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ECOLOGICAL GENETICS OF AN INDUCED PLANT DEFENSE AGAINST HERBIVORES: ADDITIVE GENETIC VARIANCE AND COSTS OF PHENOTYPIC PLASTICITY

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Abstract.—Adaptive phenotypic plasticity in chemical defense is thought to play a major role in plant-herbivore interactions. We investigated genetic variation for inducibility of defensive traits in wild radish plants and asked if the evolution of induction is constrained by costs of phenotypic plasticity. In a greenhouse experiment using paternal half-sibling families, we show additive genetic variation for plasticity in glucosinolate concentration. Genetic variation for glucosinolates was not detected in undamaged plants, but was significant following herbivory by a specialist herbivore, *Pieris rapae*. On average, damaged plants had 55% higher concentrations of glucosinolates compared to controls. In addition, we found significant narrow-sense heritabilities for leaf size, trichome number, flowering phenology, and lifetime fruit production. In a second experiment, we found evidence of genetic variation in induced plant resistance to *P. rapae*. Although overall there was little evidence for genetic correlations between the defensive and life-history traits we measured, we show that more plastic families had lower fitness than less plastic families in the absence of herbivory (i.e., evidence for genetic costs of plasticity). Thus, there is genetic variation for induction of defense in wild radish, and the evolution of inducibility may be constrained by costs of plasticity.

Key words.—Adaptive phenotypic plasticity, additive genetic variation, cost of plasticity, glucosinolates, *Pieris rapae*, plant-herbivore interactions, *Raphanus raphanistrum*.

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Phenotypic plasticity is the ability of an individual genotype to express different phenotypes in different biotic or abiotic environments. The evolution of phenotypic plasticity is thought to result from variable natural selection in ecologically variable environments, and numerous experiments now show that plasticity mediates the adaptive expression of phenotypes in nature (Via et al. 1995; Karban and Baldwin 1997; Roff 1997; DeWitt et al. 1998; Agrawal 2001a; Pigliucci 2001). Most investigations of adaptive phenotypic plasticity have not combined phenotypic manipulations with tests for a genetic basis of the traits of interest (but see Spitze 1992; Harvell 1998; Relyea 2002). For phenotypic plasticity to have evolved by natural selection, the trait must have exhibited heritable variation in plasticity (i.e., genotype $\times$ environment interaction) that affected fitness. Some plastic traits may be genetically controlled but no longer show variation in natural populations because the genes controlling the trait have been driven to fixation by selection. However, the evolution of phenotypic plasticity may be constrained by genetic correlations with other traits and costs of plasticity (Roff 1997; DeWitt 1998; DeWitt et al. 1998; Scheiner and Berrigan 1998; Dorn et al. 2000; van Kleunen et al. 2000; Agrawal 2001a; Relyea 2002). These constraints could maintain additive variation for plasticity. In particular, costs of plasticity per se (i.e., the cost of the ability to change, not the cost of the new phenotype) have recently received attention for being relatively unexplored, yet likely candidates as constraints on the evolution of adaptive phenotypic plasticity (DeWitt et al. 1998).

Inducible defense systems in animals and plants have played a central role in our understanding of phenotypic plasticity (Karban and Baldwin 1997; Agrawal et al. 1999a; Tollrian and Harvell 1999). Typically, cues associated with predators and parasites cause an induction or increase in the defensive phenotype of the prey. Given that predator and parasite loads are variable in space and time and that defenses are likely costly, inducible defenses are thought to be cost-saving adaptations that are only employed when necessary. Still, for the continued evolution of induction, genetic variation for the trait must exist. Some of the best evidence for such genetic variation comes from studies of aquatic animals, such as *Daphnia*, marine bryozoans, and tadpoles (Spitze 1992; Harvell 1998; Scheiner and Berrigan 1998; Tollrian and Harvell 1999; Relyea 2002). Water-borne cues associated with the predators are usually sufficient to cause the defensive responses in prey. In these and other systems, not only have the fitness consequences of alternate defense phenotypes been elucidated, but the ecological genetics of plasticity in defense are being studied (e.g., Relyea 2002).

