

Evolution of phenotypic plasticity: Genetic differentiation and additive genetic variation for induced plant defence in wild arugula *Eruca sativa*

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Abstract

Phenotypic plasticity is the primary mechanism of organismal resilience to abiotic and biotic stress, and genetic differentiation in plasticity can evolve if stresses differ among populations. Inducible defence is a common form of adaptive phenotypic plasticity, and long-standing theory predicts that its evolution is shaped by costs of the defensive traits, costs of plasticity and a trade-off in allocation to constitutive versus induced traits. We used a common garden to study the evolution of defence in two native populations of wild arugula *Eruca sativa* (Brassicaceae) from contrasting desert and Mediterranean habitats that differ in attack by caterpillars and aphids. We report genetic differentiation and additive genetic variance for phenology, growth and three defensive traits (toxic glucosinolates, anti-nutritive protease inhibitors and physical trichome barriers) as well their inducibility in response to the plant hormone jasmonic acid. The two populations were strongly differentiated for plasticity in nearly all traits. There was little evidence for costs of defence or plasticity, but constitutive and induced traits showed a consistent additive genetic trade-off within each population for the three defensive traits. We conclude that these populations have evolutionarily diverged in inducible defence and retain ample potential for the future evolution of phenotypic plasticity in defence.

KEYWORDS

cost of plasticity, genetic differentiation, genetic trade-off, genetic variation, herbivory, induced defence, phenotypic plasticity

1 | INTRODUCTION

Adaptive phenotypic plasticity can evolve in response to temporal variation in the environment, or when populations that experience different environments are connected by rates of gene flow that reduce genetic differentiation between them (Sultan & Spencer, 2002; Via & Lande, 1985). If two populations differ in the kind or amount of temporal variation in the environment, then genetic differentiation for locally adaptive plasticity can evolve. While the evolution of adaptive plasticity

within populations has been well studied for decades (Scheiner, 1993; Via et al., 1995), the study of adaptive genetic differentiation for plasticity is more recent [see below; (Murren et al., 2014; Relyea, 2002; Torres-Dowdall, Handelsman, Reznick, & Ghalambor, 2012)], and few studies have examined genetic variation for plasticity within and among the same populations. This local adaptation for plasticity provides some of the best evidence for adaptive plasticity itself.

Induced defences in response to attack by enemies have long provided many of our best examples of adaptive plasticity (Tollrian

& Harvell, 1999). Feeding by herbivores has led to the evolution of various plant defence mechanisms and the plastic ability of a plant to up-regulate defence following attack (Karban & Baldwin, 1997). Constitutive defences are widely thought to be adaptive under conditions of constant herbivore pressure, while induced defence strategies are expected to be favoured in environments where herbivore pressure is more variable (Bixenmann, Coley, Weinhold, & Kursar, 2016). As a means to understand the evolution of plasticity in defence strategies, within-population ecological genetic studies have considered costs and trade-offs associated with induced defence (Agrawal, Conner, Johnson, & Wallsgrave, 2002; Bingham & Agrawal, 2010; Uesugi, Poelman, & Kessler, 2013) and between species phylogenetic approaches have also recently been employed to understand the macroevolution of constitutive and induced defence (Haak, Ballenger, & Moyle, 2014; Moreira, Abdala-Roberts, Parra-Tabla, & Mooney, 2014; Rasmann & Agrawal, 2011).

Among-population studies have recently garnered attention as an intermediate method to testing adaptive plant defence hypotheses (Agrawal, 2011; Hahn & Maron, 2016; Zust et al., 2012). Indeed, when populations experience consistent differences in selection, there is the potential for local adaptation in constitutive or plastic defence strategies (Murren et al., 2014). Early work on wild parsnips revealed that differential herbivory at two nearby populations was associated with genetic differentiation in constitutive and induced levels of defensive furanocoumarins, although there was no evidence for differentiation in plasticity per se (Zangerl & Berenbaum, 1990). More recently, population divergence in induced ant attractants has been studied in a legume along an elevation gradient (Rasmann et al., 2014) and for induced trichome production in *Mimulus* as means to identify quantitative trait loci underlying defensive traits (Holeski, Chase-Alone, & Kelly, 2010). Although population divergence in defensive plasticity (and other traits) has been documented, it is largely unknown whether these populations maintain additive genetic variance in plasticity, which is a prerequisite for the future evolution of plasticity (Murren et al., 2014).

