

## THE EVOLUTION OF RESISTANCE TO HERBIVORY IN *IPOMOEA PURPUREA*. II. NATURAL SELECTION BY INSECTS AND COSTS OF RESISTANCE

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**Abstract.**—An important component of the process of coevolution between plants and their insect herbivores is the imposition of selection on plants by insects. Although such selection has been inferred from indirect evidence, little direct evidence for it exists. One goal of this study was to seek direct evidence by determining, for a single plant-herbivore system, whether insect herbivores impose selection on their host plants. A second goal was to determine whether costs are associated with genotypes that confer resistance to herbivores, as has been commonly postulated.

The annual morning glory, *Ipomoea purpurea*, exhibits genetic variation in resistance to four different types of insects. For three of these types, most of the genetic variation is additive. Removal of insect herbivores increased the number of seeds produced by *I. purpurea* by 20% and eliminated additive genetic variation for seed number (fitness). This result implies that herbivores impose selection on some trait(s) of their host plants. Coupled with selection for decreased damage by corn earworms, as revealed by a negative additive genetic covariance between damage and fitness, this result suggests that insect herbivores impose selection on resistance to corn earworms in *I. purpurea*.

Two types of cost of resistance to herbivores were sought in *I. purpurea*: 1) internal trade-offs in allocation of resources and 2) ecological trade-offs between resistances to different insects. No costs of either type were detected. This result suggests that cost-benefit arguments that attempt to predict the evolution of levels of resistance to herbivores are not applicable to *I. purpurea*.

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Although it is generally believed that plant secondary chemistry and other types of resistance to herbivores have evolved in response to selection pressures imposed by herbivores (Ehrlich and Raven, 1964; Feeny, 1976; Rhoades and Cates, 1976; Futuyma, 1983), very little direct evidence supporting this belief exists. In particular, there is very little empirical evidence indicating that herbivorous insects act as selective agents on plant characters. There are, of course, many examples of plant traits that reduce herbivory (e.g., Dirzo and Harper, 1982a; Harborne, 1982; Jones, 1983; Berenbaum et al., 1986). However, demonstrating only that a character reduces herbivory does not allow one to conclude that herbivores have exerted the selection pressures responsible for the evolution of that character, since resistance to herbivory may be a pleiotropic effect of a trait that serves some other primary function (Nowacki et al., 1976; Moran and Hamilton, 1980).

Rausher and Simms (1989) demonstrated that resistance to the corn earworm, *Heliothis zea*, in the annual morning glory *Ipomoea purpurea* is subject to directional selection for increasing resistance. One goal of the present study was to determine experimentally whether insect herbivores are, at least in part, the agents responsible for that selection. More generally, we sought to determine the extent to which insect herbivores are responsible for genetic variation in fitness in *I. purpurea*.

Even for cases in which selection by herbivores for resistance is strongly suspected, little is known about how selection determines the equilibrium level of resistance in natural plant populations (Gould, 1983). Nevertheless, several workers have suggested that the mean level of resistance may often evolve toward an intermediate optimum determined by a trade-off between the costs and benefits of resistance (Janzen, 1973a, 1973b; McKey, 1974; Feeny, 1976; Rhoades, 1979, 1983; Krischik and Denno, 1983; Coley et al., 1985). This argument essentially postulates that genotypes that allocate more resources to resistance incur a cost (i.e., an incremental reduction in fit-

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ness), because of the diversion of resources away from other fitness-enhancing functions. At the same time, these genotypes presumably incur a benefit (an incremental increase in fitness), because of a reduction in herbivore damage. Simms and Rausher (1987) presented a formal model of this argument and showed that when 1) the benefit approaches an asymptote as the allocation to resistance increases and 2) the cost is a linear or concave-upward function of allocation to resistance, an intermediate, optimal level of resistance will exist, and stabilizing selection will act to maintain the population at that level.

Rausher and Simms (1989) were unable to detect such stabilizing selection on resistance in *Ipomoea purpurea* to any of four types of herbivorous insects. It thus seems that the mean level of resistance to these insects does not reflect a trade-off of the type described above. One explanation for these results is that one or more of the assumptions underlying the cost-benefit model of Simms and Rausher (1987) are not applicable to *I. purpurea*. A second goal of this study was to evaluate this explanation. In particular, we attempted to determine whether the assumption that resistance is costly is valid for *I. purpurea*.

#### MATERIALS AND METHODS

*Measuring Costs of Resistance.*—We examined two types of cost that may be associated with resistance to a particular herbivore species. One type of cost, which we term "allocation cost," results from diverting resources to resistance and away from other functions that contribute directly to survival or seed production. The second type of cost, which we designate "ecological cost," can occur if traits that confer resistance to one type of herbivore make a plant susceptible to other herbivores. Both types of cost can lead to maximum fitness at intermediate levels of resistance, and hence to stabilizing selection (see Appendix A for ecological costs).

