Within and between generation phenotypic plasticity in trichome density of *Mimulus guttatus*

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Abstract

Mimulus guttatus (yellow monkeyflower) frequently produce glandular trichomes, a trait that may resist herbivory. Constitutive production of trichomes is variable both within and among populations of *M. guttatus* and most of this variation is genetic. This study demonstrates that damage on early leaves can induce increased trichome production on later leaves, a plastic response that is likely adaptive. Moreover, this study shows that this induction can be maternally transmitted, increasing trichome density in progeny before they experience herbivory. This transgenerational response must involve a yet undescribed epigenetic mechanism. These experiments also show genetic variation among plants in the capacity for both *within* and *between* plant generation induction. Despite the clear evolutionary importance of variation in constitutive and induced herbivory-resistance traits, few other studies have noted genetic variation in both within a plant species.

Introduction

Phenotypic plasticity is environmentally dependant phenotypic variation in the growth or development of an organism (Bradshaw, 1965; West-Eberhard, 1989; DeWitt & Scheiner, 2004). Plasticity is well-documented in plants and is commonly hypothesized to be an adaptive response of these sessile organisms to environmental heterogeneity (Bradshaw, 1965; Dudley, 2004). Plasticity is adaptive only if a particular change in growth/development improves fitness. To determine whether or to what extent phenotypic plasticity affects the evolutionary trajectory of a population or species, it is essential to know not only whether it is adaptive, but whether there is variation for plasticity and what proportion of this variation is heritable (Schlichting, 2004).

Plant trichomes are hairlike structures that extend from the plant epidermis and occur in a variety of types including glandular or nonglandular, hooked or straight. Trichomes may serve a variety of defensive and physiological functions. Leaf trichomes have been shown to reduce insect herbivory in a number of plant species

(Levin, 1973; Marquis, 1992; van Dam & Hare, 1998; Romeis *et al.*, 1999; Valverde *et al.*, 2001). They interfere with insect movement and feeding, and often secrete glandular fluids that may trap, poison, or repel herbivores (Levin, 1973; Elle & Hare, 2000). For plants in xeric habitats, trichomes reflect light and can reduce transpiration (Ehleringer, 1984; Larcher, 2001). Geographical and genetic variation in trichome density and type has been noted in a number of plant species (Grant, 1950; Levin, 1973; van Dam *et al.*, 1999; Valverde *et al.*, 2001).

Several studies have shown that leaf damage can induce increased trichome production. Both vertebrate herbivore damage and mechanical damage induced higher trichome densities in stinging nettle (*Urtica dioica*) (Pullin & Gilbert, 1989). They also found induction to depend on soil nutrient availability. Damage by cabbage butterfly larvae (*Pieris rapae*) on the fifth leaf of black mustard plants (*Brassica nigra*) induced higher trichome densities on the seventh leaf of these plants (Traw & Dawson, 2002). Adult leaf beetle grazing (*Phratora vulgatissima*) on willow (*Salix cinerea*) induced significantly greater trichome densities on later leaves, which reduced herbivory by the next generation of beetle larvae of the same species later in the season (Dalin & Bjorkman, 2003). Mechanical defoliation of a tropical shrub (*Cnidoscolus aconitifolius*) induced higher trichome density in leaves that developed between 30 days and

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3 months after the defoliation (Abdala-Roberts & Parra-Tabla, 2005). Despite the apparent frequency of the response across plant species, no studies previous to this have documented genetic variation in within-generation trichome induction.

When there is consistency of environmental conditions across generations, it may be advantageous to transmit a trait-induction signal to progeny. Transgenerational induction of a trait differs from typical maternal effects in both mechanism and effect. Maternal effects often reflect differences in the quality of the maternal environment, resulting in differential partitioning of resources to the offspring. This, in turn, affects the fitness of these offspring (Lynch & Walsh, 1998). Transgenerational plasticity, in contrast, is epigenetically based; the expression of genes influencing trait development in the offspring is maternally activated. Transgenerational induction of a plant trait has been demonstrated only once before, by Agrawal (1999, 2001) in wild radish (*Raphanus raphanistrum*). Transgenerational induction is only adaptive if environmental cues received by the parent are predictive of the fitness environment that will be experienced by their progeny.

