords: mating systems, induced responses, phytohormone, plant resistance, plant tolerance, inbreeding depression, *Solanum carolinense*.

INTRODUCTION

Biologists have been studying the fitness consequences of inbreeding and outcrossing for at least 200 years (Knight, 1799; Darwin, 1876; Husband and Schemske, 1996). These consequences (e.g. inbreeding depression, heterosis) are predicted to influence the evolution of mating system variation (Lande and Schemske, 1985), which in turn may have widespread consequences for population genetics (e.g. genetic variation) and dynamics (Charlesworth and Charlesworth, 1987; Lande, 1988). However, we still know relatively little about the effects of mating system variation on the interactions of species with their environment.

The broader ecological effects of inbreeding and outcrossing are important from several perspectives. Inbreeding increases homozygosity, and thus the expression of deleterious recessive mutations. The resulting fitness reduction (inbreeding depression) may constrain the evolution of an inbreeding mating strategy by opposing the inherent transmission and reproductive assurance advantages of selfing (Lloyd, 1979; Charlesworth and Charlesworth, 1999). Inbreeding can also influence fundamental aspects of a species' ecology, such as tolerance of abiotic stress (Hauser and Loeschcke, 1996; Kristensen *et al.*, 2008), and interactions with competitors (Darwin, 1876; Schmitt and Ehrhardt, 1990) or natural enemies (Steets *et al.*, 2007). These effects presumably arise from deleterious mutations (genetic load) at loci underlying ecologically relevant traits, although evidence for such mechanisms is sparse (Leimu *et al.*, 2012a; Campbell *et al.*, 2013). In turn, when ecological interactions alter the magnitude of inbreeding depression (Cheptou and Donohue, 2011; Campbell *et al.*, 2013), they may impose natural selection on mating strategies by making the benefits of outcrossing dependent on specific aspects of the environment (e.g. presence of enemies, resource availability). These interactions may lead to correlated evolution of mating systems and additional non-reproductive traits associated with the environment (Campbell and Kessler, 2013). Finally, inbreeding may alter responses to anthropogenic environmental change by negatively affecting tolerance to ecological stress, and such effects may be particularly severe in

fragmented habitats with limited outcrossing opportunities (Eckert *et al.*, 2010).

While evidence is accumulating that mating system variation affects ecological interactions and vice versa, almost nothing is known about the effects of mating systems on adaptive phenotypic responses to different environments, i.e. phenotypic plasticity. From an ontogenetic perspective, inbreeding has been hypothesized to negatively affect developmental stability, and thereby contribute to greater phenotypic variation under stress (e.g. fluctuating asymmetry) (Deng, 1997; Siikamaki and Lammi, 1998), i.e. inbreeding may amplify non-adaptive plasticity. However, many species exhibit adaptive phenotypic plasticity, allowing individuals and populations to tolerate environmental variation (Schlichting, 1986). Adaptive phenotypic plasticity requires coordinated expression of genes involved in the perception of environmental cues and signal transduction, together with genes for biosynthesis, any of which may harbour recessive, deleterious genetic load. However, only a few studies have explicitly examined the effect of inbreeding on adaptive phenotypic plasticity. Population studies of the correlations among heterozygosity, population size and plasticity may not have isolated the effect of inbreeding *per se* (Levin, 1970; Fischer *et al.*, 2000). A manipulative breeding study of the snail *Physa acuta* found evidence for reduced plasticity for an anti-predator trait under inbreeding (Auld and Relyea, 2010), but several other plant studies have found little to no effect on plasticity (Schlichting and Levin, 1986; O'Halloran and Carr, 2010; Murren and Dudash, 2012). Thus, the relationships between mating systems, phenotypic plasticity and ecological interactions remain unclear.

The effects of inbreeding on phenotypic plasticity may be particularly relevant for plants, which are predominantly hermaphroditic (potentially able to self-fertilize), and, as sessile organisms, must cope with local fluctuations in abiotic stresses and biotic stresses such as herbivory and disease. Accordingly, plants exhibit adaptive plasticity in a wide range of phenotypes in response to stress (Schlichting, 1986). For example, many plants up-regulate defensive and immune responses only after initial damage or infection, via complex hormone signalling pathways (Kessler and Baldwin, 2002). A diverse suite of secondary metabolites are implicated in defence in many plants, and are induced following herbivory (Mithöfer and Boland, 2011). Both theoretical arguments and empirical data indicate that an inducible defence strategy primarily operates to limit the costs of trait expression in the absence of antagonists (Karban and Baldwin, 1997; Baldwin, 1998; Agrawal *et al.*, 1999; Heil and Baldwin, 2002). Plants may also alter the expression of growth or developmental traits in order to compensate for the effects of damage (i.e. tolerance) (Núñez-Farfán *et al.*, 2007). A common set of plant phytohormones, including jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), auxin/indole acetic acids (IAA), gibberellic acid and ethylene, are produced in response to biotic and abiotic stress, and influence the expression of both chemical resistance traits and tolerance-related growth traits (Kessler and Baldwin, 2002). Phytohormones interact with one another both positively and negatively (antagonistically), and plant inducibility therefore depends on the relative production of the various signalling molecules (Robert-Seilaniantz *et al.*, 2011). Inbreeding may alter trait regulation directly, by suppressing phytohormone production, and/or indirectly, by disrupting interactions among phytohormones. However, the physiological mechanisms that govern the effects of inbreeding on growth or responses to environmental stress (e.g. drought, herbivory) remain poorly understood (Leimu *et al.*, 2012a).

A growing number of studies have examined inbreeding effects on plant resistance to herbivores (Carr and Eubanks, 2002; Leimu *et al.*, 2008; Delphia *et al.*, 2009; Bello-Bedoy and Núñez-Farfán, 2011; Campbell *et al.*, 2013) and pathogens (Stephenson *et al.*, 2004; Ivey and Carr, 2012). These studies indicate that inbreeding may be a significant factor in expression of resistance, although the magnitude of this effect probably depends on the population mating system history, and the opportunity for selective purging of load associated with defence genes. A previous study showed that inbreeding reduced the production of defence-related secondary metabolites (Campbell *et al.*, 2013), complementing work showing reduced volatile emissions and trichome production (Kariyat *et al.*, 2012, 2013), and reduced pathogenesis-related protein expression (Leimu *et al.*, 2012a) in inbred plants. Campbell *et al.* (2013) hypothesized that inbreeding also disrupts phytohormonal regulation of defence trait expression, and thereby negatively affects plant phenotypic plasticity under herbivory, i.e. the magnitude of the phenotypic change. In this study, we used experimentally inbred and outcrossed plants of *Solanum carolinense* to test two fundamental predictions of this hypothesis, specifically that inbreeding will: (i) disrupt the magnitude of phenotypic plasticity (inducibility) of defence and growth traits in response to damage, and (ii) disrupt endogenous signalling involved in mediating phenotypic responses. We began by re-analysing the phenolic data presented by Campbell *et al.* (2013), and then tested the effects of inbreeding and wounding on growth traits and production of four key phytohormones. Our phytohormone analysis quantified inducibility of hormone production in inbred and outcrossed plants; we also analysed covariances among hormones to determine whether inbreeding disrupted the relationships among these signalling molecules.

