Ant–aphid interactions on *Asclepias syriaca* are mediated by plant genotype and caterpillar damage

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The means by which plant genotypes influence species interactions and arthropod community structure remain poorly understood. One potential, but largely unstudied mechanism is that occurring through plant genetic variation in induced responses to herbivory. Here we test whether induced responses to leaf damage and genotypic variation for induction in *Asclepias syriaca* influence interactions among *Formica podzolica* ants, the ant-tended aphid *Aphis asclepiadis*, and the untended aphid *Myzocallis asclepiadis*. In so doing, we assess genetic variation in plant-mediated interactions among different herbivore guilds. We conducted a three-way factorial field experiment manipulating plant genotype, leaf damage by specialist monarch caterpillars *Danaus plexippus*, and ant presence, and documented effects on aphid and ant abundances. Leaf damage increased *Aphis* abundance in both the presence and absence of ants and *Myzocallis* abundance under ant exclusion. In the presence of ants, leaf damage decreased *Myzocallis* abundance, likely due to effects on ant–*Myzocallis* interactions; ants showed a positive association with *Myzocallis*, leaf damage increased the strength of this association (425% more ants per aphid), and this in turn fed back to suppress *Myzocallis* abundance. Yet, these aggregate effects of leaf damage on *Myzocallis* and ants were underlain by substantial variation among milkweed genotypes, with leaf damage inducing lower aphid and ant abundances on some genotypes, but higher abundances on others. As a consequence, a substantial fraction of the variation in leaf damage effects on ants ($R^2 = 0.42$) was explained by milkweed genetic variation in the strength and sign of leaf damage effects on *Myzocallis*. Although plant genetic variation influenced *Aphis* abundance, this did not translate into genetic variation in ant abundance, and leaf damage did not influence *Aphis*–ant interactions. Overall, we show that variation in induced responses to herbivory is a relevant condition by which plant genotype influences interactions in plant-centered arthropod communities and provide novel results of effects on the third trophic level.

Studies conducted over the last decade have shown that plant genotype can have a strong effect on the structure of arthropod communities (Dungey et al. 2000, Johnson and Agrawal 2005, Bailey et al. 2009, Barbour et al. 2009, Crutsinger et al. 2009). Likewise, there is a growing appreciation for the ecological and evolutionary role of intraspecific variation in plants for the outcome of biotic interactions under a multitrophic setting (Weis and Abrahamson 1986, Fritz 1995, Gassmann and Hare 2005, Mooney and Agrawal 2008). Nonetheless, the mechanisms by which plant genotypes influence biotic interactions and shape community structure at higher trophic levels remain poorly understood (Johnson 2008; reviewed by Mooney and Singer 2012).

Through induced responses to herbivory, plants may directly influence herbivores and predators, as well as indirectly mediate arthropod interactions within and between trophic levels (Agrawal 2005a, Heil 2010, Utsumi 2011). The distinction between chewing and sap-feeding herbivores is especially important in this regard, as feeding by one herbivore guild is predicted to induce resistance to future attack by the same guild, but increase susceptibility

to the opposing guild due to interference between plant resistance signaling pathways (Thaler et al. 1999, Rodriguez-Saona et al. 2005, Viswanathan et al. 2007, Zhang et al. 2009). Herbivore damage (by either guild) can also induce the attraction of predators through the production of plant volatiles or other means, and this indirect defense against herbivores can impact plant–arthropod and arthropod–arthropod interactions (Wäckers et al. 2001, Ness 2003, Kessler and Heil 2011). Therefore, by influencing herbivores and predators, plant induction has been shown to modify community structure at higher trophic levels (Bailey and Whitham 2003, Van Zandt and Agrawal 2004, Ohgushi 2005).

Genetic variation in induced plant responses to herbivory is common (Zangerl and Berenbaum 1990, Agrawal 1999, Bingham and Agrawal 2010), but its implications for higher trophic levels remain largely unexplored. While several studies have examined plant genotype and induction effects on competitive interactions between pairs of herbivores (McGuire and Johnson 2006, Smith et al. 2008, Mooney et al. unpubl.), and a few have looked at the

effect of genetic variation in constitutive plant defense on the third trophic level (e.g. parasitoids; Gols et al. 2009), relatively few have evaluated how plant genotype and induction of defenses shape multitrophic interactions or the mechanisms underlying such effects (Tamiru et al. 2011). One exception is a study by Poelman et al. (2009), which reports genetic variation in emission of parasitoid-attracting volatiles among *Brassica oleracea* genotypes. Similarly, studies on the consequences of plant genetic variation in induction for herbivore–mutualist interactions are also lacking (Savage and Peterson 2007). Given this knowledge gap, the study of genotypic variation in plant induction within a community context offers an ideal setting to mechanistically understand how plant genotype shapes arthropod interactions and diversity.