Simms and Rausher (1987) described a method for detecting and measuring the allocation costs of resistance. This method involves growing individuals of known genetic relationships in two environments: 1)

with insect herbivores present at natural densities (control) and 2) with insect herbivores eliminated by spraying insecticides (spray). With this experimental design, allocation costs of resistance may be detected by calculating the additive genetic correlation or the covariance of fitness in the spray treatment with resistance (as measured in the control treatment). In addition, ecological costs of resistance may be detected by calculating the pairwise additive genetic correlations or covariances among levels of resistance to each herbivore in the treatment where herbivores occur at natural densities. Ecological costs are manifested by negative values of these correlations or covariances (see Appendix A).

*Experimental Organisms and Study Site.*—*I. purpurea* is a self-compatible, annual vine in the Convolvulaceae that is an important weed in corn, soybeans, and cotton (Oliver et al., 1976). Several insect species feed on *I. purpurea*. Specialists on the Convolvulaceae include adults and larvae of two species of tortoise beetle, *Deloyala guttata* and *Metriona bicolor* (Coleoptera: Chrysomelidae) (Moyhuddin, 1973; Barrows, 1979), which create numerous regular holes about 5 mm in diameter in the leaves, and adults of the sweet-potato flea beetle *Chaetocnema confinis* (Coleoptera: Alticinae) (Rosenthal, 1985), which cause distinctive tracks on the leaf surface. Several generalist folivores, including grasshoppers and lepidopteran larvae, feed on *I. purpurea* and create large irregular holes in the leaves. In addition, larvae of the generalist *Heliothis zea* (Lepidoptera: Noctuidae) feed on flowers and developing pods of *I. purpurea* (Roach, 1975).

The study site was an old agricultural field in Durham County, NC, that had last been planted in soybeans in 1983, but has since only been disked every spring. The natural population of *I. purpurea* in this field provided all of the plants involved in the experimental crosses described below.

*Field Protocol.*—Seeds were obtained from a replicated partial-diallel cross involving 30 parental plants. There were three diallels, each involving a different set of 10 plants. Each plant was crossed as a female with five of the nine other plants in the same

diallel and also crossed as a male with five of the plants in the diallel. Because two plants in one of the diallels failed to produce seed in time for planting, there were ultimately 5,680 seeds from 140 full-sib families (which were in turn distributed among 30 male half-sib and 28 female half-sib families). The design also produced one reciprocal full-sib family for each parent plant.

The seeds resulting from this mating design were scarified with sandpaper and planted during 7–10 July 1985 into Root-trainers® filled with potting mix. On 24–30 July, when the seedlings had reached the two-leaf stage, they were transplanted into the field, which had just been disked. Native vegetation was not weeded after transplanting. Plants were placed into a square grid of points 0.7 m apart and allowed to climb up 1-m tall bamboo stakes to mimic growth in corn fields and to facilitate identification of individuals. The experimental design consisted of two treatments in each of four spatial blocks. Five seedlings from each full-sib family were randomly allocated to each treatment-block combination, resulting in a total of 40 seedlings per family.

The treatments were: 1) insects present at natural densities (control) and 2) herbivorous insects reduced or eliminated by spraying the *I. purpurea* plants with carbaryl (Sevin XLR®) and *Bacillus thuringiensis* (Dipel®) at approximately two-week intervals (plants other than *I. purpurea* were not sprayed). Many pesticides affect plant growth, but Simms and Bucher (unpubl.) found no effect of treatment with carbaryl and *B. thuringiensis* on *I. purpurea* flowering or seed number in the greenhouse. We usually sprayed shortly after a rain, so the treatment should not have affected water status of the plants. Insecticides were sprayed in the late afternoon, when the flowers were closed and pollinators (primarily *Bombus* spp.) were not present. We observed no effect of spraying on pollinator activity.

In keeping with common usage (Painter, 1958; Beck, 1965; Carter, 1973; Horber, 1980), we defined resistance operationally in terms of the proportion of foliage or pods damaged by herbivores (see Rausher and Simms, 1989), with more-resistant plants incurring less damage than less-resistant

plants. The amount of damage suffered by an individual plant will be influenced both by its genotype and by environmental factors such as the local density of herbivores. If variation caused by environmental factors is large, then some individual plants of resistant genotypes may exhibit more damage than some individual plants of susceptible genotypes. Nevertheless, genetic variation for the proportion of damage indicates that there exists some unknown, genetically variable character that influences damage and, hence, resistance.