RESULTS

Outcrossed plants were significantly more inducible than inbred plants in terms of the expression of defence-related

phenolics measured by Campbell *et al.* (2013). We previously showed that expression of six phenolics differed as a result of inbreeding (Campbell *et al.*, 2013). A new analysis of the plasticity across all five inducible compounds reported in that study revealed that plasticity in total phenolic expression was significantly greater in outcrossed plants ($F_{1,13} = 34.76$; $P = 0.0041$) (Figure 1).

Growth trait responses to artificial herbivory differed between inbred and outcrossed plants (Figure 2 and Table 1). Two weeks after damage, leaf number did not differ due to either inbreeding or damage level (Table 1). By the end of the growing season, the effect of damage on allocation to leaf and main root biomass differed between mating systems (breeding × damage treatment interaction, Table 1). Specifically, leaf mass (Figure 2a) and main root mass (Figure 2b) decreased in inbred plants as a function of increased damage, but increased in outcrossed plants, producing significant breeding × damage effects (Table 1). Conversely, damage induced an increase in stem biomass in inbred but not outcrossed plants: compared to outcrossed plants, the stem biomass of inbreds was significantly lower under control treatment, but increased to the level of outcrossed plants as a result of damage (Figure 2c). A similar pattern was also found for plant height (Figure 2d) measured 2 weeks after damage, although there were no statistically significant differences between inbred and outcrossed plants for any treatment.

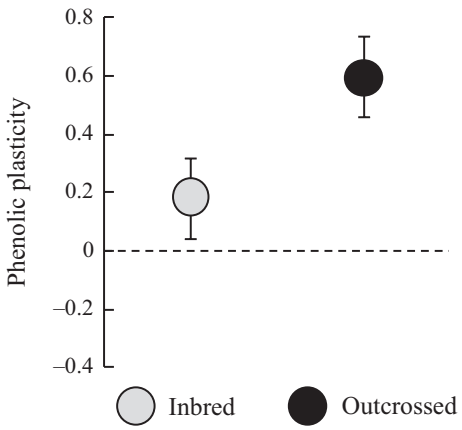

Figure 1. Plasticity in defence-related phenolics in inbred and outcrossed progeny of *Solanum carolinense* (plasticity estimates based on raw data from Campbell *et al.*, 2013).
Data points represent the proportional change in phenotype in previously damaged plants relative to control plants ±95% confidence limits.

Three of the four measured phytohormones showed significantly reduced production as a result of inbreeding, and divergent responses to the wounding treatment under each breeding condition (Figure 3 and Table 1). Salicylic acid expression was not affected by inbreeding or wounding. JA was almost undetectable in control plants, and in

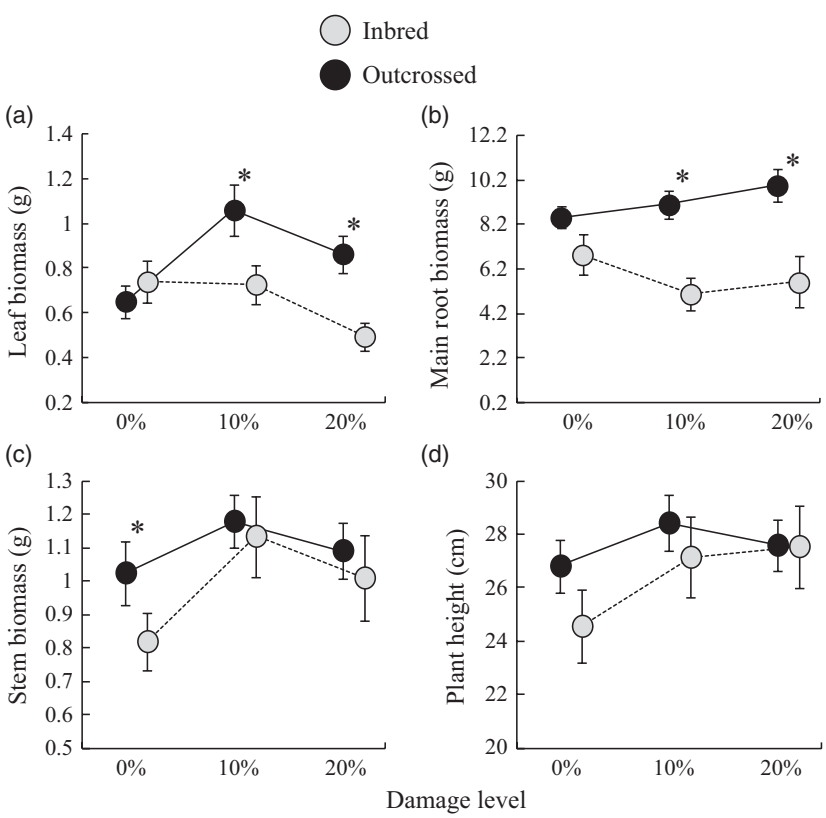

Figure 2. Growth trait responses (means ± SE) of inbred and outcrossed *Solanum carolinense* exposed to 0, 10 and 20% simulated herbivory. For clarity, inbred and outcrossed data points are offset within each treatment. Asterisks indicate significant pairwise differences between inbred and outcrossed plants within each treatment (Tukey's tests).

Table 1 Linear model results for fixed effects of breeding (inbreeding versus outcrossing) and damage/wounding treatment on plant growth trait responses, and expression of plant phytohormones

	Source of variation			
	Breeding	Treatment	Breeding × treatment	R^2
Growth trait				
Leaf mass				
F	9.411	0.574	4.071	0.20
P	**0.0023**	0.4493	**0.0445**	
Main root mass				
F	41.028	0.2659	3.934	0.25
P	**<0.0001**	0.6066	**0.0483**	
Stem mass				
F	25.562	0.852	0.103	0.37
P	**<0.0001**	0.3567	0.7482	
Height				
F	20.807	4.771	1.800	0.25
P	**<0.0001**	**0.0294**	0.1804	
Leaf number				
F	1.153	0.6795	0.9149	0.25
P	0.2828	0.4102	0.3393	
Hormone				
SA				
F	0.045	1.305	0.499	0.18
P	0.8317	0.2579	0.4826	
JA				
F	4.889	77.101	5.342	0.63
P	**0.0311**	**<0.0001**	**0.0245**	
ABA				
F	45.585	5.159	0.0209	0.54
P	**<0.0001**	**0.0268**	0.8856	
IAA				
F	4.030	0.028	0.027	0.61
P	**0.0493**	0.8689	0.8703	

SA, salicylic acid; JA, jasmonic acid; ABA, abscisic acid; IAA, indole-3–acetic acid. Significant *P* values are shown in bold.

response to wounding was differentially up-regulated 116-fold and 51-fold in outcrossed and inbred plants, respectively (Figure 3). Thus, there was a significant breeding × wounding interaction, but also significant main effects of inbreeding and wounding on JA production (Table 1). ABA levels were reduced 55% in inbred plants relative to outcrossed plants, and were up-regulated 32% in response to wounding, with similar inducibility in outcrossed and inbred plants (Figure 3 and Table 1). IAA levels were reduced significantly by 27% due to inbreeding, but were not affected by wounding (Figure 3).

