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Lynn S. Adler · Johanna Schmitt · M. Deane Bowers

Genetic variation in defensive chemistry in *Plantago lanceolata* (Plantaginaceae) and its effect on the specialist herbivore *Junonia coenia* (Nymphalidae)

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Abstract To examine genetic variation in defensive chemistry within and between natural populations of *Plantago lanceolata*, we performed a greenhouse experiment using clonal replicates of 15 genotypes from each of two populations, from a mowed lawn and an abandoned hayfield. Replicates of each genotype were harvested for determinations of aboveground biomass and leaf chemical content either at the beginning of the experiment (initial controls), after exposure to herbivory by larvae of *Junonia coenia*, a specialist on *P. lanceolata* (herbivory treatment), or at the end of the experiment without exposure to herbivory (final controls). Allocation to the iridoid glycosides aucubin and catalpol and the phenylpropanoid glycoside verbascoside displayed significant genetic variation within and between populations, and differed with leaf age. Significant genotype×treatment interactions indicated genetic variation in response of leaf chemistry to the treatments. There was no evidence for a cost of allocation to chemical defense: genetic correlations within and between chemical pathways and between defensive chemicals and aboveground growth were positive or non-significant. Although iridoid glycosides are known to be qualitative feeding stimulants for *J. coenia*, multiple regression of larval survivorship on leaf chemical content and shoot biomass indicated that larvae had lower survivorship on *P. lanceolata* ge-notypes with higher concentrations of aucubin in the leaves. Larval survivorship was unaffected by levels of catalpol and verbascoside. Thus, although specialist herbivores may respond to defensive chemicals as qualitative feeding stimulants, they do not necessarily have higher fitness on plant genotypes containing higher concentrations of these chemicals.

L. S. Adler · J. Schmitt (✉)
Department of Ecology and Evolutionary Biology, Box G-W,
Brown University,
Providence, RI 02912, USA

M. D. Bowers
University Museum, Department of Environmental,
Population and Organismic Biology, Campus Box 334,
University of Colorado,
Boulder, CO 80309, USA

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Introduction

Herbivory is generally considered detrimental to plant fitness (Belsky 1986; Crawley 1989; Marquis 1984; Morrow and LaMarche 1978; Rausher and Feeny 1980; Sacchi et al. 1988; Simms 1992), which suggests that plants may be selected for resistance to herbivory. Many studies of plant resistance to herbivory focus on the production of defensive chemicals that deter herbivores. The production of some defensive chemicals has been shown to be at least partially under genetic control (Berenbaum et al. 1986; Bowers and Stamp 1992, 1993; Bowers et al. 1992; Chew and Rodman 1979; Dolinger et al. 1973; Fajer et al. 1992; Gouyon et al. 1983; Hanover 1966; Krischik and Denno 1983; Lincoln and Murray 1978; Lincoln et al. 1986; Mitter and Futuyma 1983; Murray et al. 1980; Vrieling 1991). For defensive chemistry to evolve in response to selection, there must be genetic variation within populations associated with variation in fitness. It is therefore important to ask how much genetic variation for defense occurs within natural plant populations and how expression of this variation depends upon the environment.

Evolutionary response to selection on chemical defense may be constrained by several factors. One important possible constraint is the postulated cost of this defense. Defensive chemicals can make up a significant percentage of foliage biomass, and their production is often assumed to have some cost to the plant (Chew and Rodman 1979; Karban 1993; Simms 1992; Simms and Rausher 1987). However, studies measuring the costs of defense for various fitness parameters have yielded conflicting results (Berenbaum et al. 1986; Bowers and Stamp 1993; Brown 1988; Coley 1986; Karban 1993; Simms 1992; Simms and Rausher 1987, 1989). If costs of defense do exist, one would expect to see a tradeoff in the absence of herbivores between allocation to defense

and to other functions that increase plant fitness, such as growth or seed production. For species that produce more than one defensive chemical, tradeoffs could also exist in allocation to different chemicals.

Another possible constraint on the evolution of chemical defense is opposing selection by different herbivores (Marquis 1990; Rausher and Simms 1989). Generalist herbivores often have lower fitness on, and are deterred by, higher levels of defensive chemicals (Bowers and Puttick 1988, 1989; Ikeda et al. 1977; Jones et al. 1979; Kraft and Denno 1982; Lincoln et al. 1982). Specialists are generally unaffected or prefer higher levels of certain chemicals (Bowers 1984; Bowers and Puttick 1988, 1989; Jones et al. 1979; Kraft and Denno 1982), and some use defensive chemicals as feeding and/or oviposition stimulants (Blum 1983; Bordner et al. 1983; Bowers 1983, 1984; Metcalf et al. 1982; Pereyra and Bowers 1988; Thorsteinson 1960). Therefore plants may experience selection from different herbivores for both increased and decreased chemical levels (Rausher and Simms 1987). Moreover, the fitness of a particular herbivore may depend on the genotype of the host plant.

The herbaceous perennial *Plantago lanceolata* L. (Plantaginaceae) and the buckeye butterfly *Junonia coenia* Hübner (Nymphalidae) provide an excellent system for studying genetic variation in defensive chemistry and its impact on a specialist herbivore. *P. lanceolata* produces two types of carbon-based defensive chemicals, the iridoid glycosides aucubin and catalpol and the phenylpropanoid glycosides verbascoside and plantamajoside (Andary et al. 1988; Duff et al. 1965; El-Naggar and Beal 1980; Fajer et al. 1992; Jensen et al. 1975; Ravn and Brimer 1988). *J. coenia* is a specialist herbivore on *P. lanceolata* and many other plants containing iridoid glycosides (reviewed in Bowers 1984). Larvae of this species grow and survive better when fed artificial diet with iridoid glycosides (Bowers 1984; Bowers and Puttick 1988, 1989) or verbascoside (M. Arntz and M.D. Bowers, unpublished work) than when fed a diet without these chemicals. However, the response of these larvae to quantitative variation in defensive chemicals found in *P. lanceolata* is uncertain. Quantitative studies are necessary to determine how fitness of *J. coenia* is affected by feeding on plants with higher concentrations of defensive chemicals.