Trichomes in *Mimulus guttatus* are straight and occur mostly in the glandular form, often secreting a sticky and potentially noxious fluid. In *M. guttatus*, constitutive production of trichomes is variable both within and among populations and most of this variation is genetic (Holeski, 2007). Physiological benefits (i.e. to reduce light radiation or transpiration rates) are unlikely to explain the high trichome densities of plants in a number of the populations, as they are located in the fog belt of coastal Northern California and should not suffer extreme drought or intense sunlight. These high trichome density populations are perennial, and experience heavy herbivory by a number of insect herbivores. This contrasts with annual, alpine *M. guttatus* populations where plants only live for 6–10 weeks. Frequently, plants in these populations have few or no trichomes and experience little insect herbivory in their short lifetimes (Holeski, 2007). One might thus predict presence of inter-population genetic variation for trichome density or other putative resistance traits between the alpine annual and coastal perennial *M. guttatus* populations.

Here, I describe experiments that investigate phenotype plasticity in trichome density in *M. guttatus* (yellow monkeyflower). With these experiments, I address the following questions: (1) Is trichome production in *M. guttatus* phenotypically plastic; i.e. can foliar damage on early leaves induce increased trichome densities on later leaves? (2) Is there transgenerational plasticity, i.e. can induction be transmitted maternally? (3) Is there genetic variation for either within-generation or transgenerational trichome induction in *M. guttatus*? (4) Is constitutive production negatively related to the capacity for induction?

Materials and methods

Yellow monkeyflower [*M. guttatus*; Phrymaceae (Beardsley & Olmstead, 2002)] ranges from Mexico to Alaska in western North America and typically inhabits wet areas such as stream banks. It is a self-compatible, hermaphroditic plant that reproduces by a mixture of outcrossing and self-fertilization. Local populations differ extensively in morphology, life history and selfing rate (Ritland, 1989; Fenster & Ritland, 1994; Kelly & Arathi, 2003; Hall & Willis, 2006; Hall *et al.*, 2006; Holeski & Kelly, 2006).

The experiments described here used offspring of plants collected from two natural populations, Iron Mountain (IM) and Point Reyes National Seashore (PR). IM is an annual population in the Cascade Mountains of central Oregon. Plants have a short life span (1–2 months from germination to death) and typically experience little insect herbivory. PR is a perennial, low elevation population in the fog belt of coastal northern California. These plants experience heavy herbivory (Holeski, 2007). The IM plants were from a highly inbred line (IM 767) extracted from the natural population (see Willis, 1999 for details). The PR plants were full siblings from a single outbred family.

I developed recombinant inbred lines (RILs) from a cross between an IM 767 plant (father) and a PR plant (mother). A single F1 individual from this cross was self-fertilized to generate 1000 F2 individuals, each of which founded a distinct recombinant lineage. These lines were propagated through single-seed descent for four subsequent generations. Because of line loss, approximately 450 RILs remained in the F6 generation. Experiment 1 was conducted (in part) over the course of RIL development while Experiment 2 used only the F6 generation plants and the parentals. Unless genetically correlated, trait differences among the parental genotypes will segregate more or less independently in these hybrid RIL populations. The RILs thus encompass a wide range of constitutive and induced trichome phenotypes within more similar genetic backgrounds than those of the parental plants (Jordan, 1991; Nagy, 1997; Lexer *et al.*, 2003; Hall & Willis, 2006; Wright *et al.*, 2006).

Experiment 1: Constitutive trichome production and trichome induction

I used three categories of plants for this experiment: plants from the two parental populations (PR and IM) and RILs in the F3, F4 and F6 generations. F3 and F4 RILs used had almost complete overlap, while F3/F4 and F6 RILs used had only partial overlap. As illustrated in Table 1, replicates from each of these plant categories experienced some or all of the following treatments: (1) constitutive second leaf trichome count, (2) constitutive fifth leaf trichome count, (3) induced fifth leaf trichome count. In all cases, plants were grown in 2 in pots. Plants were randomized within and among categories and were

Table 1 Experimental design for Experiment 1, demonstrating the total number of plants evaluated within each plant type as well as the division of plants into treatment categories.

Plant type	Total no. of plants evaluated	No. evaluated for constitutive second leaf trichomes	No. evaluated for constitutive fifth leaf trichomes	No. evaluated for fifth leaf trichomes (post-second leaf damage)
IM parent	10 (inbred line replicates)	0	5	5
PR parent	25 (full siblings)	0	11	14
RILs, F3 generation	297 (99 lines, 3 replicates per line)	99 (1 replicate per line)	99 (1 replicate per line)	99 (1 replicate per line)
RILs, F4 generation	297 (99 lines, 3 replicates per line)	99 (1 replicate per line)	99 (1 replicate per line)	99 (1 replicate per line)
RILs, F6 generation	820 (82 lines, 10 replicates per line)	0	410 (5 replicates for each of 85 lines)	410 (5 replicates for each of 85 lines)

rotated daily on the greenhouse bench to minimize environmental effects.

