Genetic Constraints and Selection Acting on Tolerance to Herbivory in the Common Morning Glory *Ipomoea purpurea*

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ABSTRACT: Tolerance to herbivory minimizes the effects of herbivory on plant fitness. In the presence of herbivores, maximal levels of tolerance may be expected to evolve. In many plant species, however, tolerance is found at an intermediate level. Tolerance may be prevented from evolving to a maximal level by genetic constraints or stabilizing selection. We report on a field study of *Ipomoea purpurea*, the common morning glory, in which we measured three types of costs that may be associated with tolerance and the pattern of selection acting on tolerance to two types of herbivore damage: apical meristem damage and folivory. We used genetic correlations to test for the presence of three types of costs: a trade-off between tolerance and fitness in the absence of herbivore damage, a trade-off between tolerance and resistance, and genetic covariances among tolerance to different types of damage. We found no evidence that tolerance to apical meristem damage or tolerance to folivory was correlated with resistance, although these two types of tolerance were positively correlated with one another. Tolerance to both types of damage involved costs of lower fitness in the absence of herbivory. Selection acting on tolerance to either type of herbivory was not detected at natural levels of herbivory. Selection is expected to act against tolerance at reduced levels of herbivory and favor tolerance at elevated levels of herbivory. In addition, significant correlational selection gradients indicate that the pattern of selection acting on tolerance depends on values of resistance. Although we found no evidence for stabilizing selection, fluctuating selection resulting from fluctuating herbivore loads may be responsible for maintaining tolerance at an intermediate level.

Keywords: herbivory, tolerance, resistance, costs, *Ipomoea purpurea*.

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The examination of plant evolutionary responses to attack by herbivores has focused almost entirely on avoidance of herbivory through mechanisms of resistance. Resistance mechanisms provide a selective advantage to a plant by reducing herbivory, which, with few exceptions (e.g. Paige and Whitham 1987; Lennartsson et al. 1997), is detrimental to plant fitness (reviewed in Marquis 1992). However, resistance is only one of two strategies that plants may employ to reduce the potential impacts of herbivory. A second is the evolution of tolerance to herbivory. Tolerance, which reflects the degree to which damage or feeding by herbivores fails to reduce plant fitness, provides a selective advantage by minimizing the detrimental effects of herbivory on fitness (Painter 1958; Rosenthal and Kotenan 1994). Although plant breeders have discussed tolerance as an alternative to plant resistance for at least 40 yr (Painter 1958), the role of tolerance in the evolutionary response of plants to the selective pressure imposed by herbivores has only recently begun to be considered (Mashinski and Whitham 1989; Simms and Triplett 1994; Fineblum and Rausher 1995; Rosenthal and Welter 1995; Juenger and Bergelson 1997; Mauricio et al. 1997; Stowe 1998).

Several studies have established that genetic variation for tolerance to herbivory and disease infestation is present in natural plant populations (Simms and Triplett 1994; Fineblum and Rausher 1995; Mauricio et al. 1997), yet little is known about the pattern of selection acting on this variation. Absent any other selective forces, the benefits of tolerance would be expected to result in directional selection favoring maximal levels of tolerance. The persistence of genetic variation for tolerance indicates that such maximal levels have not been attained and that therefore some additional, opposing selective force—a cost of tolerance—is likely operating. Moreover, the persistence of intermediate levels of tolerance suggests that the interaction of costs and benefits of tolerance may be generating stabilizing selection on tolerance, much as is expected for

resistance (Rhoades 1983; Simms and Rausher 1987; Abrahamson and Weis 1997; Mauricio and Rausher 1997).