Ants, by acting as predators, mutualists or ecosystem engineers, can have substantial effects on plant and arthropod communities (Wimp and Whitham 2001, Frederickson et al. 2005). Thus, intraspecific variation in plants for ant abundance may in turn have community-wide effects. Such plant effects occur either directly, through variation in traits that attract ants (e.g. nectar; Rudgers and Strauss 2004, Ashman and King 2005), or indirectly by influencing herbivores that attract ants (Pierce 2001, Mooney and Agrawal 2008). The latter mechanism occurs in ant–hemipteran symbioses which are considered 'keystone interactions' largely due to their effects on ant abundance (Styrsky and Eubanks 2007, Wimp and Whitham 2001, 2007). Past work has shown that genetically variable plant traits can directly influence hemipteran performance (Fritz and Simms 1992, Johnson 2008) and, in so doing, indirectly influence ant abundance and arthropod community composition (Vrieling et al. 1991, Wimp and Whitham 2001, Johnson 2008, Mooney and Agrawal 2008). While herbivore-induced plant responses are known to affect ant abundance in specialized ant–plant systems (Agrawal and Rutter 1998), little is known of how plant induction following herbivory changes the nature of ant–hemipteran interactions (Ohgushi 2008) or how plant genetic variation alters such dynamics.

We have previously reported on a field experiment that measured the interactive effects of ants and common milkweed *Asclepias syriaca* genotype on the associated arthropod community (Mooney and Agrawal 2008). This work demonstrated that milkweed genotype exerted indirect genetic control over ant abundance through both direct effects on aphid abundance, i.e. plant genotypes with more aphids also had more ants (interaction chains sensu Wootton 1994), as well through effects on ant–aphid interactions, i.e. plant genotypes varied in the number of ants recruited per aphid (interaction modification, sensu Wootton 1994). Furthermore, plant effects on ants were transmitted through two aphid species, the ant-tended aphid *Aphis asclepiadis* and the untended aphid *Myzocallis asclepiadis*. This milkweed genetic variation in ant abundance was in turn negatively correlated with monarch *Danaus plexippus* caterpillar survival. Here we build upon this past work with a new field experiment in which we investigate the consequences of leaf damage by monarch caterpillars on ant–aphid interactions and whether there is plant-based genetic variation in these effects. Specifically,

we address the following questions: 1) What is the effect of leaf damage on each aphid species, and are effects contingent on ant presence? 2) Does leaf damage indirectly influence ant abundance through effects on aphid abundance and modification of ant–aphid interactions? And 3) Is there plant genetic variation for leaf damage effects on aphids and does this variation explain changes in ant abundance?

Methods

Natural history

Common milkweed *Asclepias syriaca* is a perennial herb widely distributed throughout eastern United States and Canada. Seeds from each fruit of *A. syriaca* are full siblings because of the pollination system of milkweed in which a single pollen sac (i.e. pollinium) sires all of the seeds from a flower (Gold and Shore 1995). The present work was conducted in an abandoned field near Ithaca, NY (42°30′1.44″N, 76°26′8.52″W). At this and other nearby study sites, milkweed is principally fed upon by two aphid species, *Aphis asclepiadis* and *Myzocallis asclepiadis* (hereafter *Aphis* and *Myzocallis*, respectively). In addition, the ant *Formica podzolica* (hereafter *Formica*) tends *Aphis*, while *Myzocallis* is not tended by *F. podzolica* or other ants. *Aphis nerii* also occurs at these sites (Smith et al. 2008), but only becomes abundant in mid- to late August and was thus not present during the course of this study.

Past work on this system demonstrates that the ant *Formica podzolica* (hereafter *Formica*) tends *Aphis* (i.e. it collects honeydew directly from the aphid), is positively associated with *Aphis* abundance, and that the presence of *Formica* reduces predator abundance and increases *Aphis* abundance (Mooney and Agrawal 2008, Smith 2008) or has no detectable effect on this aphid (Mooney 2011). In contrast, *Formica* does not tend or prey upon *Myzocallis*, but is positively correlated with *Myzocallis* abundance as it collectes its honeydew from leaf surfaces (Mooney and Agrawal 2008). *Formica* in turn reduces *Myzocallis* abundance (Mooney and Agrawal 2008, Smith et al. 2008), likely due to reduced *Myzocallis* fecundity or increased dispersal. While it is seemingly paradoxical that *Formica* positively correlates with, but decreases *Myzocallis* abundance, these opposing patterns are parallel to other commonly observed density-dependent associations between consumers and resources (e.g. predator and prey).

Experimental protocols

The design of this experiment is similar to that used by Mooney and Agrawal (2008). In spring 2006, we germinated milkweed seeds from each of 10 full-sib families (hereafter referred to as 'families') collected from an old field near Ithaca, NY, (42°30′1.44″N, 76°26′8.52″W, the location of our previous study). We initially grew plants in growth chambers in 500-ml pots filled with potting soil that were watered as needed and fertilized weekly for six weeks. By growing all plants for this relatively long period under hospitable conditions, we minimized maternal effects

on milkweed phenotypes (Rausher 1992). Past work with *A. syriaca* kept under such conditions showed that trait heritabilities measured in both the year of and the year after plantings were similar, suggesting limited maternal effects (Agrawal 2005b).