In this study we used 30 genotypes of *P. lanceolata* from two populations to examine the genetic variation in defensive chemistry and response of different genotypes to herbivory. We also examined how plant defensive chemistry affected performance of the host plant and herbivore. The questions addressed were:

1. Is there genetic variation within or between populations in the quantity, composition or allocation of defensive chemicals?

2. Are there negative genetic correlations between defensive chemicals, or between defense and aboveground growth, to support the hypothesis that defense is costly?

3. How are survival and pupal weight of *J. coenia* affected by defensive chemicals in the host plant?

Materials and methods

Thirty genotypes of *P. lanceolata* were originally collected in August 1987 from a field and adjacent mowed lawn at the Brown University Haffenreffer Reserve in Bristol County, Rhode Island, United States. These were subsequently grown and clonally propagated for 5 years in the Brown University greenhouse. Plants were cloned in December 1992 by clipping the leaves to approximately 7.5 cm, separating the plant into its constituent rosettes, and repotting the rosettes separately. Nine to twelve clonal replicates were made for each of the 30 genotypes for a total of 293 plants. Plants were rearranged haphazardly on three benches of the Brown University greenhouse until April 1993, when they were randomized in preparation for the experiment. Extra illumination was provided by fifteen 60-W incandescent bulbs arranged approximately 60 cm above the plants in three rows, extending the photoperiod to a 14-h day. During the period from December 1992 to April 1993, inflorescences were plucked weekly to encourage vegetative growth. In April 1993, replicates from each genotype were assigned randomly to each of three treatments: (1) initial control (three to six replicates/genotype), to examine chemical content of plant genotypes at the beginning of the experiment, (2) herbivory (three replicates/genotype), exposure to a specialist herbivore, and (3) final control (three replicates/genotype), to examine chemical content of plant genotypes that were not exposed to herbivory at the conclusion of the experiment.

On 4 and 5 April initial control plants were harvested. Five new leaves (less than halfway expanded), five intermediate leaves (completely expanded but not yet senescing), and five old leaves (beginning to senesce but at least 50% green) were collected from each of the three to six replicate plants per genotype for later chemical analysis. The rest of the plant was then collected by severing it from the root at the point where the most basal leaf attached. Roots were not collected; in this paper, "plant biomass" will refer to aboveground biomass only. All collected material was dried at 50°C for 1 week prior to weighing.

Young larvae and eggs of *J. coenia* were obtained from a laboratory population at the University of Colorado at Boulder that had been raised for many generations on *P. lanceolata*, with some generations in winter raised on artificial diet. The original stock for the population came from North Carolina. These larvae were raised in 15-cm plastic petri dishes with moistened paper toweling taped to the roof. Dishes were haphazardly arranged in a growth chamber with a 14-h day set for a 25°C/20°C day/night cycle. Larvae were fed artificial diet following the recipe of Nijhout (unpublished work) with approximately 10% dry weight ground *P. lanceolata*.

Starting on 6 April, four larvae weighing between 0.05 and 0.15 g were placed on each plant in the herbivory treatment. Due to the difficulty in getting many larvae at the same stage of development, placing larvae on the plants was a process that spanned about 4 weeks. We placed larvae on one replicate of all genotypes before beginning the next replicate, so the timing of herbivory was not confounded with genotype. Larvae were contained on each plant by an inverted cone structure 28.3 cm high made of aluminum screening and lined at the rim with duct tape. A thick line of tanglefoot (The Tanglefoot Company, Grand Rapids, Mich., USA) was put inside every cone to prevent the larvae from escaping. Identical structures were also placed around all final control plants.

Beginning on 8 May, we conducted weekly censuses of plants and larvae. If all larvae on a plant had pupated or died, five leaves of each age class were collected and the rest of the aboveground plant was harvested according to the same procedure used for initial controls. In addition, each leaf was scored for herbivore damage [categories were (0) 0%, (1) 1–25%, (2) 26–50%, (3) 51–75%, or (4) 76–100% of leaf area removed]. The number of leaves in each category was counted in order to calculate a "damage index" for the entire plant {=0×[number of leaves in (0)]+1×[number of leaves in (1)]+2×[number of leaves in (2)]+3×[number of leaves in (3)]+4×[number of leaves in (4)]/total number of leaves}. Pupae, when present, were also collected from the plant and dried in pa-

per coin envelopes at 50°C. When all the plants in the herbivory treatment had been harvested (8 June), the final control plants were harvested using the same procedure. After drying, all plants, leaves and pupae were weighed on a Mettler balance (model AE240). Relative growth rate (RGR) of each plant was calculated for the herbivory and final control treatments using the genotype mean biomass from the initial control harvest as initial biomass [RGR=(log final biomass –log initial biomass)/days from initial control harvest to harvest]. In the herbivory treatment, this measure is an estimate of realized RGR. It does not include biomass lost to herbivory, which presumably would not contribute to fitness.