As a result of these effects, the relative ratios among hormones showed striking differences among breeding and wounding treatments (Figure 3b). The significance of correlations between specific pairs of phytohormones also differed strikingly as a result of inbreeding in both control and wounded plants (Table 2). We also tested whether inbreeding disrupted the overall structure of phytohormone relationships, using a random skewers analysis of the covariance matrices (Cheverud, 1996). As predicted, the structure of the hormone variance–covariance matrix was similar in both breeding treatments, but differed between control and wounded plants when JA was included (Table 3). When JA was excluded, there was a significant correlation between the control and wounded outcrossed matrices, but not the control and wounded inbred matrices; inbred and outcrossed hormone matrices were not significantly correlated in either treatment (Table 3).

DISCUSSION

Our results support the hypothesis that inbreeding reduces adaptive plasticity in plant defence and growth traits in *Solanum carolinense*. Despite genetic and compound-specific variation (Campbell *et al.*, 2013), inbreeding reduced the magnitude of plasticity in defence-related phenolics, which may contribute to significant increases in the damage experienced by inbred *S. carolinense* in the field (Kariyat *et al.*, 2011; Campbell *et al.*, 2013), and significant herbivore-mediated inbreeding depression for fitness traits (Campbell *et al.*, 2013). As an additional test of inbreeding depression for plasticity, we performed a bioassay to test for inducibility in resistance to *Manduca sexta* (Appendix S1). which also showed that resistance traits of inbred *S. carolinense* were significantly less inducible compared to outcrossed plants ($P = 0.015$, Figure S1). Although the different goals of each experiment required different damage treatments, the chemical, phytohormonal, growth and bioassay data presented in this study show congruent responses under inbreeding regardless of damage or wounding type. Our results add to a growing appreciation that plant mating system variation may have significant effects on antagonistic species interactions such as parasitism (Ellison *et al.*, 2011), disease (Stephenson *et al.*, 2004) and herbivory (Campbell *et al.*, 2013). While previous studies on defence-related traits have shown negative effects of inbreeding on defence-related secondary metabolites (Campbell *et al.*, 2013) and trichome density (Kariyat *et al.*, 2013), the results of the current study indicate that fundamental regulatory mechanisms of plant defence also harbour deleterious genetic load. Given the effect of inbreeding on inducibility, we hypothesize that variation in inbreeding depression may alter natural selection for an inducible versus constitutive strategy of plant defence. Specifically, we hypothesize that a genotype or species that relies on both regulatory and biosynthetic loci for a successful defence (inducible strategy) may pay a greater cost of inbreeding compared to a genotype or species that relies only on the genes for metabolite biosynthesis (constitutive strategy). Inbreeding depression is significantly higher in primarily outcrossing taxa such as *S. carolinense* (Winn *et al.*, 2011), suggesting that plants that are predominantly outcrossing (and experience

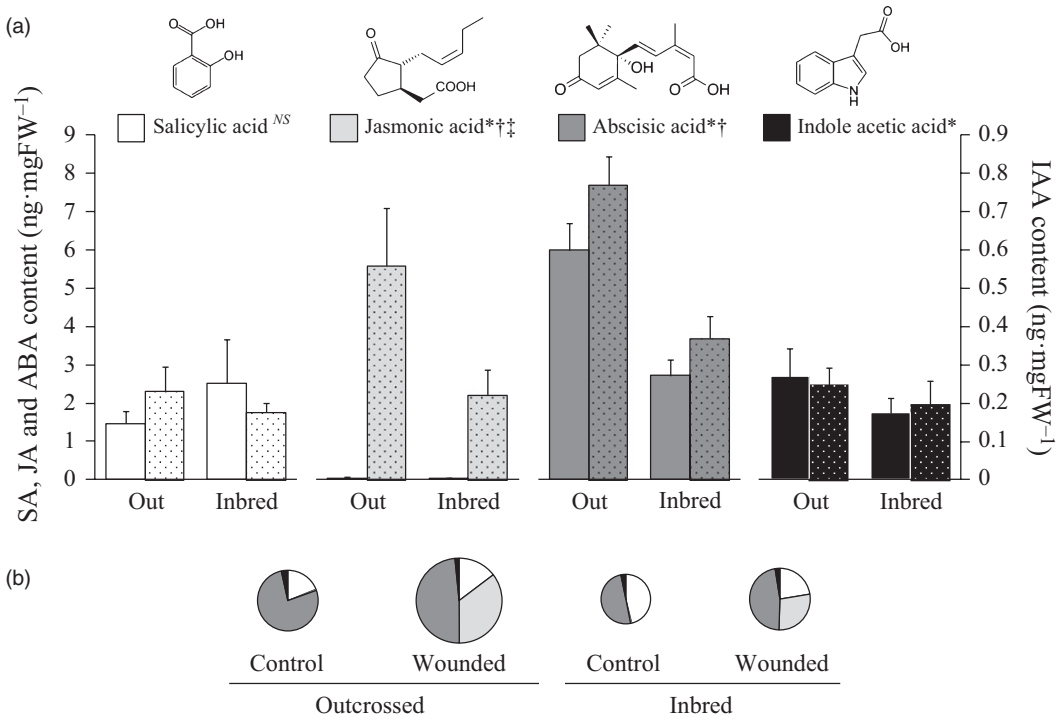

Figure 3. Phytohormone production under breeding and wounding treatments. (a) Constitutive expression (open bars) and wounding-induced expression (stippled bars) of the phytohormones salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA) and indole-3–acetic acid (IAA) in leaves of inbred and outcrossed *Solanum carolinense*. The structures of the hormones are shown at the top. Data are means ± SE. Values for IAA are shown on the right-hand axis for clarity. *, † and ‡ indicate significant effects of breeding, wounding treatment and their interaction, respectively (see Table 1); 'NS' indicates no significant effect.
(b) Relative hormone ratios within each combination of breeding and wounding treatment. Pie chart sizes are scaled to indicate relative total phytohormone production.

Table 2 Pairwise phenotypic correlation matrices for phytohormone traits in (a) control and (b) wounded plants

	SA	JA	ABA	IAA
Control				
SA	–	0.018	0.229	−0.026
JA	0.278	–	**0.535**	**0.779**[a]
ABA	**0.470**	−0.173	–	**0.671***
IAA	**0.558**	**0.541**	**0.549**	–
Wounded				
SA	–	0.316	0.263	0.187
JA	**0.466**	–	0.219	0.381
ABA	0.328	0.132	–	**0.682***
IAA	0.330	**0.612***	0.216	–

Values below the diagonals are for outcrossed plants, while values above the diagonals are for inbred plants. Correlations in bold have a P value ≤0.05; *$P \leq 0.01$; [a]$P \leq 0.001$.

inbreeding depression for inducibility) may be under stronger selection to invest in constitutive defence. This hypothesis is consistent with a phylogenetically controlled study showing elevated constitutive resistance in outcrossing compared to inbreeding Solanaceae (Campbell and Kessler, 2013).

