SHORT COMMUNICATION

# Induced defences alter the strength and direction of natural selection on reproductive traits in common milkweed

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## Abstract

Evolutionary biologists have long sought to understand the ecological processes that generate plant reproductive diversity. Recent evidence indicates that constitutive antiherbivore defences can alter natural selection on reproductive traits, but it is unclear whether induced defences will have the same effect and whether reduced foliar damage in defended plants is the cause of this pattern. In a factorial field experiment using common milkweed, *Asclepias syriaca* L., we induced plant defences using jasmonic acid (JA) and imposed foliar damage using scissors. We found that JA-induced plants experienced selection for more inflorescences that were smaller in size (fewer flowers), whereas control plants only experienced a trend towards selection for larger inflorescences (more flowers); all effects were independent of foliar damage. Our results demonstrate that induced defences can alter both the strength and direction of selection on reproductive traits, and suggest that antiherbivore defences may promote the evolution of plant reproductive diversity.

## Introduction

Understanding the ecological and evolutionary processes that have generated the astounding diversity of plant reproductive traits is an important and long-standing problem in evolutionary biology (Barrett, 2010). Although plant–pollinator interactions have played a major role in this diversification (Stebbins, 1970; Harder & Barrett, 2007), it is increasingly apparent that both herbivores and antiherbivore defences have also contributed to the evolution of plant reproductive diversity (Galen, 1999; Irwin *et al.*, 2004; Armbruster *et al.*, 2009; Hanley *et al.*, 2009; Thompson & Johnson, 2016). Although we are beginning to understand how herbivores can directly select on reproductive traits (Strauss & Whittall, 2006; Carr & Eubanks, 2014), we know relatively little about the ecological mechanisms underpinning the evolutionary interactions between plant defence and reproduction (Campbell, 2014; Johnson *et al.*, 2015). Here, we test whether

induced responses associated with chemical and physical defence traits (hereafter 'induced defences' *sensu* Karban & Baldwin (1997)) alter natural selection on plant reproductive traits.

Antiherbivore defences may alter natural selection on plant reproductive traits through two nonmutually exclusive mechanisms. First, allocation trade-offs between defence and reproduction may cause investment in defences to directly reduce reproductive fitness. Such a trade-off could drive selection for increased allocation into reproduction in favour of reduced allocation into defence, and vice versa (Thompson & Johnson, 2016). Numerous studies have shown that induced defences can change reproductive trait expression (e.g. Hoffmeister *et al.*, 2016) and fitness (e.g. Schuman *et al.*, 2012), but none have tested whether induced defences alter selection on reproductive traits. Second, because herbivores and pollinators both exert selection on reproductive traits, if defences alter interactions with either of these selective agents, then phenotypic variation for defence could be associated with patterns of natural selection on reproductive traits (Thompson & Johnson, 2016). Although there are theoretical reasons to expect that antiherbivore defences can alter natural

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selection on plant reproductive traits (Johnson *et al.*, 2015), there is currently little empirical evidence to support this hypothesis.

A previous study demonstrated that genetic variation in constitutive defences (cyanogenesis within *Trifolium repens*) can alter natural selection on reproductive traits (Thompson & Johnson, 2016), but it is unclear whether induced defences can have similar effects. Although both constitutive and induced defences increase fitness in the presence of herbivores, they differ in that constitutive defences are always present whereas induced defences increase following interactions with herbivores (Karban & Baldwin, 1997). Several studies have shown that, like constitutive defences, induced defences can reduce reproductive fitness through allocation trade-offs (Strauss, 1997; Agrawal *et al.*, 1999; Lucas-Barbosa, 2015) and can alter both floral herbivory and pollination (Heil, 2002; Strauss *et al.*, 2002; McCall & Karban, 2006; Kessler *et al.*, 2011). These observations collectively suggest that induced defences might alter natural selection on reproductive traits.

Despite the similarities between constitutive and induced defences, they have several important differences that preclude the assertion that both types of defence should similarly alter natural selection on reproductive traits. Constitutive and induced defences may differ in their associated costs, because constitutive defences are always expressed, whereas induced defences are expressed only in response to specific stimuli (Karban & Myers, 1989). Alternatively, induced responses to herbivory can trigger 'reproductive escape' whereby plants drastically increase investment into reproduction (Lucas-Barbosa *et al.*, 2013). Constitutive and induced defences may also have different effects on pollination and herbivory. Induced responses can change floral morphology and scent (Hoffmeister *et al.*, 2016), trigger the release of volatiles that attract parasitoids of herbivores (Schuman *et al.*, 2012; Gols *et al.*, 2015) and have unique effects on herbivore population dynamics and distributions (Underwood & Rausher, 2002; Underwood *et al.*, 2005; Rubin *et al.*, 2015). Because induced and constitutive defences may differ in their associated costs, and their effects on pollination and herbivory, they may differ in how they influence selection on reproductive traits. For example, relative to constitutive defences, induced defences may weakly influence selection on reproductive traits if they are less costly or have a smaller effect on resistance to herbivory. Given the differences between induced and constitutive defences, experiments that manipulate induced defence are needed to advance our general understanding of how defences alter natural selection on plant reproductive traits.

When investigating the phenotypic consequences of induced defences, it is essential to decouple the effects of tissue damage caused by herbivores from the effects of a change in defensive phenotype. This is because herbivory has two main effects on plants: first, it removes tissue that could otherwise be used in photosynthesis; second, it frequently induces chemical and physical defences. Thus, in natural systems where foliar damage induces defences, it can be difficult to attribute effects to one or the other. With respect to defence-mediated changes in selection, it is especially important to control for foliar damage, because as with defence, foliar damage can affect allocation to reproduction and attractiveness to pollinators (Strauss *et al.*, 1996). The independent effects of leaf tissue removal and induced defences can be decoupled by applying chemicals to induce the defences of a plant, and by removing leaf tissue mechanically without inducing defences. Experimental decoupling of defence and foliar damage is biologically meaningful because species can possess genetic variation for induced responses to a given amount of herbivory (Agrawal *et al.*, 2002), such that the degree to which foliar damage and defence covary differs among genotypes. In addition, there are cases where foliar herbivory does not up-regulate defences (Karban & Baldwin, 1997), and where defences are induced in the absence of damage (e.g. by volatiles; Heil & Karban (2010)). By independently manipulating foliar damage and induced defences, it becomes possible to quantify their respective contributions to defence-mediated changes in selection on reproductive traits.

