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RESEARCH ARTICLE

Patterns of constitutive and induced herbivore defence are complex, but share a common genetic basis in annual and perennial monkeyflower

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Abstract

1. Despite multiple ecological and evolutionary hypotheses that predict patterns of phenotypic relationships between plant growth, reproduction and constitutive and/or induced resistance to herbivores, these hypotheses do not make any predictions about the underlying molecular genetic mechanisms that mediate these relationships.
2. We investigated how divergent plant life-history strategies in the yellow monkeyflower and a life-history altering locus, *DIV1*, influence plasticity of phytochemical herbivory resistance traits in response to attack by two herbivore species with different diet breadth.
3. Life-history strategy (annual vs. perennial) and the *DIV1* locus significantly influenced levels of constitutive herbivory resistance, as well as resistance induction following both generalist and specialist herbivory. Perennial plants had higher total levels of univariate constitutive and induced defence than annuals, regardless of herbivore type. Annuals induced less in response to generalist herbivory than did perennials, while induction response was equivalent across the ecotypes for specialist herbivory.
4. The effects of the *DIV1* locus on levels of constitutive and induced defence were dependent on genetic background, the annual versus perennial haplotype of *DIV1* and herbivore identity. The patterns of univariate induction due to *DIV1* were non-additive and did not always match expectations based on patterns of divergence for annual/perennial parents. For example, perennial plants had higher levels of constitutive and induced defence than did annuals, but when the annual *DIV1* was present in the perennial genetic background induction response to herbivory was higher than for the perennial parent lines.
5. Patterns for multivariate defence arsenals generally echoed those of univariate, with annual and perennial monkeyflowers and those with alternative versions of *DIV1* differing significantly in constitutive and induced resistance. Like univariate resistance, induced multivariate defence arsenals were affected by herbivore identity.

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6. Our results highlight the complexity of the genetic mechanisms underlying plastic response to herbivory. While a genetic locus underlying substantial phenotypic variation in life-history strategy and constitutive defence also influences defence plasticity, the induction response also depends on genetic background. This result demonstrates the potential for some degree of evolutionary independence between constitutive and induced defence, or induced defence and life-history strategy, in monkeyflowers.

KEYWORDS
constitutive defence, defence induction, generalist herbivore, herbivory, monkeyflower, resistance, specialist herbivore

1 | INTRODUCTION

Plant evolutionary biology has long predicted phenotypic trade-offs in resource allocation among plant traits, where resource investment in one life-history trait occurs at the expense of another (Cope et al., 2021; Futuyma & Moreno, 1988; Lundgren & Des Marais, 2020). Plant defence hypotheses are premised on the assumption of finite resources available for the allocation to growth, defence, reproduction and other critical functions (Coley et al., 1985; Herms & Mattson, 1992; Rhoades, 1979). Intraspecific patterns of resource allocation are generally different from patterns among plant species, which often follow the predictions of the resource allocation hypothesis (Coley et al., 1985; Endara & Coley, 2011). Within species, levels of constitutive defence (defence in the absence of herbivory) typically are correlated with the length of a growing season (Hahn & Maron, 2016; Kooyers et al., 2017). Intraspecific differences in investment in defence could be particularly pronounced in species with both annual and perennial ecotypes. Perennial plants live and often reproduce across multiple growing seasons, giving them more time for foliar production and constitutive defence than annuals, which must complete their reproductive life cycles within one growing season (Lowry et al., 2019; Mutikainen & Wall, 1995). In addition, plants with longer periods of vegetative growth (whether within or across growing seasons) may face greater herbivore pressures than annuals (Marquis & Moura, 2021).

In addition to constitutive defence production, plants often induce higher levels of defence following herbivory (Adler & Harvell, 1990). Defence induction provides a mechanism for plants to minimize direct or ecological costs of defence production in the absence of herbivory while increasing levels of defence when they are attacked (Cipollini et al., 2003; Stamp, 2003; Strauss et al., 2002). While evolutionary defence hypotheses such as Optimal Defence Theory predict trade-offs between constitutive and induced defences within individuals (Rhoades, 1979; Stamp, 2003), empirical studies show that levels of induced resistance may be positively or negatively correlated with levels of constitutive resistance (Karban & Baldwin, 1997).

Induction of defence occurs when the feeding of insects on plants initiates the activation of multiple plant signalling pathways, including the jasmonic acid and ethylene pathways (Acevedo et al., 2019; Thaler et al., 2012). Chewing herbivores may elicit both a generalized response to cellular damage as well as a more specific response to the chemical cues generated by particular insect species (Bonaventure, 2014). This provides plants with an opportunity to tailor defence to specific herbivores, or types of herbivores. In addition, herbivores can orally secrete molecules (elicitors and effectors) that suppress or alter plant response (Erb et al., 2012; Jones et al., 2022; Musser et al., 2002). A plant's response to herbivory is thus affected by both the plant and the herbivore. These reciprocal interactions are highly complex and may be interaction-specific in their effects, with the same compound initiating opposite effects in the defence pathways of different plant species (Bonaventure, 2014; Musser et al., 2005; Tian et al., 2012). In addition, plant defence responses may benefit one herbivore species but not another, due to differential herbivore response to defence trait(s) (Cornell & Hawkins, 2003).

Many plant species are attacked by both dietary generalist and dietary specialist (hereafter generalist or specialist) herbivores. Specialist herbivores feed on plants from a narrow phylogenetic breadth and are often able to at least partially avoid or mitigate the effects of phylogenetically conserved plant defences. In comparison, generalist herbivore species tend to have a wider dietary breadth, with tremendous variability in the extent of generalization within this category (Loxdale & Harvey, 2016; Singer, 2008). The specialist–generalist paradigm predicts that specialist herbivores will be less negatively affected by the defences of their host plants than generalists (Ali & Agrawal, 2012; Rothwell & Holeski, 2019) and may also benefit from the host defences via sequestration. Predictions of specificity of plant response to herbivores are often framed in the context of generalist versus specialist herbivore, with the expectation that plant induction may differ according to herbivore diet breadth (e.g. Agrawal, 2000; Bowers & Stamp, 1993). Although this difference is not always confirmed in the literature (Ali & Agrawal, 2012), the ability of a plant to respond differently to a variable community of potential herbivores represents one type of investment in inducible defence, in addition to the magnitude of response.

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Despite the multiple ecological and evolutionary hypotheses mentioned above that postulate a *phenotypic* relationship between growth, reproduction and constitutive and/or induced resistance to herbivores, these hypotheses do not make any predictions about the underlying molecular *genetic* mechanisms that mediate these relationships. While genes or genetic regions involved in constitutive defence trait variation and/or trade-offs between constitutive resistance and growth are beginning to be identified in model systems (e.g. Campos et al., 2016; Havko et al., 2016), that research has typically been done in benign laboratory conditions and has not incorporated plastic defence trait induction. Likewise, the jasmonic signalling pathway is recognized as the core pathway involved in defence induction in response to herbivory, particularly chewing herbivores (Erb & Reymond, 2019). This pathway is known to interact with transcription factors involved in plant growth (Howe et al., 2018). However, there has been very little conceptual overlap between these molecular genetic studies and those incorporating evolutionary ecology theories of trade-offs between defence and other aspects of life-history (Züst & Agrawal, 2017). Both defence traits and life-history attributes are quantitative traits influenced by multiple genetic loci (Holeski, 2021; Kliebenstein, 2014) and with complex regulatory pathways making the translation between genetic architecture and phenotypic outcome or broad evolutionary predictions more difficult (Huot et al., 2014; Kliebenstein, 2016; Yang et al., 2012).

Previous research in yellow monkeyflower shows that trade-offs between life-history strategy and constitutive defence levels are at least in part genetically controlled (Holeski et al., 2013, 2014; Kooyers et al., 2017, 2020). Populations and ecotypes of this species are often locally adapted along a drought-induced gradient in growing season length (Hall & Willis, 2006; Kooyers et al., 2014). One major quantitative trait locus (QTL), *DIV1*, accounts for a substantial portion of the differences in life-history and constitutive defence traits between ecotypes (Lowry et al., 2019). The *DIV1* locus maps to a nested set of two large chromosomal inversions (Kollar et al., 2023) that contribute to adaptive phenotypic divergence in multiple traits between perennial and annual ecotypes of the species (Lowry & Willis, 2010).

In this article, we investigate how the genetic mechanisms that underlie differences in allocation between growth, reproduction and constitutive resistance traits influence plant defence induction in response to herbivory. We assess patterns of constitutive defence and induced defence. We focused our study on annual and perennial yellow monkeyflower plants that have evolved in response to local adaptation to divergent habitats. To study how the adaptive *DIV1* locus contributes to differences in induced defence, we included lines in our experiments where *DIV1* was reciprocally introgressed into both annual and perennial genetic backgrounds. To understand how induced responses would differ for damage by a dietary generalist versus a dietary specialist herbivore, we exposed plants to feeding by both types of herbivores. Overall, we assessed how life-history strategy, genetic locus (*DIV1*) and herbivore identity act independently and interactively to affect (1) constitutive defence (2) extent of induction and (3) specificity of induction response.

2 | MATERIALS AND METHODS

2.1 | Study system

Yellow monkeyflower (*Erythranthe guttata* (DC), GL Nelsom; synonym: *Mimulus guttatus* DC, Phrymaceae) is a forb that occurs in ephemeral and perennial water sources across western North America. This species produces phenylpropanoid glycosides (PPGs), secondary metabolites which can have negative impacts on a variety of herbivores (Holeski et al., 2013; Keefover-Ring et al., 2014; Rotter et al., 2018). Yellow monkeyflower is a model system for the study of ecology and evolutionary genetics due in part to the great deal of morphological, genetic and life-history variation across its range (Flagel et al., 2014; Friedman, 2014; Hall & Willis, 2006; Monnahan & Kelly, 2017; Wu et al., 2008). The plants used in this study are derived from natural populations in Northern California that differ in life-history strategy across a seasonal soil–water availability gradient (Lowry & Willis, 2010). Populations growing near the coast live in year-round seeps and are perennials that invest heavily in vegetative growth, including lateral stolons, and are slow to flower. In contrast, populations growing further inland live in ephemeral seeps which dry up each year with the onset of regular summer drought. These plants are short-lived annuals that invest little in vegetative growth and reproduce quickly.