We have been studying divergence among populations of wild arugula *Eruca sativa* (Brassicaceae) in Israel. Past common garden experiments revealed genetic differentiation among nine populations in AFLP markers as well as trichome density and flowering time (Westberg, Ohali, Shevelevich, Fine, & Barazani, 2013). Two of these populations, Susita in the northern Mediterranean climate and Sartaba in the southern desert, were chosen for further study; these show strong genome-wide differentiation, with an F_{ST} estimate of 0.15 from the AFLP markers (Barazani, unpub.). These populations differed in their induced resistance to a generalist herbivore (Ogran et al., 2016), perhaps due to differential induction of glucosinolates (Textor & Gershenzon, 2009). Here, we combine quantitative and ecological genetic approaches to estimate within- and between-population variation in induced defensive traits, including two chemical traits, toxic glucosinolates and anti-nutritive protease inhibitors (Ogran et al., 2016), and a physical defence (trichomes). We also monitored herbivory in the natural habitats to relate population differentiation in phenotypes to differences herbivory regimes.

Specifically, we addressed the following questions: (a) Is there temporal or spatial variation in the identity and intensity of herbivores attacking *E. sativa*? Temporal variation would select for plasticity (induction) while spatial variation for genetic differentiation for either constitutive or induced defences; (b) Is there genetic differentiation between the Mediterranean and desert populations for three defensive traits and plasticity in each? (c) Is there additive genetic variation for plasticity, that is, genotype by environment interaction, within either population? If so, this would enable a response to selection for plasticity in the future; and (d) Is there evidence for a costs of defence, costs of plasticity or a trade-off between constitutive and induced defensive traits? Although each of these costs has been addressed independently in other systems, typically for only one or two traits, by addressing them together for three defensive traits along with estimates of additive genetic variation we gain a more comprehensive picture of constraints on the evolution of defensive adaptations.

2 | MATERIALS AND METHODS

To study defence evolution, we used the two populations of *E. sativa* from Ogran et al. (2016): one from the east coast of the Sea of Galilee (32°46'39"N, 35°39'29"E) and the second from the southern Jordan Valley (32°04'49"N, 35°29'46"E). The former is characterized as a Mediterranean environment (average annual rainfall of 400–430 mm), whereas the latter site is a desert environment (average annual rainfall of ≤ 200 mm) (Goldreich, 2003).

2.1 | Field sampling of herbivory

To test for differences in the herbivore community between the desert and Mediterranean environments, we sampled a total of 17 plants at the two natural populations in 2014, nine at the desert and eight at the Mediterranean site. Plants were placed in paper bags and brought to the laboratory in a cooler. Herbivores were removed from the plants and sorted to the four most common insect taxa that were found on the plants, representing the Aphididae, Thysanoptera, Coleoptera and Lepidoptera. Aphids were most numerous, so the number per plant were counted on all but one plant from each site; the other three taxa were scored as present or absent on each plant. Plants were dried and weighed to correct for the larger size of the Mediterranean site plants. Because the main lepidopteran herbivore in these surveys was the specialist diamondback moth (*Plutella xylostella*), over the next 2 years we monitored the abundance of this species with pheromone lures that attract males (Contech Inc.). Following the manufacturer recommendations, we placed three lures in each habitat 50 m apart in March of both years, and traps were retrieved 7–10 days later. Traps were replaced three times in 2015; in 2016, the plant growing season was shortened by early high temperatures so only a single set of traps were placed. Traps were always placed and retrieved on the same days in the two locations; thus, sampling is paired in time. The number of male diamondback moths in each trap was counted.

2.2 | Common garden study

To test for genetic variation within populations and genetic differentiation among them in the traits and plasticity of defence, we grew half-sib families from both populations in a common garden. To minimize maternal effects, we created an initial grandparental generation by planting seeds from at least 30 plants from each of the two field populations in two separate screen houses at the Agricultural Research Organization experimental field site (32°46'39"N, 35°39'28"E), which is a Mediterranean environment. These plants were open pollinated within each population by introduced bumble bee hives. Plants from seeds produced in this common garden were grown in a pollinator-free greenhouse, and nested paternal half-sib families were created by randomly assigning to 20 plants from each population to be sires and 60 to be dams. Crossing each sire with three unique dams resulted in 20 paternal half-sib and 60 full-sib families per population, 120 total. In the following year, seeds from each full-sib family were germinated in 9-cm Petri dishes on moist filter paper (Whatman No. 1) and four-day-old seedlings were transplanted to 1-L pots containing a mixture of 50% peat, 30% tuff and 20% perlite (Shacham). Six seedlings from each of the full-sib families were randomly selected and grown in an insect-free net house at the same location, resulting in 360 plants per population, 720 total. Plants were grown in randomized positions and were drip-irrigated via an automatic system. Our analyses are based on these plants, which are descended from two generations of plants grown in a common environment, eliminating parental and grandparental environmental effects as a cause of population differentiation.

At the early vegetative stage, four weeks after sowing, methyl jasmonate was applied to three randomly chosen plants from each of the 120 full-sib families to stimulate induced responses to herbivory. Methyl jasmonate is a strong and consistent inducer of both glucosinolates and protease inhibitors in the Brassicaceae, and this treatment specifically stimulates an endogenous burst of the jasmonic acid hormonal signal (Cipollini & Sipe, 2001; Doughty, Kiddle, Pye, Wallsgrove, & Pickett, 1995). 150 µg of methyl jasmonate in 20 µl lanolin was applied to each of three leaves on each plant over a period of five days, one leaf per treatment day, with two-day intervals between treatments. The first induced leaf was collected 48 hr after treatment for glucosinolate and trypsin proteinase inhibitor (trypsin-PI) activity analyses (below); one leaf at the same developmental

stage was collected at the same time from noninduced control plants. Harvested leaf samples were immediately frozen in liquid nitrogen and stored at -80°C until the analyses.