Here, we report the results of a field experiment designed to test whether induced plant defences and simulated foliar damage alter natural selection on reproductive traits in common milkweed, *Asclepias syriaca* L. (Apocynaceae). Our specific research question was: Do induced plant defences alter natural selection on reproductive traits independently of foliar damage? We hypothesized that induced defences would alter selection on reproductive traits via resource allocation trade-offs between defence and reproduction, or by altering patterns of pollination and/or herbivory. Evaluating whether induced defences alter selection on reproductive traits will advance our understanding of the ecological mechanisms driving evolutionary interactions between plant defence and reproduction.

## Materials and methods

### Study system

Common milkweed, *Asclepias syriaca*, is a perennial plant native to eastern North America (Uva *et al.*, 1997). Plants reproduce vegetatively via rhizomes and sexually via highly modified flowers (Fig. S1) arranged into umbels (Kephart, 1983). Five nectar-filled hoods surround the fused male and female reproductive organs. The corolla is reflexed and obscures the calyx (Fig. S1). Rather than individual pollen grains, milkweed pollen are aggregated within highly derived

structures called pollinia. Pollinia are removed in pairs from flowers as they attach to insect legs and tongues.

Milkweeds (*Asclepias* spp.) are well known for their defensive mechanisms. The common name, milkweed, derives from the thick white latex that exudes from laticifers following mechanical damage to tissues, and it functions by physically impeding herbivores (Dussourd & Eisner, 1987; Agrawal & Konno, 2009). Milkweeds also contain cardenolides, which disrupt sodium–potassium regulation in cells and are toxic to most animals (Malcolm, 1991; Dobler *et al.*, 2012; Zhen *et al.*, 2012). Production of these defences is up-regulated following attack by herbivores, which subsequently reduces herbivory (Van Zandt & Agrawal, 2004). Induction of cardenolides and latex in milkweeds is regulated by the steroid hormone, jasmonic acid (hereafter 'JA') (Agrawal *et al.*, 2012), and exogenous JA application up-regulates latex and cardenolide production (Rasmann *et al.*, 2009; Agrawal *et al.*, 2012). Milkweed defence traits exhibit heritable variation within populations (Agrawal, 2004), are subject to selection by specialist herbivores (Agrawal, 2005b) and have diverged among species in the genus (Agrawal & Fishbein, 2008; Agrawal *et al.*, 2009). We refer to induction of cardenolides and latex following herbivory or JA application as an induced 'defence' because of the substantial evidence that these traits are involved in defence against herbivores.

## Experimental design

To determine whether induced defences alter selection on plant reproductive traits independently of foliar damage, we manipulated both induced defences and foliar damage in a 2 × 6 fully factorial field experiment (i.e. 12 treatment combinations) using 156 naturally occurring milkweed plants (13 plants/treatment) on and around the University of Toronto Mississauga campus, (approx. 1.25 km$^2$; 43.548° N, 79.663° W; 120 m a.s.l.). We only included plants with no herbivore damage, and neighbouring plants were separated by at least 1.5 m to reduce the likelihood of sampling the same genet twice (La Rosa, 2015). We covered all plants during the second week of June 2014 with spun polyester bags (Pro 17; Agrofabric, Alpharetta, GA, USA) and tied the bags at the top and bottom of each plant to prevent access by insects (Fig. S1). As plants matured and formed flower buds, we tied the upper portion of bags below the lowest buds (Fig. S1); this allowed for flower visitation while protecting vegetative parts from herbivores.

We experimentally manipulated induced plant defences using a treatment with two levels (the 'induction' treatment). Plants were either assigned to a control group with no induction ('control' plants), or to a treatment group where we used the exogenous application of JA to induce defences ('JA-induced' plants). We

prepared a 0.5 mM JA solution by dissolving 100 mg JA (Sigma-Aldrich #77026-92-7) in 2 mL of 99.5% acetone and diluting with 950 mL of distilled water; we also prepared a control solution that lacked dissolved JA but was otherwise identical to the treatment solution (Thaler *et al.*, 1996). This method has been used previously to induce defences in *A. syriaca* (e.g. Mooney *et al.*, 2008). We applied either the JA or control solution to each plant by spraying every fully expanded leaf once (ca. 1 mL leaf$^{-1}$) during each of three applications spaced at 2-week intervals (13 and 27 June, 11 July); the first application occurred while plants were still undergoing vegetative growth and before any plants had flowered. At the time of initial treatment application, mean plant height was 65.4 ± 14.4 [SD] cm and mean leaf number was 11.49 ± 2.43. We used intervals of 2 weeks to apply treatments because JA-induced chemical changes are maintained in other systems for at least 14 days (Thaler, 1999).

To decouple the effects of chemically induced defences and foliar damage, we imposed a leaf tissue removal treatment (hereafter the 'damage' treatment) that had six levels. We imposed our treatment by cutting each leaf on a plant with scissors to remove 0, 10, 20, 30, 40 or 50% of leaf tissue, which encompasses the natural range of damage that *A. syriaca* plants typically experience (Turcotte *et al.*, 2014). We imposed the damage treatment with multiple levels, so we could detect any linear or nonlinear effects of leaf removal on selection. Leaves were cut once at the start of the experiment, concurrently with the initial application of the induction treatment. We used scissors rather than herbivores because we explicitly wanted to determine the effects of defoliation in the absence of induced defences; clipping with scissors should not have caused an induced response because of an absence of herbivore saliva, which frequently elicits JA (Agrawal *et al.*, 1999; Musser *et al.*, 2002; Rodriguez-Saona *et al.*, 2002). This rationale is supported by data from Mooney *et al.* (2008), who found that exogenous JA application – but not mechanical foliar damage – up-regulated the production of induced defences in *A. syriaca* in ways that were similar to the effects of monarch (*Danaus plexippus*) caterpillar herbivory.