We have been studying the evolutionary ecology of inducible defense against herbivores in wild radish, *Raphanus raphanistrum*. Plants damaged by caterpillars of the specialist herbivore *Pieris rapae* increase production of putative defense chemicals (glucosinolates; Agrawal et al. 1999c) and produce new leaves with higher densities and total numbers of trichomes than equally aged leaves on undamaged plants (Agrawal 1999b). Several species of subsequently feeding herbivores grow more slowly on or have reduced preference for damaged plants compared to previously undamaged plants (Agrawal 1999b, 2000). Induced responses in wild radish exhibit specificity to the type of herbivore, showing differential induced resistance following attack by four lepidopteran herbivores (Agrawal 2000) and minimal induction following artificial clipping by scissors (Agrawal 1998, 1999b; Agrawal et al. 1999c). In three field experiments, plants induced by early-season herbivory had higher relative fitness

than uninduced controls (Agrawal 1998, 1999b). In the absence of later herbivory, the benefits of induced responses to early damage are countered by costs measured in terms of reduced production of male reproductive characters in damaged plants compared to controls (Agrawal et al. 1999c; Lehtilä and Strauss 1999; but see Strauss et al. 2001). Evidence for an additive genetic basis of induced defense against herbivores is lacking in *R. raphanistrum*; additive genetic variance in current populations would indicate that the trait has the potential to evolve further in response to continued selection.

In this study, we tested whether these phenotypic effects (i.e., induced chemical and morphological defense) have a genetic basis. The presence of additive genetic variation for these fitness-related traits would demonstrate the potential for the continued evolution of induced defense. We employed a greenhouse experiment with paternal half-sib families and present narrow-sense heritabilities and genetic correlations (Falconer and Mackay 1996; Roff 1997) for traits associated with induced defense and life-history traits of wild radish. Specifically we tested for genetic variation in defensive (glucosinolates, leaf toughness, trichomes) and life-history (leaf size, flower and fruit production) traits. We further tested the hypothesis that defense and phenotypic plasticity in defense have fitness costs.

Materials and Methods

Raphanus raphanistrum (Brassicaceae) is a widely distributed, self-incompatible annual plant found in disturbed sites. We bred paternal half-sib families of *R. raphanistrum* from a single wild population in upstate New York (Conner and Via 1993). To examine genetic variation in defensive and life-history traits of wild radish, we crossed each of 28 sire plants with four to five different dam plants and used progeny from four dams, resulting in 112 full-sib families and 28 paternal half-sib families. Below we present the results from two independent experiments; the second, smaller experiment was conducted as a follow-up because of unexpected results in the first experiment.

On 6 September 2000 we placed seeds from each of the 112 full-sib families together into petri dishes lined with moist filter paper. A maximum of 15 seeds were germinated per full-sib family (range = 5–15 seeds), and the petri dishes were randomized on a laboratory bench. After two days the seeds were beginning to germinate and were transferred singly to plastic pots (210-ml volume) filled with unfertilized 75% Sunshine Soil Mix #1 (Sun Grow Horticulture, Inc., Bellevue, WA) and 25% sterilized topsoil. The resulting 980 plants were completely randomized over two greenhouse benches, and treatments were randomly assigned as follows: (1) control chemistry (one plant per available full-sib family, 107 plants in total); (2) induced chemistry (one plant per available full-sib family, 107 plants in total); (3) control bioassay (two or three plants per full-sib family, 256 plants in total); (4) induced bioassay (two or three plants per full-sib family, 254 plants in total); and (5) control seed production (one to four plants per full-sib family, depending on the number of seeds germinated, 256 plants in total). Chemistry plants were used to determine profiles of glucosinolates in the plants; five full-sib families were not included in the chemical analysis because these families had too few plants. Bioassay plants were used to estimate effects of induced responses on a common folivore of wild radish (see below). Seed production plants were used to obtain estimates of fitness in undamaged control plants.

On 18 September 2000 all plants were entering the two true-leaf stage, and treatments were imposed. Plants in induced treatments received a clip-cage on the first true leaf with a fourth or fifth instar *P. rapae* larva. Control plants for the chemistry measurements and bioassays received empty clip-cages at the same time. Clip-cages were made from the tops of plastic petri dishes (5 cm) attached to a Plexiglas tube with a hair clip, with the entire top being mesh to allow for normal gas exchange. The caterpillars were allowed to feed until they consumed about 85% of the leaf (4–24 h), at which point the clip cages were removed. The use of clip-cages allowed us to precisely control for the level of leaf damage imposed by the caterpillar. In other words, we did not allow for familial variation in constitutive resistance to confound the level of damage imposed in the induced plants. All treatments were imposed by 20 September, and on 26 September all plants for the chemical analyses were destructively harvested. The total aboveground plant was cut with a razor blade, immediately immersed in liquid nitrogen, placed in a coin envelope, and transferred to a −80°C freezer. The plant samples were then lyophilized and stored for glucosinolate analysis by HPLC following the methods of Brown and Morra (1995). We report glucosinolates in two classes, indolyl and all other (non-indolyl) glucosinolates, because other research on the Brassicaceae, including our work with wild radish, has found that indolyl glucosinolates are the primary class that are induced following herbivory (Koritsas et al. 1991; Bodnaryk and Rymerson 1994; Agrawal et al. 1999c).