Plant performance was evaluated on all plants from rosette vegetative stage until flowering. The day of bolting (when the first elongated floral scape reached 1.0 cm in height) was recorded. The density of trichomes on flower buds was assessed on a scale of 0 (absence) to 5 (high density). The plant was harvested at the day the first flower opened, oven dried at 70°C for 2 days and weighed.

To test the effects of lanolin, we conducted a preliminary study in which we treated a separate set of 15 plants from the two populations with pure lanolin using the same protocol as in the main experiment. Lanolin caused nonsignificant decreases in time to bolting and total glucosinolate concentration (Figure 1), opposite to the effects of methyl jasmonate. Thus, lanolin was not applied to noninduced control plants in the half-sib experiment, and the methyl jasmonate results may be conservative due to the effects of lanolin.

2.3 | Measurement of defence metabolites

The extraction and analysis of glucosinolates and trypsin-PI activity were performed as previously described (Ogran et al., 2016). After sulfatase treatment, glucosinolates were separated and measured with an HPLC ProStar 240 high-performance liquid chromatography (Varian) equipped with an Acclaim reverse-phase C18 column (2.1 × 250 mm, 5 µm) (Dionex). Total glucosinolates were quantified using benzyl glucosinolate as an internal standard. Trypsin-PI activity measures binding potential to digestive proteases in an herbivore (thereby making nutrients unavailable to the herbivore) and was calculated as the percentage of trypsin activity relative to controls (Ogran et al., 2016; Thaler, Stout, Karban, & Duffey, 1996).

2.4 | Data analysis

Mixed-model ANOVA using REML was used on the full data set to test for genetic differentiation in the traits (population main effect), plasticity in response to the methyl jasmonate treatment averaged over both populations (treatment main effect) and genetic differentiation for plasticity (population by treatment interaction); these were

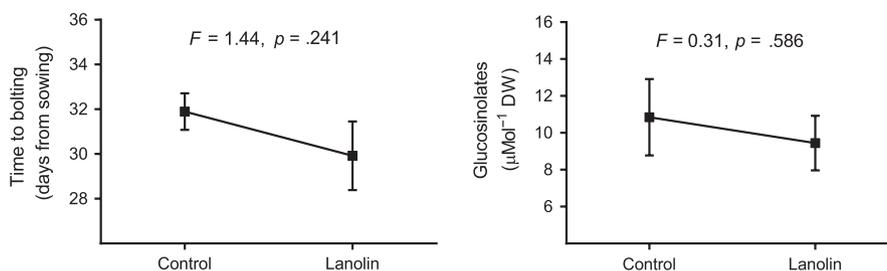


FIGURE 1 Lanolin caused nonsignificant decreases in bolting and total glucosinolate concentration in *Eruca sativa*. Mean ± SE of 15 randomly assigned plants from each of the two populations; glucosinolates were analysed in leaves of five plants collected at the vegetative stage, 48 hr after lanolin application, just as in the methyl jasmonate experiment

modelled as fixed effects. To account for the family structure in the data, the random effects of sire nested within population, dam nested within sire and the sire by treatment interaction were included in the models. For traits where the population by treatment interaction was significant at $p < .1$, separate models for each treatment level were run to test for significant genetic differentiation within treatments.

To test for genotype by environment interaction ($G \times E$) within each of the two populations, which is equivalent to additive genetic variation for plasticity, separate models were run for each trait and population, with treatment as a fixed effect and sire, the treatment by sire interaction (testing for $G \times E$) and dam nested with sire as

random effects as in the full model. Significance of the random effects was tested by running each model without each random effect and conducting a 1-tailed chi-square test on the difference in $2 \times$ log-likelihood values between the two models (Littell, Milliken, Stroup, Wolfinger, & Schabenberger, 2006). Note that the sire by treatment interaction in the full model tests for $G \times E$ averaged across both populations, which is not evolutionarily meaningful; it was included in the full model to account for this variance in the other more meaningful tests.