Table 3 Random skewers analysis comparing covariance matrices for control and wounding-induced hormone expression in outcrossed and inbred plants

	Outcrossed		Inbred	
	Control	Wound	Control	Wound
All hormones				
Outcrossed				
Control	–	0.21	**0.03**	0.28
Wound	0.470	–	0.21	**0.02**
Inbred				
Control	**0.871**	0.467	–	0.29
Wound	0.347	**0.905**	0.335	–
Without JA				
Outcrossed				
Control	–	**0.03**	0.06	**0.03**
Wound	**0.932**	–	**0.01**	0.05
Inbred				
Control	0.878	**0.972**	–	0.06
Wound	**0.943**	0.901	0.884	–

Pairwise vector correlation coefficients between matrices are given below the diagonals; P values are given above the diagonals, indicating whether two covariance matrices are significantly correlated. Significant coefficients/P values ($P < 0.05$) are in bold.

Inbreeding also altered plasticity in plant growth traits in response to damage, suggesting that mechanisms of tolerance to herbivory are also negatively affected by inbreeding. One other study has examined the effect of inbreeding on tolerance-related growth traits, and showed that re-growth in damaged *Mimulus guttatus* (Phrymaceae) was reduced to a greater extent in inbred plants (Ivey *et al.*, 2004). Compensatory reproduction (flower production) in response to apical meristem clipping in *M. guttatus* was also affected by inbreeding, and the direction and magnitude of the effect differed significantly among populations (McCall and Carr, 2013). In the present study, inbred and outcrossed plants also exhibited strongly divergent growth responses to standardized damage. Damage induced a significant increase in leaf biomass in outcrossed plants (Figure 2a), a response that is considered an adaptive mechanism to compensate for lost source (photosynthetic) tissue (Stowe *et al.*, 2000). In contrast, herbivory induced a significant decrease in leaf growth in inbred plants, suggesting that inbred plants suffered disproportionately greater resource (photosynthetic) limitation and a reduced ability to compensate for herbivory. The difference in plasticity in main root growth (Figure 2b) further supports the hypothesis that inbreeding negatively affects tolerance traits. Re-allocation of resources between tissue types (Korpita *et al.*, 2014) and increased allocation to storage roots (Gómez *et al.*, 2010; Machado *et al.*, 2013) are considered key tolerance traits after damage, particularly for perennial or facultatively asexual species such as *S. carolinense*. Moreover, outcrossed plants were able to maintain constant stem growth in response to damage. Interestingly, inbred plants showed increased stem growth (biomass and plant height) in response to damage. Stem biomass was significantly lower in inbred control plants compared to outcrossed control plants, but increased 31% in damaged inbred plants (Figure 2c). A similar pattern was found for plant height (Figure 2d), with significant main effects of breeding and damage that appear to have been driven by the response of inbred plants: separate analyses for each breeding condition revealed that the effect of damage on plant height was significant for inbred ($P = 0.038$) but not outcrossed plants ($P = 0.3725$). In contrast to leaf and root growth, the adaptive consequence of increased plasticity in inbred stem growth is less clear. Reduced resource acquisition due to inbreeding may have been predicted to cause a trade-off of investment in leaf versus stem growth in inbred plants. However, at the individual plant level, there was no evidence for such a trade-off, with significant, positive correlations among growth traits (all $P \leq 0.01$) for all treatment combinations. Thus, we conclude that inbreeding effects on these growth traits were due to independent disruption of growth regulation (see below). A few studies have examined the combined effects of herbivory and inbreeding on fitness (Campbell *et al.*, 2013), and have occasionally suggested effects of inbreeding on tolerance in the field, where simultaneous inbreeding depression for resistance traits (and differential damage between inbred and outcrossed plants) may complicate the interpretation of tolerance responses (e.g. Du *et al.*, 2008). While tolerance is conventionally defined as a reduction in the fitness costs of herbivory (Núñez-Farfán *et al.*, 2007), we suggest that progress in this area may benefit from considering the effects of inbreeding on specific herbivore-induced growth traits (or other so-called 'mechanisms' of tolerance) that, together with effects on specific resistance traits, may underlie variation in fitness.

Our results strongly implicate differential production of several key plant hormones as mechanisms for altered plasticity under inbreeding. All four measured phytohormones play important roles in regulating the expression of plant growth and defence traits (Chandler and Robertson, 1994; Creelman and Mullet, 1997; Woodward and Bartel, 2005). The significant reduction of three of these hormones in inbred plants provides evidence for negative effects of inbreeding on the regulation of gene expression. JA is critical in regulating induced resistance and up-regulation of defensive metabolites, particularly the phenolics produced by the phenylpropanoid pathway (Kessler and Baldwin, 2002). Thus, the 60% reduction in JA up-regulation under inbreeding is consistent with the significant reduction in inducible resistance and plasticity in phenolic production. This finding is also consistent with inbreeding depression for defence traits and fitness in 29 field-grown families of *S. carolinense* (Campbell *et al.*, 2013), and with a study suggesting reduced up-regulation of volatiles under inbreeding in three genotypes (Kariyat *et al.*, 2012), although neither study tested for differences in the magnitude of plasticity. JA may also mediate herbivore-induced changes to resource allocation and growth (Babst *et al.*, 2005; Zavala and Baldwin, 2006; Gómez *et al.*, 2010 though see also Schwachtje *et al.*, 2006), potentially implicating this phytohormone in regulation of the divergent root growth responses between outcrossed and inbred plants (Figure 2). Overall, we predict that a wide variety of JA-mediated phenotypes and interactions are affected by inbreeding. Consistent with other studies (Schmelz *et al.*, 2003), SA was not up-regulated under this wounding treatment, and was unaffected by inbreeding, suggesting that interactions mediated by SA (e.g. pathogen resistance) may be less affected by inbreeding, However, this interpretation is not supported by the study by Stephenson *et al.* (2004) and it may be that inbreeding effects on SA up-regulation are only obvious in response to pathogen elicitation.