We measured several traits during our experiment. During initial treatment application, we measured plant height and counted leaves and inflorescence buds. We recorded the first date of flowering for each plant and measured several aspects of flower size: hood length and width, petal length and width, and the length and width of entire flowers (see Fig. S1). We measured these traits to the nearest 0.5 mm on up to five flowers from the lowest three inflorescences, or all inflorescences for plants with ≤3 inflorescences. To obtain mean values of these measurements for individual plants, we averaged all flower size measurements within inflorescences, and then across inflorescences.

We counted the number of flowers within the inflorescences used for floral measurements and refer to the mean number of flowers within inflorescences hereafter as 'inflorescence size'. In addition, we recorded the total number of inflorescences that each plant initiated, hereafter referred to as 'inflorescence number'. We harvested the aboveground vegetative biomass of each plant into paper bags at the end of the experiment. After drying plants for 72 h at 50 °C, we removed leaves and weighed the stem tissue as a measure of aboveground biomass. Leaves were removed to avoid including direct treatment effects (mass lost due to leaf tissue removal), or confounded variation in leaf abscission between treatments, in our measure of biomass.

We recorded estimates of both male and female fitness. For male fitness, we counted the number of pollinia removed from five flowers from the lowest three inflorescences, or all inflorescences for plants with ≤3 inflorescences. Pollinia removal can be accurately determined on flowers over their entire lifetime, including on senescing flowers. We took the mean number of pollinia removed from each inflorescence and averaged that among inflorescences to estimate male fitness. Two studies have quantified the relationship between pollinia removal and seeds sired in milkweeds using genetic markers and found them to be significantly correlated ($r_{Pearson}$ range: 0.44–0.47) (Broyles & Wyatt, 1990; La Rosa, 2015). These studies indicate that a large fraction of pollinia removed by pollinators do not sire seeds. Thus, our measure of male fitness represents the upper limit of an individual's siring success; we acknowledge that this metric is an imperfect estimate of true male fitness, and a more accurate estimate would require molecular markers and exhaustive sampling of the population. As an estimate of female fitness, we harvested ripe fruits and counted the number of viable seeds. We did not include measures of fitness related to vegetative reproduction because *A. syriaca* is a long-lived plant, and it is difficult to determine the belowground rhizome connections between ramets. Nevertheless, this did not affect our ability to effectively address our research questions about whether induced defences and herbivory alter selection on reproductive traits.

### Statistical analysis

Before analysis, we assessed whether our data met assumptions of normality and homogeneity of variance. We transformed some traits to meet the assumptions of our analyses (Table S1). We calculated pairwise correlations between all measured traits to test for multicollinearity. The six flower size traits were positively correlated (mean $r_{Pearson}$ = 0.62), so we collapsed them into two principal components subject to 'varimax' rotation using the R package 'psych' (Revelle, 2015). The first principal component (PC1) was highly positively correlated with the three flower width measurements

(hereafter *floral width PC*), and the other principal component (PC2) was positively correlated with the three flower length measurements (hereafter *floral length PC*); PC1 and PC2 explained 68% and 14% of variation in the data, respectively (Table S2). All pairwise correlations between remaining traits were <0.7 after collapsing floral measurements, except between inflorescence number and biomass ($r_{Pearson}$ = 0.78); we retained both variables in spite of this correlation because of an *a priori* interest in analysing differences in defence-mediated changes in selection on both of these traits (Tables S3 and S4). We also calculated coefficients of phenotypic variation as $CV_p = 100(V_p^{0.5}/\mu_i)$, where $\mu_i$ is the untransformed mean of trait $i$ (Houle, 1992). For all subsequent analyses, we standardized traits to a mean of 0 and a standard deviation of 1.

We used univariate ANOVA to test whether the induction and damage treatments affected the expression of phenotypic traits and fitness components. This allowed us to determine whether there were any allocation costs associated with defence, and to evaluate the phenotypic effects of leaf damage. If damage or induced defences directly change the expression of a reproductive trait, this could alter selection on the trait.

To determine whether JA induction altered natural selection on plant reproductive traits independently of foliar damage, we used multivariate phenotypic selection analyses (Lande & Arnold, 1983). We first calculated relative fitness ($w_i$) across all plants, standardized trait values, and then ran a linear model regressing relative fitness values against the main effects of each treatment (induction and damage), each phenotypic trait, all trait × induction interactions and all trait × damage interactions. Induction was coded as a categorical variable with two levels, and damage was a continuous variable with six levels. The model took the following form:

$$
\begin{aligned}
w_i = {} & \text{intercept} + \text{induction treatment} \\
& + \text{damage treatment} + \text{floral width PC} \\
& + \text{floral length PC} + \text{first flower} \\
& + \text{inflorescence number} + \text{inflorescence size} \\
& + \text{biomass} + \text{trait} \\
& \times \text{induction treatment interactions} + \text{trait} \\
& \times \text{damage treatment interactions} + \text{error.}
\end{aligned}
$$

Three-way trait × induction treatment × damage treatment terms and nonlinear selection coefficients were included in preliminary models, but all were nonsignificant ($P > 0.5$) and are not reported. Significant main effects for treatments indicate that the treatment influenced fitness, and significant main effects for traits indicate that the trait was under directional selection. A significant trait × treatment interaction indicates that the treatment altered directional selection on the trait (i.e. the confidence intervals on selection gradients for different treatment levels are nonoverlapping).

We analysed multivariate selection models using a multimodel selection procedure implemented using the *dredge* function in the R package 'MuMIn' (Barton, 2015). This procedure tests models that span all possible combinations of variables (except for models containing interactions without the corresponding main effects). We took weighted averages from all models within 2 ΔAIC of the best model – where better fitting models are weighted more heavily – to determine final model-averaged parameters. The best model had 12 variables, and statistically equivalent models (within 2 ΔAIC) had between 7 and 14 variables. We interpreted the 'full' model output from the analysis because parameter estimates in the 'conditional' output are biased away from zero; thus, the 'full' model estimates are more conservative (Barton, 2015). We note that the general conclusions of the present study do not change when the multivariate selection model is analysed using standard multiple regression (see R script).