All trichome counts were performed on one leaf from the second/fifth leaf pair on a defined measuring area of 1 cm² on the basal central part of the adaxial side of the leaf, immediately after the leaves in the third/sixth leaf pair were fully expanded. For the induced fifth leaf trichome counts, I simulated insect-chewing damage (punched two holes of approximately 6 mm in diameter) on both leaves of the second leaf pair as soon as the third leaf pair was unfurled. Fifth leaf trichome counts were performed on these plants following the same method as the constitutive fifth leaf counts. Because simulated damage and all trichome counts were performed on a specific leaf when the leaf pair immediately above it was unfurled, both damage and response were measured at the same particular development stage across plants (Orians, 2005; Morris *et al.*, 2006). Fifth leaf trichome counts were used to assess induction because a pre-liminary experiment showed that induction was more pronounced in fifth leaves than in later (seventh) leaves (L. Holeski, unpublished data).

Experiment 2: Transgenerational trichome induction

I germinated two replicates from each of 90 F6 RILs, 12 IM 767 plants, and 12 PR full-siblings in 2-in pots in the greenhouse. One replicate from each RIL, six of the IM 767 plants, and six of the PR plants were in the 'No damage' category. For these, I grew, self-pollinated, and collected seed from each plant. One replicate from each RIL, six IM 767 plants, and six PR plants were in the 'Damaged' category in which I damaged leaves using the simulated insect-chewing method described in Experiment 1. I began the damage of each plant on the leaves from the second leaf pair as soon as the third leaf pair was unfurled and continued to damage the consecutive leaf pairs in the same manner during the self-pollination and seed maturation of these plants. I randomized the position of all plants at the beginning of the experiment on the greenhouse bench and rotated them daily. In the following generation (F7), I grew four offspring from each maternal plant (damaged and undamaged parents

of each genotypic class) to maturity and counted trichomes on fifth leaf pair. Preliminary results with a smaller subset of RIL indicated that maternal damage has no clear impact on the ability of damaged progeny to induce higher trichome densities when they (the progeny) receive second leaf simulated leaf damage, so this was omitted from the larger experiment (L. Holeski, unpublished data).

Analysis

Trichome counts exhibited a right-skew with a large number of zero values. For analysis, I transformed the counts as log (trichome count +1). All calculations were performed using Minitab 14.0 (SAS, Cary, NC, USA).

Experiment 1

Parental populations
The significance testing for the direct effect of treatment on the parental population was accomplished via one-way ANOVA. The IM plants had values of zero for transformed trichome density in all of the control and treatment plants, so an ANOVA was carried out only for the PR plants. The interaction effect of population × treatment was tested in a General Linear Model ANOVA (population and treatment as fixed factors).

RILs
A mean value was calculated for constitutive second, constitutive fifth, and post-damage fifth leaf trichome counts for each RIL. Induced fifth leaf counts were calculated for each RIL as the difference between the means of fifth leaf counts between damaged and undamaged plants. Significant testing was done via a General Linear Model ANOVA with generation and treatment as fixed factors, RIL as a random factor, and fifth leaf trichome density as the response variable. The direct effect of treatment was used as a test for trichome induction; the interaction effect of RIL × treatment tests for genetic variation in induction.

To evaluate the ontological relationship between second leaf constitutive trichome density and the extent of

fifth leaf trichome induction, I regressed the induced fifth leaf trichome counts onto the second leaf counts for all of the RILs. I also coded second leaf trichome counts and induced fifth leaf trichome counts into a discrete form to evaluate the relationship between presence or absence of second leaf trichomes and presence or absence of fifth leaf trichome induction through a chi-squared table analysis. Only F3 and F4 RIL data was used for the regression and table analysis, because second leaf trichome counts were not available for the F6 plants used for the fifth leaf constitutive/damaged counts.