ABA and IAA were reduced by 55 and 27%, respectively, under inbreeding. While ABA is primarily considered a growth and abiotic stress response hormone (Chandler and Robertson, 1994), ABA-deficient mutant tomato plants (*Solanum lycopersicum*) show reduced resistance to

caterpillar herbivores (Thaler and Bostock, 2004), and ABA modulates JA expression (Peña-Cortés *et al.*, 1995), implicating this hormone in inbreeding depression for induced plant resistance. ABA has also been shown to inhibit stem elongation (Arney and Mitchell, 1969), providing a possible mechanism for the increased stem growth observed in inbred plants, particularly with the correlated reduction in IAA (Woodward and Bartel, 2005). Both ABA and IAA have diverse and fundamental roles in leaf cell division and growth, and are implicated in defence trait expression (Onkokesung *et al.*, 2010), suggesting that their reduced expression in inbred plants may be linked to reduced allocation to leaf biomass following damage. While our results indicate severe inbreeding depression for phytohormone production, they do not indicate whether genetic load is localized at particular stages of damage perception and signal transduction (e.g. systemin production and hormone perception), within phytohormone biosynthetic pathways (e.g. the octadecanoid pathway), or both (Kessler and Baldwin, 2002). Moreover, our hormone experiment did not examine the time course of hormone production, or features of real herbivory such as feeding rate or salivary/chemical elicitors. If additional deleterious mutations exist at loci governing plant responses to these factors, our experiment may be a conservative indication of the magnitude of inbreeding depression for endogenous signalling. Examining these additional, specific effects of inbreeding remains goals for future studies of the effects of mating system variation on how plants physiologically respond to specific stresses.

Plant hormone signalling pathways act in a coordinated fashion (Kessler and Baldwin, 2002; Thaler *et al.*, 2012), and the effect of inbreeding on hormone antagonism and the interaction of induction pathways may be as significant as the effect on specific phytohormone quantities. Indeed, ratios among hormones clearly changed as a function of inbreeding (Figure 3b). We also tested the hypothesis that inbreeding disrupted the correlated expression of hormones. A disruption of the quantitative relationships among phytohormones would have significant consequences, as these correlations represent functional constraints on the ability of the plant to regulate downstream gene expression (Robert-Seilaniantz *et al.*, 2011). In support of this hypothesis, inbreeding altered the strength and significance of correlations for specific pairs of hormones (Table 2) as well as the overall pattern of relationships, as indicated by a comparison of covariance matrix structure (Cheverud, 1996). As predicted, when JA was included in the analysis, its strong induction appeared to drive correlations between inbred and outcrossed control matrices, and inbred and outcrossed wounded matrices (Table 3). When removed, there was a significant correlation between control and wounded trait matrices in outcrossed plants but not inbred plants (Table 3), indicating that the correlated pattern of expression of ABA, IAA and SA was conserved under wounding in outcrossed but not inbred plants. In addition, outcrossed and inbred matrices were not significantly correlated in either the control or wounded treatment, confirming that inbreeding fundamentally altered the coordinated hormonal response to wounding. The relative importance of reductions in the magnitude of hormone production versus disruption of the coordinated hormone balance remains an open question.

These results also have broader implications for the study of inbreeding depression in plants. In particular, our study provides a mechanistic hypothesis for the common observation that inbreeding reduces plant growth and vigour (Darwin, 1876; Husband and Schemske, 1996). Few studies have examined the mechanisms of inbreeding depression for growth and fitness at the level of plant physiology and metabolism (Norman *et al.*, 1995; Leimu *et al.*, 2012a). Research on maize cultivars (*Zea mays*) has suggested a role for gibberellic acid in hybrid vigour (Rood *et al.*, 1988), and our study may be the first to show severe inbreeding depression for the expression of key growth hormones such as ABA and auxin/IAA in a wild species. Inbreeding depression appears to be greater under environmental stress (e.g. drought) in many species (Cheptou and Donohue, 2011), and a mechanism for ecologically mediated inbreeding depression may be genetic load in signalling-related genes.

Finally, mating systems may influence the role of phenotypic plasticity for both dynamics at range margins (Chevin and Lande, 2011) and responses to anthropogenic environmental change (Chevin *et al.*, 2010; Leimu *et al.*, 2012b). Phenotypic plasticity is considered important for fitness in the fluctuating environment of range margins (Sexton *et al.*, 2009). Our data suggest that plants in these habitats, where mate limitation and inbreeding depression are likely to be higher (Eckert *et al.*, 2010), may be further compromised by limitations in adaptive plasticity. Thus, population genetic and demographic studies of vulnerable species may benefit from understanding the interactions among population size/isolation, phenotypic responses to ecological factors, and mating systems.

EXPERIMENTAL PROCEDURES

Study system and plant material

Horsenettle, *Solanum carolinense* L. (Solanaceae), is a short-lived perennial herb native to eastern North America. The study system has been described previously by Campbell *et al.* (2013). *Solanum carolinense* possesses a predominantly outcrossing mating system that makes it an ideal species for studies of the effects of inbreeding on ecological interactions. We collected seed from three source populations near Ithaca, New York state, USA, and grew maternal plants from these collections in a greenhouse. We experimentally self-fertilized and outcrossed 60 maternal plants to create full-sib families, and randomly sampled from these families

for subsequent experiments. Details on the breeding protocol that produced these families have been reported previously by Campbell *et al.* (2013). For the experiments described here, subsets of families were selected at random from the larger pool, and clonal replicates of two to five selfed and outcrossed offspring per family were created by sub-dividing main roots into 1.5 g segments, and placing segments in a 1:1:1 mixture of vermiculite/perlite/potting soil (Metro-Mix 360 all-purpose potting soil; Scotts-Sierra Horticultural Products, www.scotts.com) in 27-well seed flats for sprouting. Plants were grown in the greenhouse for 2 weeks, transplanted into 355 ml pots filled with soil, and grown for an additional 3 weeks under a 16 h light/8 h dark schedule, *ad libitum* watering, and weekly fertilization (21–5–20 NPK, 150 ppm), with trays being randomized to minimize greenhouse position effects. Inbred and outcrossed plants grown in this way were then used in two experiments.