Because induced defences altered selection on traits (see **Results**), we quantified patterns of selection on JA-induced and control plants separately. We restrict these analyses to traits where we detected significant trait × induction treatment interactions. To accomplish this, we analysed linear models that included all terms from the dredge model with the lowest AIC (i.e. the best-fitting individual model; see Table 1) and extracted residuals for fitness and trait values using the approach for partial variance in multiple regression analyses as explained by SAS (SAS Institute Inc, 2009). Specifically, we extracted fitness residuals from the model after removing both the focal trait and the focal trait × induction treatment interaction term. We then extracted trait residuals from an identical model with the standardized trait as the response variable rather than relative fitness. Regression of fitness residuals on trait residuals estimates the selection gradient on the focal trait while controlling for other measured traits. We partitioned the data across treatments and then analysed linear models of fitness residuals on trait residuals to estimate the selection gradient in each treatment separately. All data were analysed using R v.3.0.3. (R Core Team, 2014), and all R code used to generate results and figures is available online.

## Results

### Treatment effects on traits and fitness

We did not detect direct effects of the induction treatment or the damage treatment on the expression of trait values or fitness in our univariate analyses (Table S5). There were nearly significant effects of the induction treatment on inflorescence size (19% larger in JA-induced plants; $P = 0.07$) and biomass (10% smaller in JA-induced plants; $P = 0.10$). In our multivariate selection model, which accounted for covariance among traits, we detected a significant effect of JA induction on fitness, where JA-induced plants produced 20% fewer seeds than control plants ($\beta = -0.51 \pm 0.20$ [SEM], $P = 0.012$) (Table 1). We did not detect an effect of damage on fitness in our multivariate analysis.

### Population-wide patterns of natural selection

We observed natural selection through female fitness, but not male fitness, in our multivariate selection analyses (Table 1). At the population level, there was significant directional selection for greater inflorescence size ($\beta = 0.32 \pm 0.16$, $P = 0.047$) and biomass ($\beta = 0.57 \pm 0.16$, $P < 0.001$) via female fitness. We found no evidence that selection was operating via male fitness on any trait main effect or trait × treatment interaction (Table 1), despite large variation in pollinia removal ($CV_p = 98.8$; Table S1).

### Defence-mediated changes in natural selection

Phenotypic selection analyses revealed that the induction treatment altered the strength and direction of natural selection on plant reproductive traits via female fitness (Table 1; Fig. 1). Specifically, JA-induced plants experienced selection for more inflorescences ($\beta_{\text{JA-induced – inflorescence number}} = 2.81 \pm 0.98$, $P = 0.003$), whereas selection in control plants was nonsignificant ($\beta_{\text{control – inflorescence number}} = -0.60 \pm 0.58$, $P = 0.312$); the slopes were significantly different between the two treatments (inflorescence number × induction: $P = 0.001$). JA-induced plants also experienced selection for smaller inflorescences ($\beta_{\text{JA-induced – inflorescence size}} = -1.19 \pm 0.48$, $P = 0.017$), whereas control plants experienced a trend towards opposing selection for larger inflorescences ($\beta_{\text{control – inflorescence size}} = 0.74 \pm 0.38$, $P = 0.056$); these slopes also differed between treatments (inflorescence size × induction: $P = 0.004$). Finally, we observed significant positive selection on biomass in control plants ($\beta_{\text{control – biomass}} = 1.47 \pm 0.42$, $P < 0.001$), but found no evidence for selection on biomass in JA-induced plants ($\beta_{\text{JA-induced – biomass}} = 0.17 \pm 0.37$, $P = 0.645$); as above, these slopes were significantly different between treatments (biomass × induction: $P = 0.033$) (Fig. S1). No trait × damage treatment interactions were significant.

## Discussion

Studies seldom consider antiherbivore defences when investigating the evolution of plant reproductive diversity. Recent research, however, highlights independent roles for both herbivory and antiherbivore defences in shaping natural selection on plant reproductive traits (Campbell, 2014; Carr & Eubanks, 2014; Johnson *et al.*, 2015; Thompson & Johnson, 2016). In this study, we asked whether induced plant defences alter natural

**Table 1** Multivariate phenotypic selection analysis depicting model-averaged coefficients (*β*), *z*-values, and corresponding *P*-values from the 'dredge' multimodel averaging procedure. Significant coefficients (*P* < 0.05) and interaction terms are highlighted in bold. The model includes all plants that had data collected for all measured traits (*n* = 94 plants). Significant main effects indicate that the treatment or trait directly affected fitness, and significant trait × defence interaction terms indicate that selection on the trait is different between JA-induced and control plants. Terms with NA values were not present in any models with AIC scores within 2 units of that of the best-fitting model.

| | Female fitness* | | | Male fitness† | | |
|---|---|---|---|---|---|---|
| | *β* | *z* | *P* | *β* | *z* | *P* |
| Induction‡ | **−0.513** | **2.519** | **0.012** | 0.068 | 0.589 | 0.556 |
| Damage‡ | −0.132 | 0.954 | 0.340 | 0.049 | 0.513 | 0.608 |
| Floral width PC‡ | −0.138 | 0.937 | 0.349 | −0.178 | 0.868 | 0.385 |
| Floral length PC | 0.001 | 0.036 | 0.971 | 0.103 | 0.797 | 0.425 |
| First flower‡ | −0.090 | 0.784 | 0.433 | −0.002 | 0.086 | 0.931 |
| Inflorescence number‡ | −0.210 | 1.090 | 0.276 | 0.090 | 0.634 | 0.526 |
| Inflorescence size‡ | **0.319** | **1.985** | **0.047** | 0.251 | 1.278 | 0.201 |
| Biomass‡ | **0.574** | **3.638** | **<0.001** | 0.000 | 0.004 | 0.997 |
| Induction × Damage‡ | 0.237 | 1.215 | 0.224 | NA | | |
| Floral length PC × Induction | NA | | | 0.121 | 0.761 | 0.447 |
| Floral width PC × Induction‡ | 0.202 | 1.283 | 0.200 | 0.045 | 0.429 | 0.668 |
| First flower × Induction | −0.003 | 0.096 | 0.923 | NA | | |
| Inflorescence number × Induction‡ | **0.856** | **3.239** | **0.001** | −0.039 | 0.320 | 0.749 |
| Inflorescence size × Induction‡ | **−0.563** | **2.849** | **0.004** | −0.112 | 0.636 | 0.525 |
| Biomass × Induction‡ | **−0.438** | **2.137** | **0.033** | NA | | |
| Floral length PC × Damage | NA | | | 0.002 | 0.058 | 0.954 |
| Floral width PC × Damage | 0.019 | 0.257 | 0.797 | 0.358 | 1.766 | 0.0774 |
| First flower × Damage | −0.006 | 0.129 | 0.898 | NA | | |
| Inflorescence number × Damage | 0.044 | 0.372 | 0.710 | NA | | |
| Inflorescence size × Damage | 0.010 | 0.183 | 0.855 | −0.026 | 0.298 | 0.766 |
| Biomass × Damage | 0.015 | 0.213 | 0.832 | NA | | |

*Number of seeds produced.