Damage by folivores was measured in late August, approximately one month after transplanting and before any leaves had senesced. This damage was evaluated by means of a leaf census in which we estimated the proportion of the total leaf area missing due to feeding by each type of folivore. A clear plastic grid with 0.59 cm<sup>2</sup> squares was placed over each leaf. The number of squares that covered the leaf provided an estimate of the predamage area. The margins of leaves were seldom damaged, so this method was probably quite accurate. The damaged leaf area was measured in the same manner and categorized by the type of insect that caused it. Two types of leaf damage, that caused by flea beetles and that by tortoise beetles, are distinctive and so could be measured separately. All other damage, caused mainly by orthopteran generalists, was categorized as “other generalist folivores.” Damaged and predamage areas were summed over all leaves on each plant to yield, respectively, total damage in each of the three categories and plant size. The proportion damage was then determined by dividing total area damaged by total leaf area. The proportion damage to pods was determined in similar fashion from a census of damaged and undamaged pods in late September. All proportions were arcsine(square root)-transformed prior to analysis (Neter and Wasserman, 1974 pp. 507–508).

In the control plots, damage to every plant was measured. A random subsample of full-sib families was also measured in the sprayed plots. Approximately two-fifths of the plants in the control treatment were sampled destructively for chemical analysis and so were

not included in the pod census. The analysis of damage by corn earworms is thus based on measurements of 1,529 plants. Offspring of all families were represented in approximately equal numbers.

Fitness was measured by collecting and counting all seeds produced by plants in both treatments, including those that died before producing seeds. Because this fitness measure incorporates the effects of mortality over most of the growing season, it is probably a reasonable estimate of true fitness in this annual plant (see Rausher and Simms [1989] for further details).

*Statistical Analysis.*—Analysis of variance was used to determine whether elimination of herbivores in the spray treatment increased seed production. In this and subsequent analyses, the block effect was treated as fixed, because the experimental population covered the entire area occupied by the native *I. purpurea* population at the field site. Thus, blocks were not random samples from some larger universe about which we wished to generalize from our results.

Analysis of damage and seed number followed standard procedures for analysis of variance for factorial designs (Searle, 1971 pp. 286–318). A significant sire main effect indicates additive genetic variation for the character being analyzed (Falconer, 1981 pp. 154–159). The expected mean square associated with the dam effect includes both additive genetic variance and reciprocal effects. We examined reciprocal effects in two ways. First, we tested the ratio of dam mean square over sire mean square; a ratio significantly greater than one indicates that the dam mean square exceeds that of the sire because of maternal-effect variance. Second, we performed a separate paired analysis of variance of reciprocal full-sib families to determine whether offspring from the same full-sib family differ depending upon the identity of their seed parent. In the absence of significant reciprocal effects, a significant dam main effect indicates the presence of a significant amount of heritable genetic variation. The expected mean square for the sire  $\times$  dam interaction includes mainly nonadditive genetic variation. Environmental variation appears in the block main effect and the error sums of squares.

Preliminary analyses indicated that interactions involving block were not significant. These effects were therefore dropped from the final analyses. All analyses of variance were performed with the general linear model (GLM) procedure of the SAS statistical package using type-III sums of squares (SAS Institute, 1982).

In the control treatment, all measures of seed production and damage were performed on the same individuals. We therefore calculated estimates of additive genetic variances and covariances from the sums of squares and cross products due to sire for those traits. Because most characters did not exhibit significant reciprocal effects, we repeated this calculation using variance and covariance estimates from the dam main effect. These matrices were generated using the MANOVA option of the GLM procedure of SAS (SAS Institute, 1982). Standard errors for these values were obtained using the method of Mode and Robinson (1959). The use of these standard errors for hypothesis-testing is not strictly valid, because the sampling distribution of genetic parameters is not known. However, as a rough indication of whether a covariance deviates from zero more than expected by chance, we asked whether the ratio of the covariance to its standard error exceeded the critical  $t$  value with the appropriate degrees of freedom.

Since damage was not assessed for most plants in the spray treatment, the method described above was not appropriate. We therefore used the CORR procedure of SAS (SAS Institute, 1982) to calculate the paternal- and maternal-family-mean covariances of damage in the control treatment (herbivores present) with the fitness component measured in the spray treatment (herbivores absent), which estimate the true additive genetic covariances (Yamada, 1962). The significance of these covariances was determined using standard procedures for product-moment correlations.

Because spraying may not have completely eliminated herbivory, the additive genetic covariance between fitness in the spray treatment and damage in the control may be reduced to the extent by which damage in the two treatments is correlated. This

complication can be taken into account by solving the following system of two equations with two unknowns:

$$\text{Cov}(d_{ci}, w_{si}') = \text{Cov}(d_{ci}, w_{si}) - k\text{Cov}(d_{ci}, d_{si}) \quad (1)$$

$$\text{Cov}(d_{ci}, w_{ci}) = \text{Cov}(d_{ci}, w_{si}) - k\text{Var}(d_{ci}). \quad (2)$$