2.2 | Herbivores

To examine differences in induction due to different herbivores, we selected two caterpillar species that feed upon natural populations of *M. guttatus* in Northern California where our source plant populations occur. Common buckeye, *Junonia coenia* (Nymphalidae), specializes on plants that contain iridoid glycosides or PPGs and can sequester both (Bowers & Collinge, 1992; Rothwell, Keefover-Ring, and Holeski, unpublished data). Cabbage looper, *Trichoplusia ni* (Noctuidae), is a common agricultural pest and extreme dietary generalist that feeds on wild species across a broad range of plant families. Our study did not require ethical approval from an animal ethics committee.

2.3 | Plant materials

To test the impact of the *DIV1* locus on the induction of PPGs, we utilized near-isogenic lines (NILs) where annual and perennial *DIV1* ecotypes were reciprocally introgressed into the opposite genetic background. These lines were created using an inland annual population (LMC) and a coastal perennial population (SWB) that occur in habitats that differ dramatically in soil–water availability during summer months (Lowry & Willis, 2010). As described in more detail by Lowry and Willis (2010), a line from each ecotype was crossed to create heterozygous F1s. These were then recurrently backcrossed to the parental lines of each ecotype. After genotyping using two markers outside of *DIV1* and one within *DIV1*, heterozygous individuals were backcrossed again to both parental

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ecotype lines. After 3–4 generations of backcrosses, plants that were homozygous were selected for the annual or perennial orientation of *DIV1*. This resulted in four lines, one with the annual genetic background and the annual orientation of *DIV1* (Annual control), one with the annual genetic background and the perennial orientation of *DIV1* (Annual with perennial *DIV1*), one with the perennial background and the perennial orientation of *DIV1* (Perennial control) and one with the perennial background and the annual orientation of *DIV1* (Perennial with annual *DIV1*). These lines were then self-fertilized four times. This crossing design is illustrated in figure 3 of Lowry and Willis (2010) and in Figure S1. Annual and perennial parent lines (Annual parent and Perennial parent) were also self-fertilized for at least nine generations to account for inbreeding as a result of the breeding scheme. The control lines and lines with the *DIV1* haplotype that have the same genetic background (e.g. annual control vs. annual with perennial *DIV1*) are expected to be, on average, equivalent across all regions of the genome except at the *DIV1* locus. Note, however, that some genes from the alternative ecotype are also introgressed, at random, across the genome of all of these individuals. For example, both the annual control lines and the annual with perennial *DIV1* lines have some perennial genes introgressed.

2.4 | Induction experiment

We used caterpillar herbivores to experimentally induce changes in PPGs in the four NILs and in the annual and perennial parent lines. In the winter of 2021, we cold-stratified seeds in the dark at 4°C on soil for 1 week. We then transferred the seeds to a growth room, under full-spectrum T5-grow lights, at 21°C. We germinated seeds under humidity domes in the growth room under 16-h day-length for 1 week. We then transplanted all seedlings to individual 4″ pots and continued to grow them at 21°C with 13-h day-length, randomizing their position within the grow racks once a week. The 13-h day length is similar to the early growth conditions for the parent populations in their natural habitat. We bottom-watered the plants once a day for 30 min and fertilized with a 10-30-20 fertilizer (Peters' Professional Base Formulation) once a week. We used two soil-mixes; for germination, we used 65% peat moss, 20% perlite, 15% vermiculite, three tablespoons dolomite lime, three tablespoons wetting agent and three tablespoons silica; for the rest of the experiment, we used 55% peat moss, 30% bark, 15% perlite, three tablespoons dolomite lime, three tablespoons wetting agent and three tablespoons silica.

We tested induction in the lines discussed above (annual parent, annual control, annual with perennial *DIV1*, perennial parent, perennial control and perennial with annual *DIV1*), factorially crossed with two caterpillar species (buckeyes and cabbage loopers). These two variables resulted in 12 unique groups. For each group, we assigned 20 plants to induction treatment (feeding by caterpillars; 20 plants × 2 caterpillar species) and 10 to the control group (no herbivory; 10 plants × 2 caterpillar species). The cabbage looper and buckeye treatments (and the timing of affiliated plants) were offset by 1 month.

At day 35, we applied six neonate caterpillars to each treatment plant, three to one leaf at the second node and three to one leaf at the third node. To keep caterpillars in place, we used green mesh bags with drawstrings closed around the feeding leaves. As added protection for the leaves that we intended to sample for PPG concentration, in case of any caterpillar escape, we also placed these bags around the opposite second node leaf from the second node feeding leaf. The control plants had the same three leaves bagged, but without any caterpillars. For 10 days, we checked these plants daily, replacing any deceased caterpillars (this occurred six times in the first 3 days and then not after) with newly hatched neonates. On day 10 of the experiment, we removed all caterpillars and mesh bags from all plants. We then used a hole punch to apply additional leaf-area removal to any herbivory treatment leaves that did not reach 50% removal so that the proportion of tissue removed was standardized. On day 11, we sampled both the opposite second-node leaf and the fourth-node leaf on the same side of the plant as the second node feeding leaf for chemical analysis. We flash froze leaves with dry-ice and then lyophilized, after which we stored them in a freezer at −20°C until processing for chemical analysis.

2.5 | Quantification of PPGs

To determine the PPG concentrations in sampled leaves, we ground, extracted and prepped extract aliquots as described in Holeski et al. (2013). We then used high-performance liquid chromatography (HPLC) to quantify PPGs. The HPLC method is described in Kooyers et al. (2017) and was run on an Agilent 1260 HPLC with a diode array detector and Poroshell 120 EC-C18 analytical column (4.6 × 250 mm, 2.7 μm particle size); Agilent Technologies. We measured six different PPGs: Calceolarioside A, conandroside, verbascoside, mimuloside, unknown PPG 10 and unknown PPG 16 (Keefover-Ring et al., 2014; Kooyers et al., 2017). We calculated concentrations of PPGs as verbascoside equivalents, using a standard verbascoside solution (Santa Cruz Biotechnology, Dallas, TX), as described in Holeski et al. (2013, 2014).

2.6 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Ecotypes within a species	Ecotypes or nearly isogenic lines (NILs) within ecotypes within a species	Ten control plants (constitutive) and 20 herbivory-treatment plants (induced)

2.7 | Data analysis

We assessed phytochemical defences in both a univariate and multivariate way. Defences were characterized as sums of individual compounds (total PPG concentration), and multivariate suites of compounds (hereafter termed 'arsenals'). In some contexts, we also examined concentrations of individual PPGs.

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Yellow monkeyflower populations generally all contain the same set of PPGs in varying concentrations (e.g. Holeski et al., 2013, 2014; Kooyers et al., 2017; Rotter et al., 2018, 2019). Within species, plants with higher concentrations of secondary metabolites also tend to have a greater diversity of compounds, which suggests that, when plant samples with low-concentrations of secondary metabolites are analysed, low-concentration compounds may fall below detection limits. To compensate for limits in detection, prevent the impact of zero-inflation on our multivariate analyses and allow for data transformation, we replaced all 0s in our data set with 0.1, which represents 0.1% of the average sample concentration (Martín-Fernández et al., 2003). The data are available from the Dryad Digital Repository, https://doi.org/10.5061/dryad.w3r22810f (Blanchard et al., 2024).

2.8 | Total PPG concentration

To analyse how the summed concentration of all PPGs (total PPG concentration) changed with induction by caterpillar herbivory, and how this induction varied among NILs and between plants that were fed upon by the two caterpillar species, we fit a linear model with total PPG (mg PPGs/g of dry plant material) as the response variable. Our first model was a linear mixed-model (*lmerTest* function from the *lmerTest* package) in R (Kuznetsova et al., 2017) with four fixed effects: treatment (constitutive vs. induced), caterpillar species (buckeye vs. cabbage looper), plant line (the six NILs) and leaf node (second vs. fourth); and one random effect, plant ID, to account for the repeated-measures effect of sampling two leaves from each plant. The model included all two-way interactions. To adhere to the assumptions of the model of homoskedasticity and normality, we log-transformed the data. We found a significant interaction between leaf node and treatment, indicating differences in induction between nodes ($F_{1,347}=229.92$, $p<0.001$). We then performed pairwise comparisons (with the function *emmeans* from the package *emmeans*, Lenth et al., 2024) to determine the effect of treatment across leaf nodes, which indicated that only the fourth node leaves had significantly induced (second node: $t=1.56$, $p=0.403$; fourth node: $t=20.51$, $p<0.001$).

As only the fourth node leaves induced in response to herbivory, we focused only on the fourth node leaves for all other analyses and ran a simplified linear model (*lm* function) including only three fixed effects: treatment, caterpillar species and plant line (annual parent, annual control, annual with perennial *DIV1*, perennial parent, perennial control and perennial with annual *DIV1*), and all interactions. To adhere to the assumptions of the model of homoskedasticity, we used a weighted regression approach, with the inverse of the variance for the explanatory factors as the weighting. This approach was more compatible with our downstream analysis than transforming the results. All two-way interactions and the three-way interactions were significant, so none were dropped from the model. We then performed statistical tests to determine: (1) which groups significantly changed in total PPG concentration (or induced) in response to herbivory and (2) if the degree of induction varied by ecotype, herbivore species or *DIV1*. Induction is indicated by a significant difference in PPGs between the constitutive and control plants (treatment variable) for each group (caterpillar species × plant line combinations). As chemical defence is a complex quantitative trait, the amount and direction of change of PPGs (degree of induction) in response to herbivory is also an important measure of the impacts of ecotype, herbivore species and *DIV1* on induction. We determined degree of induction as the difference between constitutive and induced PPGs for each group and then compared this difference among groups. To statistically test for differences in degree of induction, we specified custom contrasts (generated using function *model.matrix* from the stats package and fitted with function *glht* from *multcomp* package (Hothorn, 2023), with Bonferroni adjustment for multiple comparisons).