†Mean pollinia removed per flower.

‡Term present in model with lowest AIC (i.e. best-fitting model) in *dredge* analysis and used to generate residual values for direct selection analysis (female fitness only).

selection on reproductive traits independently of foliar damage. Our results demonstrate that selection on inflorescence size and number – as well as biomass – changed following the chemical induction of plant defences, whereas manual defoliation did not alter selection on any trait. Specifically, in the absence of induced defences, natural selection favours large plants with many flowers per inflorescence. When defences are induced, however, plants with many inflorescences – but fewer flowers per inflorescence – have the highest fitness. These patterns of defence-mediated changes in selection indicate that the trait values conferring optimal fitness differ according to a plant's defensive phenotype. Taken together, our results suggest that phenotypically plastic variation in defence can alter both the strength (coefficient magnitude) and direction (coefficient sign) of natural selection on plant reproductive traits.

## Causes of defence-mediated changes in natural selection

Resource allocation trade-offs between defence and reproduction, where fitness is reduced in highly defended plants, may cause selection for increased allocation to reproduction. JA-induced plants produced fewer seeds than control plants in our experiment, suggesting that there was a direct fitness cost of inducing defences in *A. syriaca* (Strauss *et al.*, 2002). In general, reproduction through female function is more resource-limited than through male function (Haig & Westoby, 1988), and resource limitation is thought to be the most important determinant of seed production in milkweeds (Wyatt & Broyles, 1994). We found that JA-induced plants experienced selection for more inflorescences, but we did not detect selection on inflorescence number in control plants. This may represent selection for higher investment into reproduction in JA-induced plants (Herms & Mattson, 1992). Milkweeds can mature few fruits per inflorescence, and large inflorescences may be especially inefficient in JA-induced plants if many potential fruits are aborted because of insufficient resources. The observed selection for more inflorescences that are smaller in size among JA-induced plants could thus be selection to partition flower production among more inflorescences in order to increase fruit set per inflorescence. Simultaneous
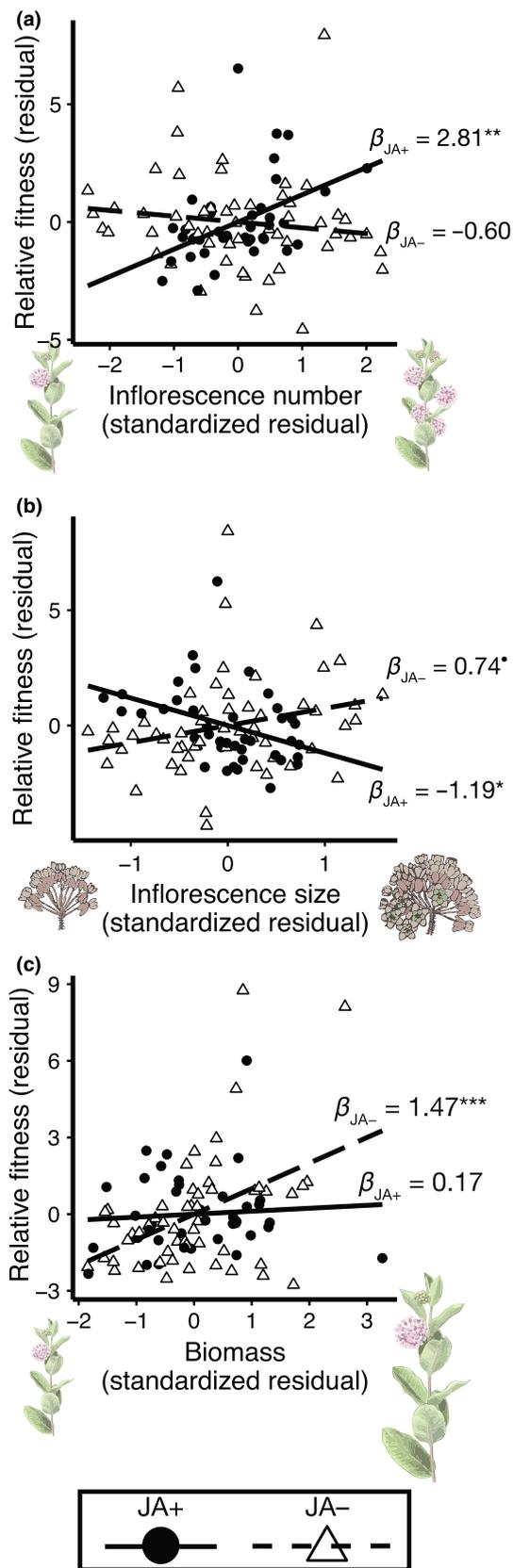
**Fig. 1** Scatterplots comparing linear selection gradients between JA-induced ($n$ = 40) and control ($n$ = 54) plants in our experiment. (a) JA-induced (JA+) plants experienced strong positive selection for more inflorescences, but selection was not acting on control (JA−) plants. (b) Inflorescence size was under selection in both treatments, but selection was negative in JA-induced plants and trended towards positive in control plants. (c) There was selection for larger biomass in control plants, but no selection in JA-induced plants. Selection gradient ($\beta$) values are reported adjacent to the corresponding selection gradient regression lines ($^{\bullet}P$ = 0.056; *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001). Residual values from regressions with all traits are plotted to visualize the direct effect of the focal trait on fitness while controlling for the effects of other traits in the model.

manipulation of defence and resource environment is required to test these hypotheses.