On 12 and 13 June 2006, plants of each family were moved to field cages and randomly assigned to one of two leaf damage treatments. Ten plants of each family were subjected to 15% leaf area consumption by monarch caterpillars *Danaus plexippus*, while 10 plants were kept as undamaged controls. For the monarch leaf damage treatment (hereafter 'damage' treatment), one to three larvae reared from locally-collected eggs were placed on each plant for between 12 to 36 h until it was visually assessed that 15% of leaf area had been removed. Control plants were maintained in the same environment as damaged plants.

On 15 June 2006, all plants were transplanted into the same field from which seeds were collected, with 12 to 16 plants each in 3 m-diameter circles around each of 15 *Formica* mounds. We excluded ants from half of these plants by burying 20 cm tall by 25 cm diameter aluminum flashing rings into the soil 5 cm deep and coating the outside surface with sticky paste. We controlled for any unintended effects of the aluminum rings by setting 15 cm tall rings loosely on the ground around control plants to allow ant access. Our experimental design was a balanced incomplete block, with ant mound as the block and plant family (10 families), leaf damage (damaged, undamaged), and ant exclusion (exclusion, control) as experimental factors. The SAS procedure OPTEX (SAS Inst.) allowed us to assign experimental treatments to each of the 200 plant positions according to the requirements of the balanced incomplete block design. We censused arthropods on each plant on 7 July, 20 July, 29 July and 14 August 2006, classifying each arthropod as *Aphis*, *Myzocallis*, or ants (all of which were *F. podzolica*). Herbivores other than the two aphid species previously mentioned were rare.

Statistical analyses

Aphids

The goal of these analyses was to evaluate whether leaf damage affected aphid abundance, as well as if such an effect was contingent on plant genotype and the presence of ants. *Aphis* and *Myzocallis* abundances were uncorrelated at both the level of plant and plant family ($R^2 = 0.009$, $p = 0.34$ and $R^2 = 0.023$, $p = 0.67$, respectively), suggesting the interactions between the aphids were weak and that the effects of ants, leaf damage and plant family acted independently on these herbivores. Because residuals for these analyses violated ANOVA assumptions of normality, generalized linear models were used as a more powerful alternative to data transformations (Bolker et al. 2009). Proc GENMOD in SAS (SAS Inst.) was used to test for effects of ant exclusion, leaf damage and plant family, as well as two-way interactions among these factors on *Aphis* and *Myzocallis* abundance per plant. The effect of ant mound was also included to control for variation among these blocks. Although these data approximated a Poisson distribution, the two models were based upon a negative

binomial distribution (log link function) because the model assuming a Poisson distribution did not converge for *Aphis*. The *Myzocallis* model that assumed a Poisson distribution did converge and provided qualitatively identical results to those using the negative bionomial distribution. Ant mound and plant family were both treated as fixed effects in these analyses. In the case of ant mound, this is appropriate as they were selected non-randomly based on size similarity. While plant family should properly be treated as a random effect (Newman et al. 1997), the validity of likelihood ratio tests for significance tests of random factors is questionable for generalized linear models (Bolker et al. 2009).

Preliminary analyses showed that ant exclusion significantly reduced ant abundance for all time points ($F \geq 27.02$, $p < 0.001$ in all cases) and repeated measures models showed that the effects of ant exclusion and leaf damage on aphids were consistent through time (non-significant time × treatment interactions: $p \geq 0.26$ in all cases). Accordingly, all analyses are based upon values per plant averaged across all censuses. Three-way interactions were excluded and type III SS were used in all cases. Means and SE are presented as descriptive statistics.

Ants

The goal of this analysis was to evaluate whether leaf damage affected ant abundance, as well as if such an effect was contingent on plant genotype. These indirect effects might occur through changes in aphid abundance, i.e. direct effect of leaf damage or family on aphid abundance which in turn indirectly influences ant abundance (interaction chains sensu Wootton 1994), as well as through changes in ant–aphid interactions, i.e. leaf damage or family influences the number of ants recruited per aphid (interaction modification, sensu Wootton 1994). Analyses of ant abundance were based upon only those plants for which ants were not excluded. Because residuals were normally distributed, Proc GLM in SAS was used to test for effects of leaf damage, plant family (treated again as fixed), *Myzocallis* abundance, and *Aphis* abundance (both covariates) and all two-way interactions on ant abundance. The inclusion of the aphid terms tests for an overall influence of aphid abundance on ant abundance. The inclusion of terms for the interactions between leaf damage or plant family and aphid abundance test for interaction modification, where the slope of ant abundance regressed on aphid abundance is modified (contingent upon) leaf damage or plant family. Ant mound was included as an additional fixed effect. Preliminary analyses showed that the magnitude of the leaf damage effect on ants was consistent across all time points (non-significant time × leaf damage interaction based on repeated measures model: $F_{6,604} = 0.76$, $p = 0.60$). Accordingly, this analysis was based upon values per plant averaged across censuses. Three-way interactions were excluded and type III SS were used in all cases. Means and SE are provided as descriptive statistics.