Growth and defence-related trait responses to herbivory and inbreeding

We performed a greenhouse experiment to measure the effects of inbreeding on responses to damage, focussing on plasticity in defence and growth-related traits. Plant induced responses may be highly sensitive to the rate of feeding, and, because we had previously shown differential consumption rates by herbivores on inbred and outcrossed *S. carolinense* (Campbell *et al.*, 2013), we chose to use controlled, simulated herbivory to test plant growth and metabolite responses. Inbred and outcrossed plants ($n = 20$ families) were randomly assigned to 0, 10 or 20% manual tissue removal ($n = 520$ plants). Damage levels were based on visual estimation, and damage was imposed on every leaf using a standard paper hole-punch, with holes evenly distributed over the leaf avoiding the mid-rib. Additional details on the experimental design are provided by Campbell *et al.* (2013). For 14 of these families ($n = 234$), we quantified the amounts of six phenolic compounds that correlate negatively with damage in field-grown *S. carolinense* (Campbell *et al.*, 2013), implicating them as defence-related secondary metabolites in this system. The raw phenolic data (for each compound) were presented by Campbell *et al.* (2013); here, we analyse these data to explicitly examine the inducibility of all six metabolites in response to damage. In brief, phenolic expression was quantified by excising a single leaf from each plant with a razor blade, and taking a 100 mg sample of fresh tissue (excluding mid-vein), which was weighed, flash-frozen in liquid N_2, and stored at −80°C. Samples were then extracted in ice-cold 40% aqueous methanol containing 0.5% acetic acid, and analysed for secondary metabolites by HPLC using a standard method targeted at phenolic compounds (Keinanen *et al.*, 2001). Plasticity in phenolic expression was calculated as the mean of the proportional change in expression in damaged (20%) relative to control plants across five of the six quantifiable phenolics. One phenolic compound (compound 'C'; an hydroxycinnamic acid derivative with retention time 9.58 min) was excluded because it showed no inducibility in either outcrossed or inbred plants. We compared plasticity in outcrossed versus inbred plants for each family (to control for genetic variation among families, Campbell *et al.*, 2013), in JMP® version 9.0 (SAS, www.sas.com) using an *F* test, i.e. a matched-pairs design. At the time of tissue sampling (2 weeks after damage), we also recorded plant height and leaf number. Plants were then moved outside at Cornell University, Ithaca, New York on 4 September 2011, where they were watered daily and fertilized weekly (as above) until the first frost (2 months). This allowed us to examine plant growth under semi-natural conditions following damage. No pests were observed on the plants. Aboveground plant parts were harvested, dried and weighed. Main roots (the source of asexual reproduction in this species) were harvested, and the fresh weight was measured to allow subsequent propagation. Box plots and distributions of growth traits were examined for outliers, normality and heteroscedasticity. Data were log-transformed, and analysed using JMP® version 9.0 with linear mixed models in a restricted maximum likelihood framework. Genetic family, breeding status, treatment and breeding × treatment were specified as model terms. Family was set as a random effect, and treatment was modelled as a continuous variable. We also performed pairwise multiple comparisons (Tukey's tests) among all breeding and damage treatment combinations, with experiment-wise $\alpha = 0.05$.

Phytohormone analysis

We performed a controlled artificial wounding experiment to compare inbred and outcrossed plants for their constitutive and induced expression of four key plant hormones that regulate plant growth, defensive metabolite expression, and responses to environmental stresses such as herbivory. In the greenhouse, leaves on inbred and outcrossed plants ($n = 8$ maternal families, two to five replicates per family) were wounded by applying rows of punctures parallel to the mid-rib at 5 mm spacing using a fabric pattern wheel, thereby providing a wound that was standardized to the leaf area of all plants. This wounding technique specifically allows us to conservatively examine endogenous hormone production independently of mating system differences in the effects of physical damage (tissue removal), herbivore physiology (e.g. saliva) and behaviour (e.g. feeding rate). This approach is particularly advantageous because hormone production may be affected by plant resource status, and inbred plants may show greater resource limitation than outbred plants under the same level of tissue removal (see Results). A limitation of this approach is that responses may not be directly comparable between experiments; however, we note that the multiple damage types had similar effects, which may mitigate this limitation (see also Appendix S1). Damaged leaves were sampled exactly 60 min after wounding based on a conservative estimate of the plateau in hormone expression. Jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA) and auxin/indole-3–acetic acid (IAA) were extracted from the damaged leaves using the protocol described by Thaler *et al.* (2010). In brief, 1 ml of an isopropanol/H_2O/concentrated HCl (2:1:0.005) extraction buffer was added to approximately 300 mg of frozen tissue, and 80 ng each of d_4-SA, d_5-JA, d_6-ABA and d_5-IAA (C/D/N Isotopes Inc., www.cdnisotopes.com/) were added as internal standards. Samples were homogenized on a FastPrep® homogenizer (MP Biomedicals, www.mpbio.com) at 6 m sec^{-1} for 45 sec using 0.9 g grinding beads (zirconia/silica 2.3 mm; Biospec, www.biospec.com), re-extracted with dichloromethane, dried and dissolved in 200 μl methanol. A 10 μl aliquot of each sample was analysed on a triple-qadrupole LC-MS/MS (Thermo Scientific, www.thermoscientific.com) equipped with a C_{18} reverse-phase HPLC column (Gemini–NX, 3 μm particle size, 150 × 2.00 mm; Phenomenex, www.phenomenex.com), using the method described by Thaler *et al.* (2010). Auxin/IAA was analysed by positive electrospray ionisation, and JA, SA and ABA were analysed by negative electrospray ionization (spray voltage 3.5 kV; sheath gas 15 arb. unit; auxiliary gas 15 arb. unit; capillary temperature 350°C), collision-induced dissociation (argon CID gas pressure 1.3 mTorr, CID energies 16 V for JA and SA, 13 V for ABA and 18 V for IAA), and selected reaction monitoring of compound-specific transitions [parent → product ion]: SA [137 → 93]; d_4-SA [141 → 97]; JA [209 → 59]; d_5-JA [214 → 62]; ABA [263 → 153]; d_6-ABA [269 → 159]; IAA [176 → 129]; d_5-IAA [181 → 134].

Analyte quantities were normalized to the mass of fresh sample tissue, log-transformed to improve residual normality and heteroscedasticity, and analysed identically to the growth traits. In addition, we performed a test of whether inbreeding altered coordinated hormone expression in both wounded and control plants. Plant hormones are known to interact with one another in their effects on gene expression (Kessler and Baldwin, 2002), and as the treatments may alter both the magnitude of the traits and the relationships among them, we compared the phenotypic variance–covariance matrices of the four combinations of breeding and wounding treatment. Covariance matrices were estimated using the restricted maximum likelihood framework in JMP® version 9.0, and compared using the random skewers method (Cheverud, 1996). The random skewers method compares the overall structure of matrices by calculating the mean vector correlation of the products of random vectors and the two covariance matrices being compared. The method produces vector correlation coefficients that correspond to a test of whether the structures of two covariance matrices are correlated with one another. We used the SKEWERS software, courtesy of L. Revell (http://faculty.umb.edu/liam.revell/programs/index.html), with 10^6 skewers (Cheverud and Marroig, 2007). We performed the analysis with and without JA, as this hormone is often detectable only after wounding, and its inclusion was predicted to bias the analysis in favour of finding correlations based on wounding alone rather than breeding status. For each treatment/breeding combination, we also estimated product-moment correlations for all pairs of hormones.

ACKNOWLEDGEMENTS

Phytohormone analyses were performed at the Chemical Ecology Group Core Facility (Cornell University, Ithaca, NY). We thank M. del Campo, P. Cooper, M. Freedman, L. Decker, K. Chalkowski for assistance in the greenhouse or lab, the US National Science Foundation (grant NSF-IOS 0950225 to A.K.), the Andrew Mellon Foundation (Cornell Graduate Research Grant) and the College of Agriculture and Life Sciences (Cornell University) for funding, and M. Geber, R. Raguso, S. Cardinal, S. Deleon, K. Morrell, A. Uesugi, the Cornell Plant–Insect Interactions Group, and two anonymous reviewers for feedback on this project and/or drafts of this manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Results of the induced resistance experiment.

Appendix S1. Description of the induced resistance experiment.