One unexpected event may have influenced the results of this experiment. In late May there was an infestation of green peach aphids (*Myzus persicae*) that rapidly spread to almost all the plants. Chemical treatment would have adversely affected the *J. coenia* larvae. The aphids were controlled using the aphid predator *Aphidoletes aphidimyza*, but aphid damage was visible on some plants. Upon harvest each plant was scored as having none (0), few (1), medium (2), or many (3) aphids. Plants with few aphids had aphids on less than half the leaves, none on the scapes, and no visible aphid damage. Medium plants had aphids on more than half but not all the leaves, a few or no aphids on the scapes, and no visible aphid damage. Plants with many aphids had aphids on most leaves and scapes, and visible aphid damage.

After drying and weighing, the five leaves within each age class collected from each plant were combined and ground using a Krups coffee grinder (type 208A) and stored in glass scintillation vials until analysis. Due to constraints of time and equipment availability, leaves from the three replicate plants for each genotype and treatment combination were pooled within age class for chemical analysis. Equal weights of ground leaf from each clonal replicate were combined in each sample to provide an estimate of the genotype mean. For each leaf age and genotype combination, there were one to two pooled samples for the initial controls and one sample for the herbivory and final control treatments, for a total of 312 samples. Aucubin and catalpol quantities were determined by gas chromatography following the procedure of Bowers and Stamp (1992), originally developed by Gardner and Stermitz (1988). The phenylpropanoid glycosides were quantified by fluorometry using the procedure of Fajer et al. (1992). The fluorescence spectra of plantamajoside and verbascoside are indistinguishable, so fluorometry provided a measure of the total phenylpropanoid glycosides. However, plantamajoside represents a very small amount of the total phenylpropanoid glycosides in our populations of *P. lanceolata* (Fajer et al. 1992), and we will equate verbascoside with total phenylpropanoid glycosides. For each sample, the percent biomass made up of aucubin, catalpol, verbascoside, and total defensive chemicals was determined. These percentages were converted to proportions and arcsine square root transformed for all analyses.

We tested for differences in aucubin, catalpol, verbascoside, and total defense content of genotypes from field and lawn populations using a hierarchical mixed-model ANOVA (SAS GLM procedure, type III sums of squares; SAS Institute 1982), with treatment, leaf age, and population as fixed effects and genotype as a random effect nested within population. *F*-tests were constructed using the expected mean squares generated by the SAS RANDOM statement. A three-way genotype×leaf age×treatment interaction term could not be included in the model due to lack of replication within cells. Genotype×treatment, genotype×leaf age, and treatment×leaf age interactions therefore were tested over the three-way interaction (remainder) sums of squares as an error term. To examine patterns of genetic variation within each population and treatment, we also performed separate two-way ANOVAs, with genotype and leaf age as main effects. In these analyses, genotype×leaf age interactions could not be tested due to lack of within-treatment replication, and main effects were tested over the remainder sum of squares. In all these analyses, tests of random effects would be equivalent to the SAS model (rather than the Scheffé model) for mixed-model ANOVA (e.g., Ayres and Thomas 1990; Fry 1992) if the remainder term included a significant interaction component. We used sequential Bonferroni tests (Rice 1989) to determine tablewide significance at $P<0.05$ for the multiple two-way tests.

Results

Genetic variation in defensive chemistry

Field genotypes had a significantly higher percentage biomass of all three chemicals than lawn genotypes (Fig. 1; aucubin: *df* 1,28, $F=6.4$, $P=0.017$; catalpol: *df* 1,28, $F=11.7$, $P=0.002$; verbascoside: *df* 1,28, $F=10.6$, $P=0.003$; total: *df* 1,28, $F=17.5$, $P=0.0003$). The concentration of aucubin was highest in the intermediate leaves, while the concentrations of catalpol and verbascoside were highest in the new leaves (Figs. 1, 2). The final controls had the highest mean concentration of all three chemicals and initial controls had the lowest (Figs. 2, 3). Populations did not differ significantly in effects of leaf age or response to treatments; all population×leaf age and population×treatment interactions were nonsignificant. However, there were significant genotype×treatment interactions for aucubin (*df* 56,158, $F=2.5$, $P<0.0001$), catalpol (*df* 56,158, $F=3.6$, $P=0.0001$), verbascoside (*df* 56,153, $F=2.7$, $P<0.0001$) and total defense (*df* 56,153, $F=3.2$, $P<0.0001$). There also was a significant genotype×leaf age interaction for catalpol ($F=1.8$, $P=0.002$) but not for aucubin ($F=1.1$, $P=0.25$), verbascoside ($F=0.9$, $P=0.71$) or total defense ($F=1.22$, $P=0.18$).

Separate two-way ANOVA revealed significant effects of leaf age for all chemicals in all treatments and both populations (Table 1). Effects of genotype on chemistry varied between populations and treatments (Table 1, Fig. 3). In the initial control and herbivory treatments, percentages of catalpol, verbascoside and total defensive chemistry differed significantly among genotypes in the field but not the lawn populations. Thus, the ability to detect genetic variation differed among populations and depended on treatment. For catalpol, the results from the three-way model suggest that a significant genotype×leaf-age interaction component may contribute to the remainder variance. The resulting tests of the random genotype effect for catalpol should therefore be interpreted in light of the SAS model rather than the Scheffé model for mixed-model ANOVA (Ayres and Thomas 1990; Fry 1992).