We further explored the mechanisms of leaf damage and plant family effects on ants using genetic correlations. We performed correlations between ant and aphid abundance using undamaged family means to test for indirect plant genetic effects on ant abundance through genetic

variation in constitutive resistance to each aphid (i.e. interaction chain effect of family on ants). To evaluate the consequences of plant genetic variation in induced responses for ant abundance via changes in aphid abundance (i.e. whether the effect of leaf damage on ants via an interaction chain varies among plant families), we regressed family mean leaf damage effect size on ants on family mean leaf damage effect size on aphids using simple linear regression, and separately for each aphid. Effect sizes were quantified as log response ratios (ln [family mean for damaged plants/family mean for undamaged plants]) using plants from which ants were not excluded.

Results

Effects of leaf damage, plant family and ants on aphids

Effects on Myzocallis

We found that the effects of leaf damage and ant exclusion interacted significantly (leaf damage × ant effect, Table 1, Fig. 1A), which can be viewed from two perspectives. First, ants had a negative effect on *Myzocallis* abundance on damaged plants ($F_{1,57} = 22.03$, $p < 0.0001$) but no effect on undamaged plants ($F_{1,62} = 0.59$, $p = 0.44$) (Fig. 1A). Second, leaf damage induced susceptibility to *Myzocallis* in the absence of ants ($F_{1,62} = 9.40$, $p = 0.002$) but resistance in the presence of ants ($F_{1,57} = 7.01$, $p = 0.008$) (Fig. 1A). These effects of ants and leaf damage were in turn underlain by significant variation among plant families. The effect of leaf damage was contingent upon plant family (leaf damage × family effect, Table 1), with plant families varying in both the magnitude and direction of changes in *Myzocallis* abundance in response to leaf damage (Fig. 2A, B), i.e. some families showed induced susceptibility while others showed induced resistance to *Myzocallis*. In addition, the effect of ants was also contingent upon plant family (ant × plant family effect, Table 1).

Table 1. Results from generalized linear models testing for main effects and interactions on *Aphis* and *Myzocallis* abundance. The two models used Proc GENMOD assuming a negative binomial error distribution. Damage = leaf damage treatment; ant = ant exclusion treatment; family = plant family; mound = ant mound. Significant effects are in bold.

| Dependent variable	Source	DF	χ^2	p-value
Myzocallis abundance	Family	9,142	26.77	**0.001**
	Damage	1,142	1.19	0.274
	Ant	1,142	8.58	**0.003**
	Damage × Family	9,142	22.12	**0.008**
	Ant × Family	9,142	16.77	**0.052**
	Ant × Damage	1,142	17.54	**<0.0001**
	Mound	14,142	83.63	**<0.0001**
Aphis abundance	Family	9,141	40.57	**<0.0001**
	Damage	1,141	7.74	**0.005**
	Ant	1,141	0.13	0.720
	Damage × Family	9,141	12.81	0.171
	Ant × Family	9,141	14.04	0.121
	Ant × Damage	1,141	0.15	0.695
	Mound	14,141	47.37	**<0.0001**
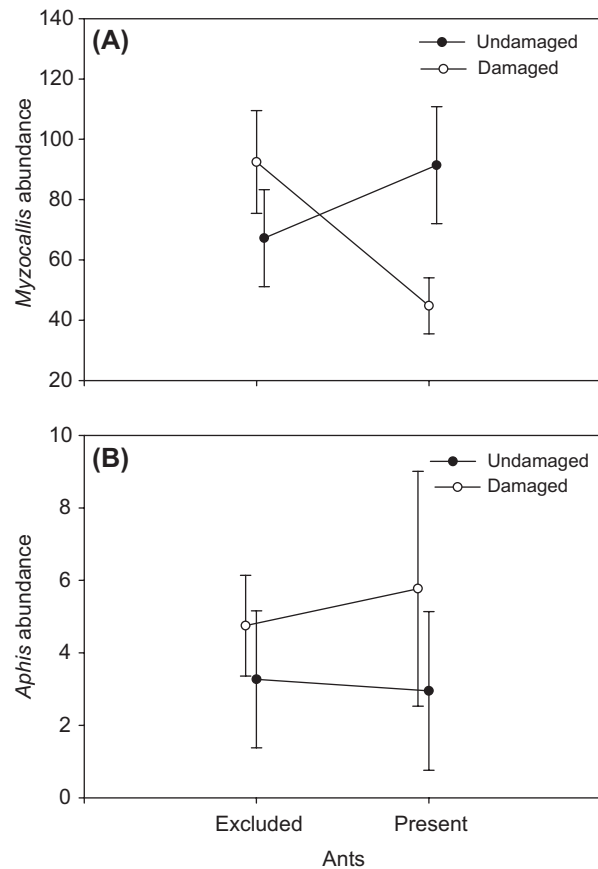
Figure 1. Effect of leaf damage on *Myzocallis* (A) and *Aphis* (B) abundances in the presence and absence of *Formica* ants. Shown are means ± SE. For *Myzocallis* (A), there was a significant interaction ($p < 0.0001$, Table 1) between the effects of leaf damage and ants. Leaf damage increased aphid abundance under ant-exclusion ($p = 0.002$) but decreased aphid abundance in the presence of ants ($p = 0.008$). Ants decreased aphid abundance on damaged plants ($p < 0.0001$) but had no effect on undamaged plants ($p = 0.44$). For Aphis (B), leaf damage increased aphid abundance ($p = 0.005$) but there was no effect of ants ($p = 0.72$) (Table 1).

