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Diffuse Selection on Resistance to Deer Herbivory in the Ivyleaf Morning Glory, *Ipomoea hederacea*

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ABSTRACT: Recent work defines coevolution between plants and herbivores as pairwise when the pattern of selection on resistance traits and the response to selection are both independent of the presence or absence of other herbivores. In addition, for a pairwise response to selection, resistance to a focal herbivore must have the same genetic basis in the presence and absence of other herbivores. None of these conditions were satisfied for the ivyleaf morning glory, *Ipomoea hederacea*, and its insect, fungal, and mammalian natural enemies with a quantitative genetics field experiment. A significant negative genetic correlation exists between resistance to deer and generalist insect herbivory that would preclude an independent response to selection. In addition, resistance loci under selection differ depending on the composition of the natural enemy community as indicated by genetic correlations between deer resistances in the presence and absence of other natural enemies that differ substantially from 1. Finally, selection on deer resistance depends on the presence or absence of insects; in the presence of insects, greater deer resistance is favored, but in the absence of insects, deer resistance is effectively neutral. These results indicate that the composition of the natural enemy community can alter both the pattern of selection and the likely response to selection of resistance traits.

Keywords: herbivory, resistance, *Ipomoea hederacea*, diffuse coevolution.

Coevolution is usually defined as successive, reciprocal evolutionary change in each of two species in response to selection imposed by the other species (Janzen 1980; Futuyma and Slatkin 1983; Rausher 1992*b*; Thompson 1994; Clayton et al. 1999). Although this definition of coevolution includes only a pair of species, we know that most pairs of interacting species are only subsets of multispecies interactions.

The prevalence of multispecies interactions has led to a distinction between two types of coevolution: pairwise and diffuse. In the strictest definition of these terms, pairwise coevolution implies that the coevolutionary dynamics of each pair of species can be understood without considering additional species (Janzen 1980; we term these additional species "auxiliary species" to distinguish them from the focal pair of species). "Diffuse coevolution," by contrast, implies that interactions with auxiliary species need to be examined to understand the coevolutionary dynamics between a focal pair of species (Janzen 1980; Fox 1981). The distinction between pairwise and diffuse coevolution, therefore, reflects in part the degree to which ecological complexity—namely, the presence of other interacting species—influences the direction and rate of evolutionary change between two interacting species.

Despite suggestions that the distinction between pairwise and diffuse coevolution be abandoned (e.g., Thompson 1997), the distinction remains useful because each view generates markedly different predictions about the evolutionary dynamics of ecologically important traits. Under pairwise coevolution, both the nature of selection and the likely response to selection would be independent of the presence or absence of auxiliary species. Under diffuse coevolution, however, the evolutionary dynamics of ecologically important traits would be constrained by genetic correlations or the community context, or both (Iwao and Rausher 1997). Moreover, under diffuse coevolution, geographical variation in the abundance of auxiliary species can lead to divergent patterns of coevolution of a focal pair of species (the "geographic mosaic" of Thompson 1997), whereas such divergence will not occur under pairwise coevolution.

Though the conceptual distinction between pairwise and diffuse coevolution has existed for 20 yr, there is little direct, empirical evidence indicating which alternative is more common in plant–natural enemy interactions (Hougen-Eitzman and Rausher 1994; Rausher 1996; Iwao and Rausher 1997). One way to address this issue is to

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Table 1: Proposed conditions to distinguish between pairwise and diffuse coevolution in plant–natural enemy systems

Condition	Potential mechanisms	Implication for coevolution
1. Genetic correlations between resistance to different natural enemies:		
Absent	NA	Pairwise
Present	Linkage disequilibrium or pleiotropy	Diffuse
2. Genetic correlations between resistances to the same natural enemies in the presence and absence of other enemies:		
$r = 0$	Two completely independent resistance traits	Diffuse
$0 < r < 1$	Partially independent traits	Diffuse
$r = 1$	Same genetic basis in two environments	Pairwise
3. Pattern of natural selection in the presence and absence of other natural enemies:		
Changes	Changes in the amount of damage[a]	Diffuse
	Nonadditive effects of damage on fitness[a]	Diffuse
Does not change	NA	Pairwise

Note: NA, not applicable.

[a] These are actually criteria 2 and 3 from Hougen-Eitzman and Rausher (1994) and Iwao and Rausher (1997). We consider them simply to be potential mechanisms that could alter the pattern of selection in the presence or absence of other natural enemies and therefore subsume them under condition 3.

examine the patterns of selection exerted by natural enemies on their host plants and their likely response, and vice versa. If coevolution is pairwise, then the patterns of selection exerted by an herbivore on its host and by the host on its herbivore, as well as the responses to that selection, will be independent of the presence or absence of auxiliary species, whereas if any of those patterns depend on the presence of auxiliary species, coevolution is diffuse. In this article, we use this approach to ask whether the patterns of selection exerted by herbivores on their host plants, and the potential responses to that selection, are pairwise or diffuse. We also describe a new criterion for distinguishing between pairwise and diffuse responses to selection.

Recent experimental and theoretical advances have made it possible to determine whether diffuse or pairwise selection and response are more likely to be occurring within a given system. In particular, Rausher and coworkers (Rausher 1992*b*; Hougen-Eitzman and Rausher 1994; Iwao and Rausher 1997) proposed a general method for distinguishing between pairwise and diffuse selection and response. There are two primary conditions that suggest pairwise coevolution: first, there are no genetic correlations between resistance to the focal herbivore and resistances to auxiliary herbivores, and second, selection on resistance to any natural enemy does not depend on the presence or absence of other natural enemies (Hougen-Eitzman and Rausher 1994; Iwao and Rausher 1997). If either of these conditions are not met, then coevolution is considered diffuse. There are two nonexclusive mechanisms that can cause violation of the second criterion (Hougen-Eitzman and Rausher 1994). On the one hand, the presence or absence of auxiliary herbivores could alter the amount of damage imposed by the focal herbivore in a manner that would alter the overall pattern of selection. On the other hand, it is possible that the fitness consequences of focal herbivore damage depend on the presence or absence of auxiliary herbivores (we term these interactions "nonadditive effects of damage"). In these cases, the presence or absence of auxiliary herbivores can alter the pattern of selection imposed by the focal herbivore.