In the above equations,  $w_{ci}$  and  $w_{si}$  are, respectively, the breeding values of an individual of genotype  $i$  for fitness with insects present (control) and with insects eliminated completely;  $w_{si}'$  is the breeding value of fitness in the spray treatment;  $d_{ci}$  and  $d_{si}$  are, respectively, the breeding values for damage in the control and spray treatment for an individual of genotype  $i$ ;  $c_i$  is the breeding value for cost of resistance; and  $k$  is the reduction in fitness per unit of herbivore damage (see Appendix B for derivation of these equations).  $\text{Cov}(d_{ci}, w_{ci})$  and  $\text{Cov}(d_{ci}, w_{si}')$  were calculated as described above. We estimated  $\text{Cov}(d_{ci}, d_{si})$  from the family-mean covariance between levels of damage in the two treatments, using the random subsample of families for which damage was measured in both treatments. The value calculated for the unknown  $\text{Cov}(d_{ci}, w_{si})$  is the estimated covariance between damage and fitness with herbivores completely eliminated. If resistance is costly, the sign of this covariance will be positive. It was not possible to obtain standard errors for these covariances using the method of Mode and Robinson (1959). Instead, averages were obtained by summing the values obtained from sire and dam components of covariance, and standard errors of these averages were calculated. Because the sire and dam covariance components are correlated, this standard error underestimates the true standard error.

## RESULTS

*Genetic Variation for Damage.*—In our experimental *I. purpurea* population, we detected additive genetic variation for the proportion of leaves damaged by generalist folivores and tortoise beetles (sire effects in Table 1). Neither the sire  $\times$  dam effects nor the reciprocal effects were significant for these classes of herbivores. Consequently, the significant dam effects also indicate the

TABLE 1. Analysis of variance on the proportion damage in control treatment. Reciprocal-1 is calculated by dividing the dam (nested within diallel) mean square by the sire (nested within diallel) mean square. Reciprocal-2 is the result of a separate ANOVA comparing reciprocal full-sib families. Mean squares used for  $F$  tests are shown in Table 2.

Source	d.f.	Corn earworms		Flea beetles		Tortoise beetles		Generalist folivores	
		SS	F	SS	F	SS	F	SS	F
Block	3	1.015	24.19***	237.11	521.35***	10.69	48.76***	33.63	189.53***
Diallel	2	0.080	1.70	0.29	1.10	0.70	1.60	0.10	0.36
Sire	27	0.460	1.22	3.66	0.89	3.20	1.62*	2.59	1.62*
Dam	25	0.540	1.55*	5.12	1.35	4.31	2.36***	2.48	1.68*
Sire $\times$ dam	85	1.318	1.11	17.33	1.34*	6.50	1.05	4.92	0.98
Error	2,611 <sup>a</sup>	19.382		395.68		190.72		154.45	
Reciprocal-1	25, 27		1.26		1.52		1.45		1.03
Reciprocal-2	13, 288 <sup>b</sup>		0.99		1.51		0.64		1.03

<sup>a</sup> For pod damage, error d.f. = 1,386.

<sup>b</sup> For pod damage, reciprocal-2 d.f. = 13, 256.

\*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .

TABLE 2. Analysis of variance on seed number. The reciprocal analyses are as described in Table 1. The MS column designates name for mean square for each effect; the *F* test column indicates mean squares used to form *F* ratio to test each effect.

Source	d.f.	MS	<i>F</i> test	Control treatment		Spray treatment	
				SS	<i>F</i>	SS	<i>F</i>
Block	3	MS6	MS6/MS1	26,975.1	4.76***	917,386.6	43.69***
Diallel	2	MS5	MS5/(MS4 + MS3 - MS2)	4,064.0	0.34	1,106.8	0.08
Sire	27	MS4	MS4/MS2 <sup>b</sup>	102,243.6	2.00**	140,970.2	0.75
Dam	25	MS3	MS3/MS2 <sup>b</sup>	105,732.7	2.24***	177,826.1	1.02
Sire × dam	85	MS2	MS2/MS1	180,554.6	1.12	485,818.1	0.82
Error	1,507/2,056 <sup>a</sup>	MS1	—	2,846,391.2	—	14,391,602.7	—
Reciprocal-1	25, 27	—	MS3/MS4	—	1.11	—	1.37
Reciprocal-2	13, 288	—	—	—	1.17	—	1.22

<sup>a</sup> d.f. = 1,507 for control treatment; d.f. = 2,056 for spray treatment.

<sup>b</sup> When the sire × dam effect was not significant, the dam and sire mean squares were retested over the pooled MS1 and MS2 to obtain the *F* values shown. \*\* *P* < 0.01; \*\*\* *P* < 0.001.

presence of genetic variation and suggest that most of it is additive. In addition, we found evidence of additive genetic variation for the proportion of pods damaged by corn earworms, although the sire effect was not significant. The tests for reciprocal effects were not significant, suggesting that maternal effects are minimal and that the significant dam effect indicates the presence of additive genetic variation for damage. The lack of a significant sire × dam interaction indicates an absence of detectable nonadditive genetic variation for pod damage. For flea beetles, genetic variation was present but was primarily nonadditive (significant sire × dam effects and nonsignificant sire and dam effects; Table 1).