In addition to resource allocation trade-offs, altered interactions with agents of selection on plant reproductive traits could cause the observed defence-mediated changes in natural selection. A previous study in *A. syriaca* found that herbivores destroyed >90% of the smallest inflorescences but negligibly damaged large inflorescences (Willson & Rathcke, 1974). Thus, large inflorescences may allow milkweed plants to tolerate herbivory (Strauss & Agrawal, 1999). Our data indicate that JA-induced plants experienced selection for smaller inflorescences, whereas control plants experienced a trend towards selection for larger inflorescences. Induced defences can reduce florivory (McCall & Karban, 2006), and thus, selection for larger inflorescences in (undefended) control plants may be selection for tolerance to herbivore damage. Experimental manipulation of both florivory and defence is needed to test this hypothesis.

## Novel contributions of this study

Our study is the second to demonstrate that antiherbivore defences can alter natural selection on plant reproductive traits. Thompson & Johnson (2016) showed that the cyanogenesis polymorphism of *Trifolium repens* alters natural selection on petal and inflorescence size. The present study builds on this result in two key ways. First, whereas the previous study manipulated genetic variation for a constitutive defence, we experimentally manipulated induced defences. That our results are qualitatively similar indicates that both constitutive and induced defences can alter natural selection on reproductive traits. Second, we experimentally manipulated foliar damage, whereas Thompson & Johnson (2016) measured natural herbivore damage and included it as a covariate. Defence and herbivory were significantly correlated in their study, and thus, our study provides a stronger test of the hypothesis that defence-mediated changes in selection on reproductive traits are independent of foliar damage. Thus, our study builds on that of

Thompson & Johnson (2016) and provides additional evidence in support of the hypothesis that plant defences can alter natural selection on reproductive traits.

Common milkweed, *Asclepias syriaca*, is a model plant system for studying the evolutionary ecology of induced responses to herbivory (Malcolm & Zalucki, 1996; Van Zandt & Agrawal, 2004; Mooney *et al.*, 2008). Identifying costs associated with induced responses is a major goal of research on induced responses (Agrawal, 2005a), and our study provides the first evidence of fitness costs associated with induced responses in milkweeds. Our results generate testable hypotheses aimed at addressing the ecological mechanisms underlying costs – and ecological consequences – of induced responses in this system. Testing these hypotheses will advance our understanding of the evolutionary ecology of induced responses by furthering the development of milkweeds as a model system for the study of plant–insect interactions.

### Caveats

We acknowledge several limitations of our experiment and analyses. First, the most accurate estimates of selection utilize breeding values (e.g. Etterson & Shaw (2001)), rather than phenotypic variation among individuals, because environmental covariance between traits and fitness can bias estimates of selection in the field (Rausher, 1992; Stinchcombe *et al.*, 2002). Although our use of phenotypic data could bias overall estimates of selection, we applied our treatments randomly and it is unlikely that environmental effects would influence our conclusion that induced defences alter selection. Second, *A. syriaca* is a highly clonal perennial, and our estimates of fitness likely capture a small portion of lifetime fitness. However, our methods still allow us to accurately quantify defence-mediated changes in selection within a generation. Third, although we found no evidence that natural selection was operating through male fitness, our male fitness metric may be too coarse to detect more subtle patterns. Fourth, we did not verify differences in defensive properties across JA-induced and control plants; other studies have confirmed the efficacy of identical treatments in *A. syriaca* (e.g. Mooney *et al.*, 2008), and the strong effects of induction treatment on fitness and selection further indicate that our treatments altered plant physiology. Last, manual defoliation did not affect traits or fitness, which suggests that our damage treatment was not severe enough to impose fitness costs on *A. syriaca*. We note that our result is consistent with other studies demonstrating low fitness consequences of defoliation in *A. syriaca* (e.g. Hochwender *et al.*, 2000) and that higher levels of damage would have been biologically unrealistic for *A. syriaca* (mean % leaf herbivory in the field = 4.0 ± 1.5%, min = 0%,

max = 24.2%; Turcotte *et al.* (2014)). Despite these caveats, our field experiment provides strong evidence that induced defences can alter natural selection on reproductive traits via female fitness in a natural milkweed population.

## Conclusions

Understanding the factors that underlie the diversification of plant reproductive structures is an important area of research in evolutionary biology (Barrett, 2010). Recent evidence demonstrates that both herbivores and antiherbivore defences promote plant reproductive diversity on both macroevolutionary (Armbruster, 1997; Armbruster *et al.*, 2009; Hanley *et al.*, 2009) and microevolutionary timescales (Thompson & Johnson, 2016). Our results demonstrate that induced plant defences can alter both the strength and direction of natural selection on reproductive traits independently of foliar damage. Although the available evidence suggests that antiherbivore defences can alter natural selection on plant reproductive traits, very little is known about the mechanisms responsible for these patterns. It is also currently uncertain whether disruptive selection associated with defence can ultimately drive phenotypic divergence within or among populations (Marquis *et al.*, 2016). Experimental studies that manipulate defence and resource limitation, and defence and pollinators and/or herbivores, are needed to fully understand the ecological mechanisms through which antiherbivore defences alter natural selection on plant reproductive traits.

## Author contributions

MTJJ and KAC conceived and designed the experiment. KAC collected the data. KAT and MTJJ analysed the data. KAT wrote the first draft of the manuscript, and KAT and MTJJ revised the manuscript.

## References

Agrawal, A.A. 2004. Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. *Ecology* **85**: 2118–2133.

Agrawal, A.A. 2005a. Future directions in the study of induced plant responses to herbivory. *J. Chem. Ecol.* **115**: 97–105.

Agrawal, A.A. 2005b. Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evol. Ecol. Res.* **7**: 651–667.

Agrawal, A.A. & Fishbein, M. 2008. Phylogenetic escalation and decline of plant defense strategies. *Proc. Natl. Acad. Sci. USA* **105**: 10057–10060.

Agrawal, A.A. & Konno, K. 2009. Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annu. Rev. Ecol. Evol. Syst.* **40**: 311–331.