2.9 | Multivariate PPG arsenals

To determine how the composition of all six PPG compounds changed with induction by caterpillar herbivory, and how this varied among NILs and between caterpillar species, we used permutational multivariate analysis of variance (PERMANOVA, function *adonis2* from package *vegan*, Oksanen et al., 2022, with Bray–Curtis distance). For our model, which also focused only on the fourth node leaves, we included the same three fixed effects as for the univariate model: treatment, caterpillar species and plant line, and all interactions. All two-way interactions were significant, but the three-way interaction was not. Therefore, we simplified the model by dropping the three-way interaction. We then made pairwise comparisons (using function *adonis. pair* from the package *EcolUtils*, Salazar, 2021) to further investigate how our variables influenced multivariate PPG composition. We specified custom contrasts (fitted with PERMANOVA) to test for differences in the degree of induction in multivariate arsenals depending on herbivory identity.

To visualize how multivariate PPG composition is influenced by our factors, we used non-metric multidimensional scaling (NMDS) (*MetaMDS* function in *vegan* package with Bray–Curtis distance to determine dissimilarity) and added standard error ellipses at 95% confidence around the centroid of each cluster (function *ordiellipse* from package vegan).

3 | RESULTS

3.1 | Parent ecotypes

As in previous studies with annual and perennial yellow monkeyflower ecotypes (Holeski et al., 2013; Lowry et al., 2019), the annual parent line tended to have lower constitutive levels of defence, with approximately half of the total PPG concentrations as perennial parent lines (Figure 1).

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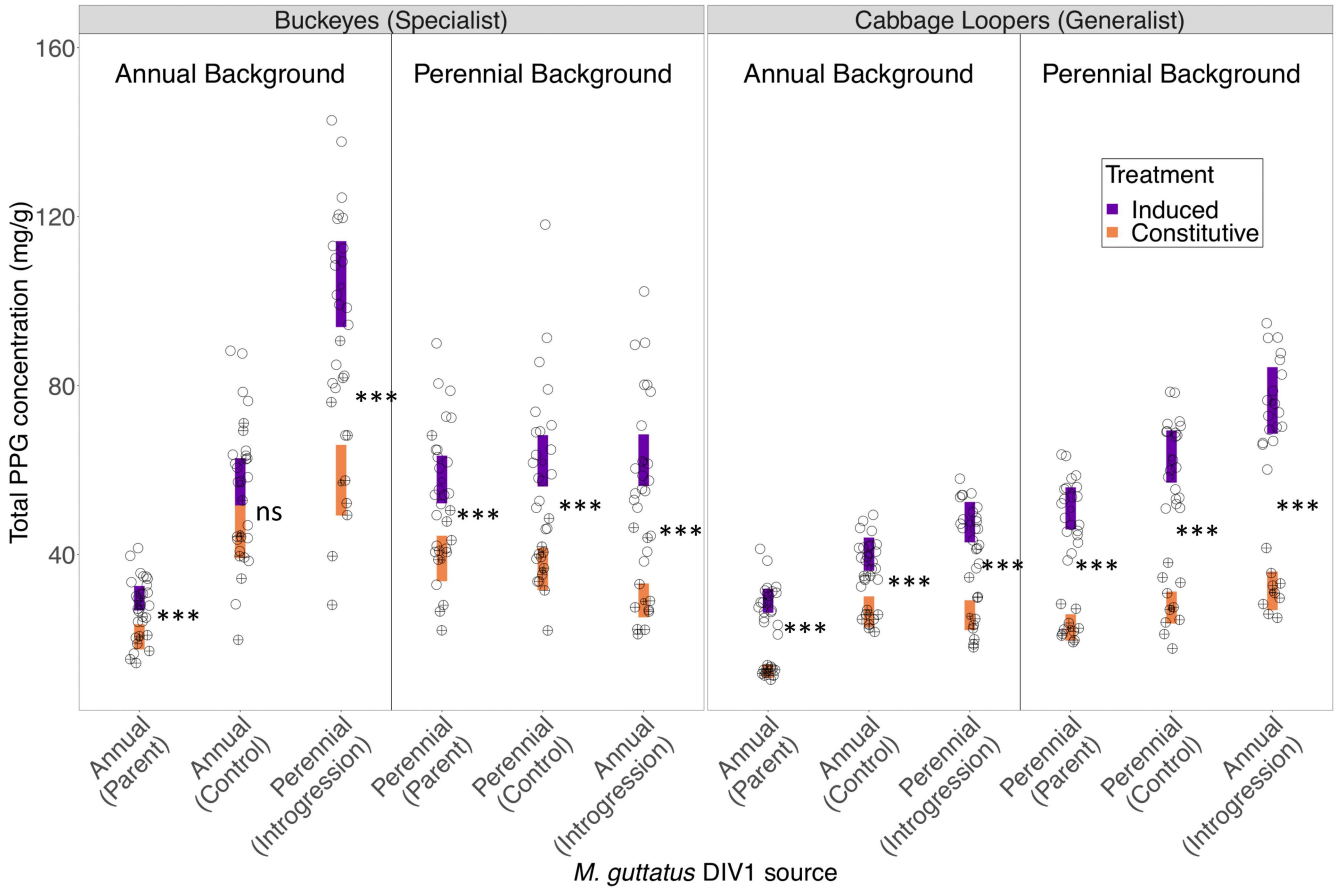

FIGURE 1 Constitutive and induced total foliar produces phenylpropanoid glycosides (mg/g dry foliar tissue). Results include annual and perennial parent lines and annual/perennial lines with *DIV1* or other genetic regions from the alternative ecotype introgressed. Induction was done via buckeye or cabbage looper herbivory. Significant differences between constitutive and induced values for each category are indicated with asterisks. Bars indicate 95% confidence intervals.

We found significant induction of Total PPGs across all plant lines and herbivory treatments. We also found significant two-way and three-way interactions for each independent variable, indicating differential responses of plant lines to herbivore identity (Table 1). We thus subsequently explored the effects of custom-specified contrasts used to test for significant induction in response to herbivory and to compare the degree of that induction.

Total PPGs increased significantly following herbivory in both annual and perennial parent lines, regardless of herbivore identity (Table 2A). Within ecotypes, annual parent lines induced marginally less in response to the specialist than to the generalist herbivore, while perennial parent lines did not differ in extent of induction by the two herbivore species (Figure 2; Table S1). Across ecotypes, annual and perennial parent lines did not differ significantly from one another in the extent of induction from the specialist herbivore ($t = -2.04$, $p = 0.588$), but the annual parent lines induced less than perennial parents in response to the generalist herbivore ($t = -3.43$, $p = 0.009$).

3.2 | DIV1 locus

Induction in response to feeding by both herbivores was influenced by *DIV1*, as well as introgression of non-target genes. All introgression lines had significant induction in response to both specialist and generalist herbivores except for the annual lines with perennial non-*DIV1* genes introgressed ('annual control'; Figure 1; Table 2B,C).

The impact of *DIV1* on the extent of induction was context-dependent, depending on both the origin of *DIV1*/genetic background, and herbivore identity. The response to herbivory by the specialist herbivore was most pronounced in annual plants with the perennial *DIV1*, while response to herbivory by the generalist herbivore was most pronounced in perennial plants with the annual *DIV1* (Table 2C; Figure 2).

Introgression of the perennial *DIV1* locus into the annual genome significantly increased the amount of induction in response to buckeye (specialist) herbivory, relative to annual parent lines or annual lines with other perennial genes introgressed ('annual control'; Table 3A). Annual lines with the introgressed perennial *DIV1* locus induced more than a fourfold increase in total PPGs relative to the annual parent lines in response to buckeye herbivory (Figure 2). While these lines almost doubled in total PPG levels following cabbage looper herbivory (Figure 1), this extent of induction was not significantly greater than that of the other annual lines following cabbage looper herbivory (Figure 2; Table 3A).

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TABLE 1 The effect of treatment (constitutive vs. induced), plant line (six lines that differ in genetic background and *DIV1*), caterpillar species (specialist buckeye vs. generalist cabbage looper) and all two-way and three-way interactions on (A) the univariate trait foliar total produces phenylpropanoid glycosides (PPGs) concentration, and (B) the multivariate trait PPG defence arsenals.

		(A) Univariate analyses (total PPGs)		(B) Multivariate analyses (PPG defence arsenals)	
Factor	**df**	***F***	***p*-Value**	**Pseudo *F***	***p*-Value**
Treatment (control vs. herbivory)	1330	608.1	**<0.001**	289.5	**0.001**
Plant line	5330	118.9	**<0.001**	63.7	**0.001**
Caterpillar species	1330	123.4	**<0.001**	142.6	**0.001**
Treatment:plant line	5330	7.0	**<0.001**	11.0	**0.001**
Treatment:caterpillar species	1330	26.4	**0.028**	32.1	**0.001**
Plant line:caterpillar species	5330	30.2	**<0.001**	24.4	**0.001**
Treatment:plant line:caterpillar species	5330	2.0	**0.003**	1.3	0.199

Note: Significant *p*-values are in bold text.

TABLE 2 Induction of foliar total produces phenylpropanoid glycosides, tested with custom contrasts following the analysis reported in Table 1 and corrected with Bonferroni adjustment. Control lines have some alleles from the alternative ecotype but maintain the *DIV1* orientation that matches their genetic background. Positive *T* statistics indicate induced concentrations that are higher than constitutive concentrations.