Next, we tested for costs of constitutive levels of the defensive traits by estimating the genetic correlations between each trait and

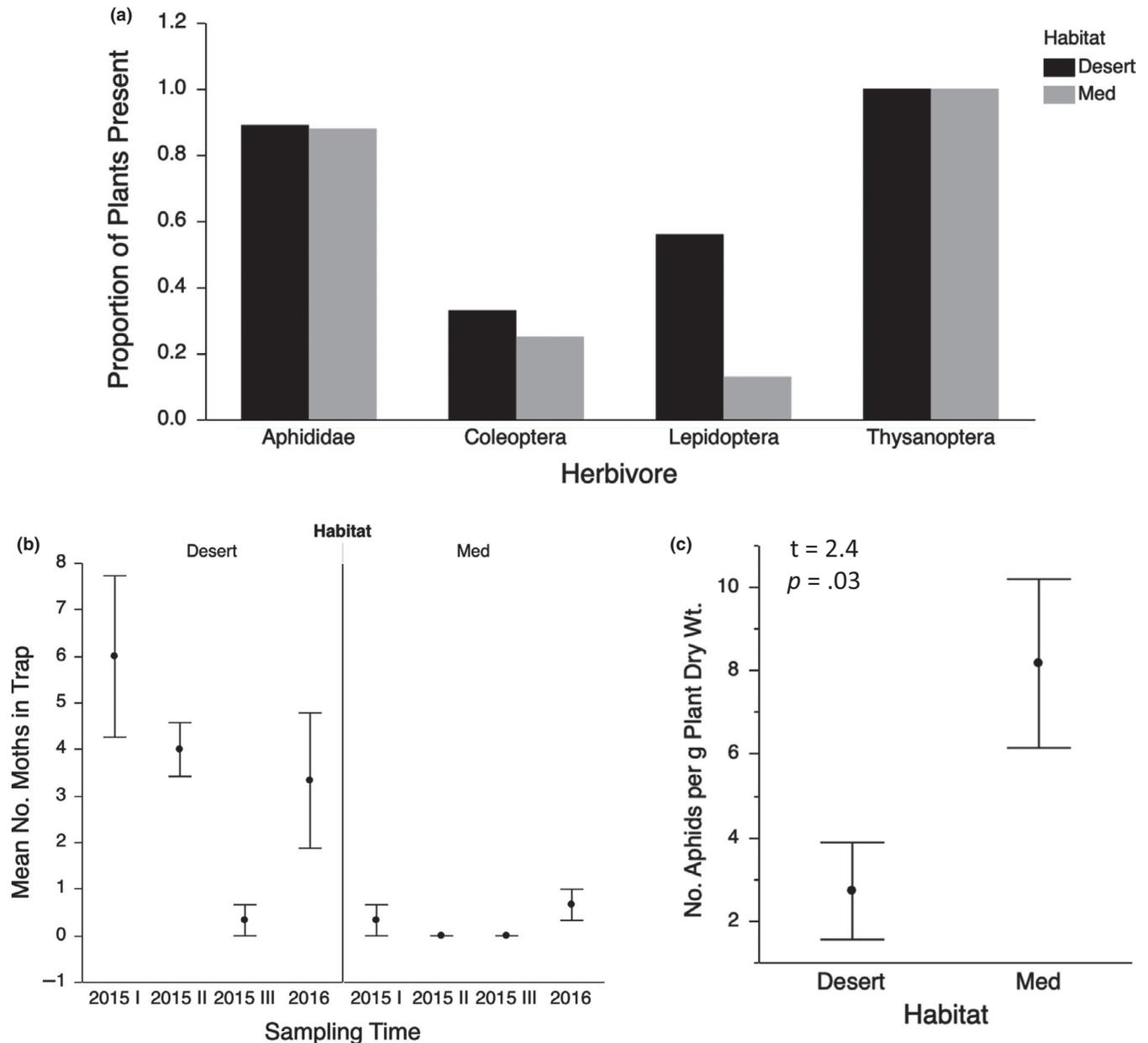


FIGURE 2 Herbivory survey at the natural populations in 2014. (a) Proportion of plants at each site with at least one individual from each order of herbivores. (b) Mean \pm SEM numbers of male diamondback moths per pheromone lure at the desert and Mediterranean populations. Two-way ANOVA showed the main effects of habitat was significant at $p < .0001$, and the sampling time and their interaction was significant at $p < .04$. $N = 9$ lures per site in 2015 and $N = 3$ in 2016. (c) Mean \pm SEM number of aphids per plant at each site in 2014, corrected for the dry weight of the plant. $N = 9$ desert and 8 Mediterranean plants sampled

dry weight using half-sib means. We also tested for a cost of plastic induction of defence, defined as the reduction in fitness in the absence of herbivores that results from having the ability to be plastic. Evidence for a cost would be a significant negative genetic correlation between inducibility for a given defence trait and the residuals of the regression of dry weight (as an estimate of fitness) on the constitutive levels of the same defence trait (DeWitt, Sih, & Wilson, 1998; Van Tienderen, 1991). Inducibility of each half-sib family within each population was estimated as the absolute value of the induced minus the control family mean values.