Bioassays of constitutive and induced plant resistance were conducted with neonate *P. rapae* larvae from a laboratory colony maintained on artificial diet. The colony was started with wild individuals and had been in the laboratory for less than five generations. Every two days (over a 10-day period) immediately preceding the experiment we collected all the eggs from the colony and stored them at 5°C. On 24 September, two days before the bioassay was to be started, the eggs were placed in a room at ambient temperature (≈ 22°C). On 26 September, the eggs were kept at 30°C until 510 larvae hatched, at which point a single larva was placed on each bioassay plant. The bioassay plants were completely mixed over two greenhouse benches and spaced so that the leaves were not touching. No cages were employed for the bioassay because previous results had shown that *P. rapae* is generally sluggish and does not wander off a suitable host-plant. After 7 days of feeding, we removed caterpillars from plants and placed them in plastic tubes. Tubes were stored in a standard freezer before larvae were weighed to the nearest microgram on a Mettler-Toledo UMT-2 balance (Hightstown, NJ).

We took measurements of plant performance only on unmanipulated control plants (one to four plants from each full-sib family) to test for costs of phenotypic plasticity. The first flower was observed on 4 October. Because the natural day length was rapidly declining, 400-watt sodium vapor lights

were employed on a 16:8 day:night cycle to enhance natural light. To promote maximum seed set, plants were hand pollinated every other day using a soft-haired brush. The brush was loaded with pollen collected from haphazardly chosen plants and was dabbed across every open flower. As a measure of early-season flowering, we counted total flower number on 19 October, approximately two weeks after the first flower appeared. Early season flowering is a disproportionately important component of male fitness for wild radish plants in the field, because early flowering plants sire more seeds than later flowering plants (Ashman et al. 1993). Thus, phenological differences in flower number between families were measured as a surrogate for male fitness. As each plant naturally senesced, fruits were collected in paper bags and allowed to dry. Total fruit mass was measured as an indicator of female fitness. Lifetime fruit mass is a good predictor of the number of seeds produced ($n = 624$, $r^2 = 0.80$, $P < 0.05$; combined data from Agrawal 1998; Agrawal et al. 1999c), and also takes into account variation in individual seed mass among plants. Because seed mass can strongly influence seedling competitive ability and adult fitness in wild radish (Stanton 1984), we use total fruit mass as our estimate of female fitness.

We also measured leaf toughness and trichome number, two putative physical defenses of wild radish leaves (Coley 1983; Raupp 1985). Toughness was measured on the set of control plants used for reproductive measures; it was measured by using a force gauge penetrometer (Type 516, Chatillon Corp., Kew Gardens, NY) that measures the grams of force needed to penetrate a surface. The fourth true leaf on each intact plant was sandwiched between two pieces of Plexiglass, each with a 0.5-cm hole. The probe of the penetrometer was then pushed through the hole and we recorded the maximum force required for penetration. Two measures were taken for each leaf, one on each side of the midrib, and these data were averaged and used as a single datapoint per plant. Trichomes were counted on the newest fully expanded leaf of the bioassay plants. Leaves were digitized on a flatbed scanner and the area of the leaves and density of trichomes was calculated using image analysis software (Scion Image, Scion Corp., Frederick, MD).

Analyses

Proc Mixed (SAS ver. 8, SAS Institute, Cary, NC) was used for all analyses, using restricted maximum likelihood (REML) estimation (Littell et al. 1996). Genetic variances and heritability within each treatment were calculated with models including only sire and dam nested within sire (both random effects). For the data with both control and caterpillar-induced treatments, a second set of mixed models were run with treatment as a fixed effect and sire, dam nested with sire, and the interactions of both with treatment as random effects. Equality of variance across treatments was tested in all cases; significant deviations from equal variances were found only for glucosinolate measures (see Results). Sire breeding values were also estimated with these models using best linear unbiased prediction (BLUP; Littell et al. 1996, ch. 6). BLUP estimates are more accurate than sire family means because they use all available information, and thus are not biased by dominance and environmental effects, as are family means (Shaw et al. 1995). Genetic correlations were estimated among all traits with nonzero variance components with the following exceptions. Only trichome density was used in correlations (not leaf area or total trichome number) because density is likely the resistance trait that matters to herbivores. Correlations with caterpillar mass are also not shown because this variable exhibited low variation and was unaffected by any of our treatments.