Testing for a cost of defense

Genotype mean correlations (e.g., Geber 1991) of all defensive chemicals and aboveground plant biomass were determined for each treatment using the SAS CORR procedure (SAS Institute 1982). Aphid severity and RGR were additional variables in the herbivory and final control treatments, and larval survival and damage from herbivory were added for the herbivory treatment only. Sequential Bonferroni tests were used to determine

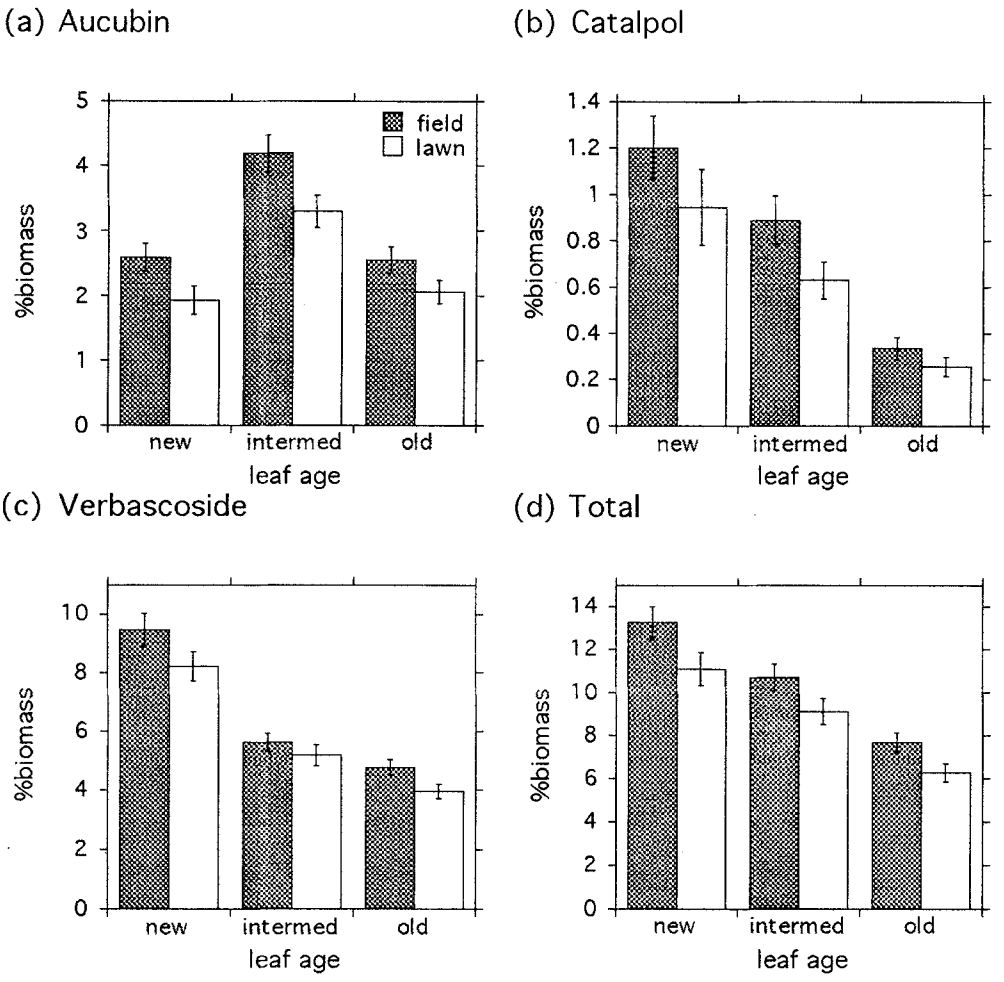

Fig. 1 Percent biomass of **a** aucubin, **b** catalpol, **c** verbascoside and **d** total defensive chemistry in new, intermediate and old leaves of *P. lanceolata* from field (*hatched*) and lawn (*open*) populations. Measurements were pooled over the three treatments (initial control, herbivory, and final control). *Bars* represent SE. Arcsine square root transformed proportions of all chemicals were used for analysis

tablewide significance ($P<0.05$). All correlations were analyzed separately for each leaf age class, and patterns of correlation were similar. For simplicity, only the results for intermediate leaves (the most common leaves on the plant) are shown (Table 2).

Genetic tradeoffs between allocation to different defensive chemicals were not detected; genotype mean correlations of percentage biomass were positive for all the defensive chemicals. Correlations were significant tablewide in the initial control treatment (Table 2). Allocations to all chemicals were also positively correlated with aboveground biomass and RGR, although these correlations were not significant tablewide. Thus, no tradeoffs between allocation to aboveground growth and to defense were detected.

To test for direct effects of allocation to each chemical on subsequent plant performance, we performed multiple regressions of genotype means of plant RGR and biomass at harvest in the herbivory and final control treatments on genotype means for aucubin, catalpol, and verbascoside content at harvest and initial biomass (genotype mean of biomass from initial control treatment). Analyses were performed separately for each leaf age class and results were similar; only results from the intermediate leaves are shown (Table 3). We also tested for direct effects of initial chemical content (genotype mean of chemical content from initial control treatment) and initial biomass on plant performance using a similar multiple regression (Table 3). In both of these analyses, initial biomass positively affected biomass at harvest and negatively affected RGR. None of the defensive chemicals had any significant effect on biomass or RGR, indicating no evidence for tradeoffs between growth and defense.