Effects on Aphis

Overall, leaf damage caused a 40% increase in *Aphis* abundance (Fig. 1B), while ants did not affect *Aphis* abundance and these two effects did not interact significantly (Table 1, Fig. 1B). In addition, the effects of ants and leaf damage were not contingent upon plant family (ant × family and leaf damage × family interactions; Table 1).

Effects of plant genotype, leaf damage and aphids on ants

Effects mediated by Myzocallis

Although ant abundance was positively associated with *Myzocallis* abundance (ants = $0.042 \times$ *Myzocallis* + 0.091, $R^2 = 0.48$; *Myzocallis* effect, Table 2), this effect was contingent upon leaf damage (*Myzocallis* × leaf damage effect, Table 2). Indeed, the rate of ant recruitment (i.e. regression slope) was 425% steeper for damaged plants (ants = $0.017 \times$ *Myzocallis* − 0.094) compared to undamaged plants (ants = $0.004 \times$ *Myzocallis* + 0.35) (Fig. 3). The slope, R^2 and significance of these individual regressions is largely
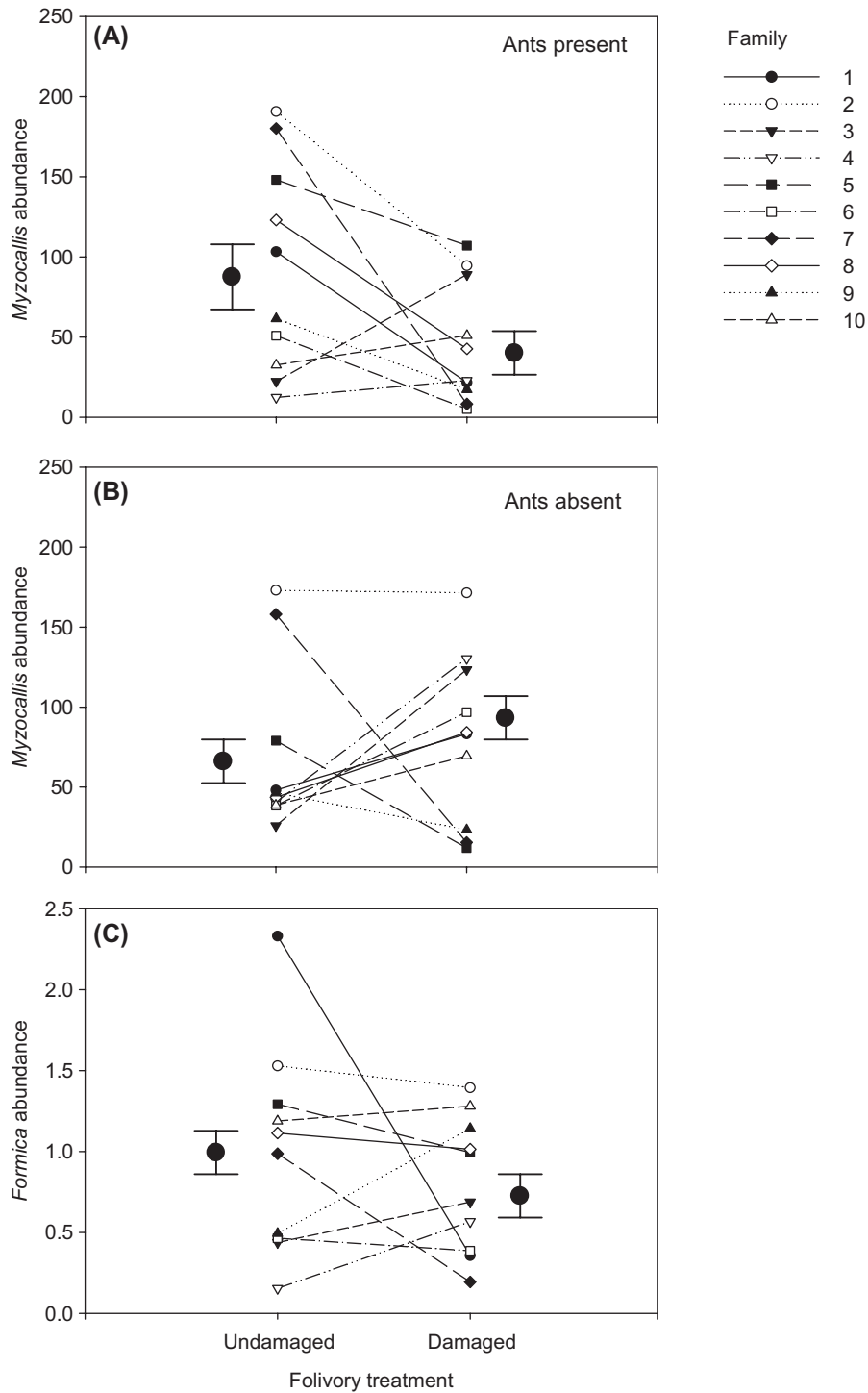
Figure 2. Plant family means for damaged and undamaged treatments for *Myzocallis* abundance with (A) and without (B) ants, as well as *Formica* (C) abundances (the latter case included only plants not excluded from ants). Overall means ± SE for each leaf damage level are presented beside family means on each side.