Finally, to test for trade-offs between constitutive and induced responses to herbivory, we used a bias-corrected Monte Carlo procedure which accounts for sampling variation and measurement error implemented in Matlab (Ver. R2018a) (Morris, Traw, & Bergelson,

2006). We tested for trade-offs in each of the three defensive traits within each population (six tests) using paternal half-sib means for constitutive and induced trait values. All other statistical tests were performed with the JMP v. 10 package (SAS Institute, 2012).

3 | RESULTS AND DISCUSSION

3.1 | Herbivore pressure in the natural habitats of *Eruca sativa*

Most or all plants at both sites had sucking herbivores (aphids) as well as cell-feeding thrips, while on average less than half of plants had chewing herbivores (beetles and Lepidoptera; Figure 2a). For three

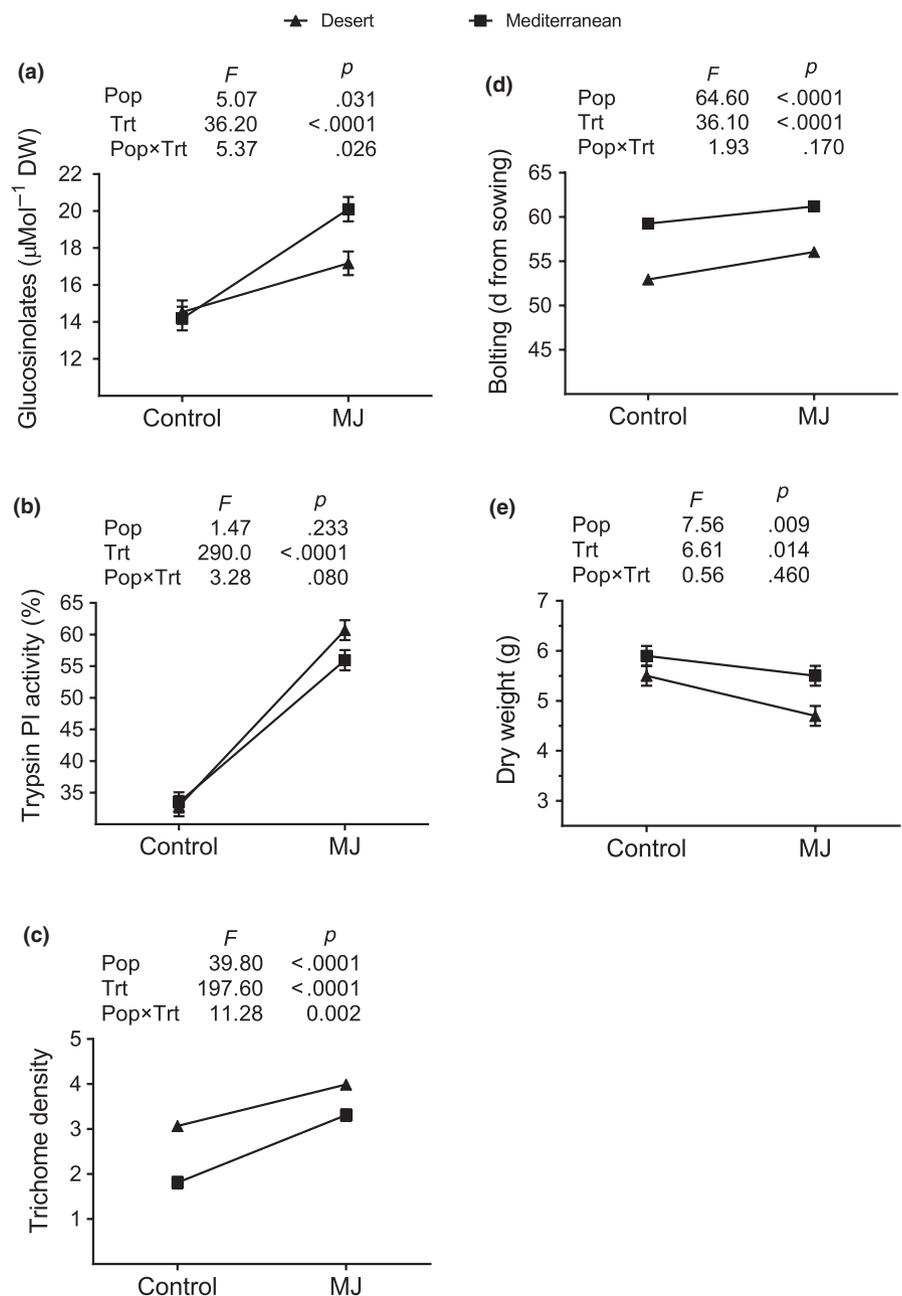


FIGURE 3 Genetic differentiation of traits and trait plasticity. The means ($\pm 2\text{SEM}$) of each of the five traits from each of the two populations expressed in the control and methyl jasmonate-induced treatments are shown, with summaries of the fixed-effects tests from the mixed models (details in Table 1). (a) Glucosinolate concentration, (b) trypsin-PI activity, (c) trichome density, (d) bolting date and (e) dry biomass on the day the first flower opened. $N = 550\text{--}622$. The SEM for trichome density (c) and bolting date (d) are obscured by the data point