REFERENCES

Agrawal, A.A., Strauss, S.Y. and Stout, M.J. (1999) Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution*, **53**, 1093–1104.

Arney, S.E. and Mitchell, D.L. (1969) Effect of abscisic acid on stem elongation and correlative inhibition. *New Phytol.* **68**, 1001–1015.

Auld, J.R. and Relyea, R.A. (2010) Inbreeding depression in adaptive plasticity under predation risk in a freshwater snail. *Biol. Lett.* **6**, 222–224.

Babst, B.A., Ferrieri, R.A., Gray, D.W., Lerdau, M., Schlyer, D.J., Schueller, M., Thorpe, M.R. and Orians, C.M. (2005) Jasmonic acid induces rapid changes in carbon transport and partitioning in *Populus*. *New Phytol.* **167**, 63–72.

Baldwin, I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc. Natl Acad. Sci. USA*, **95**, 8113–8118.

Bello-Bedoy, R. and Núñez-Farfán, J. (2011) The effect of inbreeding on defence against multiple enemies in *Datura stramonium*. *J. Evol. Biol.* **24**, 518–530.

Campbell, S.A. and Kessler, A. (2013) Plant mating system transitions drive the macroevolution of defense strategies. *Proc. Natl Acad. Sci. USA*, **110**, 3973–3978.

Campbell, S.A., Thaler, J.S. and Kessler, A. (2013) Plant chemistry underlies herbivore-mediated inbreeding depression in nature. *Ecol. Lett.* **16**, 252–260.

Carr, D.E. and Eubanks, M.D. (2002) Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). *Evolution*, **56**, 22–30.

Chandler, P.M. and Robertson, M. (1994) Gene expression regulated by abscisic acid and its relation to stress tolerance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 113–141.

Charlesworth, D. and Charlesworth, B. (1987) Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**, 237–268.

Charlesworth, B. and Charlesworth, D. (1999) The genetic basis of inbreeding depression. *Genet. Res.* **74**, 329–340.

Cheptou, P.O. and Donohue, K. (2011) Environment-dependent inbreeding depression: its ecological and evolutionary significance. *New Phytol.* **189**, 395–407.

Cheverud, J.M. (1996) Quantitative genetic analysis of cranial morphology in the cotton-top (*Saguinus oedipus*) and saddle-back (*S. fuscicollis*) tamarins. *J. Evol. Biol.* **9**, 5–42.

Cheverud, J.M. and Marroig, G. (2007) Comparing covariance matrices: random skewers method compared to the common principal components model. *Genet. Mol. Biol.* **30**, 461–469.

Chevin, L.M. and Lande, R. (2011) Adaptation to marginal habitats by evolution of increased phenotypic plasticity. *J. Evol. Biol.* **24**, 1462–1476.

Chevin, L.M., Lande, R. and Mace, G.M. (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* **8**, e1000357.

Creelman, R.A. and Mullet, J.E. (1997) Biosynthesis and action of jasmonates in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 355–381.

Darwin, C. (1876) *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. London: J. Murray.

Delphia, C.M., De Moraes, C.M., Stephenson, A.G. and Mescher, M.C. (2009) Inbreeding in horsenettle influences herbivore resistance. *Ecol. Entomol.* **34**, 513–519.

Deng, H.W. (1997) Increase in developmental instability upon inbreeding in *Daphnia*. *Heredity*, **78**, 182–189.

Du, D.L., Winsor, J.A., Smith, M., Denicco, A. and Stephenson, A.G. (2008) Resistance and tolerance to herbivory changes with inbreeding and ontogeny in a wild gourd (Cucurbitaceae). *Am. J. Bot.* **95**, 84–92.

Eckert, C.G., Kalisz, S., Geber, M.A. *et al.* (2010) Plant mating systems in a changing world. *Trends Ecol. Evol.* **25**, 35–43.

Ellison, A., Cable, J. and Consuegra, S. (2011) Best of both worlds? Association between outcrossing and parasite loads in a selfing fish. *Evolution*, **65**, 3021–3026.

Fischer, M., van Kleunen, M. and Schmid, B. (2000) Genetic Allee effects on performance, plasticity and developmental stability in a clonal plant. *Ecol. Lett.* **3**, 530–539.

Gómez, S., Ferrieri, R.A., Schueller, M. and Orians, C.M. (2010) Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. *New Phytol.* **188**, 835–844.

Hauser, T.P. and Loeschcke, V. (1996) Drought stress and inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae). *Evolution*, **50**, 1119–1126.

Heil, M. and Baldwin, I.T. (2002) Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends Plant Sci.* **7**, 61–67.

Husband, B.C. and Schemske, D.W. (1996) Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution*, **50**, 54–70.

Ivey, C.T. and Carr, D.E. (2012) Tests for the joint evolution of mating system and drought escape in *Mimulus*. *Ann. Bot.* **109**, 583–598. Erratum *Ann. Bot.* **109**, 1381.

Ivey, C.T., Carr, D.E. and Eubanks, M.D. (2004) Effects of inbreeding in *Mimulus guttatus* on tolerance to herbivory in natural environments. *Ecology*, **85**, 567–574.

Karban, R. and Baldwin, I. (1997) *Induced Responses to Herbivory*. Chicago, IL: University of Chicago Press.

Kariyat, R.R., Scanlon, S.R., Mescher, M.C., De Moraes, C.M. and Stephenson, A.G. (2011) Inbreeding depression in *Solanum carolinense* (Solanaceae) under field conditions and implications for mating system evolution. *PLoS One*, **6**, 7.

Kariyat, R.R., Mauck, K.E., Moraes, C.M., Stephenson, A.G. and Mescher, M.C. (2012) Inbreeding alters volatile signalling phenotypes and influences tri-trophic interactions in horsenettle (*Solanum carolinense* L.). *Ecol. Lett.* **15**, 301–309.

Kariyat, R.R., Balogh, C.M., Moraski, R.P., De Moraes, C.M., Mescher, M.C. and Stephenson, A.G. (2013) Constitutive and herbivore-induced structural defenses are compromised by inbreeding in *Solanum carolinense* (Solanaceae). *Am. J. Bot.* **100**, 1014–1021.

Keinanen, M., Oldham, N.J. and Baldwin, I.T. (2001) Rapid HPLC screening of jasmonate-induced increases in tobacco alkaloids, phenolics, and diterpene glycosides in *Nicotiana attenuata*. *J. Agric. Food Chem.* **49**, 3553–3558.

Kessler, A. and Baldwin, I.T. (2002) Plant responses to insect herbivory: the emerging molecular analysis. *Annu. Rev. Plant Biol.* **53**, 299–328.

Knight, T.A. (1799) An account of some experiments on the fecundation of vegetables. *Philos. Trans. R. Soc. Lond.* **89**, 195–204.

Korpita, T., Gómez, S. and Orians, C.M. (2014) Cues from a specialist herbivore increase tolerance to defoliation in tomato. *Func. Ecol.* **28**, 395–401.