Another aspect of pairwise coevolution that has not previously been considered is whether resistance to the focal herbivore in the presence of auxiliary herbivores has the same genetic basis as resistance in the absence of auxiliary herbivores. If there is an absolute genetic correlation between resistance to the focal herbivore in the presence of auxiliary herbivores and resistance in the absence of auxiliary herbivores, the same loci contribute to resistance in each case. However, if the genetic correlation differs from 1, at least some loci that confer resistance to the focal herbivore differ in the presence and absence of auxiliary herbivores. Consequently, different loci are under selection in different conditions and the response to selection will be diffuse. We provide a summary of these conditions for distinguishing between pairwise and diffuse coevolution in table 1; hereafter, we refer to the conditions presented in table 1 as criteria 1, 2, and 3.

While it seems intuitively likely that this criterion for pairwise response would commonly be met, there is some evidence that it may not always be satisfied. For example, Pilson (1992) determined that genetic variation for resistance to aphids in goldenrod depended on the presence of other natural enemies. Consequently, there was no genetic correlation for resistance to aphids across the two environments (presence and absence of other enemies). Thus, the expression of resistance to aphids, and presumably its costs and benefits, and the nature of selection on resistance to aphids, were all dependent on the presence or absence of auxiliary enemies.

While some studies have examined whether some of these conditions are met (reviewed by Rausher 1996; also see Juenger and Bergelson 1998), none have attempted to assess the validity of the new condition for distinguishing between pairwise and diffuse coevolution. Moreover, the paucity of studies examining all three criteria has made it impossible to determine how often the evolution of resistance traits in plant–natural enemy systems are constrained by genetic correlations or ecological interactions, or both. In this study, we evaluated all of the conditions described above for the ivyleaf morning glory, *Ipomoea hederacea*, and its taxonomically disparate suite of natural enemies. We utilized a quantitative genetics field experiment, independent manipulations of the presence or absence of auxiliary herbivores, and genetic selection analysis (Rausher 1992*a*) to evaluate whether selection imposed by natural enemies on their host is likely to be pairwise or diffuse in this system.

Material and Methods

Natural History

The ivyleaf morning glory, *Ipomoea hederacea* (L.) Jacquin (Convolvulaceae), is a weedy annual vine common to roadsides and agricultural fields in the southeastern United States. Seeds typically germinate in June or July, and plants die with the first fall frost, usually in late October or early November. Plants begin flowering 4–6 wk after germination, and seeds mature in papery capsules about 4 wk later. Flowers are tubular and range from light to dark blue in color and are often visited by bumblebees (Ennos 1981; Stucky 1985), though the selfing rate for one population has been estimated to be 93% (Ennos 1981). Leaf shape in *I. hederacea* exhibits a genetic polymorphism—all plants have either entire, heart-shaped leaves or leaves with three distinct lobes ("entire" and "lobed" hereafter). Elmore (1986) and Bright (1998) determined that leaf shape is inherited as a single Mendelian locus.

In Durham and Orange Counties, North Carolina, *I. hederacea* is attacked by a variety of natural enemies—insect, mammalian, and fungal. Damage imposed by each of these natural enemies is distinctive, and each can be quantified independently (Bright 1998). Common insect herbivores include grasshoppers (Orthoptera: Acrididae), fleahoppers (Hemiptera: Miridae), and lepidopteran larvae. Mammalian herbivores typically include deer (*Odocoileus virginianus*), occasionally rabbits (*Sylvilagus pennsylvanicus*), and woodchucks (*Marmota monax*). Two fungal pathogens are ubiquitous on *I. hederacea*: a white rust (*Albugo ipomoeae-panduranae* [Oomycetes: Peronosporales: Albuginaceae]) and orange rust (*Coleosporium ipomoea* [Uredinales: Coleosporaceae]). Damage at our study site was limited to insect herbivores, fungal pathogens, and deer.

Experimental Design

Our experimental approach was to determine the effect of altering the abundance of certain natural enemies (auxiliary enemies) on the patterns of selection exerted by focal natural enemies on *I. hederacea* and to estimate the genetic correlations between resistances to focal and auxiliary enemies. We treat resistances as quantitative traits and employ standard methods of quantitative genetics to estimate genetic correlations and selection (Rausher 1992*a*; Falconer and Mackay 1996).

To generate our experimental seeds, we utilized 18 parental plants grown simultaneously in the greenhouse to equalize maternal effects. Each parental plant had been collected as a seed from a unique plant in the field, such that an equal number of the two leaf shapes were sampled from each of two Durham County populations of *I. hederacea*. Each of the 18 parental plants were allowed to self in the greenhouse to produce the experimental seeds. Hereafter, we refer to all the selfed progeny of a single parental plant as a "family."

On July 23 and 24, 1998, 1,440 seeds, consisting of 80 seeds from each family, were planted into a randomized block design consisting of 10 spatial blocks in an agricultural field in Orange County, North Carolina. The field used was plowed twice to reduce the abundance of native vegetation. Seeds were scarified by nicking the seed coat with a razor blade; nicked seeds were stored in seed envelopes until suitable weather conditions allowed planting. Each seed was planted into a square grid with rows and columns such that neighboring plants were separated by 1.25 m. In contrast to much previous work with *Ipomoea* species (e.g., Rausher and Simms 1989; Simms and Rausher 1989; Tiffin and Rausher 1999), plants were not staked. Two electric fences were installed around the plot to exclude deer (see Bright 1998), though they proved to be ineffective. For the duration of the experiment, nonexperimental *I. hederacea* plants were removed but other colonizing vegetation was not disturbed.