Experiment 2

For Experiment 2, I used a General Linear Model ANOVA (maternal treatment, fixed factor; RIL random factor; fifth leaf trichome density response variable) to determine whether offspring of damaged maternal plants had higher constitutive fifth leaf trichome densities than do offspring of undamaged maternal plants. As in Experiment 1, the direct effect of treatment tests for (maternal) induction, while the treatment × RIL interaction tests for genetic variation in induction.

Results

Experiment 1

Simulated insect damage on the second leaf pair of plants significantly induced higher fifth leaf trichome densities in the PR plants ($P = 0.001$) and RILs (Table 2), but not in the IM plants (Fig. 1). There was a significant population by treatment interaction and a non-significant RIL by treatment interaction (Table 2).

Table 2 ANOVA table of factors affecting fifth leaf trichome density in the parental populations (PR and IM) and RILs from Experiment 1.

Source	d.f.	MS	F	P
Population (PR or IM)	1	28.315	257.83	**< 0.001**
Second leaf damage?	1	0.621	5.65	**0.024**
Population × second leaf damage?	1	0.621	5.65	**0.024**
Error	31	0.110		
Total	34			
Generation	2	1.423	2.42	0.091
RIL	87	3.724	6.29	**< 0.001**
Second leaf damage?	1	6.335	10.73	**0.001**
RIL × second leaf damage?	87	0.592	1.01	0.470
Error	247	0.587		
Total	424			

Explanatory variables include the direct effects of RIL generation, direct effects of population or RIL, direct effects of treatment (presence or absence of second leaf damage), and a population or RIL × treatment interaction. Significant *P*-values are in bold type.
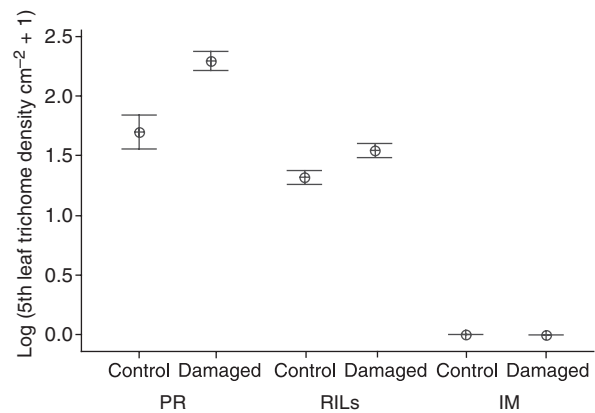

Fig. 1 Mean fifth leaf trichome count per cm² for parental (PR and IM) and RIL plants in Experiment 1 that did ('Damaged') or did not ('Control') receive second leaf damage. In this and subsequent figures, error bars represent 1 standard error (SE).

The mean constitutive second leaf trichome density was significantly lower than the mean constitutive fifth leaf trichome density in RILs ($F_{1, 313} = 35.43$, $P < 0.001$; Fig. 2a). Constitutive second leaf and constitutive fifth leaf trichome densities were positively related, although this trend was nonsignificant ($P = 0.443$; $r^2 = 0.4\%$). There was also no significant relationship between constitutive trichome density (second leaf) and induced fifth leaf trichome density when data were treated as continuous ($P = 0.401$; $r^2 = 0.6\%$; Fig. 2b) or as discrete ($\chi^2_1 = 1.001$, $P = 0.317$).

Experiment 2

Within the RILs and within the Point Reyes population, offspring of damaged maternal plants had significantly higher constitutive fifth leaf trichome densities than did offspring of undamaged maternal plants (Fig. 3; RILs: $F_{1, 647} = 4.13$, $P = 0.043$; PR: $F_{1, 35} = 4.48$, $P = 0.042$). There was a significant RIL by treatment interaction (Table 3). There was no significant difference in constitutive fifth leaf trichome production between offspring of damaged and undamaged IM 767 maternal plants (Fig. 3; $F_{1, 38} = 0.31$, $P = 0.58$).

Only eight RILs were used in both experiments 1 and 2, so I cannot test for a relationship between induction within and across generations for a particular genotype. For these eight RILs, there was no preliminary relationship apparent between within and across generation induction.