Comparison—induced versus constitutive	**Buckeyes (specialist)**				**Cabbage loopers (generalist)**			
Plant line	**Estimate**	**SE**	***T***	***p*-Value**	**Estimate**	**SE**	***T***	***p*-Value**
(A)								
Annual parent	9.33	2.08	4.49	**<0.001**	16.96	1.61	10.54	**<0.001**
Perennial parent	18.60	4.04	4.61	**<0.001**	28.48	2.94	9.70	**<0.001**
(B)								
Annual control	11.32	4.44	2.55	0.134	13.64	2.66	5.13	**<0.001**
Perennial control	27.52	4.06	6.78	**<0.001**	35.64	3.65	9.77	**<0.001**
(C)								
Annual with perennial *DIV1*	44.97	6.82	6.60	**<0.001**	21.48	2.99	7.19	**<0.001**
Perennial with annual *DIV1*	34.32	3.75	9.14	**<0.001**	45.27	4.58	9.90	**<0.001**

Note: Significant *p*-values are in bold text and indicate significant induction.

Contrary to expectations, introgression of the annual *DIV1* locus into the perennial background also increased the amount of induction in response to herbivory relative to perennial parent lines (Figure 2; Table 3B). Perennial lines with the annual *DIV1* locus introgressed induced at least twofold in response to feeding by each herbivore (Figure 1). This induction was only marginally significantly greater than that of perennial parents for the specialist buckeye herbivore, although significant for this comparison for the generalist cabbage looper (Figure 2; Table 3B). Perennial lines with the annual *DIV1* locus introgressed did not induce more in response to either herbivore than did the perennial lines with other annual genes introgressed ('perennial control'; Table 3B).

3.3 | Multivariate arsenal defence induction

Multivariate defence arsenals changed following herbivory, for all herbivore types and across all plant lines (Table 1B). There were also significant interactions for all two-way permutations of treatment, plant line and herbivore type in their effects on the multivariate defence arsenal.

3.4 | Parent ecotypes

As with the univariate metric of total PPGs, constitutive multivariate PPG arsenals of annual parent lines differed from those of perennial parent lines (pseudo $F = 35.46$, $p(\text{perm}) = 0.001$; Figure 3). The arsenal composition of annual and perennial parent lines significantly changed relative to constitutive arsenals following herbivory (annual: pseudo $F = 64.35$, $p(\text{perm}) = 0.001$; perennial: pseudo $F = 36.20$, $p(\text{perm}) = 0.001$; Figure 3).

Herbivory by the specialist herbivore resulted in a different defence arsenal than did herbivory by the generalist, within both annual and perennial parent lines (pseudo $F = 50.40$, $p(\text{perm}) = 0.001$). Herbivory by the generalist herbivore also resulted in a greater change in

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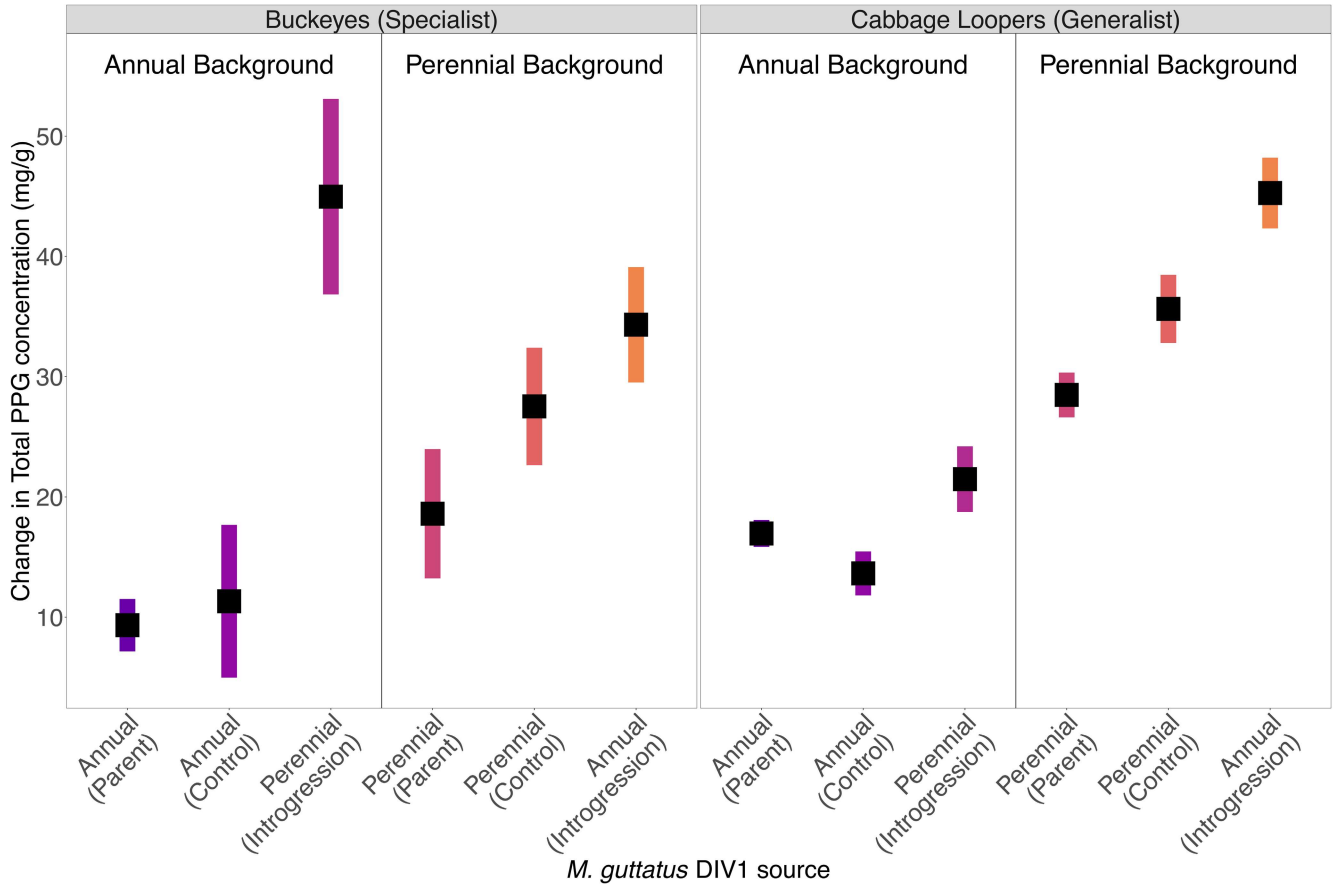

FIGURE 2 The change in concentration of foliar total produces phenylpropanoid glycosides in response to herbivory. Results include annual and perennial parent lines and annual/perennial lines with *DIV1* or other genetic regions from the alternative ecotype introgressed. Induction was done via buckeye or cabbage looper herbivory. Mean change is indicated by black squares with ±1 standard error bars in colour. All plant lines showed significant induction except annual control plants with buckeye herbivory.

TABLE 3 The effects of the *DIV1* locus on degree of total produces phenylpropanoid glycoside induction, tested with custom contrasts following the analysis reported in Table 1 and corrected with Bonferroni adjustment.

Degree of induction (induced—constitutive)	Buckeyes				Cabbage loopers			
Comparison	Estimate	SE	*T*	*p*-Value	Estimate	SE	*T*	*p*-Value
(A)								
Annual with perennial *DIV1* versus annual parent	35.64	7.13	5.00	**<0.001**	4.51	3.39	1.33	>0.999
Annual with perennial *DIV1* versus annual control	33.65	8.13	4.14	**<0.001**	7.84	4.00	1.96	0.714
(B)								
Perennial with annual *DIV1* versus perennial parent	15.72	5.51	2.85	*0.065*	16.80	5.44	3.09	**0.030**
Perennial with annual *DIV1* versus perennial control	6.79	5.53	1.23	>0.999	9.64	5.85	1.65	>0.999

Note: Significant *p*-values are in bold text and indicate a difference in the degree of induction between plant lines.

defence arsenals than did herbivory by the specialist herbivore (pseudo $F=16.024$, $p(\text{perm})=0.001$). Regardless of herbivory type, annual and perennial parent lines were also significantly different from one another in induced PPG arsenal composition (pseudo $F=54.19$, $p(\text{perm})=0.001$).

Plant lines did not differ in the specificity of their multivariate defence arsenal response to induction by the specialist versus the generalist, as shown by the lack of three-way interaction between treatment, plant line and herbivore type (Table 1B).