Individual plants were randomly assigned to receive one of four treatments for the duration of the experiment: natural levels of herbivores and fungi (sprayed with water), insect herbivores but no fungal pathogens (through the application of the fungicide Ridomil Gold Copper), fungal pathogens but no insect herbivores (through the application of a mixture of the insecticides Carbaryl and *Bt*), and neither fungal pathogens nor insect herbivores (through the application of both insecticide and fungicide). Thus, there were 20 plants per family in each of these four treatments. Deer herbivory occurred in all treatments. Spraying treatments commenced when plants had four true leaves and were imposed at approximately 2-wk intervals such that only one pesticide was sprayed on a given day.

Germination and survival were monitored throughout the summer, and 7 wk after emergence, we recorded the total number of leaves and the number of leaves removed by deer (determined by leaf scars on stems or the presence of the petiole but no leaf). Our damage survey occurred before leaf senescence. By placing a clear plastic grid over four focal leaves, we measured leaf length and the amount of leaf area missing due to each type of insect herbivore, as well as the amount of leaf area covered by each fungus. We chose focal leaves systematically so that they would be evenly spaced throughout the plant—for example, if a plant had 100 leaves we sampled every twenty-fifth leaf. Leaf length was converted to leaf area through use of a regression calculated on 100 leaves of each leaf shape genotype ($r^2 = 0.955$ and 0.942 for entire and lobed genotypes, respectively). Average leaf area of the four focal leaves was then multiplied by the total number of leaves to estimate total leaf area. Estimates of total leaf area calculated by this method are highly correlated with estimates of total leaf area based on exhaustive measurements of every leaf of *I. hederacea* ($r = 0.96$, $N = 60$ plants; J. M. Kniskern, unpublished data).

Because we failed to exclude deer, we measured resistance to deer herbivory on all plants. The proportion of leaves damaged by deer was calculated as the number of leaves removed by deer divided by the total number of leaves. For example, if a plant had 25 leaf scars and 75 extant leaves, proportion deer damage was scored as 0.25 (proportion damaged = 25/[25 + 75]). This method is conservative in that it assumes that all current leaves were present at the time of herbivory. Therefore, if any regrowth or new growth occurred after deer herbivory and before our damage survey, it would cause our calculated value of the proportion leaves eaten by deer to be lower than the actual value. In contrast, proportion insect and fungal damage was calculated by dividing by the area of damaged leaf tissue by leaf area at the time of the survey. These measures were not corrected for leaves missing due to deer herbivory because we assumed that missing leaves were damaged to the same extent as remaining leaves. For insect, fungal, and deer damage, we defined resistance as 1 − proportion damaged (Simms and Rausher 1989).

All seeds produced by the experimental plants were gathered on a daily basis, as soon as capsules began to mature, and stored in individual envelopes for each plant until killing frosts on October 24 and November 4, 1998, ended the experiment. Seeds were counted at a later date; obviously inviable seeds—ones with incomplete or missing seed coats or abnormally small, shriveled seeds—were not included in analyses (previous experiments indicated 0% germination for such seeds; J. Stinchcombe, unpublished data).

Data Analysis

Overview. The 18 families used in the field experiment form the basis of our genetic analysis. These families represent a sample of the variation present in each leaf-shape genotype and in the two subpopulations from which they were collected. We do not present data on the effects of leaf shape or source population per se because our goal was not to generalize about differences in leaf-shape genotypes, but rather to include a representative sample of the variation present in local populations of *I. hederacea.* Furthermore, preliminary analyses indicated that neither of these effects were significant ($F \leq 0.13$, $P \geq .72$ for both effects).

Because all of the experimental seeds are the products of selfed lines, the between-family variation reflects broad-sense genetic variation (additive and nonadditive) plus maternal effects. Though we have no estimates of the potential magnitude or nature of maternal effects in our system, we attempted to equalize their influence on our results by raising all parental plants in a common greenhouse environment. While the evolution of traits in outcrossing species depends specifically on additive genetic variation (Falconer and Mackay 1996), using total genetic variation is appropriate for *I. hederacea* because of its high selfing rate (93%). In populations with such a high selfing rate, natural selection acts primarily on genetic variation in the broad sense, rather than just the additive genetic variation (Roughgarden 1979). Furthermore, the crosses necessary to discern the additive and nonadditive components of genetic variation would create an experimental population of seeds with artificially high levels of heterozygosity and thus would be of questionable relevance to natural populations of this species (Bright 1998; Mauricio 1998).

Genetic Variation for Resistance. We utilized the GLM procedure of SAS (SAS Institute 1990) to analyze our data with ANOVA and ANCOVA using Type III sums of squares. In all of our analyses, we considered family to be a random effect and all other effects to be fixed; we report results of analyses using the appropriate mean squares used as error terms (Sokal and Rohlf 1995). Values for resistance were arcsine–square root transformed to improve the normality of residuals. We performed this analysis on all of our data and evaluated a full model that included all possible interactions.

Genetic Correlations. We calculated the Pearson correlation coefficients and evaluated their significance using the CORR procedure of SAS. We first used ANOVA on data from just control plants to remove the effects of block and total leaf area, which reflect large- and small-scale environmental variation, respectively, on resistance. The residuals from this analysis were saved and averaged by family. This procedure was repeated for all types of natural enemy damage; afterward, we evaluated the significance of the correlation between the family means of the residuals to each type of damage. We then applied a sequential Bonferroni correction for multiple comparisons (Rice 1989). We obtained 95% confidence intervals (CIs) for genetic correlations by standard jackknifing procedures (Sokal and Rohlf 1995).