Effects of defensive chemistry on herbivores

To examine the effects of defensive chemistry on larval performance, we used multiple regressions of mean larval survival and pupal weight on plant genotype means of percentage aucubin, catalpol, and verbascoside, and plant biomass in the herbivory treatment. Larval survival was significantly lower on genotypes with higher percentages of aucubin in the intermediate leaves (Table 4, Fig. 4). The relationship of aucubin to survival was marginally non-significant in the new and old leaves, and no other variables had significant effects on survival. There

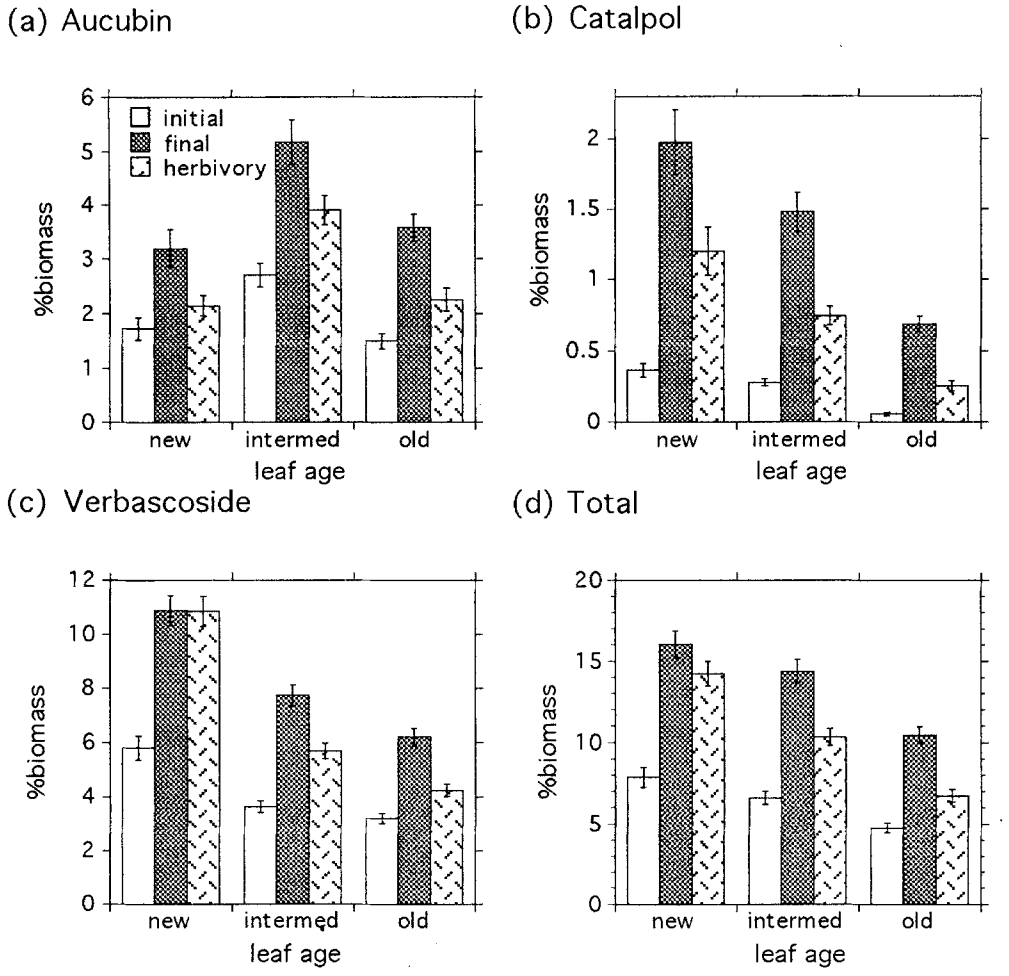

Fig. 2 Percent biomass of **a** aucubin, **b** catalpol, **c** verbascoside and **d** total defensive chemistry in new, intermediate and old leaves of *P. lanceolata* exposed to initial control (*open*), final control (*densely hatched*), and herbivory (*lightly marked*) treatments. Measurements were pooled over the two populations (field and lawn). *Bars* represent SE. Arcsine square root transformed proportions of all chemicals were used for analysis

Table 1 The effects of genotype (geno) and leaf age (lfage) on the transformed percentage aucubin (Auc), catalpol (Cat), verbascoside (Verb) and total defensive chemistry (Total) in *P. lanceolata*) within each combination of population and treatment. Initial=initial control, Herb=herbivory, Final=final control. F values in bold type are significant at P<0.05 tablewide by sequential Bonferroni criterion

*1 $P<0.05$, *2 $P<0.01$, *3 $P<0.001$, *4 $P<0.0001$

	Source	*df*	*F* value			
			Auc	Cat	Verb	Total
Initial, Field	Geno	14	**3.62** *3	**3.43** *3	**5.16** *4	**5.40** *4
	Lfage	2	**13.23** *4	**42.22** *4	**19.36** *4	**13.30** *4
Initial, Lawn	Geno	14	**6.98** *4	1.51	1.45	2.74 *2
	Lfage	2	**13.63** *4	**14.69** *4	**19.36** *4	**11.44** *4
Herb, Field	Geno	14	**7.11** *4	**5.89** *4	**4.81** *3	**5.79** *4
	Lfage	2	**32.33** *4	**63.46** *4	**132.34** *4	**86.07** *4
Herb, Lawn	Geno	14	2.38 *1	1.91	2.00	2.38 *1
	Lfage	2	**12.76** *4	**12.88** *4	**45.35** *4	**25.01** *4
Final, Field	Geno	14	2.07 *1	2.83 *2	1.33	1.65
	Lfage	2	**4.97** *1	**23.50** *4	**17.68** *4	**8.47** *2
Final, Lawn	Geno	14	1.46	2.64 *1	2.77 *1	1.64
	Lfage	2	**6.18** *2	**8.54** *2	**28.18** *4	**12.88** *4

were no significant effects on pupal weight, but due to high mortality of the larvae, *n* was very small for this test. A one-way ANOVA indicated that plant genotypes did not differ significantly in damage from herbivory, larval survival, or pupal weight, although power to detect these effects was low. Mean damage to the plants was approximately 13% (SD=4%) of leaf area [damage index=0.52 (SD=0.16)]. Pupal dry weight varied widely amongst surviving larvae, with a mean of 43.9 mg (SD=12.2 mg).