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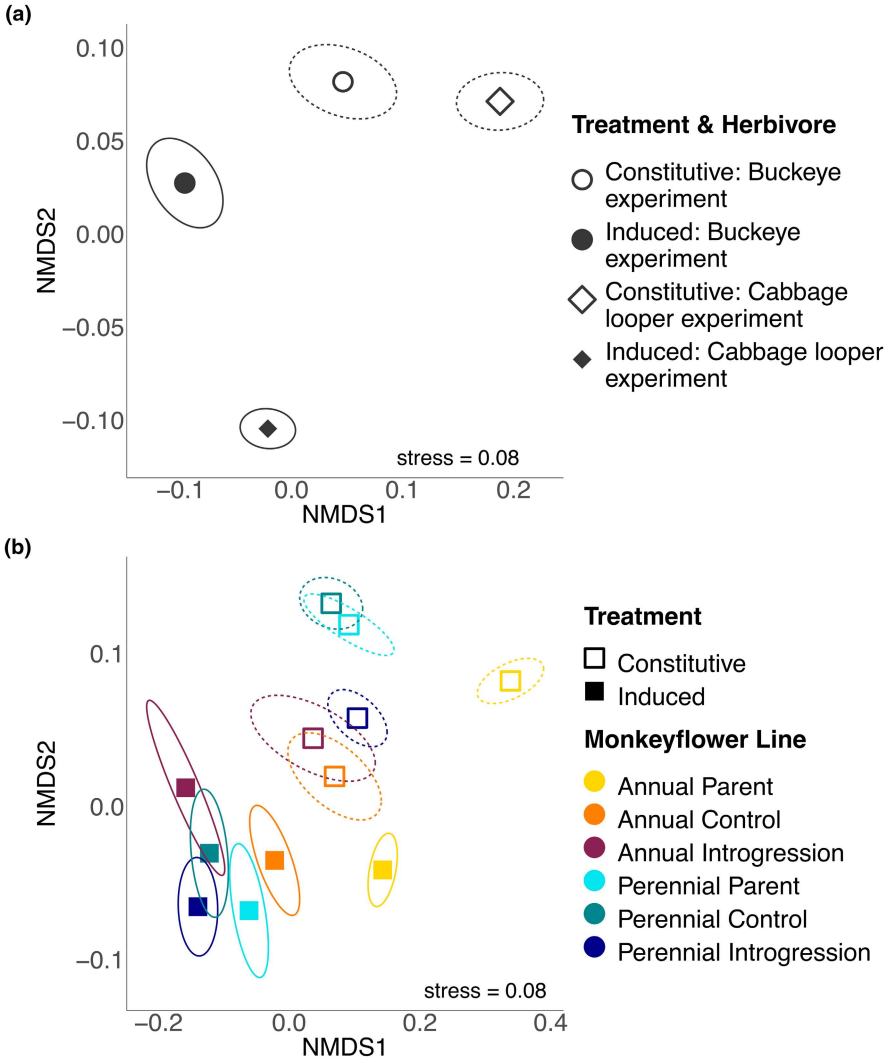

FIGURE 3 Foliar PPG multivariate arsenals in constitutive and induced plants. (a) represents the two-way interaction between treatment and caterpillar species and (b) shows the interaction between treatment and plant line. Centroids of groups of plants visualized using non-metric multidimensional scaling with Bray–Curtis distance. Control lines have some alleles from the alternative ecotype but maintain the *DIV1* orientation that matches their genetic background. 'Annual Introgression' lines have the annual genetic background and the perennial *DIV1* locus introgressed, and vice versa for 'Perennial Introgression'. Ellipses represent 95% confidence; dotted ellipses are used for constitutive produces phenylpropanoid glycoside arsenals and solid for induced.

3.5 | DIV1 locus

Constitutive and induced PPG multivariate arsenals were significantly influenced by the *DIV1* locus (Figure 3; Table 4). Both reciprocal introgressions of *DIV1* (introgression of the perennial *DIV1* locus into the annual genome and introgression of the annual *DIV1* locus into the perennial genome) resulted in significant shifts in constitutive and induced PPG arsenal composition when compared with parent lines.

While that result is relatively straightforward, comparisons of constitutive and induced multivariate arsenals between lines introgressed with *DIV1* versus other genes illuminates the complex genetic interactions underlying the phenotype patterns. The constitutive composition did not differ between annual lines introgressed with perennial *DIV1* versus those introgressed with other perennial genes, though the induced composition did differ between those lines (Figure 3; Table 4). Conversely, the induced composition did not differ between perennial lines introgressed with annual *DIV1* versus those introgressed with other annual genes, though the constitutive composition did differ between those lines (Figure 3; Table 4).

3.6 | Response across individual PPGs

A closer look at changes in levels of individual PPGs following herbivory by the specialist buckeye or generalist cabbage looper highlights some of the trends underlying broader patterns in the univariate and multivariate responses. These patterns are shown in Figure 4. We did not do significance testing on individual PPG levels due to the very high number of combinations of individual PPGs, but here we highlight a few trends. First, there is very clearly specificity in response to herbivory, which is particularly apparent for calceolarioside A (calcA) and conandroside, the two most abundant PPGs across the study (Figure 4a).

There is a substantially greater increase in calcA in most plants induced by cabbage loopers relative to buckeyes, with the extent ranging from nearly the same (in perennial parents) to a ninefold increase in annuals with perennial *DIV1*. Likewise, most plants induced by buckeyes have a relatively greater increase in conandroside, ranging nearly the same (in perennials with annual *DIV1*) to 13-fold (in annuals with perennial *DIV1*) relative to cabbage looper induction.

Second, concentrations of individual PPGs tended to increase in response to herbivory, with a few exceptions (Figure 4). Plants with

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TABLE 4 The influence of the *DIV1* locus on constitutive (A) and induced (B) multivariate produces phenylpropanoid glycoside arsenal composition, tested with PERMANOVA pairwise comparisons following the analysis presented in Table 1.

	(A) Constitutive arsenal composition		(B) Induced arsenal composition	
Comparisons	**Pseudo *F***	**Corrected *p*(perm)**	**Pseudo *F***	**Corrected *p*(perm)**
Annual with perennial *DIV1* versus annual parent	34.01	**0.001**	54.74	**0.001**
Annual with perennial *DIV1* versus annual control	0.49	0.549	11.42	**0.003**
Perennial with annual *DIV1* versus perennial parent	3.70	**0.035**	5.50	**0.007**
Perennial with annual *DIV1* versus perennial control	44.10	**0.001**	2.10	0.129

Note: Significant *p*-values are in bold text.

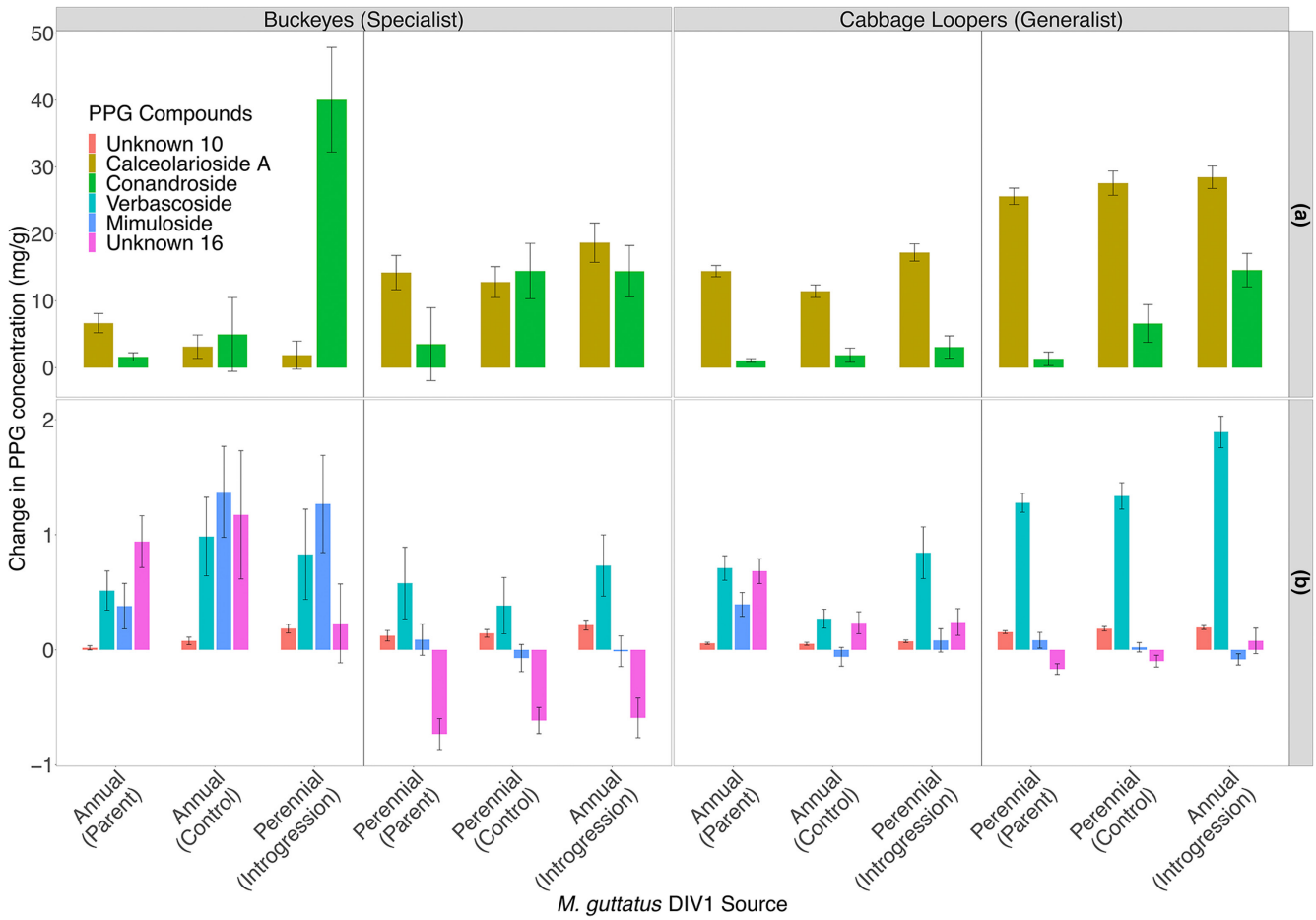

FIGURE 4 Induced changes in plant foliar concentrations (mg/g dry weight) of individual produces phenylpropanoid glycosides (PPGs) in response to herbivory by buckeyes and cabbage loopers. Bars represent mean change in concentration and error bars show ±1 standard error. Note the differences in *y*-axis scale between panels (a) (PPGs with higher concentrations) and (b) (PPGs with lower concentrations).

the perennial background (perennial parent lines, perennial control lines and perennial lines with the annual *DIV1* locus) had reduced levels of some compounds following herbivory. This is most apparent for the compound unknown PPG 16 and is most pronounced following buckeye herbivory.