To determine whether resistance to the focal herbivore had the same genetic basis in the presence and absence of other natural enemies, we calculated genetic correlations between resistance to the same herbivore across treatments involving other natural enemies. We tested the null hypothesis that the correlation coefficient was equal to 1 by calculating the Pearson correlation coefficients for family means and then determining whether the 95% CIs of these correlation coefficients included 1. As before, we calculated our 95% CIs using Fisher's *Z*-transformation and standard jackknifing procedures (Sokal and Rohlf 1995). We interpret correlation coefficients that do not have a 95% CI that includes 1 as significant evidence of the absence of an absolute correlation (D. S. Burdick, personal communication).

Selection Analysis. Selection analyses were carried out using Rausher's (1992*a*) genetic selection analysis. This analysis is similar to the Lande and Arnold (1983) approach, but it utilizes breeding or genotypic values for a trait rather than phenotypic values of individuals. By measuring selection acting on genotypic instead of phenotypic values, this approach eliminates potential biases introduced by environmental correlations between resistance traits and fitness (Mitchell-Olds and Shaw 1987; Mauricio and Mojonnier 1997). Measuring selection on family means is a close approximation to measuring selection on genotypes since each family represents a highly inbred line (Mauricio et al. 1997).

For all selection analyses, we used least squares family means of resistance values after the effects of block had been removed and standardized these values to a mean of 0 and a variance of 1. We calculated relative fitness for each plant by dividing its viable seed production by the mean viable seed production for all plants. We also removed the effects of block on relative fitness before analysis. Directional selection gradients were estimated for resistance to each type of natural enemy in each treatment from the partial regression coefficients of a linear regression of the family mean of relative fitness on the family mean of the character. Stabilizing/disruptive selection gradients were estimated from the second-order coefficients of a quadratic regression of the family mean of relative fitness on the character. To determine whether the presence or absence of other natural enemies altered the pattern of selection on resistance characters, we utilized standard ANCOVA techniques to evaluate the significance of the treatment × resistance term on relative fitness (e.g., Iwao and Rausher 1997; Juenger and Bergelson 1998).

To assess whether deer imposed natural selection on resistance, ideally we would have excluded deer from a portion of our experiment to see whether the pattern of natural selection changed significantly depending on the presence or absence of deer herbivory (e.g., Simms and Rausher 1989; Wade and Kalisz 1990; Mauricio and Rausher 1997; Juenger and Bergelson 1998). However, since we were unable to effectively exclude deer from any portion of this experiment (85% of the surviving plants suffered deer damage), our assumption that any selection gradients detected on deer resistance were in fact due to deer herbivory should be considered hypotheses rather than concrete demonstrations. We believe, however, that the value of considering how selection imposed by mammalian herbivores could change in the presence and absence of insect herbivores and pathogenic fungi outweighs this limitation.

Individuals that did not germinate or survive to the damage census were excluded from this and other analyses. Individuals that survived to the damage census but did not set any viable seed were assigned a fitness value of 0.

Possible Inadvertent Effects of Pesticides. Our experimental manipulations are based on the assumption that any differences between spraying treatments are due to the absence of a given suite of natural enemies, rather than inadvertent effects of the chemicals themselves on fitness. In a separate greenhouse experiment, we replicated our spraying regime on 144 plants randomly selected from families used in the experiment (36 plants per treatment × 4 treatments) to determine whether there were any effects of pesticides on lifetime reproductive success. After 4 mo, we counted the number of seeds set by each plant and evaluated the effects of treatments on seed set using ANOVA. There were no significant insecticide, fungicide, or insecticide × fungicide effects on seed set ($P = .14$, .98, and .44, respectively).

We also evaluated the possibility that fungicide treat-

Table 2: ANOVA for deer resistance showing genetic variation for resistance to deer herbivory

Source of variation	df	Type III sums of squares	Mean square	F	P
Block	**9**	**47.254**	**5.250**	**72.79**	**.0001**
Insecticide treatment	1	.216	.216	3.00	.0837
Fungicide treatment	1	.024	.024	.33	.5674
Family	**17**	**2.613**	**.154**	**2.13**	**.0047**
Insecticide × fungicide	1	.008	.008	.11	.7406
Family × insecticide	17	1.086	.064	.89	.5916
Family × fungicide	17	.892	.052	.73	.7766
Block × insecticide	9	.509	.057	.78	.6306
Block × fungicide	9	.775	.086	1.19	.2954
Block × insecticide × fungicide	9	.520	.058	.80	.6150
Family × insecticide × fungicide	17	1.185	.070	.97	.4948
Error	1,117	80.574			

Note: Significant effects are shown in bold.

ments might alter the palatability of plants to insect herbivores (or that insecticide might alter suitability to fungal spores), perhaps because of residues from the chemicals. We raised 80 plants (again, randomly selected from the families used during the experiment) free of damage in the greenhouse for 4 wk, randomly assigned each of them to a spraying treatment, and then immediately brought them into the field for 2 wk. After 2 wk, we measured the total amount of damage due to insect herbivory or fungal pathogens. Since all plants were damage free and sprayed only before leaving the greenhouse, any treatment effects would indicate that the pesticides altered the palatability or suitability of the plants, rather than the absence of a given natural enemy. We found no evidence of fungal infection of these plants. We also found no evidence that residues from the insecticides or fungicides altered insect herbivory ($P > .4$ for insecticide, fungicide, and insecticide × fungicide effects). These results indicate that any subsequent treatment effects in the main experiment could be ascribed to the elimination of the targeted natural enemies rather than inadvertent side effects of the chemicals themselves. To address the possibility that the spraying regime might have a significant impact in survivorship of plants, we evaluated whether or not pesticide treatments had significant effects on survival in the field (see "Results").