To test for costs of plasticity per se (i.e., the cost of the ability to change or carrying the genetic and sensory machinery, not the cost of producing the new phenotype), we examined the relationship between the BLUP values for inducibility (absolute value of induced minus constitutive levels of glucosinolates) and residuals from the relationship between constitutive levels of glucosinolates and lifetime fruit mass (van Tienderen 1991; DeWitt 1998; DeWitt et al. 1998; Scheiner and Berrigan 1998; Dorn et al. 2000; van Kleunen et al. 2000; Relyea 2002). Here the residuals are used to correct for the relationship between constitutive levels of glucosinolates and fitness in the undamaged environment (van Tienderen 1991; DeWitt et al. 1998). This analysis was conducted as an additive genetic correlation (28 half-sib families), whereby our a priori prediction was that families with large induction indices would have relatively lower fitness estimates, even in the absence of induction (DeWitt et al. 1998; Agrawal 2001a). We examined the costs of plasticity for glucosinolate production because this was the only trait for which we detected genetic variation in plasticity.

Induced Resistance Experiment

Because our main experiment unexpectedly showed no effects of glucosinolate variation on *P. rapae* (high constitutive glucosinolate levels and its induction are usually correlated with reduced *P. rapae* performance: Stowe 1998a; Agrawal 2000), we conducted an additional experiment in February 2002. We selected seeds from four sires from the original experiment, each crossed with five dams (20 full-sib families). The families were selected to span a range of chemical induction observed in the first experiment. We planted six to 10 seeds from each full-sib family and the resulting 100 plants were divided into control and herbivore-induced groups. We employed similar methods to those described above except as follows: (1) we used larger pots (500 ml) because previous studies have shown that pot-bound plants fail to induce resistance (Karban and Baldwin 1997); (2) we used Sunshine Soil Mix #1 with $\approx$ 0.6 g of slow-release Nutricote fertilizer (13:13:13 N:P:K, Vicksburg Chemical, Vicksburg, MS; we fertilized our plants because a recent study showed that unfertilized plants failed to induce proteinase inhibitors in related *Brassica* plants; Cipollini and Bergelson 2001); and (3) we allowed damaging caterpillars in the induction treatment to freely roam over the plants and damage all leaves (no clip-cages were employed), which resulted in 20–50% leaf damage. This approach more closely reflects natural conditions where caterpillars are able to freely damage the whole plant and does not restrict the original feeding to one leaf. Treatments were randomly assigned and plants were completely randomized on a greenhouse bench.

TABLE 1. Descriptive statistics, variance components, and heritabilities within treatments. Significance levels for heritabilities are from log-likelihood tests of the sire variance component. SEM is the standard error of the mean, CV_A is the coefficient of additive genetic variation or evolvability (Houle 1992), SireVC is the sire variance component, DamVC is the dam variance component, and ErrVC is the error variance component. Bold values are significant at least at the $P < 0.05$ level.

Trait	Mean	SEM	CV_A	SireVC	DamVC	ErrVC	h^2
Trichome density (control)	46.49	2.69	28.98	45.38	164.28	779.98	0.18
Trichome density (induced)	41.05	1.64	0.00	0.00	110.54	653.63	0.00
Leaf area (control)	19.31	0.35	8.25	0.63	2.77	19.17	0.11
Leaf area (induced)	19.45	0.46	15.48	2.27	4.06	17.23	**0.38***
Total trichomes (control)	866.49	53.35	42.15	33347.00	40099.00	253510.00	**0.41***
Total trichomes (induced)	780.40	35.01	0.00	0.00	33114.00	235196.00	0.00
Caterpillar mass (control)	13.93	0.51	18.53	1.67	0.22	47.60	0.13
Caterpillar mass (induced)	14.28	0.41	0.00	0.00	1.96	50.13	0.00
Indolyl glucosinolates (control)	325.10	26.90	0.00	0.00	73461.00	1.00	0
Indolyl glucosinolates (induced)	742.40	78.40	99.83	137320.00	510217.00	1.00	**0.85***
Non-indolyl glucosinolates (control)	1354.00	107.20	0.00	0.00	1143218.00	1.00	0
Non-indolyl glucosinolates (induced)	1855.90	148.20	65.71	371847.00	1918862.00	1.01	**0.65***
Leaf toughness	144.01	2.94	11.40	67.33	61.42	1450.12	0.17
Early flower number	10.92	1.40	108.43	35.06	11.65	89.42	**1.03*****
Lifetime fruit mass	0.46	0.01	25.02	0.0034	0.00	0.02	**0.58****

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

After 5 days of treatment (i.e., feeding on the induced plants) by third to fourth instar caterpillars, damaging caterpillars were removed and all plants received a single freshly hatched *P. rapae* larva. After 7 days of feeding we weighed all caterpillars. We selected our sire families from the original subset and therefore treated the effects of sire, induction treatment, and sire × treatment interaction on caterpillar growth as fixed effects and treated dam(sire) as a random effect employing Proc Mixed in SAS.