*Effects of Herbivores on Seed Production.* — The number of seeds produced by plants in the spray treatment was 20% greater than in the control treatment (mean ± SE = 53.2 ± 1.8 vs. 44.5 ± 1.1;  $F_{[1, 3,841]} = 14.98$ ,  $P < 0.0001$ ). Spraying thus increased fitness, presumably by reducing the density of herbivores.

The difference between treatments in the amount of additive genetic variation for seed number indicates the extent to which herbivores are responsible for fitness differences among plant genotypes and, hence, indicates the potential for insects to cause evolutionary change in plant characters that affect fitness (Fisher, 1958; Roughgarden, 1979). In the control treatment, a significant sire main effect indicated the presence of additive genetic variance in seed number. Reciprocal effects were not significant, so the significant dam main effect also indicates heritable variation in seed number (Table 2). In contrast, in the spray treatment we detected no additive variation for fitness (Table 2). Moreover, all the genetic variance components for seed number were negative and/or zero (Table 2: *F* values ≤ 1.0), which suggests that additive genetic variance is truly absent and that our methods were not simply lacking sensitivity. Hence, reducing herbivory eliminated all measurable heritable variation in seed number.

*Selection on Genetic Variation for Damage.* — A significant additive genetic covariance between a character and fitness indicates selection on that character (Price, 1970; Emlen, 1984; Uyenoyama, 1986). For corn

TABLE 3. Selection on additive genetic variation for damage by herbivores on *Ipomoea purpurea* in the control treatment.  $\text{Cov}(d_{ci}, w_{ci})$  is the additive genetic covariance between fitness and damage in the control treatment. A negative covariance indicates selection for reduced damage; a positive covariance indicates selection for increased damage. Values in parentheses are standard errors. Table entries for covariance component indicate whether the covariance component was calculated from the sire or dam mean cross-product in a multivariate analysis of variance. The average values of  $\text{Cov}(d_{ci}, w_{ci})$  for sire and dam components are also shown. Statistical significance of each covariance was assessed using the probability ( $P$ ) corresponding to the  $t$  value estimated from the ratio of the covariance to its standard error.

Damage type	Covariance component	$\text{Cov}(d_{ci}, w_{ci})$	$P$
Corn earworm	sire	-0.423 (0.210)	0.05
	dam	-0.356 (0.188)	0.075
	average	-0.390	
Flea beetle	sire	0.045 (0.089)	ns
	dam	-0.122 (0.100)	ns
	average	-0.038	
Tortoise beetle	sire	0.134 (0.078)	ns
	dam	0.066 (0.085)	ns
	average	0.100	
Generalist folivore	sire	0.010 (0.076)	ns
	dam	-0.057 (0.080)	ns
	average	-0.024	

earworms in the control treatment, the magnitude of this covariance was large relative to its standard error (Table 3). The sire and dam covariance estimates agree in sign and magnitude, but only the former is statistically different from zero ( $P = 0.05$ ;  $P = 0.075$  for the dam component; Table 3). We interpret this result as indicating the presence of directional selection for less damage by these insects. In contrast, we detected no significant selection on genetic variation for damage caused by the other three types of herbivores (Table 3). These results are in agreement with those reported by Rausher and Simms (1989) for family-mean covariances.

*Costs of Resistance.* — We found little evidence of costs of resistance in *I. purpurea*. Except for damage by tortoise beetles, the half-sib family-mean covariances between each class of damage and seed number in the spray treatment did not differ significantly from zero (Table 4). Although the dam covariance for tortoise-beetle damage is statistically significant ( $P < 0.05$ ) when considered alone, it is not significant when multiple testing is taken into account (e.g., Timm, 1975 p. 402). Moreover, the sire covariance is opposite in sign. We conclude that the covariance of damage by tortoise beetles with seed number in the spray treatment probably does not differ from zero.

Correcting these covariances for incomplete removal of herbivores does not alter the conclusion that resistance to herbivory involves no detectable allocation costs (Table 5). The corrected covariances were small relative to their respective standard errors and thus were judged not to differ from zero. Moreover, the signs of all the average values but one were negative. This trend suggests that alleles that reduce damage, rather than being costly, may have pleiotropic effects that enhance fitness even when herbivores are absent. This trend did not hold for damage by tortoise beetles.

Our experimental population showed no evidence of trade-offs in resistance to different types of insects. Most of the pairwise additive genetic covariances between amounts of damage caused by different herbivores are positive (Table 6), and all but one had relatively large standard errors, indicating that they do not differ significantly from zero. The covariance between generalist folivores and corn earworms was large in comparison with its standard error, but it was positive. Thus, we found no evidence for ecological costs of resistance in *I. purpurea*.