Results

Preliminary Analyses

Germination and Early Survival. Of the 1,440 seeds planted, a total of 1,290 germinated. Germination occurred in two distinct cohorts after two heavy summer thunderstorms a week apart. Of the 1,290 plants that germinated, 1,225 survived to the damage survey, and 1,040 set viable seed. Likelihood ratio χ^2 tests indicated that families did not differ in their germination percentages or survivorship to the damage survey ($P = .17$ and .53, respectively). Likewise, there were no effects of pesticide treatment on survival to the time of the damage survey ($P = .86$).

Effectiveness of Pesticides. To analyze the effectiveness of each treatment in reducing damage by natural enemies, we utilized a two-way ANOVA, with presence or absence of insects or fungi as main effects, and with the effects of block removed. The insecticide treatment reduced the proportion of generalist insect damage by 65% ($F = 38.94$, df = 1, $P = .0001$). However, the insecticide treatment did not alter the level of fleahopper damage ($F = 0.23$, df = 1, $P = .63$). There were also no significant fungicide or insecticide × fungicide interactions for the amount of damage caused by either fleahoppers or generalist insects.

The fungicide treatment was also effective, significantly reducing the prevalence of the orange rust by 84% and the white rust by 93% ($F = 15.42$, df = 1, $P = .0001$ and $F = 229.55$, df = 1, $P = .0001$, respectively). For fungal damage, however, there were significant main effects of the insecticide treatment and the insecticide × fungicide interaction term. In general, the presence of insects significantly increased the prevalence of fungal infection but significantly less so on plants that had been sprayed with fungicide. Based on these results, we concluded that the pesticide-spraying regime was effective at reducing damage by all types of natural enemies except fleahoppers.

Genetic Variation for Resistance. Genetic variation for resistance to fungal pathogens and herbivores was indicated by a significant family effect on resistance in an ANOVA. We found direct evidence of genetic variation by ANOVA for resistance to deer herbivory (table 2) and indirect evidence for genetic variation for resistance to generalist insect herbivores (see below). Furthermore, this analysis reveals that deer herbivory was distributed evenly across treatments, as neither the treatment terms nor the treatment × block interaction terms are significant. Though the block effect indicates significant spatial variation in patterns of deer herbivory, we account for this in subsequent results by presenting analyses that utilized either least squares means or residuals for deer resistance after block effects had been removed.

Testing the Criteria for Pairwise Selection and Response

Criterion 1: No Genetic Correlation between Resistance Traits. A pairwise evolutionary response to selection imposed by herbivores requires that separate resistance traits be genetically uncorrelated. Resistance to deer herbivory exhibited a significant negative genetic correlation with resistance to generalist insect damage ($r = -0.72$, $P = .0007$; fig. 1). The 95% confidence limits of this correlation range from $r = -0.89$ to $r = -0.37$. It thus appears that these two types of resistance will not evolve independently, and thus the first criterion for pairwise resistance evolution appears to be violated.

This negative correlation is not an artifact of the deer avoiding fungal or insect-damaged plants because there was no difference in deer damage between control and fungicide-sprayed plants ($F = 0.04$, df = 1, $P = .8413$), or between control and insecticide-sprayed plants ($F = 0.10$, df = 1, $P = .7544$). Furthermore, there is no difference in insect damage between plants with and without deer herbivory ($F = 0.62$, df = 1, $P = .43$), indicating that the negative correlation is not likely due to insects avoiding deer-damaged plants.

These results also suggest that our experimental population was genetically variable for generalist insect resistance. In particular, the presence of a significant correlation among family means suggests that our failure to detect genetic variation in resistance to generalist insect herbivory with ANOVA was due to the lack of statistical power, rather than a lack of genetic variation for resistance (see Tiffin and Rausher 1999 for a similar example).