of these four insect orders, the percentage of plants with herbivores differed little between the two habitats, but many more plants had Lepidoptera at the desert site compared with the Mediterranean. This pattern was confirmed over the next two years by pheromone lures, which commonly captured male diamondback moths (*P. xylostella*) in the same desert site, but moths were rarely captured in the Mediterranean site (Figure 2b). In addition to this spatial variability, there was a large and significant temporal difference in the number of moths across sampling times. However, the opposite spatial pattern occurred for the mean number of aphids per plant, which was three times higher at the Mediterranean site after correcting for differences in plant size (Figure 2c). Thus, there was evidence for spatial variation in the herbivore guilds between sites, and some evidence for temporal variation across years in moth abundance in both habitats, suggesting that there could be selection for genetic differentiation in plant defence among habitats as well as selection for inducibility within habitats.

3.2 | Genetic differentiation in traits and trait plasticity

We found genetic differentiation (population main effect) for all traits except trypsin-PI activity, significant plasticity (treatment main effect) for all traits and significant genetic differentiation for plasticity (population by treatment interaction) for glucosinolate concentration and trichome density (Figure 3, Table 1). These overall patterns are similar to those in the Murren et al. (2014) meta-analysis, where they report over twice as much mean trait differentiation ("offset") than differentiation in plasticity ("slope"). There was no significant difference in glucosinolate concentration between populations in the control treatment (Table 1), but Mediterranean plants induced more strongly in response to the methyl jasmonate treatment. In

contrast, desert plants produced more trichomes in both treatments but induced less strongly than Mediterranean plants. These two traits, in particular, were shown to be favoured by natural selection imposed by herbivorous insects and to be costly (in the absence of herbivory) in the related mustard *Arabidopsis thaliana* (Mauricio & Rausher, 1997). Thus, it is possible that the differentiation in defensive traits observed here may be local adaptations to the differences in the herbivore communities. The match between more moths at the desert site with high trichomes is consistent with their role in resistance against chewing herbivores, including *P. xylostella* (Agren & Schemske, 1993; Sletvold, Huttunen, Handley, Karkkainen, & Agren, 2010). Although glucosinolates are typically not effective against the specialist diamondback moth and may even attract them (Li, Eigenbrode, Stringam, & Thiagarajah, 2000; Sun, Sønderby, Halkier, Jander, & Vos, 2009), their high inducibility in the Mediterranean site may be driven by defensive effects against the attacking aphids. Wagner and Mitchell-Olds (2018) presented evidence for adaptive plasticity in glucosinolate induction across four common gardens in the mustard *Boechera stricta*.

We found strong induction of trypsin-PI in both populations, with marginally significant greater induction in desert plants (Figure 3). Inducible trypsin-PIs have been well studied as an anti-nutritive defence, including work in the Brassicaceae (Cipollini, Busch, Stowe, Simms, & Bergelson, 2003; Cipollini & Sipe, 2001), with some evidence for fitness costs and benefits (Glawe, Zavala, Kessler, Dam, & Baldwin, 2003; Zavala & Baldwin, 2004). Although the relative effects of protease inhibitors on moths versus aphids are unclear, both groups can be negatively impacted (Habib & Fazili, 2007). To understand the diversity of defences in most plants, studies of natural selection by individual agents, in this case herbivore species, is critical (cf. Sahli & Conner, 2011; terHorst et al., 2015). The methyl jasmonate treatment was phenotypically costly, as both treated populations bolted later but at a lower dry weight compared

TABLE 1 The results of the fixed effect tests for genetic differentiation in traits and plasticity (cf. Figure 3); the full model also included the random effects of Sire, Sire × treatment and Dam(sire)

Phenotypic trait	Population		Treatment		Population × treatment		Overall R ²	N
	F	p	F	p	F	p		
Total glucosinolates	5.07	.03	36.20	<.0001	5.37	.026	.21	598
Control	0.15	.70						
Treatment	9.9	.003						
Trypsin-PI activity (%)	1.47	.23	290.0	<.0001	3.28	.08	.64	579
Control	0.06	.81						
Treatment	3.58	.07						
Trichome density	39.8	<.0001	197.6	<.0001	11.28	.002	.69	550
Control	48.7	<.0001						
Treatment	36.1	.0006						
Bolting time	64.6	<.0001	36.1	<.0001	1.93	.17	.61	622
Dry weight (g)	7.56	.009	6.61	.014	0.56	.46	.34	615

Note: Glucosinolates were expressed as $\mu\text{mol/g DW}$, trichome density was scored on a scale of 0 (glabrous) to 5 (high density), and bolting time is days from germination. For the three defence traits, results of models within each treatment are also shown.