## DISCUSSION

*Selection Imposed by Herbivores.* — Twenty-five years ago, Ehrlich and Raven

TABLE 4. Half-sib mean covariances between seed number in the spray treatment and damage in the control treatment. All values have been multiplied by 1,000. Table entries for covariance component indicate whether covariances were calculated using sire or dam half-sib family means. The average covariances over sire and dam family means are also shown. The significance of covariances was determined using usual procedures for product-moment correlations.

Covariance component	Type of damage			
	Corn earworms	Flea beetles	Tortoise beetles	Generalist folivores
Sire	-0.3	-86.2	-16.3	0.7
Dam	348.0	102.6	172.3*	-97.5
Average	174.1	8.2	78.0	-48.4

\*  $P < 0.05$ , but this value does not differ significantly from zero when significance levels are corrected for multiple comparisons.

(1964) proposed that plants and their insect herbivores have commonly undergone the process of coevolution. A central component of this process is the exertion of selection by herbivorous insects on plants. Such selection is commonly believed to have caused the evolution of plant traits that confer resistance to herbivory (Ehrlich and Raven, 1964; Feeny, 1976; Rhoades and Cates, 1976; Rausher, 1981; Futuyma, 1983). Selection by herbivores has never been demonstrated directly, however. Instead, its operation has been inferred from indirect evidence such as correlations between herbivore abundance and levels of resistance (e.g., Dolinger et al., 1973; Sturgeon, 1979) or the effects of particular resistance factors on herbivores (Gilbert, 1971, 1975; Erickson and Feeny, 1974; Berenbaum, 1978, 1983; Rausher, 1981). This inference is not necessarily valid, however, since resistance may be a pleiotropic effect of a trait that

performs other ecological functions (Nowacki et al., 1976; Jermy, 1976, 1984; Moran and Hamilton, 1980).

Our results provide the first direct demonstration of herbivorous insects imposing selection on a natural population of their host species. Such selection is indicated by the disappearance of additive genetic variation for fitness (seed number) when insects are removed with insecticides. Fisher's fundamental theorem of natural selection (Fisher, 1958) states that the rate of increase of mean fitness in a population is proportional to the amount of additive genetic variation for fitness. Populations lacking additive genetic variation in fitness cannot evolve. Our results thus suggest that *I. purpurea* would evolve in an environment with herbivores but not in an environment lacking herbivores. Because mean fitness will increase only if traits contributing to fitness evolve, our results also imply that the her-

TABLE 5. Estimates of additive genetic covariance between damage in control treatment and fitness in spray treatment, corrected for incomplete removal of herbivores by insecticide. Table entries for covariance component indicate whether the covariance was calculated from the sire or dam mean cross-product in a multivariate analysis of variance. The average values of sire and dam  $\text{Cov}(d_{si}, w_{si})$  values are also shown, with standard errors in parentheses (see text); the cost of herbivory ( $k$ ) is defined in Appendix B.

Damage type	Covariance component	$\text{Cov}(d_{si}, w_{si})$	$k$
Corn earworms	sire	-0.210	113.1
	dam	-0.171	279.3
	average	-0.191 (0.0274)	196.2
Flea beetles	sire	0.045	7,921.2
	dam	-0.122	-13,636.4
	average	-0.039 (0.118)	-2,857.6
Tortoise beetles	sire	0.036	-147.9
	dam	0.153	55.2
	average	0.094 (0.083)	-46.3
Generalist folivores	sire	-0.003	-20.7
	dam	-0.119	-132.5
	average	-0.061 (0.082)	-76.6

TABLE 6. Pairwise additive genetic covariances between amounts of damage caused by different herbivores in the control treatment. All values have been multiplied by 1,000. For each pair of table entries, the top numbers are based on the sire component of covariance, and the bottom numbers are based on dam components of covariance. Standard errors are in parentheses. Statistical significance of each covariance was assessed using the ratio of the covariance to its standard error and the corresponding probability for this *t* value.

Herbivore	Herbivore		
	Corn earworms	Flea beetles	Tortoise beetles
Flea beetles	-19.17 (10.4) 6.99 (12.8)	—	
Tortoise beetles	10.18 (11.20) 19.99 (12.96)	0.18 (3.99) 8.13 (4.99)	—
Generalist folivores	26.02 (11.18)* 3.02 (11.87)	-1.23 (3.72) -3.78 (4.71)	6.10 (4.22) 8.04 (4.74)

\*  $P < 0.05$ .

bivorous insects in this study imposed selection on some plant trait or traits.