Discussion

Experiment 1 demonstrates that trichome density is inducible in *M. guttatus* (within generation plasticity) and that there is genetic variation for the extent of induction (Fig. 1, Table 2). Perhaps the most striking result of this
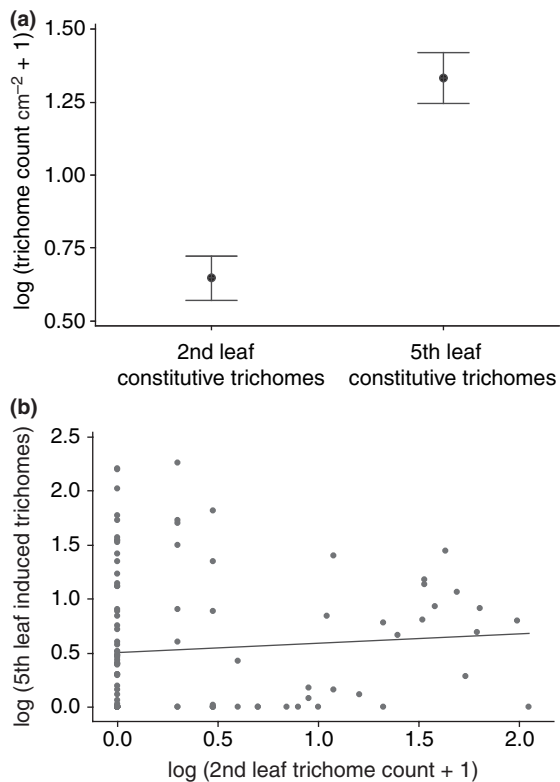
Fig. 2 (a) Mean second leaf and fifth leaf constitutive trichome densities for F3 and F4 RILs. (b) Fifth leaf induced trichomes regressed on second leaf constitutive trichomes for RILs in the F3 and F4 generations; $r^2 = 0.6\%$. Induced fifth leaf trichomes are calculated as: log [fifth leaf trichome count (treatment) + 1] – log [fifth leaf trichome count (control) + 1]. All trichome counts are per cm^2.

study is from Experiment 2, which shows that trichome induction can be maternally transmitted and that there is genetic variation in the capacity for maternal transmission. While genetic variation for induced chemical defences has been demonstrated (Agrawal *et al.*, 1999c), previous studies of trichome induction have usually failed to show genetic variation. However, this may be due to the fact that most of these experiments were not designed to detect it. Genetic variation is a prerequisite for evolution of a trait, and therefore should be a focus in studies assessing constitutive and induced defence.

The genetic variance demonstrated in this study is inter-populational. The RILs are derived from a cross between two divergent populations, the trichome dense PR and trichome depauperate IM. Not only does the IM genotype produce no trichomes constitutively, it does not respond to damage either within or between generations (Figs 1 and 3). Herbivory levels are low in the natural IM population, and it is hypothesized that they escape herbivory through rapid development. Rapid development has likely evolved as a method of drought escape in the IM population (Franks *et al.*, 2007; Holeski, 2007). The absence of trichomes in the IM population may be due to the fact that they are costly to produce or simply because selective pressures have not necessitated them.

In contrast, the PR population plants constitutively produce trichomes and greatly increase production in response to damage (Figs 1 and 3). Induction of plant defences (chemical or physical) has been shown to reduce subsequent herbivory in a number of field studies (Denno *et al.*, 1995 and references therein). In the present study, plants responded to leaf punctures that mimic the effect of chewing insects. Herbivores can emit chemicals that prevent or delay signaling pathways in the
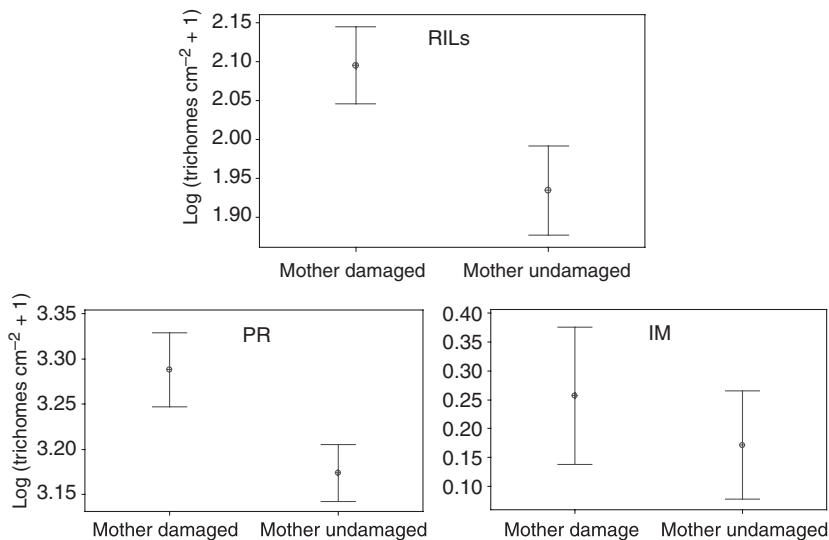

Fig. 3 Fifth leaf trichome density for parental population (PR and IM) and RIL progeny with damaged versus undamaged mothers. Differences between progeny types are significant within both the PR and RIL population. It is non-significant within the IM population. Note differences in *y*-axis scale between population plots.