TABLE 2 Additive genetic correlations testing costs and trade-offs of resistance traits

Trait	r_{constit}	p	r_{plastic}	p	$r_{\text{trade-off}}$	p
Desert						
Glucosinolate	.24	.31	-.32	.17	-.79	<.001
Trypsin-PI activity	-.66	.002	.08	.76	-.66	<.01
Trichomes	.29	.21	.08	.74	-.35	n.s.
Mediterranean						
Glucosinolate	-.18	.47	.32	.20	-.75	<.01
Trypsin-PI activity	-.45	.047	-.07	.76	-.48	<.1
Trichomes	.11	.65	-.35	.16	-.60	<.1

Note: r_{constit} are the correlations between plant dry weight and each trait in the control treatment, testing the cost of constitutive production of that defence. r_{plastic} test for the cost of plasticity in defence using the correlations between inducibility (absolute value of induced minus constitutive level) of each defensive trait versus the residuals of the regression of dry weight on constitutive levels of the defensive trait (see Materials and Methods). $r_{\text{trade-off}}$ test for trade-offs between constitutive and induced defences using the bias-corrected Monte Carlo procedure developed by Morris et al. (2006). Values are Pearson correlations among half-sib family means; $N = 17$ –20 half-sib families depending on missing data. Bold values are significant at $p < .05$.

with controls (Figure 3, Table 1). The test for costs of constitutive levels and plasticity for the defensive traits were negative except for constitutive levels of trypsin-PI, that is, we found few significant negative genetic correlations between trait values or level of induction and dry weight (first four columns of Table 2). Thus, it seems that the cost of methyl jasmonate treatment is mediated through an

unmeasured trait, which could be defensive or nondefence related. For example, methyl jasmonate could directly act to delay flowering and induce some unmeasured trait that causes the reduced dry weight in the treatment group. We note that a consequence of using methyl jasmonate to induce plants is that we provided a homogenous signal to all plants, unimpacted by variation in constitutive resistance

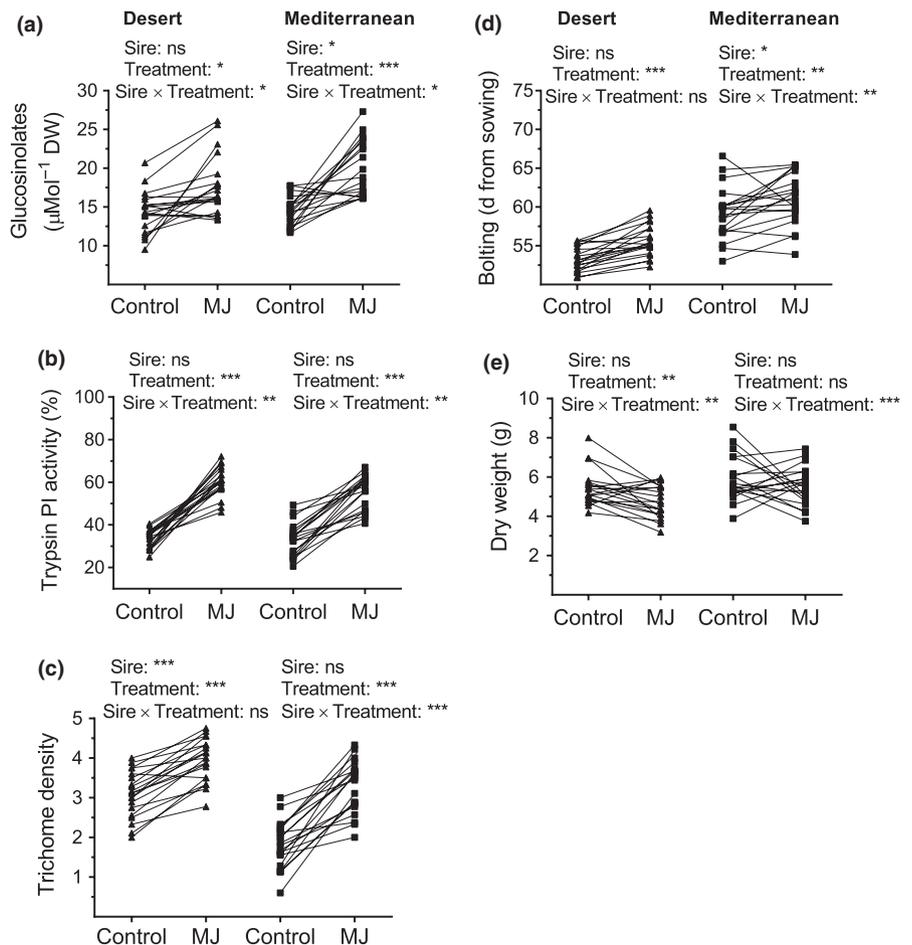
**FIGURE 4** Genetic variation for traits and plasticity within populations. Reaction norm plots show the effect of methyl jasmonate induction on sire family means for the five traits. A summary of the statistical results are given; full results in Table 3