The disappearance of genetic variation for fitness that was observed when insects were removed does not by itself indicate which traits of *I. purpurea* experienced selection imposed by herbivores. However, when coupled with the observed directional selection on damage by corn earworms in the control treatment (Rausher and Simms, 1989), this result does imply that insects imposed selection on characters contributing to resistance. Since the same families were used in both treatments, the additive genetic variation for resistance to corn earworms that was present in the control treatment was presumably also present in the spray treatment. The absence of additive variation for fitness in the spray treatment, though, implies that the additive covariance between fitness and resistance level was zero. In other words, removing insects eliminated selection on genetic variation influencing damage by corn earworms. Presumably this selection was due to corn earworms themselves. Since spraying eliminated all insects, however, we can not rule out the possibility that selection on corn-earworm resistance was imposed in part by other insects.

*Costs of Resistance.*—In a companion study (Rausher and Simms, 1989) we found no evidence for stabilizing selection favoring an intermediate level of resistance to any of the insects examined here. Those results are not consistent with the expectations of cost-benefit arguments that predict intermediate optima for levels of resistance (see Introduction for references; see Simms and

Rausher [1987] and Rausher and Simms [1989] for a formal model embodying these arguments and for a discussion of the assumptions underlying it). One possible explanation for this difference between our results and the predictions of the cost-benefit arguments is that one of the assumptions underlying the arguments is incorrect.

One assumption is that resistance is costly (i.e., that resistant genotypes have lower fitness than nonresistant [or less-resistant] genotypes in the absence of herbivores, due to diversion of more resources away from other fitness-enhancing traits by resistant genotypes). The results presented here suggest that allocation costs of this type do not exist for resistance to any of the insects examined. Moreover, ecological costs (caused by resistance to one herbivore being negatively correlated with resistance to another herbivore) also do not appear to exist. It thus seems that one of the reasons for Rausher and Simms' (1989) failure to detect the expected stabilizing selection is that costs that could produce such selection are absent.

Two caveats must be added to our conclusion that costs of resistance are absent in *Ipomoea purpurea*. One is that our experimental approach detects allocation costs only if resistance is constitutive. If resistance is inducible (e.g., Carroll and Hoffman, 1980; Karban and Carey, 1984; Faeth, 1986), costs might be a function of the level to which resistance was induced. Since induction would not occur in the spray treatment, fitness differences would not arise in that treatment, and we would therefore not

have detected costs. In subsequent experiments, however, we have been unable to detect any evidence that resistance is inducible (Rausher et al., unpubl.).

A second caveat is that our conclusion regarding lack of costs applies only to the genetic variation for resistance that existed in the native population at our study site at the time we sampled it. This variation represents underlying genetic variation in one or more as yet unidentified characters that influence susceptibility to herbivores. Our results do not preclude the possibility that costs might be associated with resistance characters that were genetically variable in the past or that may become genetically variable in the future.

The assumption that resistance can only be produced by mobilizing resources away from other vital functions dominates most current explanations about how types and degrees of defenses against herbivores evolve (Janzen, 1974; Feeny, 1976; Rhoades and Cates, 1976; Futuyma, 1983; Coley et al., 1985). However, very few studies have demonstrated the existence of any costs of resistance in natural plant populations (Fox, 1981). Of the studies examining costs of resistance (Hanover, 1966; Foulds and Grime, 1972; Dirzo and Harper, 1982b; Berenbaum et al., 1986), only Berenbaum et al. (1986) attempted specifically to measure allocation costs of resistance. Although they found large costs in *Pastinaca sativa*, the interpretation of these costs is obscured, because they were measured in a greenhouse. There is thus very little positive evidence for the existence of allocation costs associated with resistance to herbivores.

Our study suggests that, for at least one plant species, allocation costs do not exist. This result implies that a cost-benefit framework is inappropriate for understanding the evolution of resistance in *Ipomoea purpurea*. Whether this conclusion also applies to other plant species will depend on whether costs of resistance are generally absent in plants.

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#### APPENDIX A

Here, we demonstrate that stabilizing selection may favor intermediate levels of resistance to herbivores if levels of resistance to two types of insects are negatively correlated genetically. Let  $r_i$  be the expected value of the concentration of some secondary compound in the foliage of a plant of genotype  $i$ . Without loss of generality, we assume that  $0 < r_i < 1$ . Consider two insect species, one a generalist that is repelled by the compound and the other a specialist that is attracted by the compound. Suppose that the expected amount of feeding damage to a plant of genotype  $i$  by the specialist is given by

$$D_{si} = ar_i^b \quad (A1)$$

where  $a$  and  $b$  are constants defining the relationship between  $D_{si}$  and  $r_i$ , and that the expected amount of damage by the generalist is given by

$$D_{gi} = a(1 - r_i)^b \quad (A2)$$

Suppose further that genetic variation for  $r_i$ ,  $D_{si}$ , and  $D_{gi}$  is primarily additive, so that the expected values of these quantities are also the breeding values. Then because, over the range of  $r_i$ ,  $D_{si}$  is a monotonically increasing function of  $r_i$  while  $D_{gi}$  is a monotonically decreasing function, amounts of damage by the two insects are negatively correlated genetically.