Criterion 2: Resistance to Focal Herbivore Has the Same

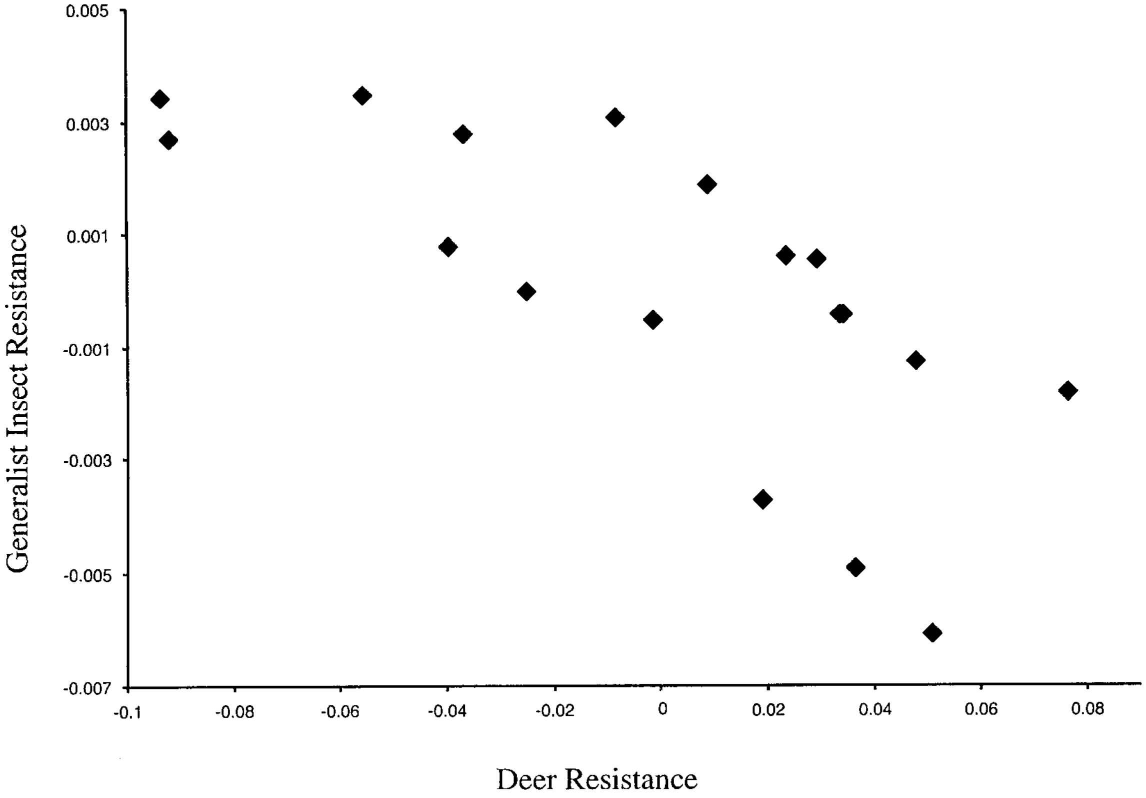

Figure 1: Negative genetic correlation ($r = -0.72024$, $P = .0007$) between resistance to deer herbivory and resistance to generalist insect herbivory. Plotted points are family means of the residuals after the effects of block and total leaf area on resistance had been removed.

Genetic Basis in Presence and Absence of Auxiliary Herbivores. For the response of resistance to selection to be pairwise, resistance to the focal herbivore must have the same genetic basis in both the presence and absence of auxiliary herbivores; that is, there must be a genetic correlation of 1 between resistance to the focal herbivore in the two contexts. Criterion 2 was violated for resistance to generalist insect damage. The family mean correlation between generalist resistance in the presence and absence of fungi was $r = 0.27$ ($P = .28$), with a 95% confidence interval that differed substantially from 1 ($r = 0.64$ and -0.24 for upper and lower limits, respectively).

This criterion was also violated for resistance to deer damage. In particular, deer resistance in the control and insecticide treatments exhibited no detectable family mean correlation ($r = 0.33$, $P = .17$) and the confidence interval of the correlation coefficient differed substantially from 1 ($r = 0.72$ and -0.32 for the upper and lower limits). Similarly, the family means of deer resistances in the control and fungicide treatments were uncorrelated ($r = 0.016$, $P = .95$), with confidence interval again differing markedly from 1 ($r = 0.45$ and -0.43 for the upper and lower limits). Finally, although resistance to deer herbivory in the control environment was significantly correlated with resistance to deer herbivory in the absence of both insects and fungi (dual-spray treatment; $r = 0.49$, $P = .04$), the confidence interval once again did not approach 1 ($r = 0.73$ and 0.09 for the upper and lower limits).

Criterion 3: The Pattern of Selection on Resistance to the Focal Herbivore Is the Same in the Presence and Absence of Auxiliary Herbivores. We confined our selection analyses to only those traits—resistance to deer herbivory and resistance to generalist insects—for which we found either direct or indirect evidence for genetic variation. Moreover, we present only the results for directional selection because we found no evidence for stabilizing/disruptive selection or correlational selection in any of the treatments.

For resistance to generalist insects, separate analyses by treatment revealed significant negative selection only in the dual-spray treatment, with no evidence of selection in the other treatments (table 3). To determine whether the magnitude of selection differed significantly among treatments, we employed ANCOVA to determine whether the slope of fitness on generalist insect resistance differed among treatments. In a full model, which contained both deer and generalist insect resistances as covariates, treatments as class variables, and all possible interactions, there was no significant interaction effect between generalist insect resistance and fungicide treatment ($F = 0.95$, $P = .33$). We thus cannot reject the hypothesis that the presence or absence of fungi does not alter the pattern of selection on generalist insect resistance.

Table 3: Standardized regression analysis of selection on resistance to deer herbivory and generalist insect herbivory in the four experimental treatments

Treatment/trait of interest	Directional selection gradients (β)
Control:	
Deer resistance	.195 (.0901)*
Generalist resistance	−1.075 (65.92)
Fungicide:	
Deer resistance	.146 (.0995)
Generalist resistance	.041 (.1004)
Insecticide:	
Deer resistance	.026 (.0882)
Generalist resistance	−.034 (.0895)
Insecticide and fungicide:	
Deer resistance	.020 (.0672)
Generalist resistance	−.147 (.0664)*

Note: Shown are regression estimates (with standard errors in parentheses); β was estimated from regressions containing only the linear terms.

* $P < .05$.

For resistance to deer herbivory, separate analyses by treatment revealed significant, positive directional selection in the control treatment but in none of the other treatments (table 3). To determine whether the magnitude of selection differed significantly among treatments, we initially analyzed a full model that contained both deer and generalist insect resistances as covariates, insecticide and fungicide treatments as class variables, and all possible interactions. The results of this full model indicated that there was no significant main effect of generalist insect resistance on fitness and that none of the interactions between insect resistance and treatments were significant ($P > .94$ for all terms involving insect resistance). To examine how the pattern of selection on deer resistance differed among treatments, we analyzed a reduced model that did not include insect resistance or interactions between insect resistance and treatment. In the resulting model, there is a marginally significant difference in the pattern of natural selection on resistance to deer herbivory, depending on the presence and absence of insects (deer resistance × insecticide term; table 4; fig. 2). It thus appears that criterion 3 is violated for resistance to deer damage since the magnitude of directional selection on this character depends on whether generalist insects are present.