unchanged by the removal of the data point with the highest ant and aphid abundance, although the *Myzocallis* × leaf damage effect is no longer significant (p = 0.46). Accordingly, more ants were recruited per aphid on damaged plants, showing that leaf damage modifies the form of *Myzocallis*–ant interactions on milkweed. The net effect of these two contrasting dynamics – an increase in the rate

of ant recruitment to *Myzocallis*, but a decrease in *Myzocallis* abundance, was a lower ant abundance on damaged than undamaged plants (Fig. 2C) because of the simultaneous decrease in *Myzocallis* abundance with leaf damage in the presence of ants (Fig. 1A).

In agreement with our previous work (Mooney and Agrawal 2008), we found a positive genetic correlation

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Table 2. Results from a generalized linear model used to test for main effects and interactions on *F. podzolica* abundance. The model was performed in Proc GLM and assumed a normal error distribution. Damage = leaf damage treatment; ant = ant exclusion treatment; family = effect of plant family; mound = ant mound. Significant effects are in bold.

Source	DF	F-value	p-value
Family	9,89	1.47	0.198
Damage	1,89	11.41	**0.001**
Damage × Family	9,89	2.67	**0.017**
Myzocallis	1,89	43.96	**<0.0001**
Aphis	1,89	0.17	0.680
Myzocallis × Family	9,89	1.76	0.111
Aphis × Family	8,89	1.65	0.147
Myzocallis × Damage	1,89	26.03	**<0.0001**
Aphis × Damage	1,89	0.01	0.967
Mound	14,89	2.72	**0.008**

Figure 4. (A) Genetic correlation between plant family undamaged mean abundance for *Myzocallis* and *Formica* and (B) relationship between *Formica* and *Myzocallis* caterpillar leaf damage effect sizes. For panel B, values plotted are effect sizes for each plant family calculated as ln[damage mean/undamaged mean] for each family. Numbers next to each dot represent plant families.

between ant and *Myzocallis* abundance (ants = 0.007 × *Myzocallis* + 0.44, R^2 = 0.54; Fig. 4A), thus documenting an indirect effect of plant genetic variation for aphid abundance on ants. Contrary to our past study, however, plant families did not vary significantly in the rate of ant recruitment to *Myzocallis* (*Myzocallis* × family effect, Table 2). Nonetheless, leaf damage effects on ants varied among plant families (leaf damage × plant family effect, Table 2), with some families showing an increase and others a decrease in ant abundance due to leaf damage (Fig. 1C). Much of this plant genetic variation in leaf damage effects on ants could in turn be traced to parallel variation in effects on *Myzocallis*: Plant family means for leaf damage effect size for ants were positively associated with leaf damage effect sizes for *Myzocallis* (p = 0.04, Fig. 4B). Hence, leaf damage indirectly influenced ant abundance through effects on *Myzocallis* abundance, but the magnitude and direction of this indirect effect varied among plant families.

Effects mediated by *Aphis*

Contrary to findings for *Myzocallis*, variation in *Aphis* abundance did not influence ant abundance (*Aphis* effect, Table 2), and the rate of ant recruitment to *Aphis* was not
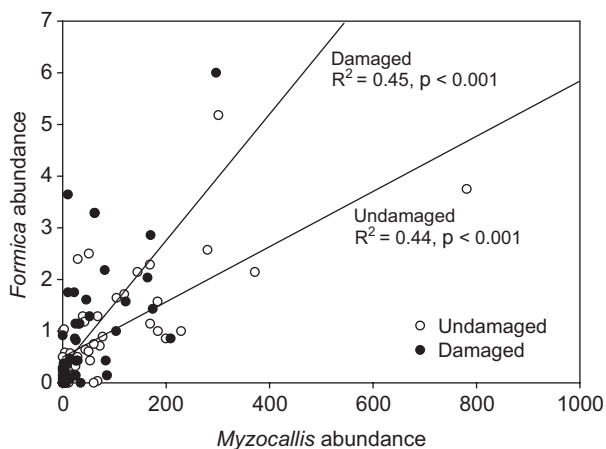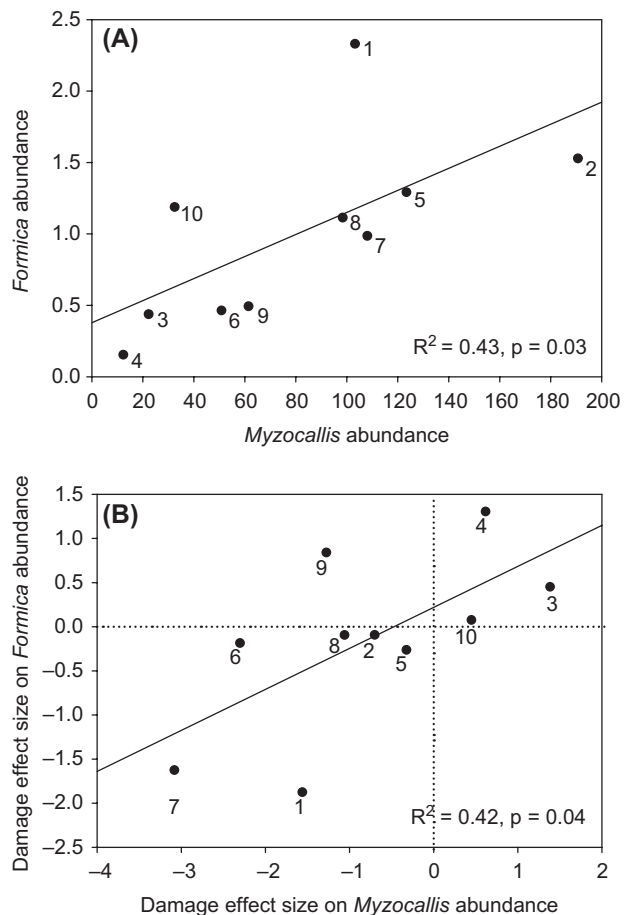