Finally, among compounds present in lower concentrations (Figure 4b), plants with the perennial background invest the most in verbascoside in response to induction, regardless of herbivore type. Plants with the annual background invest more evenly in a variety of these lower concentration compounds; this trend is especially

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pronounced in plants with the annual background following buckeye herbivory.

4 | DISCUSSION

In this study, we investigated how divergent plant life-history strategies and a life-history altering locus influence plasticity of phytochemical herbivory resistance traits in response to attack by two herbivores with different diet breadth. We found that plant life-history strategy (annual vs. perennial) and the *DIV1* locus significantly influenced levels of constitutive herbivory resistance, as well as resistance induction. Perennial plants had higher total levels of univariate constitutive and induced defence than annuals, regardless of herbivore identity. Annual lines induced less in response to herbivory by the generalist than did perennial lines, while induction response was equivalent across parent ecotypes for herbivory by the specialist.

The effects of the *DIV1* locus on levels of constitutive and induced defence were dependent on genetic background, the haplotype of *DIV1* and herbivore identity. The patterns of univariate induction due to *DIV1* were non-additive and did not always match expectations based on patterns from annual/perennial parents. For example, while perennial plants had higher levels of constitutive and induced defence than did annuals, when the annual *DIV1* locus was present in the perennial genetic background, induction response to herbivory was higher than for the perennial parent lines. *DIV1* also influenced the specificity of univariate induction; for example, the introgression of perennial *DIV1* into the annual background caused a reversal of the relative response to the two herbivores.

Patterns for multivariate defence arsenals generally echoed those of univariate, with annual and perennial plants and those with different haplotypes of *DIV1* differing significantly in constitutive and induced resistance. Like univariate resistance, we found specificity of response, whereby induced multivariate defence arsenals differed based on herbivore inducer. However, specificity of multivariate response did not differ among plant lines.

4.1 | Genetic basis of resistance and life-history strategy trade-offs

The evolution of resource allocation to growth, reproduction and defence depends on the nature of abiotic and biotic selection pressures faced by each local population as well as trait genetic architecture and prevalence of genetic constraints. While classic plant defence theory focuses on phenotypic patterns and selection pressures (Coley et al., 1985; Herms & Mattson, 1992; Rhoades, 1979), plant evolutionary response to these pressures is dependent on trait genetic architecture and the extent to which traits are genetically correlated (Gardner & Latta, 2007; Kooyers et al., 2020).

Our results finding higher constitutive levels of phytochemical defence in perennial versus annual plants are consistent with prior results in yellow monkeyflowers (Holeski et al., 2013; Lowry et al., 2019) as well as other systems (e.g. Mutikainen & Wall, 1995; but see Wallis & Galarneau, 2020). In Northern California, as well as other regions of its range, yellow monkeyflower populations differ dramatically in life-history strategy along a soil–water availability gradient that governs growing season length (Gould et al., 2018; Hall & Willis, 2006; Lowry et al., 2008; Lowry et al., 2019). Along with this local adaptation in life-history traits, there is a trade-off between growth, reproduction and constitutive defence (Kooyers et al., 2017, 2020). While prior studies in yellow monkeyflower showed that perennial plants invest more heavily in defence in the form of constitutive levels of PPGs than do annuals (Holeski et al., 2013; Lowry et al., 2019; Rotter et al., 2019), and that this difference is substantially influenced by the *DIV1* locus, relative levels of phytochemical induction were previously unknown. Here, we show that perennial plants, in addition to being more highly constitutively defended, also induce more than annuals. Further, yet-to-be-determined genes in the *DIV1* locus underlie part of this differential induction response by annuals and perennials. The co-localized genetic control of constitutive and induced resistance illustrates a genetic mechanism underlying the phenotypic predictions in plant defence theory for relationships between these traits. A small amount of previous work in monkeyflower and other systems has also shown genetic loci (QTL) that co-localize for constitutive and induced defences (Bloomer et al., 2014; Holeski et al., 2010; Kliebenstein et al., 2002).

Likewise, in support of a genetic mechanism underlying phenotypic predictions of plant defence theory, genetic correlations between plant reproduction or growth and constitutive phytochemical defence have been found in multiple plant species (e.g. Defossez et al., 2018; Koricheva, 2002). Previous work in yellow monkeyflower identified loci (QTLs) that co-localize for constitutive defence and life-history aspects and underlie differences in allocation among traits (Kooyers et al., 2020; Lowry et al., 2019). In comparison, while genetic variation for induced defence is widespread (Agrawal et al., 2002; Holeski et al., 2010, 2012; Kliebenstein et al., 2002; Ogran et al., 2020), assessment of genetic correlations or trade-offs involving induction are relatively rare. We know of no genetic mapping studies that examine differences in allocation to induced defence and life-history traits among populations.

Over the past decade, multiple studies have emphasized the role of complex regulatory control pathways in shaping both growth and resistance, with multiple interactions between these pathways influencing the phenotypic outcome (Huot et al., 2014; Kliebenstein, 2016; Yang et al., 2012). An elegant study in *Arabidopsis* demonstrated that when genetic-based regulatory switches are manipulated, growth-defence trade-offs can be uncoupled (Campos et al., 2016). While the genes and regulatory pathways responsible for trade-offs among growth, reproduction and constitutive resistance are beginning to be elucidated in model systems (Züst &

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Agrawal, 2017), the role of these in shaping patterns of defence induction remains largely unassessed.

4.2 | The role of DIV1 and gene interactions in shaping resistance

Our use of plants with perennial and annual background genomes with introgressed regions from the alternative background allows us to fill in knowledge gaps of the genetic mechanisms underlying resistance associated with different life-history strategies. Prior work in yellow monkeyflower demonstrated that the *DIV1* chromosomal inversion contributes to the difference in life-history strategy between annual and perennial populations (Friedman, 2014; Friedman et al., 2015; Lowry et al., 2019; Lowry & Willis, 2010). The perennial haplotype of *DIV1* contributes to greater long-term growth and less short-term fecundity than does the annual orientation. While *DIV1* locus also affects constitutive PPG concentrations (Lowry et al., 2019), the influence of this on defence induction was previously unknown.

Our comparisons of the *DIV1* introgression lines with the 'control' lines, that have the same genetic background, demonstrate that the induction of both univariate and multivariate resistance phenotypes is context-dependent, with genetic background, *DIV1* locus and type of herbivory influencing the outcome. Our experiment was not designed to specifically test for the role of epistasis in the induction of resistance, but both univariate and multivariate induced resistance phenotypes do not fit patterns of additive combinations of alleles from a particular genetic background and *DIV1* combination. This is most apparent in the induction results for plants with a perennial background and the annual orientation of *DIV1*; these plants showed a greater degree of induction than perennial parent lines with the perennial orientation of *DIV1*.

The genetic architecture of quantitative traits, such as life-history strategy and/or resistance traits, is often complex, with non-additive effects of multiple genetic loci (Falconer & Mackay, 1996). The extent to which epistasis affects within-population trait variation and thus evolution is largely unknown (notable exceptions include Kelly, 2005; Kelly & Mojica, 2011). Previous studies in yellow monkeyflower have found substantial pleiotropy (single gene/region influencing multiple traits) as well as epistasis underlying life-history traits and defence traits. For example, pleiotropic effects influencing life-history traits that differ between annual and perennial ecotypes have been found in multiple studies (Friedman, 2014; Hall & Willis, 2006; Hall et al., 2010; Lowry & Willis, 2010). Previous work with the *DIV1* introgression lines did show epistasis for life-history traits such as flowering time (Friedman, 2014). Unlike our results, which showed the greatest phenotypic effect of the perennial *DIV1* allele on PPG levels when present in the annual background, Friedman (2014) found the greatest effects on life-history traits in lines with the annual *DIV1* locus in the perennial background. We hypothesize that these differences across studies could reflect differences in traits of focus or could be due to differences in experimental day length between studies. Here, we use a relatively short day length (3 h shorter than Friedman, 2014) that mimics the growing season in the natural populations from which our lines were derived. This may lead to different allocation outcomes to growth, flowering and resistance traits than in experiments with longer day lengths.

4.3 | Specificity of induction response

Patterns across plant lines with different *DIV1* loci indicate that plant specificity to herbivory is genetically complex. Both our univariate and multivariate phytochemical resistance results show some differential responses to herbivory by the generalist cabbage looper versus the specialist buckeye, but this result was not uniform across plant lines. Specificity is complex in that it can be affected by both plant detection of herbivore identity and/or herbivore-specific suppression of plant response (Bonaventure, 2014; Jones et al., 2022), and can also reflect local adaptation. For example, as shown in Table 2, perennial parent and control lines tend to induce more in response to the specialist herbivore than do annual parent/control lines. This pattern is reversed in the perennial plants with the annual *DIV1* locus. In addition, both DIV1 lines induce drastically more than do the parent and control lines in response to herbivory by the specialist. The consistency of this pattern across both *DIV1* lines indicates a potential disruption of the genetic pathways influencing induction in response to this herbivore that occurs simply with the swapping of the *DIV1* region. The same pattern is not seen in response to the generalist herbivore. It is not clear from our study what aspect of specificity is driving the patterns observed; the interaction between *DIV1* and genetic background might underlie detection of herbivore identity and/or be involved with suppression of induction by particular herbivores.

Our results with individual PPGs highlight some of the patterns underlying the changes in Total PPG concentration and the multivariate arsenal composition following herbivory across plant lines. Herbivores can respond to suites of compounds within a subclass of defensive metabolites (such as PPGs) in several ways. They can respond to the combined concentration of all compounds, to the concentration of individual compounds and to mixtures of compounds (Agrawal & Fishbein, 2006; Wetzel & Whitehead, 2019). Prior work in yellow monkeyflower showed that performance of dietary generalist and specialist Lepidoptera (including the two species used in this study) is correlated with concentrations of individual PPGs, as well as with total PPG concentration (Rotter et al., 2019). The performance of the specialist buckeye is positively correlated with several individual PPGs; this herbivore sequesters PPGs and may be using them as feeding stimulants (Holeski et al., 2013; Rotter et al., 2018). Our results show patterns of plant response that would be expected with specificity of induction in response to specific herbivores. One compound, conandroside, that is negatively associated with buckeye performance was one of the most induced by buckeyes across plant lines in our study. The performance of the generalist herbivore

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(cabbage looper) used in our study is negatively associated with calceolarioside A, which was one of the individual PPGs most induced by cabbage looper herbivory in this study.