Agrawal, A.A., Strauss, S.Y. & 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.

Agrawal, A.A., Conner, J.K., Johnson, M.T.J. & Wallsgrove, R. 2002. Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution* **56**: 2206–2213.

Agrawal, A.A., Fishbein, M., Halitschke, R., Hastings, A.P., Rabosky, D.L. & Rasmann, S. 2009. Evidence for adaptive radiation from a phylogenetic study of plant defenses. *Proc. Natl. Acad. Sci. USA* **106**: 18067–18072.

Agrawal, A.A., Kearney, E.E., Hastings, A.P. & Ramsey, T.E. 2012. Attenuation of the jasmonate burst, plant defensive traits, and resistance to specialist monarch caterpillars on shaded common milkweed (*Asclepias syriaca*). *J. Chem. Ecol.* **38**: 893–901.

Armbruster, W.S. 1997. Exaptations link evolution of plant-herbivore and plant-pollinator interactions: a phylogenetic inquiry. *Ecology* **78**: 1661–1672.

Armbruster, W.S., Lee, J. & Baldwin, B.G. 2009. Macroevolutionary patterns of defense and pollination in *Dalechampia* vines: adaptation, exaptation, and evolutionary novelty. *Proc. Natl. Acad. Sci. USA* **106**: 18085–18090.

Barrett, S.C.H. 2010. Understanding plant reproductive diversity. *Philos. Trans. R. Soc. B Biol. Sci.* **365**: 99–109.

Barton, K. 2015. MuMIn: Multi-model inference. R package version 1.115.1. http://CRAN.R-project.org/package=MuMIn.

Broyles, S. & Wyatt, R. 1990. Paternity analysis in a natural population of *Asclepias exaltata*: multiple paternity, functional gender, and the "pollen-donation hypothesis". *Evolution* **44**: 1454–1468.

Campbell, S.A. 2014. Ecological mechanisms for the coevolution of mating systems and defence. *New Phytol.* **205**: 1047–1053.

Carr, D.E. & Eubanks, M.D. 2014. Interactions between insect herbivores and plant mating systems. *Annu. Rev. Entomol.* **59**: 185–203.

Dobler, S., Dalla, S., Wagschal, V. & Agrawal, A.A. 2012. Community-wide convergent evolution in insect adaptation to toxic cardenolides by substitutions in the Na, K-ATPase. *Proc. Natl. Acad. Sci. USA* **109**: 13040–13045.

Dussourd, D.E. & Eisner, T. 1987. Vein-cutting behavior: insect counterploy to the latex defense of plants. *Science* **237**: 898–901.

Etterson, J.R. & Shaw, R.G. 2001. Constraint to adaptive evolution in response to global warming. *Science* **294**: 151–154.

Galen, C. 1999. Why do flowers vary? *Bioscience* **49**: 631–640.

Gols, R., Wagenaar, R., Poelman, E.H., Kruidhof, H.M., van Loon, J.J.A. & Harvey, J.A. 2015. Fitness consequences of indirect plant defence in the annual weed, *Sinapis arvensis*. *Funct. Ecol.* **29**: 1019–1025.

Haig, D. & Westoby, M. 1988. On limits to seed production. *Am. Nat.* **131**: 757–759.

Hanley, M.E., Lamont, B.B. & Armbruster, W.S. 2009. Pollination and plant defence traits co-vary in Western Australian *Hakea*s. *New Phytol.* **182**: 251–260.

Harder, L.D. & Barrett, S.C.H. 2007. *Ecology and Evolution of Flowers*. Oxford University Press, Oxford, UK.

Heil, M. 2002. Ecological costs of induced resistance. *Curr. Opin. Plant Biol.* **5**: 345–350.

Heil, M. & Karban, R. 2010. Explaining evolution of plant communication by airborne signals. *Trends Ecol. Evol.* **25**: 137–144.

Herms, D.A. & Mattson, W.J. 1992. The dilemma of plants: to grow or defend. *Q. Rev. Biol.* **67**: 283–335.

Hochwender, C.G., Marquis, R.J. & Stowe, K.A. 2000. The potential for and constraints on the evolution of compensatory ability in *Asclepias syriaca*. *Oecologia* **122**: 361–370.

Hoffmeister, M., Wittköpper, N. & Junker, R.R. 2016. Herbivore-induced changes in flower scent and morphology affect the structure of flower-visitor networks but not plant reproduction. *Oikos* **125**: 1241–1249.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* **130**: 195–204.

Irwin, R.E., Adler, L.S. & Brody, A.K. 2004. The dual role of floral traits: pollinator attraction and plant defense. *Ecology* **85**: 1503–1511.

Johnson, M.T.J., Campbell, S.A. & Barrett, S.C.H. 2015. Evolutionary interactions between plant reproduction and defense against herbivores. *Annu. Rev. Ecol. Evol. Syst.* **46**: 191–213.

Karban, R. & Baldwin, I.T. 1997. *Induced Responses to Herbivory*. University of Chicago Press, Chicago, IL.

Karban, R. & Myers, J.H. 1989. Induced plant responses to herbivory. *Annu. Rev. Ecol. Syst.* **20**: 331–348.

Kephart, S.R. 1983. The partitioning of pollinators among three species of *Asclepias*. *Ecology* **64**: 120–133.

Kessler, A., Halitschke, R. & Poveda, K. 2011. Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant-pollinator interactions. *Ecology* **92**: 1769–1780.

La Rosa, R.J. 2015. *Floral Evolution in Milkweeds: Evidence for Selection Past and Present*. Michigan State University, East Lansing.

Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.

Lucas-Barbosa, D. 2015. Integrating studies on plant-pollinator and plant-herbivore interactions. *Trends Plant Sci.* **21**: 125–133.

Lucas-Barbosa, D., Van Loon, J.J.A., Gols, R., Van Beek, T.A. & Dicke, M. 2013. Reproductive escape: annual plant responds to butterfly eggs by accelerating seed production. *Funct. Ecol.* **27**: 245–254.