RESULTS AND DISCUSSION

Plant Chemistry

The main result from our half-sib analysis is that we find significant additive genetic variation for concentrations of glucosinolates when wild radish plants are damaged by herbivores; there was no detectable additive variation in constitutive levels of these same compounds in undamaged plants. In the undamaged environment heritabilities for both classes of glucosinolates were not distinguishable from zero, whereas in the damaged state heritabilities were substantial (Table 1). Because we found significantly unequal variances among sires in the control and induced treatment groups, violating the key assumption of equality of variances among groups, we employed an unequal variance model in which sire effects were estimated separately for the two treatment groups (Littell et al. 1996, p. 162; S. Remold, pers. comm.; Table 2). We interpret the unequal variance between damaged and undamaged plants as additive genetic variance for inducibility. If we ignore the inequality of variance (although this is statistically inappropriate), our analyses are qualitatively the same (i.e., $P < 0.05$ for sire × treatment interaction).

The finding of no variance in glucosinolate content of undamaged plants suggests that wild radish plants allocate little to constitutive or basal levels of chemical defense relative to damaged plants. High costs of defense could have favored such low allocations. When damaged, however, plants increase their levels of defense, albeit to different levels. All half-sib families increased glucosinolate concentrations following herbivory, with the increase ranging from 7% to 140% (Fig. 1). On average, we found 132%, 37%, and 56%, induction (increase) of indolyl, non-indolyl, and total glucosinolates, respectively. Here again, variation in the herbivore-induced levels of glucosinolates may be maintained by costs of glucosinolates or costs of plasticity (see below). An alternative scenario would be for plants to have genetic variation for constitutive levels of defense, but to lack variation in herbivore-induced levels of defense. Indeed, the theoretically proposed trade-off between constitutive and induced levels of defense suggests such a relationship (Brody and Karban 1992). However, little evidence exists for such a trade-off (Zangerl and Berenbaum 1990; Brody and Karban 1992; Thaler and Karban 1997; Siemens and Mitchell-Olds 1998), suggesting that our finding of genetic variation only in herbivore-induced levels of defense may be common.

Although we know quite a lot about the genetics of phenotypic plasticity in general (Roff 1997), there is almost no data on additive genetic variance of induced defenses. In both animals and plants, most studies have employed clones or maternal families (e.g., Spitze 1992; Harvell 1998; but see Relyea 2002). Given the potential influences of nonadditive genetic effects and maternal environment on defense expression (Agrawal et al. 1999b; Gilbert 1999; Shine and Downes 1999; Agrawal 2001b, 2002), paternal half-sib designs are the best for detecting additive genetic variation (Falconer and Mackay 1996). In hound's tongue, *Cynoglossum officinale*, van Dam and Vrieling (1994) used selfed progeny to demonstrate family-level variation in inducibility of alkaloids following artificial leaf damage. In contrast to our results, they found a diversity of responses (from negative to positive), ranging from about a 50% decrease to an 80% increase in alkaloid levels following damage. Using maternal half-sib families, Zangerl and Berenbaum (1990) report an upper limit to the heritability of induced production of furanocoumarins following artificial herbivory in wild parsnip. Of their 24 estimates of heritabilities of induction of different chemicals, 11 were significantly different from zero, ranging

TABLE 2. Variance components (VarComp) with significance tests for the induced defense treatments. Treatment is a fixed effect (*F*-values are shown) and the rest are random effects. Tests of variance components are one-tailed (Littell et al. 1996, p. 44); the chi-squared values shown are the differences in two times the log likelihood of that factor included versus excluded from the model. Because we found significantly unequal variances among sires between control and induced treatment groups for glucosinolates, violating the assumption of equal variance, we employed an unequal variance model in which sire effects were estimated separately for the two treatment groups (Littell et al. 1996, p. 162; S. Remold, pers. comm.). Bold values are significant at least at the $P < 0.05$ level.