Prior work in other systems has shown variation in the magnitude and specificity of induction on an interspecific scale (Campbell & Kessler, 2013). However, very few studies have investigated differences in specificity among plant populations (Bingham & Agrawal, 2010; Uesugi et al., 2013), particularly in a context with known differences in herbivore pressure (Liu et al., 2018) and/or allocation to constitutive defence. Our findings of specificity of response, potential disruption of the pathways underlying this specificity and differing patterns of induction of PPGs that are known to correlate with herbivore performance, present potentially fruitful avenues for future research inquiries into the genetic basis of and constraints on specificity of induction.

5 | CONCLUSIONS

We found differences in phytochemical resistance induction across annual and perennial ecotypes of yellow monkeyflower. These patterns were governed to some extent by the *DIV1* genetic region, which also contributes substantially to differences between ecotypes in life-history traits and constitutive defence levels. This co-localization of traits may influence trade-offs in yellow monkeyflower defence and highlights the importance of incorporating molecular genetic studies into plant defence theory. The context-dependence of the effects of the *DIV1* locus on induction (i.e. variation due to genetic background as well as herbivore identity) provides support for the complexity of the regulatory pathways underlying trait plasticity.

AUTHOR CONTRIBUTIONS

LMH and MLB conceptualized the work and designed the experiment. DBL developed and supplied introgression lines for the experiments. MLB implemented the experiment and analysed the data. LMH led the writing of this article, with contributions from MLB and DBL. LMH and DBL obtained funding for this research.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare. Liza Holeski is the Commissioning Editor of Functional Ecology, but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

The data are available from the Dryad Digital Repository, https://doi.org/10.5061/dryad.w3r22810f (Blanchard et al., 2024).

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REFERENCES

Acevedo, F. E., Smith, P., Peiffer, M., Helms, A., Tooker, J., & Felton, G. W. (2019). Phytohormones in fall armyworm saliva modulate defense responses in plants. *Journal of Chemical Ecology*, *45*, 598–609.

Adler, F. R., & Harvell, C. D. (1990). Inducible defenses, phenotypic plasticity, and biotic environments. *Trends in Ecology & Evolution*, *5*, 407–410.

Agrawal, A. A. (2000). Specificity of induced resistance in wild radish: Causes and consequences for two specialist and two generalist caterpillars. *Oikos*, *89*, 493–500.

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, F. (2006). Plant defense syndromes. *Ecology*, *87*, S132–S149.

Ali, J. G., & Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, *17*, 293–302.

Bingham, R. A., & Agrawal, A. A. (2010). Specificity and trade-offs in the induced plant defence of common milkweed *Asclepias syriaca* to two lepidopteran herbivores. *Journal of Ecology*, *98*, 1014–1022.

Blanchard, M. L., Lowry, D. B., & Holeski, L. M. (2024). *Data from: Patterns of constitutive and induced herbivore defense are complex, but share a common genetic basis in annual and perennial monkeyflower.* https://doi.org/10.5061/dryad.w3r22810f

Bloomer, R. H., Lloyd, A. M., & Symonds, V. V. (2014). The genetic architecture of constitutive and induced trichome density in two new recombinant inbred line populations of Arabidopsis thaliana: Phenotypic plasticity, epistasis, and bidirectional leaf damage response. *BMC Plant Biology*, *14*, 119.

Bonaventure, G. (2014). Plants recognize herbivorous insects by complex signaling networks. *Annual Plant Reviews: Insect-Plant Interactions*, *47*, 1–36.

Bowers, M. D., & Collinge, S. K. (1992). Fate of iridoid glycosides in different life stages of the buckeye, *Junonia coenia* (Lepidoptera, Nymphalidae). *Journal of Chemical Ecology*, *18*, 817–831.

Bowers, M. D., & Stamp, N. (1993). Effects of plant-age, genotype, and herbivory on *Plantago* performance and chemistry. *Ecology*, *74*, 1778–1791.

Campbell, S. A., & Kessler, A. (2013). Plant mating system transitions drive the macroevolution of defense strategies. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 3973–3978.

Campos, M. L., Yoshida, Y., Major, I. T., de Oliveira Ferreira, D., Weraduwage, S. M., Froehlich, J. E., Johnson, B. F., Kramer, D. M., Jander, G., Sharkey, T. D., & Howe, G. A. (2016). Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. *Nature Communications*, *7*, 12570.

Cipollini, D., Purrington, C. B., & Bergerlson, J. (2003). Costs of induced responses in plants. *Basic and Applied Ecology*, *4*, 79–85.

Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant antiherbivore defense. *Science*, *230*, 895–899.

Cope, O. L., Keefover-Ring, K., Kruger, E. L., & Lindroth, R. L. (2021). Growth-defense trade-offs shape population genetic composition in an iconic forest tree species. *Proceedings of the National Academy of Sciences of the United States of America*, *118*, 7e2103162118.

Cornell, H., & Hawkins, B. (2003). Herbivore responses to plant secondary compounds: A test of phytochemical coevolution theory. *American Naturalist*, *161*, 507–522.

Defossez, E., Pellissier, L., & Rasmann, S. (2018). The unfolding of plant growth form-defence syndromes along elevation gradients. *Ecology Letters*, *21*, 609–618.