As described above, criterion 3 for pairwise selection can fail for either of two reasons. One possibility is that the presence or absence of auxiliary enemies affects the amount of damage caused by the focal enemy and does so to a different extent for different families. To evaluate

Table 4: ANCOVA for relative fitness showing that the pattern of selection on deer resistance changes depending on the presence or absence of insects

Source of variation	df	Type III sums of squares	Mean square	*F*	*P*
Deer resistance	**1**	**.573**	**.573**	**5.13**	**.0269**
Insecticide	1	.00001	.00001	<.0001	.9900
Fungicide	1	.00004	.00004	<.0001	.9843
Insecticide × fungicide	1	.001	.001	.01	.9471
Deer resistance × insecticide	**1**	**.428**	**.428**	**3.83**	**.0546**
Deer resistance × fungicide	1	.033	.034	.30	.5857
Deer resistance × insecticide × fungicide	1	.0004	.004	<.0001	.9471
Error	64	8.190			

Note: Significant and marginally significant effects are shown in bold.

this possibility for deer damage, we analyzed a factorial model that included block, insecticide and fungicide treatments, family, and their interactions. In this analysis, we did not detect any significant effects of the presence or absence of either insect herbivores or fungal pathogens on the overall amount of damage imposed by deer: mean deer resistance in different treatments ranged from 0.73 to 0.75 (table 2, insecticide and fungicide terms, respectively). More important, we did not detect any evidence that the effect of insecticide or fungicide treatment on deer damage varied among families (table 2, family × insecticide and family × fungicide terms). The first potential explanation for diffuse selection on deer resistance, therefore, appears not to hold.

A second possible explanation for the change in the pattern of selection on deer resistance is that the presence/absence of insects alters the relationship between fitness and deer damage. We examined this possibility by using ANCOVA to determine whether the slope of the regression between proportion of leaf area damaged by deer and fitness for a given enemy differed among insecticide or fungicide treatments. This analysis revealed that the slope of the this relationship differed according to whether insects were present or absent (proportion deer damage × insecticide interaction: $F = 4.50$, $\mathrm{df} = 1$, $P = .034$). Deer damage was always negatively related to fitness but more so in the absence of insects. These results are consistent with our selection analyses; families that are more resistant (and hence suffer less damage) have higher fitness in the presence of insects.

Discussion

Two fundamental issues are raised by the results we have presented: the variable nature of the expression of quantitative traits in differing environments, and the violation of all of the criteria for pairwise selection and a pairwise response to selection. In the sections below, we discuss each of these issues in turn and conclude with a discussion of the importance of the distinction between pairwise and diffuse coevolution.

Expression of Quantitative Traits in Differing Environments

Our finding that resistances to the same natural enemy in two different biological environments are often uncorrelated, separate traits poses new challenges to the study of resistance. On the methodological level, this result indicates that while it is possible to operationally measure resistance to damage to a single natural enemy in exactly the same manner in presence and absence of auxiliary species, it does not necessarily follow that it is genetically the same trait or that it will even have similar evolutionary dynamics.

To gain an intuitive understanding of how this could be possible, consider the following hypothetical example. If damage by insect herbivores results in wounds that fungi can infect, resistance to fungi could be conferred by systemic acquired resistance or induced resistance. In the absence of insect herbivores, however, resistance to fungi could be conferred by properties of the plant's cuticle. If systemic acquired resistance or induced resistance and the properties of the plant's cuticle are genetically uncorrelated, then resistance to the same natural enemy, fungi, would be uncorrelated across the presence and absence of insect herbivores. Thus, which traits confer resistance, the pattern of selection on resistance, and costs of resistance could all depend on the presence or absence of other components of the community (e.g., Pilson 1992).

Despite the intuitive appeal of this example, it is unclear whether our results would have been the same had we measured a resistance character such as trichome density (e.g., Mauricio and Rausher 1997). For instance, it is doubtful that plants have a unique set of trichome genes for each biological or physical environment. However, it

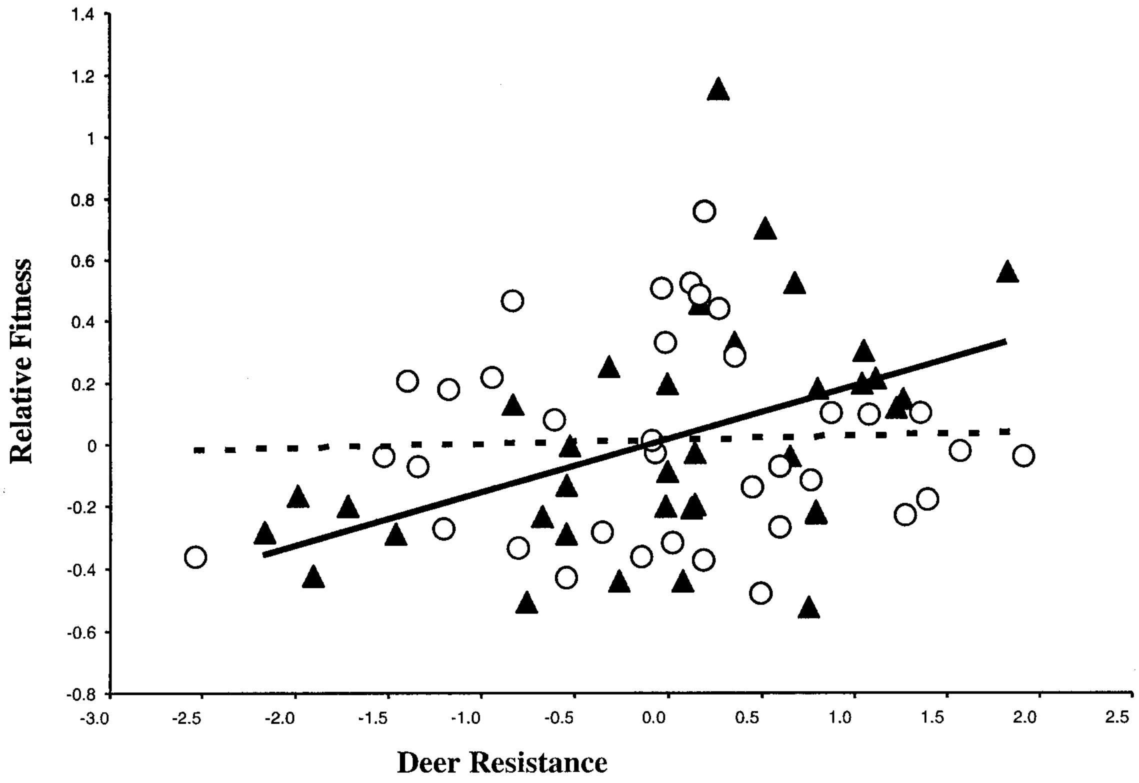

Figure 2: Estimates of selection on deer resistance in the presence and absence of insects. Plotted points are family means of resistance to deer herbivory that have been standardized to a mean of 0 and a variance of 1 and the residuals of relative fitness after the effects of block had been removed. Filled triangles represent data from treatments where insects were present (control and fungicide treatments); open circles represent data from treatments where insects were absent (insecticide and dual-spray treatments). The slopes of the two lines are marginally different based on ANCOVA ($F = 3.83$, df = 1, $P = .0546$).