Table 3 ANOVA table of factors affecting fifth leaf trichome density in the offspring of damaged or undamaged maternal plants in Experiment 2.

Source	d.f.	MS	F	*P*
Maternal treatment	1	1.052	2.79	0.096
RIL	79	4.310	11.42	**< 0.001**
Maternal treatment × RIL	79	0.926	2.45	**< 0.001**
Error	488	0.377		
Total	647			

Explanatory variables include the direct effects of maternal treatment (damage or no damage), direct effects RIL, and a RIL × maternal treatment interaction. Significant *P*-values are in bold type.

plant responsible for induction response (Agrawal, 1998, 1999, 2000; Schultz & Appel, 2004). However, other studies have found induction in defence traits with insect damage but no induction with simulated damage (Agrawal, 1998). Thus, the induction response to particular forms of simulated or insect damage seems to often be species/ herbivore specific (Agrawal, 1998, 1999, 2000, 2005; Traw & Dawson, 2002).

Despite the disadvantage of using simulated insect damage in these experiments, insofar as the experimental results are not directly applicable to the natural world, the use of simulated damage has several advantages. First, I was able to precisely control the quantity and location of tissue removed among plants in the treatment category. In contrast, the use of multiple insect herbivores across plants has potential to be less precise (Tiffin & Inouye, 2000; Inouye & Tiffin, 2003; but see Lehtilä, 2003). Secondly, in plant species such as *M. guttatus*, where many populations experience a number of generalist, chewing-insect herbivores (Holeski, 2007), simulated damage can be used to evaluate the effects of plant tissue loss independent of chewing actions or salivary compounds particular to a single chewing herbivore species (Inouye & Tiffin, 2003).

Plasticity and defence against herbivory

I found no relationship between the level of constitutive trichome production and the capacity for induction across Recombinant Inbred Lines (Fig. 2b). This result is inconsistent with the prediction of optimal defence theory, but is not incompatible with models of generalist/specialist herbivory trade-off. Optimal defence theory (Rhoades, 1979; Zangerl & Bazzaz, 1992; Zangerl & Rutledge, 1996) predicts patterns of defence based on the costs and benefits of defence, as well as the probability of attack. Assuming that defence carries a cost (because of the allocation of resources to the development of the trait that would otherwise be used for growth or reproduction), a negative correlation is expected between plant constitutive and induced levels of defence. To maximize resource use, plants that frequently and predictably

experience herbivory are predicted to have high constitutive levels of defence and low levels of induction. Populations subject to infrequent herbivory are predicted to have low constitutive levels of defence and higher levels of induction.

Although induction in response to damage may be dependent on a number of factors including host plant species, herbivore species and type of damage (simulated vs. insect herbivore), data from other studies utilizing both mechanical damage (Zangerl & Berenbaum, 1990; Lewinsohn *et al.*, 1991) and herbivore damage (Traw, 2002) support the predictions of optimal defence theory. In contrast, my results do not support the predictions of optimal defence theory (Figs 1 and 3), nor do the results of other studies using various plant species and types of damage (Brody & Karban, 1992; Agrawal *et al.*, 1999c; Havill & Raffa, 1999; Alpert & Simms, 2002). However, because of the limitations of simulated damage in its direct relevance to the natural world, I would advocate the use of these results as preliminary evidence, rather than conclusive rejection of optimal defence theory in its applicability to *M. guttatus*.

The generalist/specialist trade-off model suggests that variation in levels of constitutive and induced resistance in a particular trait is maintained because of differential effects of the trait on different herbivores. For example, some specialist herbivores feed with increased frequency on plants with high levels of induction (Chambliss & Jones, 1966; Da Costa & Jones, 1971; van Dam & Hare, 1998; Agrawal *et al.*, 1999c; Holeski, 2007). My results indicate such variation in constitutive and induced trichomes: trichome induction cannot be predicted by levels of constitutive trichomes (Fig. 2b). While this is consistent with the generalist/specialist trade-off model, generalist/specialist herbivores could not be attributed as a causal factor of the observed pattern without more information. Variation in levels of constitutive and induced resistance may also be explained in part by complex interactions between the resistance trait and insect pollinators or other mutualists (Agrawal & Karban, 1999).