	Trichome density		Leaf area		Total trichomes		Caterpillar mass	
	VarComp	χ^2 or *F*	VarComp	χ^2 or *F*	VarComp	χ^2 or *F*	VarComp	χ^2 or *F*
Treatment	—	2.35	—	0.02	—	2.12	—	0.25
Sire	1.21	0	1.63	3.7*	12970	1.4	0.66	0.4
Treatment × sire	0	0	0	0	2495	0.1	0	0
Dam(sire)	131.2	**7.9*** **	3.5	**10.4**** **	42168	**10.0**** **	0.99	0.2
Treatment × dam(sire)	38.4	0.7	0.08	0	0	0	0.59	0.1
Residual	708.0		17.9		239549		48.53	

	Indolyl glucosinolates		Non-indolyl glucosinolates		Total glucosinolates	
	VarComp	χ^2 or *F*	VarComp	χ^2 or *F*	VarComp	χ^2 or *F*
Treatment	—	**14.91**** **	—	**5.79***	—	**13.98**** **
Sire control	0	0	0	0	0	0
Sire induced	225345	**4.7***	483720	**3.4***	817277	**4.2***
Dam(sire)	15366	0.3	137860	0.8	162296	0.7
Residual	241516		1354938		1706729	

*$P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$.

from $h^2 = 0.378$ to 0.871. In *Brassica rapa*, there was no evidence for genetic variation in inducibility of glucosinolates following challenge by herbivores or pathogens among lines of plants selected for high and low levels of myrosinase (Siemens and Mitchell-Olds 1998). In several crop systems, there is evidence for varietal variation in inducibility (reviewed in Agrawal 1999a). Thus, there is strong evidence that organisms show genetic variation for induced defense. If a significant proportion of this variation is additive genetic variation and is found in natural populations, as we found in wild radish, then this will allow for the continued evolution of induced defense.

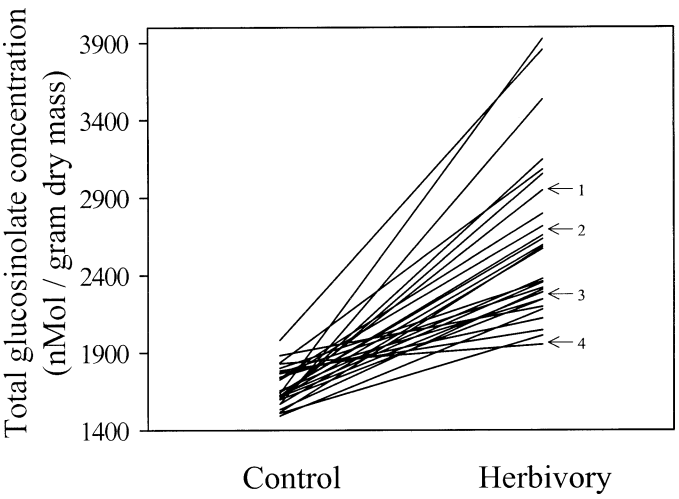

FIG. 1. Reaction norm plot for effects of controlled caterpillar herbivory on total glucosinolate concentration in 28 paternal half-sib families of wild radish. Values are calculated from within each treatment alone using best linear unbiased prediction (BLUP) produced by SAS Proc Mixed (Littell et al. 1996). Lines marked by arrows and numbers indicate the four paternal half-sib families selected for the second induced resistance experiment (Fig. 2). Separate plots of indolyl and non-indolyl glucosinolates are qualitatively similar.

Other Traits and Induced Resistance to Pieris rapae

In addition to glucosinolates, we measured two additional putative resistance traits; trichomes and leaf toughness. We found some support for heritability of trichomes. For example, total trichomes per leaf had a heritability of 0.41, but this was only detected in undamaged plants (Table 1). We did, however, detect strong dam effects, with dam families varying 10-fold in the mean density (10–102 trichomes/cm^2) and total numbers (161–1750) of trichomes per leaf (Table 2). Thus, nonnuclear genes, dominance, and maternal effects (Agrawal et al. 1999b; Agrawal 2001b) may contribute to variance in trichome number. We found no evidence for induction of trichomes in this experiment (no significant treatment effects, Table 2). Overall our morphological traits (trichomes, leaf toughness, and leaf area) showed lower heritabilities than our chemical and reproductive traits (glucosinolates, flower number, lifetime fruit mass). This result contrasts with Mousseau and Roff's (1987) review of the (mostly zoological) literature that showed generally higher heritabilities for morphological traits than behavioral, physiological, and life-history traits.