The expected total amount of damage ( $D_{Ti}$ ) is simply the sum of the damage by each type of insect:

$$D_{Ti} = D_{si} + D_{gi} \\ = ar_i^b + a(1 - r_i)^b \quad (A3)$$

To find the secondary compound concentration,  $r_i$ , that yields a maximum or minimum amount of damage, one differentiates this equation with respect to  $r_i$ , sets the resulting expression equal to zero, and solves for  $r_i$ . This procedure yields

$$r_i = \frac{1}{2}.$$

The second derivative of (A3) is

$$\frac{d(D_{Ti})}{d(r_i)} = ab(b - 1) \\ [r_i^{(b-2)} + (1 - r_i)^{(b-2)}].$$

This expression is positive (corresponding to a minimum of damage at  $r_i = 1/2$  if and only if  $b > 1$ , i.e., if and only if (A1) and (A2) are concave-upward functions of  $r_i$ ). As long as this criterion is met, minimal total damage will occur at an intermediate level of  $r_i$  and, hence, at intermediate levels of damage by the two herbivores. As long as expected fitness is inversely proportional to total damage, this result implies that the genotype with maximal fitness corresponds to  $r_i = 1/2$  and, hence, that stabilizing selection will favor intermediate levels of resistance to the two insects.

This example shows that negative genetic correlations between amounts of damage by two insects can lead to stabilizing selection on levels of resistance. In general, negative genetic correlations of this type are a necessary, but not sufficient, condition for stabilizing selection to arise in this manner. Another necessary, but not sufficient, condition is that at least one of the relationships between  $r_i$  and damage must be concave upward, i.e., its second derivative with respect to  $r_i$  must be positive.

#### APPENDIX B

Here, we demonstrate how we calculated allocation costs of resistance when herbivores were not totally eliminated from the spray treatment in our experiment. We assume that dominance and epistatic variance are minimal and that, therefore, the expected value of a character for an individual is equal to its breeding value (Falconer, 1981 pp. 106-108). Let the expected value of fitness in the control treatment for an individual of genotype  $i$  be

$$w_{ci} = w_{\max} - c_i - d_{ci}k$$

where  $w_{\max}$  is the expected fitness of the least-resistant genotype in the absence of herbivores,  $c_i$  is the expected cost of resistance for an individual of genotype  $i$ ,  $d_{ci}$  is the expected amount of damage for an individual of genotype  $i$  in the control treatment, and  $k$  is the unit reduction in fitness per unit damage. If we could eliminate all herbivory by spraying, then the expected fitness in that treatment for an individual of genotype  $i$  would be

$$w_{si} = w_{\max} - c_i.$$

If herbivory is reduced but not totally eliminated by spraying, fitness in the spray treatment is

$$w_{si}' = w_{\max} - c_i - d_{si}k$$

where  $d_{si}$  is the expected value for damage in the spray treatment.

The additive genetic covariance of the mean seed number with the mean damage in the control plot is then

$$\text{Cov}(d_{ci}, w_{ci}) = \text{Cov}(d_{ci} [w_{\max} - c_i - d_{ci}k]) \\ = \text{Cov}(d_{ci} [w_{\max} - c_i]) - k\text{Var}(d_{ci}). \\ = \text{Cov}(d_{ci}, w_{si}) - k\text{Var}(d_{ci}). \quad (B1)$$

The additive genetic covariance of seed number in the sprayed plot with damage in the control plot is

$$\text{Cov}(d_{ci}, w_{si}') = \text{Cov}(d_{ci} [w_{\max} - c_i - d_{si}k]) \\ = \text{Cov}(d_{ci} [w_{\max} - c_i]) - k\text{Cov}(d_{ci}, d_{si}) \\ = \text{Cov}(d_{ci}, w_{si}) \\ - k\text{Cov}(d_{ci}, d_{si}). \quad (B2)$$

If there were no damage in the sprayed plot, then  $\text{Cov}(d_{ci}, d_{si})$  would equal zero and  $\text{Cov}(d_{ci}, w_{si}')$  would equal  $\text{Cov}(d_{ci}, w_{si})$ .

Four of the quantities in Equations (B1) and (B2) can be estimated from the sire and dam components of variance and covariance in a standard analysis of variance, as described in the Materials and Methods.

These quantities are the additive genetic covariances  $\text{Cov}(d_{ci}, w_{si})$ ,  $\text{Cov}(d_{ci}, w_{ci})$ , and  $\text{Cov}(d_{ci}, d_{si})$  and the additive variance  $\text{Var}(d_{ci})$ . Once these values have been estimated, Equations (B1) and (B2) constitute a system of two equations with two unknowns [ $k$  and  $\text{Cov}(d_{ci}, w_{si})$ ], which can be solved by standard methods. The value obtained for  $\text{Cov}(d_{ci}, w_{si})$  provides an estimate of the additive genetic covariance between damage in the control treatment and fitness with all herbivores removed.