In several plant species, resistance to herbivores changes as plants develop, although the direction of this change is variable (Price *et al.*, 1987; Kearsley & Whitham, 1989; Karban & Thaler, 1999). Several species have increased chemical or physical resistance in the juvenile stage relative to the adult (Price *et al.*, 1987; Kearsley & Whitham, 1989), whereas others have increased adult resistance relative to their juvenile condition (Karban & Thaler, 1999). Juvenile true leaves and adult true leaves usually have different patterns of cellular differentiation and are anatomically and biochemically different (Poethig, 1997; Mauricio, 2005; Donaldson *et al.*, 2006; Rehill *et al.*, 2006). Here, I show that constitutive fifth leaf (adult stage) trichome density is significantly higher than second leaf (juvenile stage) constitutive trichome density, indicating that at least

insofar as trichomes affect herbivory, resistance is higher in adult *M. guttatus* plants relative to juveniles (Fig. 2a).

Experiment 2 demonstrates transgenerational induction of trichomes (Table 3). There is also genetic variation in the capacity for this response. A well-documented example of this general phenomenon is passive acquired immunity in human infants. During pregnancy, the mother passes antibodies through the placenta to the infant, so that the infant has high levels of antibodies at birth (Saji *et al.*, 1999). Although the specific mechanism differs between the transgenerational induction in plants seen here and human acquired immunity, the general effect is similar: progeny have increased resistance against common enemies in a particular environment before they have actually encountered them.

Transgenerational induction has been shown in only one other plant species (Agrawal *et al.*, 1999a; Agrawal, 2001, 2002). Agrawal performed a series of experiments with wild radish (*R. raphanistrum*), and demonstrated that both caterpillar (*Pieris rapae*) damage, and jasmonic acid treatment to maternal plants increased progeny resistance relative to control plants. Hydroxylated glucosinulate concentration increased in the progeny of damaged plants, whereas other classes of glucosinulates declined in concentation in these progeny. In another experiment with the same plant and herbivore species, indole glucosinulates were induced significantly by maternal damage (Agrawal *et al.*, 1999c). In these experiments, Agrawal found no genetic variation for transgenerational glucosinulate induction, but did find genetic variation for transgenerational trichome induction (Agrawal *et al.*, 1999c; Agrawal, 2001).

The mechanism for transgenerational induction is not known for either *R. raphanistrum* or *M. guttatus*. Epigenetic inheritance allows organisms to respond to a particular environment through changes in gene expression (Jaenisch & Bird, 2003; Rakyan & Whitelaw, 2003). Possible mechanisms include post-translational modification of DNA or proteins through processes such as demethylation and the effects of such processes could persist across more than one progeny generation (Jaenisch & Bird, 2003; Rakyan & Whitelaw, 2003). The genomic sequence of *M. guttatus*, in combination with extensive collection of candidate genes for trichome development in *Arabidopsis* and *Antirrhinum*, may allow mechanistic studies of transgenerational plasticity in this system.

Ecological implications

In a review of almost 200 phytophagous insect species pair-wise interactions, Denno *et al.* (1995) found that host plants mediated competitive interactions more frequently than did natural enemies, physical factors, or interspecific competition. Which herbivores are affected by a particular induction response is determined by the lag time between plant damage and defence induction,

the length of time that the defence is expressed in its induced/heightened form, and by the life history of the herbivore(s). This combination of factors creates potentially complex influences of induction on arthropod community structure (Moran & Whitham, 1990; Dalin & Bjorkman, 2003; Van Zandt & Agrawal, 2004a,b; Agrawal, 2005; McGuire & Johnson, 2006).

Transgenerational induction of defence introduces a new consideration to studies of plant defence and host-mediated competitive interactions. If densities of a particular herbivore species are consistent across seasons and maternal plants experience heavy damage, inducing higher constitutive defences provides offspring with a fitness advantage. If herbivore communities vary across seasons, transgenerational induction of a defence trait could provide plant-mediated indirect competition, albeit with a longer time lag than within-generation defence trait induction. Transgenerational induction could thus alter herbivore community dynamics and species interactions on a plant genotype *across* seasons, in a manner similar to within generation induction.

Acknowledgments

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