A two-way ANOVA revealed significant effects of plant genotype and treatment (herbivory vs. final control) on severity of aphid infestation, but there was no significant genotype×treatment interaction (Table 5). Aphid severity was greater in the final controls than in the herbivory treatment, but this was probably due to

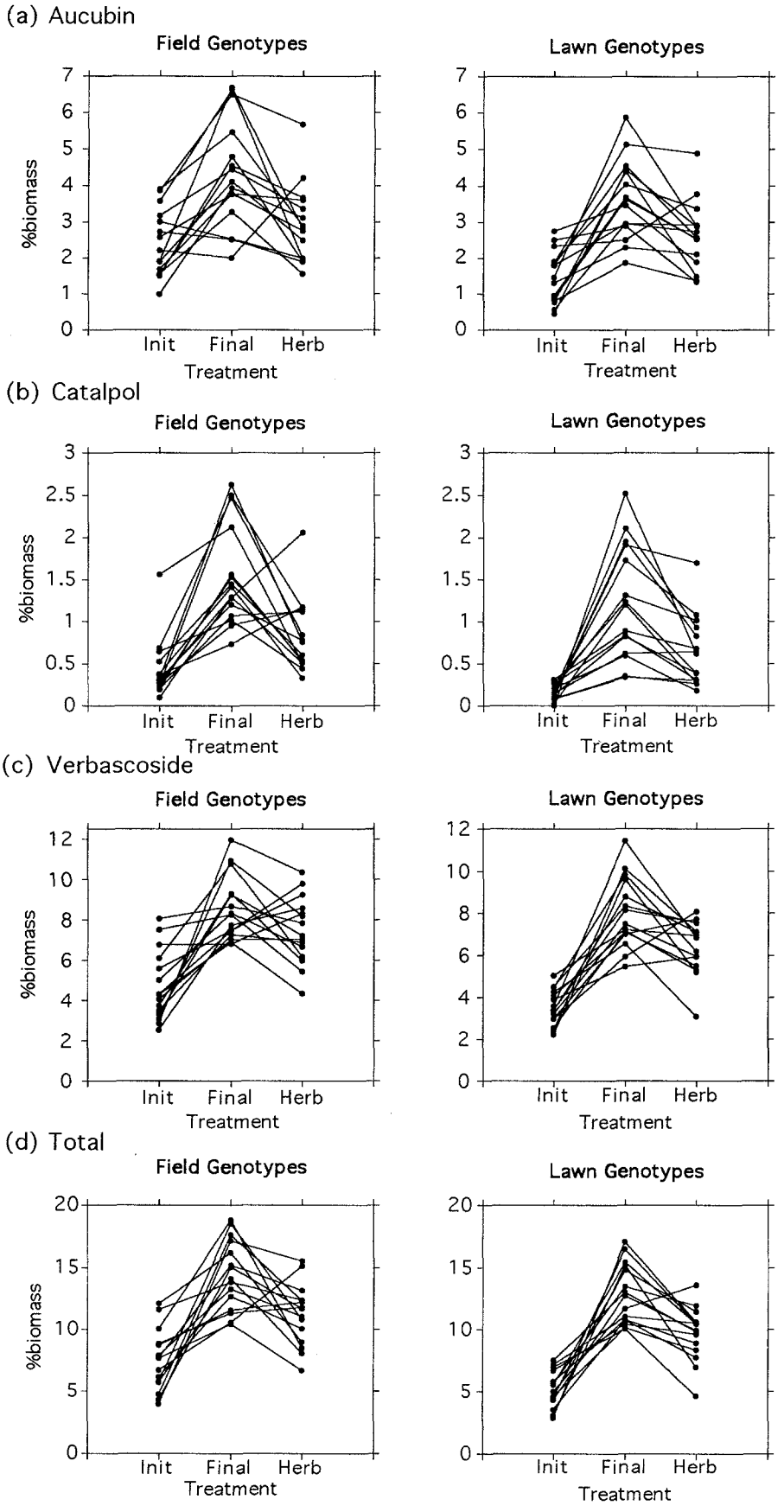

Fig. 3 Genetic variation in two populations of *P. lanceolata* in the percent biomass of **a** aucubin, **b** catalpol, **c** verbascoside and **d** total defensive chemicals in initial control, final control and herbivory treatments. Each point represents a genotype mean obtained by combining equal parts of samples from three replicates for chemical analysis. Arcsine square root transformed proportions of all chemicals were used for analysis

Table 2 Correlation of transformed percentage aucubin (Auc), catalpol (Cat), verbascoside (Verb), plant aboveground biomass (Weight), aphid severity (Aphid), relative growth rate (RGR), larval survival (Larvae), and damage from larval herbivory (Damage) for intermediate leaves of the initial control (Initial), herbivory, and final control (Final) treatments. Correlation coefficients in bold type are significant at P<0.05 tablewide by sequential Bonferroni criterion