Kristensen, T.N., Barker, J.S.F., Pedersen, K.S. and Loeschcke, V. (2008) Extreme temperatures increase the deleterious consequences of inbreeding under laboratory and semi-natural conditions. *Proc. Biol. Sci.* **275**, 2055–2061.

Lande, R. (1988) Genetics and demography in biological conservation. *Science*, **241**, 1455–1460.

Lande, R. and Schemske, D.W. (1985) The evolution of self-fertilization and inbreeding depression in plants. 1. Genetic models. *Evolution*, **39**, 24–40.

Leimu, R., Kloss, L. and Fischer, M. (2008) Effects of experimental inbreeding on herbivore resistance and plant fitness: the role of history of inbreeding, herbivory and abiotic factors. *Ecol. Lett.* **11**, 1101–1110.

Leimu, R., Kloss, L. and Fischer, M. (2012a) Inbreeding alters activities of the stress-related enzymes chitinases and β–1,3-glucanases. *PLoS One*, **7**, e42326.

Leimu, R., Muola, A., Laukkanen, L., Kalske, A., Prill, N. and Mutikainen, P. (2012b) Plant–herbivore coevolution in a changing world. *Entomol. Exp. Appl.* **144**, 3–13.

Levin, D.A. (1970) Developmental instability and evolution in peripheral isolates. *Am. Nat.* **104**, 343–353.

Lloyd, D.G. (1979) Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* **113**, 67–79.

Machado, R.A.R., Ferrieri, A.P., Robert, C.A.M., Glauser, G., Kallenbach, M., Baldwin, I.T. and Erb, M. (2013) Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. *New Phytol.* **200**, 1234–1246.

McCall, A.C. and Carr, D.E. (2013) Does inbreeding affect tolerance to inflorescence damage in *Mimulus guttatus*?. *Open Ecol. J.* **6**, 1–6.

Mithöfer, A. and Boland, W. (2011) Plant defense against herbivores: chemical aspects. *Annu. Rev. Plant Biol.* **63**, 431–450.

Murren, C.J. and Dudash, M.R. (2012) Variation in inbreeding depression and plasticity across native and non-native field environments. *Ann. Bot.* **109**, 621–632.

Norman, J.K., Sakai, A.K., Weller, S.G. and Dawson, T.E. (1995) Inbreeding depression in morphological and physiological traits of *Schiedea lydgatei* (Caryophyllaceae) in two environments. *Evolution*, **49**, 297–306.

Núñez-Farfán, J., Fornoni, J. and Valverde, P.L. (2007) The evolution of resistance and tolerance to herbivores. *Annu. Rev. Ecol. Evol. Syst.* **38**, 541–566.

O'Halloran, L.R. and Carr, D.E. (2010) Phenotypic plasticity and inbreeding depression in *Mimulus ringens* (Phrymaceae). *Evol. Ecol. Res.* **12**, 617–632.

Onkokesung, N., Galis, I., von Dahl, C.C., Matsuoka, K., Saluz, H.P. and Baldwin, I.T. (2010) Jasmonic acid and ethylene modulate local responses to wounding and simulated herbivory in *Nicotiana attenuata* leaves. *Plant Physiol.* **153**, 785–798.

Peña-Cortés, H., Fisahn, J. and Willmitzer, L. (1995) Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. *Proc. Natl Acad. Sci. USA*, **92**, 4106–4113.

Robert-Seilaniantz, A., Grant, M. and Jones, J.D.G. (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate–salicylate antagonism. *Annu. Rev. Phytopathol.* **49**, 317–343.

Rood, S.B., Buzzell, R.I., Mander, L.N., Pearce, D. and Pharis, R.P. (1988) Gibberellins: a phytohormonal basis for heterosis in maize. *Science*, **241**, 1216–1218.

Schlichting, C.D. (1986) The evolution of phenotypic plasticity in plants. *Annu. Rev. Ecol. Syst.* **17**, 667–693.

Schlichting, C.D. and Levin, D.A. (1986) Effects of inbreeding on phenotypic plasticity in cultivated *Phlox*. *Theor. Appl. Genet.* **72**, 114–119.

Schmelz, E.A., Engelberth, J., Alborn, H.T., O'Donnell, P., Sammons, M., Toshima, H. and Tumlinson, J.H. (2003) Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. *Proc. Natl Acad. Sci. USA*, **100**, 10552–10557.

Schmitt, J. and Ehrhardt, D.W. (1990) Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. *Evolution*, **44**, 269–278.

Schwachtje, J., Minchin, P.E.H., Jahnke, S., van Dongen, J.T., Schittko, U. and Baldwin, I.T. (2006) SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. *Proc. Natl Acad. Sci. USA*, **103**, 12935–12940.

Sexton, J.P., McIntyre, P.J., Angert, A.L. and Rice, K.J. (2009) Evolution and ecology of species range limits. *Annu. Rev. Ecol. Evol. Syst.* **40**, 415–436.

Siikamaki, P. and Lammi, A. (1998) Fluctuating asymmetry in central and marginal populations of *Lychnis viscaria* in relation to genetic and environmental factors. *Evolution*, **52**, 1285–1292.

Steets, J.A., Wolf, D.E., Auld, J.R. and Ashman, T.L. (2007) The role of natural enemies in the expression and evolution of mixed mating in hermaphroditic plants and animals. *Evolution*, **61**, 2043–2055.

Stephenson, A.G., Leyshon, B., Travers, S.E., Hayes, C.N. and Winsor, J.A. (2004) Interrelationships among inbreeding, herbivory, and disease on reproduction in a wild gourd. *Ecology*, **85**, 3023–3034.

Stowe, K.A., Marquis, R.J., Hochwender, C.G. and Simms, E.L. (2000) The evolutionary ecology of tolerance to consumer damage. *Annu. Rev. Ecol. Evol. Syst.* **31**, 565–595.

Thaler, J.S. and Bostock, R.M. (2004) Interactions between abscisic-acid-mediated responses and plant resistance to pathogens and insects. *Ecology*, **85**, 48–58.

Thaler, J.S., Agrawal, A.A. and Halitschke, R. (2010) Salicylate-mediated interactions between pathogens and herbivores. *Ecology*, **91**, 1075–1082.

Thaler, J.S., Humphrey, P.T. and Whiteman, N.K. (2012) Evolution of jasmonate and salicylate signal crosstalk. *Trends Plant Sci.* **17**, 260–270.

Winn, A.A., Elle, E., Kalisz, S. *et al.* (2011) Analysis of inbreeding depression in mixed-mating plants provides evidence for selective interferance and stable mixed mating. *Evolution*, **65**, 3339–3359.

Woodward, A.W. and Bartel, B. (2005) Auxin: regulation, action, and interaction. *Ann. Bot.* **95**, 707–735.

Zavala, J.A. and Baldwin, I.T. (2006) Jasmonic acid signalling and herbivore resistance traits constrain regrowth after herbivore attack in *Nicotiana attenuata*. *Plant Cell Environ.* **29**, 1751–1760.