Surprisingly, our bioassay in the first experiment revealed no heritability, induction, or family-level variation in caterpillar growth on our plants. This result is contradictory to many of our previous experiments; we do not have an explanation for this lack of an effect other than the general low variance in caterpillar growth. In our follow-up experiment with four sires and 20 full-sib families, we found strong evidence for induced resistance, with *P. rapae* caterpillars gaining 29% less mass on induced plants compared to control plants (Fig. 2; treatment: $F_{1,69} = 23.67$, $P < 0.001$; sire: $F_{3,69} = 0.30$, $P = 0.828$; dam(sire): log likelihood ratio test for random effects, $\chi^2 = 1.0$, $P = 0.159$). Despite only using

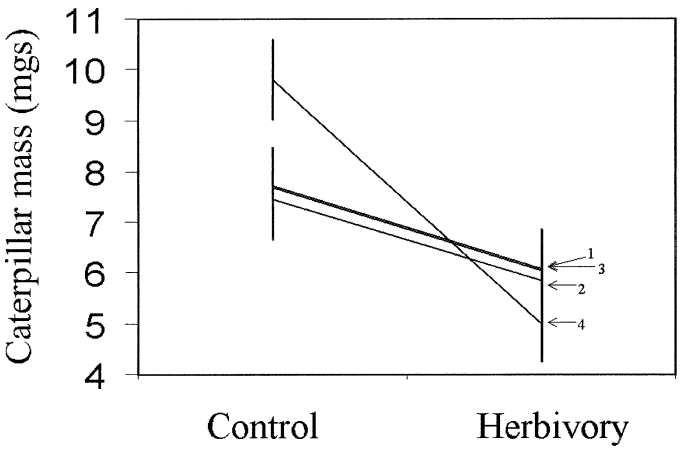

FIG. 2. Reaction norm plot for effects of controlled caterpillar herbivory on growth of specialist *Pieris rapae* caterpillars on four paternal half-sib families of wild radish. Numbers indicate the four paternal half-sib families selected from the pool of 28 shown in Figure 1. Shown are the mean ± SE in two environments.

four sires, we also found evidence for additive genetic variance in effects of induction on caterpillars (Fig. 2; sire × treatment: $F_{3,69} = 2.94$, $P = 0.039$). Although there was not a correspondence between chemical induction in experiment 1 (Fig. 1) and biological resistance in experiment 2 (Fig. 2) in the four families assayed, this should be interpreted with caution because the plants were grown in quite different environments in the two experiments. Although other studies have quantified family-level variation in inducibility of chemical compounds, few have reported effects of additive genetic variation in induction on herbivores.

Genetic Correlations and Costs of Plasticity

We measured three correlates of fitness in undamaged plants, area of a single leaf, early season flower number, and lifetime fruit mass, each of which had significant narrow-sense heritabilities (Table 1). We found limited evidence for genetic correlations among the traits we measured. For example, although both types of glucosinolates (in damaged plants) and lifetime fruit mass were heritable, they showed no significant negative genetic correlation (Table 3). The correlation between induced non-indolyl glucosinolates and fruit mass was negative but only marginally significant by a two-tailed test ($P = 0.10$, Table 3). This result suggests a cost and is consistent with other studies that found costs of constitutive glucosinolates in *Arabidopsis thaliana* (Mauricio 1998) and *B. rapa* (in terms of reduced tolerance to herbivory; Stowe 1998b). However, given that there was no detectable variation in glucosinolate levels of our undamaged plants (Table 1), we were unable to test for costs of constitutive levels of defense. Costs of defense have, in general, been difficult to measure, and their detection often seems to be dependent on environmental conditions (Simms 1992; Purrington 2000).

Our test for costs of plasticity per se involved regressing the absolute value of induced minus constitutive levels of glucosinolates versus residuals from the relationship between lifetime fruit mass and constitutive glucosinolates. We found suggestive evidence for a cost of plasticity in the induction of non-indolyl glucosinolates ($R^2 = 0.10$, $F_{1,26} = 2.883$, $P = 0.101$, Fig. 3). Given our a priori hypothesis outlined in the Materials and Methods (see also Scheiner and Berrigan 1998; van Kleunen et al. 2000), a one-tailed interpretation of the analysis ($P = 0.051$) provides somewhat stronger support for a cost of plasticity. Although statistical power was not particularly low in our study, we did lack plant families with very strong inducibility (Fig. 3). Of the growing number of tests for costs of plasticity (Newman 1988; Nguyen et al. 1989; Krebs and Feder 1997; DeWitt 1998; Scheiner and Berrigan 1998; Dorn et al. 2000; van Kleunen et al. 2000; Relyea 2002), few provide strong evidence for costs. Each of these studies presented some support for costs of plasticity in some traits; however, uniformly strong evidence for costs of plasticity is still lacking.

Costs of phenotypic plasticity may be extremely difficult to detect for several reasons (Agrawal 2001a). One problem is that attempts are being made to measure the cost of carrying

TABLE 3. Additive (half-sib; r_A) genetic correlations among selected pairs of traits (see text) calculated as Pearson product-moment correlations among BLUP breeding values. $N = 28$ for r_A. Glucosinolate correlations are only for damaged plants. The phenotypic correlations do not exist for many of these traits. Because analysis of chemistry was destructive, we were unable to measure the other phenotypic characters from the same plants. No corrections for multiple correlations were applied.