*1 $P<0.05$, *2 $P<0.01$, *3 $P<0.001$, *4 $P<0.0001$

	Auc	Cat	Verb	Weight	Aphid	RGR	Larvae	Damage
Initial								
Auc	–	**0.762** *4	**0.586** *3	0.326				
Cat		–	**0.588** *3	0.365 *1				
Verb			–	0.198				
Herbivory								
Auc	–	0.224	0.455 *1	0.110	0.008	0.312	–0.556 *2	0.006
Cat		–	0.501 *2	0.423 *1	0.508 *2	0.269	–0.207	0.079
Verb			–	0.157	0.368 *1	0.252	–0.260	0.192
Weight				–	0.541 *2	0.563 *2	–0.152	–0.377 *1
Aphid					–	0.428 *1	–0.079	–0.279
RGR						–	–0.168	–0.316
Larvae							–	0.363 *1
Final								
Auc	–	0.541 *2	0.329	0.048	0.140	0.150		
Cat		–	0.460 *1	0.111	0.072	0.121		
Verb			–	0.089	0.298	0.315		
Weight				–	0.507 *1	**0.640** *4		
Aphid					–	0.401 *1		

Table 3 Regression coefficients for multiple regressions of aboveground plant biomass and RGR on (1) initial aboveground plant biomass and chemistry in intermediate leaves at time of harvest (herbivory and final control treatments only), and (2) initial aboveground plant biomass and initial chemistry in intermediate leaves

* $P<0.05$, ** $P<0.01$

Parameter	Treatment				
	Initial	Herbivory		Final	
	Biomass	Biomass	RGR	Biomass	RGR
(1) Init. Biomass	–	0.57 **	–0.0005 *	0.56 *	–0.0003
Aucubin	–	9.41	0.0152	3.57	0.0005
Catalpol	–	42.21	0.0535	–4.14	0.0031
Verbascoside	–	–1.63	–0.0023	15.27	0.0110
r^2	–	0.397	0.299	0.206	0.225
(2) Init. biomass	–	0.60 **	–0.0005 *	0.49 *	–0.0004
Init. aucubin	8.35	–15.65	–0.0144	–7.02	–0.0077
Init. catalpol	44.77	51.62	0.0634	–0.48	–0.0003
Init. verb	–3.75	–5.02	–0.0072	11.28	0.0066
r^2	0.140	0.323	0.178	0.175	0.189

Table 4 Multiple regression showing effects of transformed percentage aucubin, catalpol, and verbascoside in the intermediate leaves of the herbivory treatment and aboveground plant biomass (Weight) on larval survival of *J. coenia*

Parameter	*df*	Regression coefficient	*f*
Aucubin	1	–3.200	8.76 **
Catalpol	1	–0.840	0.13
Verbascoside	1	0.237	0.03
Weight	1	–0.005	0.13

** $P<0.01$

differences in the time of harvest in the two treatments. Because of this time difference and the possible effects of aphids, we could not address the question of whether induction of defenses occurred in plants in the herbivory treatment.

To determine whether aphid severity was affected by defensive chemistry, we performed two different multi-

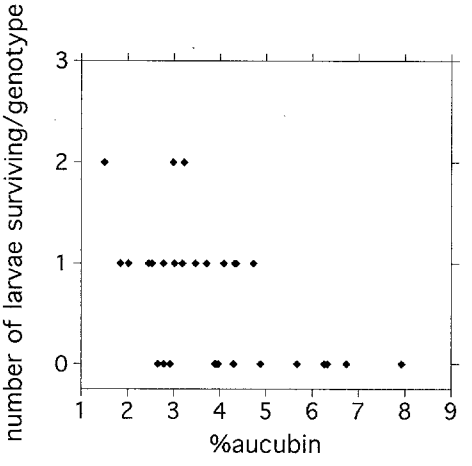

Fig. 4 Relationship of number of surviving *J. coenia* larvae (out of 12 per genotype) to the percentage of aucubin in the intermediate leaves of *P. lanceolata*. Arcsine square root transformed proportions were used for analysis

Table 5 Two-way ANOVA showing the effects of genotype and treatment on aphid severity in *Plantago lanceolata*. Genotype × treatment interaction was nonsignificant and was pooled with the error

Source	*df*	SS	*F*
Genotype	29	42.50	2.19 *2
Treatment	1	34.38	51.31 *4
Error	148	99.16	

*2 $P<0.01$, *4 $P<0.0001$

ple regressions on genotype means in the final control treatment with aphid severity as the dependent variable and the following independent variables: (1) initial shoot biomass and initial percentage aucubin, catalpol, and verbascoside; and (2) shoot biomass and percentage aucubin, catalpol, and verbascoside at harvest. In both these analyses, only shoot biomass at harvest had a significant effect; larger plants displayed higher levels of aphid infestation (analysis 2, intermediate leaves: regression coefficient=0.0815, P=0.0001). Defensive chemistry did not have any significant effects on aphid severity.

Discussion

This study detected clear genetic variation within and between natural populations in the quantity, composition and allocation of defensive chemicals in *P. lanceolata*. Genotypes also responded differently to herbivory and temporal variation (Fig. 3), suggesting that there is the potential not only for the evolution of defensive chemistry, but also for the evolution of response of chemical production to environmental variation.

Field genotypes had higher mean concentrations of all defensive chemicals than lawn genotypes (Fig. 1). These population differences are not attributable to differential availability of nutrients or light in the two environments, since all genotypes were grown and haphazardly rotated for several years in uniform greenhouse conditions prior to this study. The underlying cause of the observed genetic variation is unknown. It is possible that different levels of herbivory in the two environments have resulted in adaptive differentiation of defensive chemistry. However, the observed population differences may result from correlated selection on some other trait, or may be simply the result of random genetic differentiation due to restricted gene flow. More ecological studies of the two environments are necessary to understand the selective pressures on defensive chemistry in these populations.