is possible that different natural enemies activate or induce different biochemical pathways that could interact with the trichome pathway, possibly in a competitive or facilitative manner. If this is the case, it still would be possible to detect a genetic correlation between trichome density in the presence and absence of auxiliary species that was significantly less than 1. Indeed, many quantitative trait loci (QTL) studies of quantitative traits find significant QTL × environment interactions (e.g., Xia et al. 1999) or environment-specific QTLs (e.g., Vieira et al. 2000), both of which imply a genetic correlation across environments less than 1 for these traits. These results suggest that our findings are not due to the method that we used to measure resistance but are instead consistent with an emerging trend about the expression of quantitative traits in multiple environments.

Diffuse Selection and Response

Our results clearly indicate that to understand the evolution of resistance in *Ipomoea hederacea* it is necessary to consider multiple natural enemies and the community context in which herbivory is occurring. In particular, both the pattern of selection on resistance and the expected response to selection, as determined by the correlations between resistance to different herbivores, are diffuse rather than pairwise. Whether these findings would have been the same for different levels of deer herbivory remains unknown. Nevertheless, because we failed to detect any evidence that deer herbivory was not evenly distributed across experimental treatments, there is no reason to believe that these results are not valid for the observed levels of deer herbivory.

For example, the negative family mean correlation between deer and generalist insect resistance indicates that the evolution of resistance to these natural enemies will not be independent. These findings are contrary to an emerging consensus that negative genetic correlations between plant resistances to insect herbivores do not frequently constrain the evolution of resistance (see Rausher 1996 for a review). One possible explanation for this discrepancy is that trade-offs between resistances to mammalian and insect herbivores might be more common than trade-offs between resistance to different types of insect herbivores. Whether this is a general phenomenon is an empirical question, but to answer it will require considering mammalian and insect herbivory jointly rather than each in isolation.

Further evidence for the diffuse interaction between *I. hederacea* and its natural enemies comes from the selection analyses. It is not surprising that selection favors increased resistance to deer herbivory in the presence of insects: the fitness benefits of resisting deer herbivory are obvious in this system. In previous experiments, it was not uncommon for us to document cases in which deer consumed 99% of the aboveground biomass of an individual or even consumed an entire experiment over a few nights. Moreover, the few plants in our experiment that escaped deer damage entirely set 33% more seeds than those that suffered any amount of deer damage. Our failure to detect any selection on deer resistance in the absence of insects, therefore, suggests that the benefits of resisting deer herbivory are decreased in these environments and that the benefits and costs are approximately equal.

Diffuse selection appears to be common in the few systems investigated to date. For example, Simms and Rausher (1993), Pilson (1996), and Juenger and Bergelson (1998) have also detected diffuse components of selection (though see Hougen-Eitzman and Rausher 1994 for an exception). In all four of the systems in which diffuse selection has been detected, nonadditive effects of damage on fitness appear to be the primary mechanism. These data and other reports of nonadditive effects of damage (e.g., Strauss 1991; Wise and Sacchi 1996) suggest that nonadditive effects are likely to be widespread and thus commonly lead to diffuse selection. Moreover, despite many previous reports that the amount of damage imposed by one herbivore or natural enemy often alters the amount of damage imposed by another natural enemy (e.g., Harrison and Karban 1986; Strauss 1991; Pilson 1992; Hougen-Eitzman and Rausher 1994; Karban and Baldwin 1997; Juenger and Bergelson 1998), there is little or no evidence that these interactions modify the actual pattern of selection on resistance traits. Thus, whether changes in the amount of damage imposed by a focal herbivore in the presence or absence of auxiliary enemies actually alters the evolutionary dynamics of resistance traits remains a major unanswered empirical question of evolutionary ecology.

Conclusions

Despite Darwin's (1859) early insight that plants and insects might adapt to each other and nearly 4 decades of recent work (e.g., Ehrlich and Raven 1964; Gilbert and Raven 1973; Janzen 1980; Fox 1981; Futuyma and Slatkin 1983; Thompson 1994), little progress has been made in characterizing coevolution in systems with multiple interacting species. Though the term "diffuse coevolution" has been criticized as vague, imprecise, and outdated (Gould 1988; Thompson 1994, 1997), only recently have criteria and methods become available for distinguishing diffuse coevolution from pairwise coevolution (Rausher 1992*b*; Hougen-Eitzman and Rausher 1994; Iwao and Rausher 1997; this article). It seems premature to abandon the distinction between pairwise and diffuse coevolution for two reasons. First, documenting diffuse coevolution gives direct insight into the relative degree to which ecological and genetic constraints govern the evolution of ecologically important traits, a central and fundamental issue in evolutionary ecology. Second, the growing body of literature on the genetics of diffuse coevolution yields empirical data on how many interspecific interactions must be considered to understand the evolution of resistance traits in plants. The results presented here, along with the work of others (Strauss 1991; Pilson 1992, 1996; Simms and Rausher 1993; Wise and Sacchi 1996; Juenger and Bergelson 1998), suggest that if we are to truly understand the evolution of resistance traits, we will need to take a "community genetics" perspective (sensu Antonovics 1992).

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