Malcolm, S.B. 1991. Cardenolide-mediated interactions between plants and herbivores. In: *Herbivores: Their Interactions with Secondary Plant Metabolites, Vol. I: The Chemical Participants* (G.A. Rosenthal & M.R. Berenbaum, eds), pp. 251–296. Academic Press, San Diego, CA.

Malcolm, S.B. & Zalucki, M.P. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. *Entomol. Exp. Appl.* **80**: 193–196.

Marquis, R.J., Salazar, D., Baer, C., Reinhardt, J., Priest, G. & Barnett, K. 2016. Ode to Ehrlich and Raven or how herbivorous insects might drive plant speciation. *Ecology* **97**: 2939–2951.

McCall, A.C. & Karban, R. 2006. Induced defense in *Nicotiana attenuata* (Solanaceae) fruit and flowers. *Oecologia* **146**: 566–571.

Mooney, K.A., Jones, P. & Agrawal, A.A. 2008. Coexisting congeners: demography, competition, and interactions with cardenolides for two milkweed-feeding aphids. *Oikos* **117**: 450–458.

Musser, R.O., Hum-Musser, S.M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J.B. *et al.* 2002. Herbivory: caterpillar saliva beats plant defences. *Nature* **416**: 599–600.

R Core Team. 2014. *R: A Language and Environment for Statistical Computing*. R Core Team, Vienna, Austria. http://www.R-project.org/

Rasmann, S., Johnson, M.D. & Agrawal, A.A. 2009. Induced responses to herbivory and jasmonate in three milkweed species. *J. Chem. Ecol.* **35**: 1326–1334.

Rausher, M.D. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* **46**: 616–626.

Revelle, W. 2015. *psych: Procedures for Personality and Psychological Research. v 1.5.6*. Northwestern University, Evanston, Illinois, USA. https://cran.r-project.org/web/packages/psych/index.html.

Rodriguez-Saona, C., Crafts-Brandner, S.J., Williams, L.I. & Paré, P.W. 2002. *Lygus hesperus* feeding and salivary gland extracts induce volatile emissions in plants. *J. Chem. Ecol.* **28**: 1733–1747.

Rubin, I.N., Ellner, S.P., Kessler, A. & Morrell, K.A. 2015. Informed herbivore movement and interplant communication determine the effects of induced resistance in an individual-based model. *J. Anim. Ecol.* **84**: 1273–1285.

SAS Institute Inc. 2009. *SAS/STAT 9.2 User's Guide*. SAS Institute Inc, Cary.

Schuman, M.C., Barthel, K. & Baldwin, I.T. 2012. Herbivory-induced volatiles function as defenses increasing fitness of the native plant *Nicotiana attenuata* in nature. *Elife* **2012**: e00007.

Stebbins, G.L. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annu. Rev. Ecol. Syst.* **1**: 307–326.

Stinchcombe, J.R., Rutter, M.T., Burdick, D.S., Tiffin, P., Rausher, M.D. & Mauricio, R. 2002. Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *Am. Nat.* **160**: 511–523.

Strauss, S.Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**: 1640–1645.

Strauss, S.Y. & Agrawal, A.A. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.* **14**: 179–185.

Strauss, S.Y. & Whittall, J.B. 2006. Non-pollinator agents of selection on floral traits. In: *Ecology and Evolution of Flowers* (L.D. Harder & S.C.H. Barrett, eds), pp. 120–138. Oxford University Press, Oxford, UK.

Strauss, S.Y., Conner, J.K. & Rush, S.L. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *Am. Nat.* **147**: 1098–1107.

Strauss, S.Y., Rudgers, J.A., Lau, J.A. & Irwin, R.E. 2002. Direct and ecological costs of resistance to herbivory. *Trends Ecol. Evol.* **17**: 278–285.

Thaler, J.S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* **399**: 686–688.

Thaler, J.S., Stout, M.J., Karban, R. & Duffey, S.S. 1996. Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. *J. Chem. Ecol.* **22**: 1767–1781.

Thompson, K.A. & Johnson, M.T.J. 2016. Antiherbivore defenses alter natural selection on plant reproductive traits. *Evolution* **70**: 796–810.

Turcotte, M.M., Thomsen, C.J.M., Broadhead, G.T., Fine, P.V.A., Godfrey, R.M., Lamarre, G.P.A. *et al.* 2014. Percentage leaf herbivory across vascular plant species. *Ecology* **95**: 788.

Underwood, N. & Rausher, M. 2002. Comparing the consequences of induced and constitutive plant resistance for herbivore population dynamics. *Am. Nat.* **160**: 20–30.

Underwood, N., Anderson, K. & Inouye, B.D. 2005. Induced vs. constitutive resistance and the spatial distribution of insect herbivores among plants. *Ecology* **86**: 594–602.

Uva, R., Neal, J. & DiTomaso, J. 1997. *Weeds of the Northeast*. Comstock Publishing Associates, Ithaca.

Van Zandt, P.A. & Agrawal, A.A. 2004. Specificity of induced plant responses to specialist herbivores of the common milkweed *Asclepias syriaca*. *Oikos* **104**: 401–409.

Willson, M.F. & Rathcke, B.J. 1974. Adaptive design of the floral display in *Asclepias syriaca* L. *Am. Midl. Nat.* **92**: 47–57.

Wyatt, R. & Broyles, S.B. 1994. Ecology and evolution of reproduction in milkweeds. *Annu. Rev. Ecol. Syst.* **25**: 423–441.

Zhen, Y., Aardema, M.L., Medina, E.M., Schumer, M. & Andolfatto, P. 2012. Parallel molecular evolution in an herbivore community. *Science* **337**: 1634–1637.

## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Table S1** Mean trait values and standard deviations, coefficients of phenotypic variation, and data transformations for all traits and fitness components included in the multivariate selection analysis.

**Table S2** Summary of principal components analysis used to compress floral measurements into two principal components.

**Table S3** Phenotypic correlation matrix for all measured traits.

**Table S4** Phenotypic correlation matrix for all traits after collapsing the six floral traits into two principal components *P*-values are below the diagonal and Pearson's *r* values are above the diagonal.

**Table S5** Mean values and standard deviations for JA-induced and control plants.

**Figure S1** Photographs of the experiment and floral details of the common milkweed, *Asclepias syriaca*, study system.