No evidence was found in this study to support the hypothesis that tradeoffs occur between allocation to aboveground growth and defense in *P. lanceolata*. A multiple regression analysis, based on genotype means, indicated no direct effects of aucubin, catalpol, or verbascoside concentrations on RGR or plant biomass (Table 3), and there were no significant negative correlations of defensive chemistry and biomass or RGR (Table 2). There was also no evidence for tradeoffs in allocation to different defensive chemicals. These results provide no support for the hypothesis that defense is costly, although the failure to detect negative genetic correlations does not necessarily indicate that underlying physiological tradeoffs are absent. If variation among *P. lanceolata* genotypes in proportional allocation of carbon to growth or defense is low relative to large genetic variation in ability to assimilate carbon, genetic correlations would display the positive relationship between biomass and chemical defense seen here (Houle 1991; van Noordwijk and de Jong 1986). Similarly, there may be little genetic variation in allocation of carbon to different defensive chemicals, so that genetic correlations do not detect tradeoffs within or between pathways. It is also possible that some resource other than carbon is limiting (Abrahamson and Caswell 1982); in this case the production of carbon-based defensive chemicals might not be costly to the plant. However, plants were fertilized regularly in our experiment, so nutrient limitation seems unlikely. Another possibility is that a cost of chemical defense is only detectable in allocation to belowground biomass, which was not measured in this study. Although *P. lanceolata* allocates less to root biomass under high nutrient conditions such as those in our study (Troelstra and Brouwer 1991), the possibility remains that a cost of defense for belowground growth and storage might be important, especially in a low-nutrient field situation. Whether or not there is a physiological cost of defense, our results indicate that response to selection on defensive chemistry will not be constrained by negative genetic correlations with the performance traits we measured.

The differences in defensive chemistry found in leaves of different ages are consistent with previous studies on iridoid glycosides in *P. lanceolata* (Bowers and Stamp 1992, 1993) and with numerous studies demonstrating that new leaves are well-defended relative to older leaves (reviewed in Krischik and Denno 1983). These results support models which predict that under certain circumstances new leaves are of greatest value to the plant (Harper 1989).

The effects of the herbivory and final control treatments on defensive chemistry may be due to temporal variation as well as herbivory by *J. coenia* larvae. Temporal variation in defensive chemistry has been demonstrated in *P. lanceolata* and many other plant species (Bowers and Stamp 1993; Bowers et al. 1992; Dement and Mooney 1974; Feeny 1970; Mooney et al. 1980, 1981; Schultz et al. 1982). Although one of the original goals of this study was to determine if induction of defensive chemistry occurred, the confounding effects of time and aphid infestation made this impossible. Plants from the final control treatment were harvested on average later than those in the herbivory treatment. The high

percent biomass of defensive chemicals in the final control plants may be the result of an increase in defensive compounds over time. Despite these complications, there were clear interactions between genotype and treatment in this study, indicating that evolution of defensive chemistry in response to selection pressure may depend on the environment in which selection occurs (Bowers et al. 1992).

Although this was not the original purpose of the study, we found that the severity of attack by the aphid *Myzus persicae* was significantly greater on some genotypes than others (Table 5). This is consistent with other studies demonstrating genetic variation in resistance to aphids (Maddox and Cappuccino 1986; Moran 1981; Pilson 1992; Service 1984). Resistance to aphids in *P. lanceolata* appears to be unrelated to iridoid glycosides or verbascoside; whether these chemicals occur in the phloem is unknown. Aphid severity was positively correlated with plant biomass, indicating that aphids may have preferred larger plants.

In this study, host plants with higher levels of aucubin produced lower larval survival in the specialist herbivore *J. coenia* (Table 4, Fig. 4). Catalpol and verbascoside concentrations had no significant effects on larval survival. This is the only defensive chemical in *P. lanceolata* that has been shown to have a negative effect on a specialist herbivore. Catalpol and aucubin both have negative effects on a generalist herbivore (Bowers and Puttick 1988), and the effects of verbascoside are largely unknown but may be a deterrent to slugs and snails (Molgaard 1986).

Aucubin and catalpol both act as feeding stimulants for *J. coenia*; artificial diet with either of these iridoid glycosides is preferred over plain diet (Bowers and Puttick 1988). However, no studies have determined the effect of increasing quantities of these chemicals on larval growth and survival. One study demonstrated that *J. coenia* sequesters catalpol twice as efficiently as aucubin, and aucubin was detected in the frass in only trace amounts (Bowers and Collinge 1992). This suggests that the remaining aucubin is metabolized or altered in some way (Bowers and Collinge 1992), which may be costly to the larvae. If increased concentrations of aucubin do not stimulate an increased feeding rate in larvae, the costs of metabolizing more aucubin may result in decreased larval fitness.

The results of this study indicate that broad-sense genetic variation in defensive chemistry of *Plantago lanceolata* exists both between and within natural populations, and that genotypes vary in their chemical defenses over time and in response to different environments. There is no evidence that negative genetic correlations would constrain response to selection on this variation. If herbivores can discriminate among genotypes in natural populations on the basis of defensive chemistry, and if such selective herbivory has an impact upon fitness, then the genetic potential exists for chemical defenses to evolve in response to such selection. However, the observed genetic variation in plant susceptibility to aphid attack suggests that phloem-feeding herbivores may also select on plant traits other than the defensive compounds we measured. Our results also suggest that fitness of the herbivore *Junonia coenia* may be affected by genetic variation in host plant defenses. Quantitative field studies are clearly needed to examine the impact of genetic variation in plant chemical defense on the fitness of plants and herbivores in natural populations.

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