Trait *x*	Trait *y*	r_A	*P*
Indolyl glucosinolates	Non-indolyl glucosinolates	0.16	0.42
Indolyl glucosinolates	Trichome density	0.15	0.44
Indolyl glucosinolates	Early flower number	0.10	0.61
Indolyl glucosinolates	Leaf toughness	−0.30	0.12
Indolyl glucosinolates	Lifetime fruit mass	0.06	0.73
Non-indolyl glucosinolates	Trichome density	−0.01	0.98
Non-indolyl glucosinolates	Early flower number	−0.17	0.38
Non-indolyl glucosinolates	Leaf toughness	−0.19	0.33
Non-indolyl glucosinolates	Lifetime fruit mass	−0.32	0.10
Trichome density	Early flower number	−0.26	0.19
Trichome density	Leaf toughness	0.15	0.44
Trichome density	Lifetime fruit mass	0.06	0.75
Early flower number	Leaf toughness	−0.43	0.02
Early flower number	Lifetime fruit mass	0.32	0.10
Leaf toughness	Lifetime fruit mass	0.24	0.22

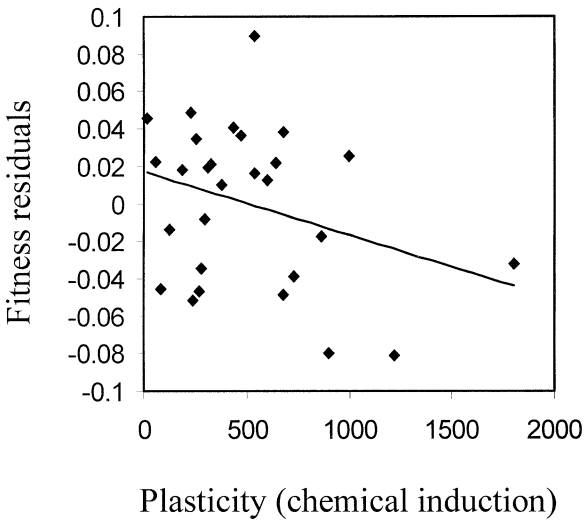

FIG. 3. A test for costs of plasticity in defense ($R^2 = 0.10$, one-tailed $P = 0.051$). The x-axis is an index of plasticity calculated as the absolute value of the concentration of non-indolyl glucosinolates in the induced state minus non-indolyl glucosinolates in the undamaged state for each paternal family. Values are calculated from within each treatment alone using best linear unbiased prediction (BLUP) produced by SAS Proc Mixed (Littell et al. 1996). The y-axis represents the residuals from the relationship between constitutive levels of glucosinolates and lifetime fruit mass. Thus, the cost of plasticity relationship is not biased by any relationship between constitutive levels of glucosinolates and fitness (van Tienderen 1991; DeWitt et al. 1998).

genetic or sensory machinery for phenotypic plasticity. Without knowledge of the mechanism of sensing the environment and responding, it will be difficult to know whether this machinery has other primary functions. More importantly, it is exceedingly unclear whether genotypes have high (or low) plasticity simply because of variable investment in the genetic or sensory machinery or have invested in the machinery but have altered plasticity for other reasons (Agrawal 2001a). When using breeding designs like ours and that of many of the other studies, variation in phenotypic plasticity is most likely caused by several factors, including variation in investment in genetic or sensory machinery. However, genotypes that are variable in plasticity are also likely affected by genes that act after investment in the genetic and sensory machinery. This latter mechanism for creating genetic variation in plasticity will interfere with our ability to detect costs of investing in genetic or sensory machinery. Thus, only when we can measure both the level of phenotypic plasticity and the mechanistic traits that are responsible for plasticity will we be able to precisely and accurately assess costs of plasticity.

In conclusion, we present evidence for additive genetic variance in induction of glucosinolates, satisfying the Darwinian criteria for the continued evolution of plasticity in defense. In companion studies we have demonstrated the fitness benefits of such induction, suggesting that past selection has likely favored the evolution of induced defense (Agrawal 1998, 1999b; Agrawal et al. 1999c). Nevertheless, we speculate that the persistence of genetic variation in inducibility may be due, in part, to constraints on this evolution from costs of plasticity. The challenge will now be to empirically determine the conditions under which inducibility is favored over constitutive strategies; this depends on the net cost-benefit ratio of each strategy. Given that we did not find strong costs of inducibility, it appears that the past evolution of induction in wild radish may be due to its cost-benefit ratio being lower than that for constitutive defense.

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