Figure 3. Relationship between *Myzocallis* abundance and *Formica* abundance for damaged and undamaged milkweed plants. R^2- and p-values reported are from simple regression models performed separately for damaged and undamaged plants.

influenced by leaf damage (*Aphis* × leaf damage effect, Table 2), nor did it vary among plant families (*Aphis* × family effect, Table 2). In addition, the genetic correlation between *Aphis* and ants was not significant (R^2 = 0.004, p = 0.86) and we did not find a relationship between family leaf damage effect sizes for ants and *Aphis* (R^2 = 0.12, p = 0.31) i.e. no influence of leaf damage on ant abundance via changes in *Aphis* abundance across plant families.

Discussion

Leaf damage consistently increased *Aphis* abundance (i.e. in both the presence and absence of ants; Fig. 1), but this did not translate into an indirect effect on ant abundance. In contrast, leaf damage increased *Myzocallis* abundance only in the absence of ants (Fig. 1). In the presence of ants, leaf damage decreased both *Myzocallis* and ant abundance, likely due to effects on ant–*Myzocallis* interactions; leaf damage increased the number of ants recruited per *Myzocallis* by 425% (i.e. Fig. 3 change in slopes) and ants had a negative effect on *Myzocallis* only on damaged plants (Fig. 2). Our results thus suggest a feedback, where

leaf damage increased ant recruitment to *Myzocallis*, which in turn decreased *Myzocallis* abundance. Such opposing effects of leaf damage on ant–*Myzocallis* interactions – increasing the attractiveness but decreasing the abundance of *Myzocallis* – resulted in a net decrease in ant abundance.

The aggregate effects of leaf damage on *Myzocallis* and ants were in turn underlain by substantial variation among milkweed genetic families. While plant families varied in resistance to both aphids, only for *Myzocallis* did such effects translate into indirect plant genetic effects on ant abundance. Similarly, there was milkweed genetic variation in damage-induced effects on *Myzocallis* but not *Aphis*, with leaf damage inducing *Myzocallis* resistance on some genotypes, but inducing susceptibility on others. Plant genetic variation for induced effects on *Myzocallis* in turn translated into parallel effects on ants; those plant families with induced resistance to *Myzocallis* had fewer ants following leaf damage, while those with induced susceptibility had more. In summary, plant genetic variation in both constitutive and induced resistance to *Myzocallis* resulted in strong linkages between leaf damage, milkweed genetic family, and ant abundance.

Effects of leaf damage on ant–aphid interactions

Results from this work indicated that leaf damage can lead to induced susceptibility to aphids in *A. syriaca*. Specifically, damaged plants showed induced susceptibility to *Aphis*, and *Myzocallis* showed a similar pattern in the absence of ants. These findings are consistent with the emergent understanding of cross-talk between jasmonate and salicylate plant resistance signaling pathways (van Dam 2009), where induction of resistance by chewing herbivores suppresses what would otherwise be induced resistance to subsequent sap-feeding herbivores (and vice versa). In the present case, monarch leaf damage may suppress salicylate responses in milkweed (Agrawal unpubl.), potentially inducing susceptibility to aphids. Accordingly, previous studies with tomato *Solanum lycopersicum* and wild cabbage *Brassica oleracea* have shown parallel positive effects of sap-feeders on chewing herbivores (Rodriguez-Saona et al. 2005, Soler et al. 2012).