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Endara, M. J., & Coley, P. D. (2011). The resource availability hypothesis revisited: A meta-analysis. *Functional Ecology*, *25*, 389–398.
Erb, M., Meldau, S., & Howe, G. A. (2012). Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science*, *17*, 250–259.
Erb, M., & Reymond, P. (2019). Molecular interactions between plants and insect herbivores. *Annual Review of Plant Biology*, *70*, 527–557.
Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). Addison Wesley Longman.
Flagel, L. E., Willis, J. H., & Vision, T. J. (2014). The standing pool of genomic structural variation in a natural population of *Mimulus guttatus*. *Genome Biology and Evolution*, *6*, 53–64.
Friedman, J. (2014). Genetic determinants and epistasis for life-history trait differences in the common monkeyflower, *Mimulus guttatus*. *Journal of Heredity*, *105*, 816–827.
Friedman, J., Twyford, A. D., Willis, J. H., & Blackman, B. K. (2015). The extent and genetic basis of phenotypic divergence in life-history traits in *Mimulus guttatus*. *Molecular Ecology*, *24*, 111–122.
Futuyma, D. J., & Moreno, G. (1988). The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, *19*, 207–234.
Gardner, K. M., & Latta, R. G. (2007). Shared quantitative trait loci underlying the genetic correlation between continuous traits. *Molecular Ecology*, *16*, 4195–4209.
Gould, B. A., Chen, Y., & Lowry, D. B. (2018). Gene regulatory divergence between locally adapted ecotypes in their native habitats. *Molecular Ecology*, *27*, 4147–4188.
Hahn, P. G., & Maron, J. L. (2016). A framework for predicting intraspecific variation in plant defense. *Trends in Ecology & Evolution*, *31*, 646–656.
Hall, M. C., Lowry, D. B., & Willis, J. H. (2010). Is local adaptation in *Mimulus guttatus* caused by trade-offs at individual loci? *Molecular Ecology*, *19*, 2739–2753.
Hall, M. C., & Willis, J. H. (2006). Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evolution*, *60*, 2466–2477.
Havko, N. E., Major, I. T., Jewell, J. B., Attaran, E., & Howe, G. A. (2016). Control of carbon assimilation and partitioning by jasmonate: An accounting of growth–defense tradeoffs. *Plants*, *5*, 7.
Herms, D. A., & Mattson, W. J. (1992). The dilemma of plants: To grow or defend. *Quarterly Review of Biology*, *67*, 283–335.
Holeski, L. M. (2021). The genetic basis of plant-herbivore interactions. In K. Del-Claro & H. M. Torezan-Silingardi (Eds.), *Plant-animal interactions* (pp. 59–92). Springer Nature.
Holeski, L. M., Chase Alone, R., & Kelly, J. K. (2010). The genetics of phenotypic plasticity in plant defense: Trichome production in *Mimulus guttatus*. *American Naturalist*, *175*, 391–400.
Holeski, L. M., Hillstrom, M. L., Whitham, T. G., & Lindroth, R. L. (2012). Relative importance of plant species identity, genotype, ontogeny, induction, and temporal variation in producing a mosaic of defenses by a foundation tree species. *Oecologia*, *170*, 695–704.
Holeski, L. M., Keefover-Ring, K., Bowers, M. D., HarnEnz, Z. T., & Lindroth, R. L. (2013). Patterns of phytochemical variation in *Mimulus guttatus* (yellow monkeyflower). *Journal of Chemical Ecology*, *39*, 525–536.
Holeski, L. M., Monnahan, P., Koseva, B., McCool, N., Lindroth, R. L., & Kelly, J. K. (2014). A high-resolution genetic map of yellow monkeyflower identifies chemical defense QTLs and recombination rate variation. *G3: Genes, Genomes, Genetics*, *4*, 813–821.
Hothorn, T. (2023). *Additional multcomp examples*. https://cran.r-project.org/web/packages/multcomp/vignettes/multcomp-examples.pdf
Howe, G. A., Major, I. T., & Koo, A. J. (2018). Modularity in jasmonate signaling for multistress resilience. *Annual Reviews in Plant Biology*, *69*, 387–415.
Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth-defense tradeoffs in plants: A balancing act to optimize fitness. *Molecular Plant*, *7*, 1267–1287.
Jones, A. C., Felton, G. W., & Tumlinson, J. H. (2022). The dual function of elicitors and effectors from insects: Reviewing the "arms race" against plant defenses. *Plant Molecular Biology*, *109*, 427–445.
Karban, R., & Baldwin, I. T. (1997). *Induced responses to herbivory*. University of Chicago Press.
Keefover-Ring, K., Holeski, L. M., Bowers, M. D., Clauss, A., & Lindroth, R. L. (2014). Phenylpropanoid glycosides of *Mimulus guttatus* (yellow monkeyflower). *Phytochemistry Letters*, *10*, 132–139.
Kelly, J. K. (2005). Epistasis in monkeyflowers. *Genetics*, *171*, 1917–1931.
Kelly, J. K., & Mojica, J. P. (2011). Interactions among flower-size QTL of *Mimulus guttatus* are abundant but highly variable in nature. *Genetics*, *189*, 1461–1471.
Kliebenstein, D. J. (2014). Quantitative genetics and genomics of plant resistance to insects. In C. Voelckel & G. Jander (Eds.), *Insect-plant interactions* (pp. 235–262). Wiley Blackwell.
Kliebenstein, D. J. (2016). False idolatry of the mythical growth versus immunity tradeoff in molecular systems plant pathology. *Physiological and Molecular Plant Pathology*, *95*, 55–59.
Kliebenstein, D. J., Pedersen, D., Barker, B., & Mitchell-Olds, T. (2002). Genetic architecture of plastic methyl jasmonate responses in *Arabidopsis thaliana*. *Genetics*, *161*, 1685–1696.
Kollar, L. M., Stanley, L. E., Raju, S. K. K., Lowry, D. B., & Niederhuth, C. E. (2023). The role of breakpoint mutations, supergene effects, and ancient nested rearrangements in the evolution of adaptive chromosome inversions in the yellow monkeyflower, *Mimulus guttatus*. *bioRxiv*. https://doi.org/10.1101/2023.12.06.570460
Kooyers, N. J., Blackman, B. K., & Holeski, L. M. (2017). Optimal defense theory explains deviations from latitudinal herbivory defense hypothesis. *Ecology*, *98*, 1036–1048.
Kooyers, N. J., Donofrio, A., Blackman, B. K., & Holeski, L. M. (2020). The genetic architecture of plant defense tradeoffs in a common monkeyflower. *Journal of Heredity*, *111*, 333–345.
Kooyers, N. J., Greenlee, A. B., Colicchio, J. M., Oh, M., & Blackman, B. K. (2014). Replicate altitudinal clines reveal that evolutionary flexibility underlies adaptation to drought stress in annual *Mimulus guttatus*. *New Phytologist*, *206*, 152–165.
Koricheva, J. (2002). Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. *Ecology*, *83*, 176–190.
Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1–26.
Lenth, R., Bolker, B., Buerkner, B. P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., & Sigmann, H. (2024). *emmeans: Estimated marginal means, aka least-squares means*. R package version 1.10.0. https://cran.r-project.org/web/packages/emmeans/index.html
Liu, M., Zhou, F., Pan, X., Zhang, Z., Traw, M. B., & Li, B. (2018). Specificity of herbivore-induced responses in an invasive species, *Alternanthera philoxeroides* (alligator weed). *Ecology and Evolution*, *8*, 59–70.
Lowry, D. B., Popovic, D., Brennan, D. J., & Holeski, L. M. (2019). Mechanisms of a locally adaptive shift in allocation among growth, reproduction, and herbivore resistance in *Mimulus guttatus*. *Evolution*, *73*, 1168–1181.
Lowry, D. B., Rockwood, R. C., & Willis, J. H. (2008). Ecological reproductive isolation of coast and inland races of Mimulus guttatus. *Evolution*, *62*(9), 2196–2214. https://doi.org/10.1111/j.1558-5646.2008.00457.x
Lowry, D. B., & Willis, J. H. (2010). A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. *PLoS Biology*, *8*, e1000500.
Loxdale, H. D., & Harvey, J. A. (2016). The 'generalism' debate: Misinterpreting the term in the empirical literature focusing on dietary breadth in insects. *Biological Journal of the Linnean Society*, *119*, 265–282.

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Lundgren, M., & Des Marais, D. (2020). Life-history variation as a model for understanding trade-offs in plant–environment interactions. *Current Biology*, *30*, R180–R189.

Marquis, R. J., & Moura, R. F. (2021). Escape as a mechanism of plant resistance against herbivores. In K. Del Claro & H. M. Torezan-Silingardi (Eds.), *Plant-animal interactions, sources of biodiversity* (pp. 40–60). Springer Nature.

Martín-Fernández, J. A., Barceló-Vidal, C., & Pawlowsky-Glahn, V. (2003). Dealing with zeros and missing values in compositional data sets using nonparametric imputation. *Mathematical Geology*, *35*, 253–278.

Monnahan, P. J., & Kelly, J. K. (2017). The genomic architecture of flowering time varies across space and time in *Mimulus guttatus*. *Genetics*, *206*, 1621–1635.

Musser, R. O., Cipollini, D. F., Hum-Musser, S. M., Williams, S. A., Brown, J. K., & Felton, G. W. (2005). Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. *Archives of Insect Biochemistry and Physiology*, *58*, 128–137.

Musser, R. O., Hum-Musser, S. M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J. B., & Felton, G. W. (2002). Herbivory: Caterpillar saliva beats plant defences: A new weapon emerges in the evolutionary arms race between plants and herbivores. *Nature*, *416*, 599–600.

Mutikainen, P., & Wall, M. (1995). Growth, reproduction and defence in nettles: Responses to herbivory modified by competition and fertilization. *Oecologia*, *104*, 487–495.

Ogran, A., Conner, J., Agrawal, A. A., & Barazani, O. (2020). Evolution of phenotypic plasticity: Genetic differentiation and additive genetic variation for induced plant defense in wild arugula *Eruca sativa*. *Journal of Evolutionary Biology*, *33*, 237–246.

Oksanen, J., Simpson, G. L., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Henry, M., Stevens, Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., … Weedon, J. (2022). *vegan: Community ecology package*. R package version 2.5–7. https://cran.r-project.org/web/packages/vegan/index.html

Rhoades, D. F. (1979). Evolution of plant chemical defense against herbivores. In G. A. Rosenthal & D. H. Janzen (Eds.), *Herbivores: Their interaction with secondary plant metabolites* (pp. 1–55). Academic Press.

Rothwell, E. M., & Holeski, L. M. (2019). Phytochemical defences and performance of specialist and generalist herbivores: A meta-analysis. *Ecological Entomology*, *45*, 396–405.

Rotter, M. C., Couture, J. J., Rothwell, E. M., Garcia, J., & Holeski, L. M. (2018). Evolutionary ecology of plant resistance traits across the herbivore diet spectrum: A test in the model plant *Mimulus guttatus*. *Evolutionary Ecology Research*, *19*, 423–440.

Rotter, M. C., Vallejo-Marin, M., & Holeski, L. M. (2019). A test of the evolution of increased competitive ability in two invaded regions. *Evolutionary Ecology*, *33*, 713–735.

Salazar, G. (2021). *EcolUtils: Utilities for community ecology analysis*. R package Version 0.1. https://github.com/GuillemSalazar/EcolUtils

Singer, M. S. (2008). Evolutionary ecology of polyphagy: Specialization, speciation, and radiation. In K. J. Tilmon (Ed.), *The evolutionary biology of herbivorous insects* (pp. 29–42). University of California Press.

Stamp, N. (2003). Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology*, *78*, 23–55.

Strauss, S. Y., Rudgers, J. A., Lau, J. A., & Irwin, R. E. (2002). Direct and ecological costs of resistance to herbivory. *Trends in Ecology & Evolution*, *17*, 278–285.

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

Tian, D., Peiffer, M., Shoemaker, E., Tooker, J., Haubruge, E., Francis, F., Luthe, D. S., & Felton, G. W. (2012). Salivary glucose oxidase from caterpillars mediates the induction of rapid and delayed-induced defenses in the tomato plant. *PLoS One*, *7*(4), e36168.

Uesugi, A., Poelman, E. H., & Kessler, A. (2013). A test of genotypic variation in specificity of herbivore-induced responses in *Solidago altissima L*. (Asteraceae). *Oecologia*, *173*, 1387–1396.

Wallis, C. M., & Galarneau, E. R.-A. (2020). Phenolic compound induction in plant-microbe and plant-insect interactions: A meta-analysis. *Frontiers in Plant Science*, *11*, 580753.

Wetzel, W. C., & Whitehead, S. R. (2019). The many dimensions of phytochemical diversity: Linking theory to practice. *Ecology Letters*, *23*, 16–32.

Wu, C. A., Lowry, D. B., Cooley, A. M., Wright, K. M., Lee, Y. W., & Willis, J. H. (2008). Mimulus is an emerging model system for the integration of ecological and genomic studies. *Heredity*, *100*, 220–230.

Yang, D. L., Yao, J., Mei, C. S., Tong, X. H., Zeng, L. J., Li, Q., Xiao, L. T., Sun, T. P., Li, J., Deng, X. W., Lee, C. M., Thomashow, M. F., Yang, Y., He, Z., & He, S. Y. (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, E1192–E1200.

Züst, T., & Agrawal, A. A. (2017). Trade-offs between plant growth and defense against insect herbivory: An emerging mechanistic synthesis. *Annual Review of Plant Biology*, *68*, 513–534.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Breeding design for the creation of near-isogenic introgression lines used in this study.

Table S1. The effects of herbivore identity on degree of total produces phenylpropanoid glycoside induction, tested with custom contrasts following the analysis reported in Table 1 and corrected with Bonferroni adjustment.

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