TABLE 3 Tests for genetic variation in traits and plasticity within populations

Phenotypic trait	Desert						Mediterranean					
	Sire		Treatment		Sire × Treatment		Sire		Treatment		Sire × Treatment	
	χ^2	<i>p</i>	<i>F</i>	<i>p</i>	χ^2	<i>p</i>	χ^2	<i>p</i>	<i>F</i>	<i>p</i>	χ^2	<i>p</i>
Total glucosinolates	0.02	.44	5.79	.03	4.57	.02	2.73	.05	45.1	<.0001	4.07	.02
Trypsin-PI activity	0.81	.18	148.6	<0.0001	7.61	.003	1.76	.09	140.0	<.0001	8.21	.002
Trichome density	11.36	.0004	107.4	<.0001	0.01	.46	0.06	.40	93.8	<.0001	12.01	.0003
Bolting time	0.81	.18	52.7	<.0001	0.47	.25	2.83	.046	7.33	.01	7.65	.003
Dry weight	0.47	.25	7.8	.01	5.41	.01	0.55	.23	1.29	.27	10.12	.0007

Note: Results of mixed-model analysis testing the fixed effect of treatment (induction) and the random effects of sire and their interaction on the five traits. Chi-squared values for the random effects are from the difference in two times the log-likelihood for the full model compared with the model with that term deleted; *p*-values are 1-tailed (Littell et al., 2006).

or differential endogenous jasmonate production. Consistent with previous work (Aronson, Kigel, Shmida, & Klein, 1992), desert plants appear to be adapted to a shorter growing season, as they bolted earlier at a smaller size in both treatments (Figure 3). Thus, the delayed flowering and even smaller size induced by methyl jasmonate should have a larger negative effect in the desert compared with the Mediterranean environment.

3.3 | Additive genetic variance for plasticity (G × E)

Because all traits were plastic, and two or perhaps all three of the defence traits have evolved genetic differentiation between populations, we next tested whether these populations maintain additive genetic variance for future evolution of plasticity. The answer was yes with two exceptions, as the sire by treatment interaction was significant for all trait and population combinations except the number of trichomes and bolting time in desert plants (Figure 4, Table 3). Thus, it is possible that the lower plasticity for trichome number in the desert compared with the Mediterranean population (Figure 3c) is due to a lack of genetic variance in the desert; the same pattern was significant at *p* = .17 for bolting time. Overall however, there is ample genetic variance for future evolution of plasticity in most traits in both populations, suggesting that population differentiation has not eroded its evolutionary potential.

3.4 | Trade-offs between constitutive and induced defences

Overall, we found strong evidence for trade-offs between constitutive and jasmonate-induced glucosinolates (both populations) as well as trypsin-PI (both populations, although marginal in the Mediterranean), while evidence for a trade-off in trichomes was not evident in the desert population and marginal in the Mediterranean (Table 2). Although this trade-off between constitutive and induced defensive traits has been reported several times (Koricheva,

Nykänen, & Gianoli, 2004), it is variable within versus between populations (or species) and among traits (Bingham & Agrawal, 2010; Kempel, Schädler, Chrobok, Fischer, & Kleunen, 2011; Rasmann & Agrawal, 2011; Zhang et al., 2008). To our knowledge, only one previous study compared populations in terms of this trade-off. Introduced populations of the common milkweed (*Asclepias syriaca*) in Europe, where most herbivores were lacking, lacked the trade-off and showed a general loss of inducibility compared with native North American populations (Agrawal et al., 2015). However, this differentiation could have been caused by founder effects causing drift in the European populations, and the extent of adaptive differentiation in plasticity was not examined.

4 | CONCLUSION

Among our two populations of wild arugula from distinct environments, we found more evidence for genetic differentiation in defensive trait means than in trait plasticity, in agreement with a recent meta-analysis (Murren et al., 2014). Within these populations, most traits lacked significant additive genetic variation but did show significant additive genetic variance for plasticity. Taken together, these results suggest that there has been strong divergent selection in the past on the trait means, leading to local adaptation to the divergent herbivores in the two environments, as the traits have diverged and additive variance has been depleted. Selection on these defensive traits has been shown for other plant species (Mauricio & Rausher, 1997; Sato & Kudoh, 2017; Zavala & Baldwin, 2004). In contrast, selection on plasticity may have been weaker, as there is less divergence in plasticity and more within-population variance remaining. This scenario fits with our herbivory and genetic marker data, which show consistent differences between the populations in aphids and moths and low gene flow between them, and less variability across years within sites in the composition of the herbivore community. It also fits with our evidence for trade-offs between constitutive and induced defences, which make the evolution of differentiation in both trait means and plasticity less likely.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

OB and JC: conceived this study. AO: conducted the experiment and analysed the data together with JC and AAA. All authors approved submission to Journal of Evolutionary Biology.

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DATA AVAILABILITY STATEMENT

All relevant data supporting our findings are provided in the article <https://doi.org/10.5061/dryad.b2rbnzs93>.

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