Interestingly, the effects of leaf damage on *Myzocallis* were contingent upon ant presence. Leaf damage caused induced susceptibility to *Myzocallis* on plants without ants, while a reduction in this aphid's abundance was observed when ants were present. The mechanism behind this result was likely an increase in ant recruitment to *Myzocallis* on damaged plants (i.e. modification of ant–*Myzocallis* interaction driven by damage; Fig. 3), followed by a negative effect of this increase in ant abundance on the aphid. The increase in ant recruitment can be seen in the steeper slope for the relationship of ant abundance regressed on *Myzocallis* for damaged than undamaged plants (Fig. 3). Thus, even though ants were also positively associated with *Myzocallis* on undamaged plants, they had a negative effect on the aphid in association with this damage-induced increase in ant recruitment, which apparently crosses some threshold where ant effects become negative. This negative effect was probably due to elicitation of aphid dispersal or reduced fecundity, as predation by ants has not been

observed during several hundred of hours of observation (Mooney and Agrawal 2008, Smith et al. 2008). We propose that monarch leaf damage makes *Myzocallis* more attractive to ants, presumably through changes in the quantity or quality of aphid honeydew secretions (Volkl et al. 1999, Woodring et al. 2004; but see Hembry et al. 2006) which ants collect from from leaves beneath aphid colonies (Mooney and Agrawal 2008).

Plant genetic variation in effects of leaf damage on ant–aphid interactions

Consistent with previous findings in this system (Mooney and Agrawal 2008), plant families varied substantially in resistance to both aphid species, and plant genotypic variation for constitutive resistance to *Myzocallis* translated into an indirect genetic effect on ant abundance (Fig. 4A). Building on this result, we also demonstrate an effect of leaf damage on *Myzocallis* abundance that was contingent upon plant family, with some families showing induced susceptibility and others induced resistance. This latter finding contributes to the growing body of evidence for high levels of genetic variation in induced resistance to insect herbivores (Agrawal 1999, Underwood et al. 2000).

While our results for *Myzocallis* were relatively consistent with our previous study, those for *Aphis* differed substantially. Contrary to our previous findings (Mooney and Agrawal 2008, Smith et al. 2008), we found no effect of ants on *Aphis*. In contrast, ants had a negative effect on, and were positively associated with *Myzocallis* in both this study and past studies (Mooney and Agrawal 2008, Smith et al. 2008). These contrasting results highlight two relevant points: First, the context-dependency of ant–aphid interaction outcomes, which appears to be common for different types of mutualism (Bronstein 1994); and second, that the outcomes of ant–aphid interactions can be highly dependent on the identity of the interacting species, with ant–*Myzocallis* interactions being more temporally consistent in our study system. In turn, such variation in ant–aphid interactions will have relevant implications for plant genotype indirect effects on ants transmitted by aphids. In our previous study (Mooney and Agrawal 2008), we found that plant genotype effects on ants were transmitted mainly via *Aphis* while here we found this was not the case. Instead, milkweed genotype effects on ants will presumably be more predictable when trasmitted via *Myzocallis* rather than *Aphis*.

Although a few studies have tested for plant genetic variation in induction effects on multiple herbivores (McGuire and Johnson 2006, Manzaneda et al. 2010), this study is the first, to our knowledge, to investigate the cascading consequences of such effects for higher trophic levels, in particular mutualists of herbivores. Here we found that milkweed genotype differences in response to leaf damage for *Myzocallis* abundance were correlated with genotype responses to leaf damage for ant abundance. Specifically, those families for which monarch leaf damage resulted in induced resistance to *Myzocallis* also showed a reduction in ant abundance in response to damage, while an increase in ant abundance due to leaf damage was observed for families which showed induced susceptibility to this aphid (Fig. 4B). This finding represents a novel contribution to

the understanding of the mechanisms by which plant genotypes may vary in their arthropod communities, as well as the consequences of genetic variation of induced responses for higher trophic levels.

Because milkweed genetic variation for resistance to *Myzocallis* resulted in an indirect effect of plant genotype on ant abundance, this may lead to variable outcomes of selection for plant resistance under a multitrophic setting. For instance, if aphids carry a fitness cost to milkweed, as has been reported in other systems (Minks and Harrewijn 1998, Renault et al. 2005), then selection would work against plant genotypes which exhibit constitutively low resistance and induced susceptibility to aphids in response to leaf damage. However, aphids also recruit predatory ants which provide protection against other potentially more damaging herbivores (Styrsky and Eubanks 2007, 2010), selecting for plant genotypes with constitutively low resistance or induced susceptibility to aphids (see also Stenberg et al. 2011 for example on ecological tradeoffs in direct and indirect plant defense). Milkweed genotypes may thus be found at different points along a continuum of tradeoffs between negative effects of aphids and positive effects of ants, contingent upon presence and impacts of other herbivores (Styrsky and Eubanks 2007, 2010).

Conclusions

We have shown that both plant genotype and leaf damage influence the abundance of sap-feeding herbivores, and that such direct effects in turn indirectly modulate the abundance of predatory ants associated with *A. syriaca*. Moreover, by evaluating the effects of milkweed genotype, we show that the effects of induction on the second and third trophic level are contingent on plant genotypic variation in both constitutive and induced resistance to herbivory. These findings provide evidence of and contribute to the understanding of the mechanisms by which plant genotypes influence multitrophic interactions and arthropod community structure.

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