Although the operation of stabilizing selection is an attractive hypothesis for explaining the common observation of intermediate levels of tolerance, little experimental evidence is available to support this hypothesis. Costs in the form of negative genetic correlations between tolerance and resistance (ecological costs) have been predicted by several authors (van der Meijden et al. 1988; Herms and Mattson 1992; Belsky et al. 1993) and have been reported for two systems (Fineblum and Rausher 1995; Stowe 1998). However, models of the joint evolution of tolerance and resistance predict that such costs should engender selection that is either directional or disruptive (van der Meijden et al. 1988; Fineblum and Rausher 1995) and, thus, do not seem to be able to account for intermediate levels of tolerance. Published models incorporating physiological costs of tolerance, reflected in negative genetic correlations between tolerance and fitness in the absence of herbivores (Abrahamson and Weis 1997), similarly indicate that only directional selection is expected to act on tolerance and, thus, suggest that these types of costs are also unlikely to account for intermediate levels of tolerance. As we show in appendix A, however, this prediction depends critically on the assumption that costs increase linearly with increasing tolerance. When this assumption is relaxed, physiological costs can yield stabilizing selection on tolerance. Nevertheless, because only one previous investigation has reported the existence of physiological costs of tolerance (Simms and Triplett 1994), it is not clear how generally they contribute to maintaining intermediate levels of tolerance.

The primary objective of this investigation was to address two issues: whether tolerance to herbivory in the common morning glory *Ipomoea purpurea* (L.) Roth is costly, and whether such costs generate stabilizing selection favoring intermediate levels of tolerance. We first present experimental evidence indicating that tolerance to folivores, and probably insects that damage apical meristem tissue, is genetically variable in this species. We then analyze whether costs are associated with tolerance and what form of selection is expected, given the nature of the costs. Last, we document the pattern of selection acting on tolerance and resistance to these two types of herbivores to determine whether the pattern is expected to favor an intermediate level of tolerance.

Material and Methods

Experimental System

Ipomoea purpurea (L.) Roth (Convolvulaceae), the common morning glory, is a self-compatible, annual vine commonly found in agricultural fields and disturbed areas throughout the southeastern United States. In North Carolina, plants emerge between May and August and die with the first frost, usually in October or November. Flowers are generally produced within 6 wk of emergence. Plants can bear multiple flowers daily, and individual flowers last only 1 d. Plants flower continuously until they begin senescing or are killed by frost. Dehiscent fruits, each of which typically contains from five to six seeds, mature within approximately 4 wk of fertilization. Mature fruits typically remain on plants for longer than 1 wk before dehiscing.

Morning glory apical meristems, leaves, and seed capsules are damaged by several different herbivores (Rausher and Simms 1989). Apical meristem damage (AMD) is caused by flea beetles (Coleoptera: Alticinae), grasshoppers, and lepidopteran larvae. Leaves are consumed by three specialists on the family Convolvulaceae (two species of tortoise beetles, *Deloyla guttata* and *Charidatilla* [=*Metriona*] *bicolor* [Coleoptera: Chrysomelidae], and the sweet potato flea beetle *Chaetocnema confinis* [Coleoptera: Alticinae]), as well as generalist lepidopteran larvae, grasshoppers, and an unidentified weevil (Coleoptera: Curculionidae). Seed capsules and leaves are consumed by *Helicoverpa zea*, the corn earworm, and other lepidopteran larvae.

Experimental Methods

Seeds for experimental plants were obtained from a half-sib breeding design in which 35 pollen parents were each crossed to three randomly selected seed parents. Separate seed parents were selected for each pollen parent. All parental plants were selected from a collection of 39 tenth-generation single-seed-descent inbred lines. These inbred lines were derived from seeds initially collected from a field in Durham County, North Carolina, in the fall of 1989. Flowers of seed parents were emasculated the evening before flower opening to prevent self-pollination and were hand pollinated the following morning. All crosses were made from January through March of 1996, using plants growing in a greenhouse.

Because estimates of tolerance cannot be made on an individual (see below), inbred lines, rather than outbred individuals, were used as parental plants in order to minimize within-family variance and thus increase our power to detect genetic variation for tolerance while avoiding a prohibitively large number of experimental plants. Although using inbred lines minimized the amount of genetic variation within paternal half-sib families, it also resulted in several potential biases or departures from standard methods. First, we had only 39 inbred lines, and our experimental population was composed of 35 half-sib

families; thus, lines were used as both pollen and seed parents, and the 105 maternal full-sib families are represented by only 39 maternal genotypes. Our design is therefore actually a very incomplete diallel, without selfs, that was analyzed as a half-sib design (Lynch and Walsh 1998). Although the biases of analyzing this design in this way are not clear, we suspect it should result in a conservative test for the presence of additive genetic variation because paternal half-sib families will be more closely related than they would be if each of the seed parents were taken from a different line. In particular, repeated sampling of maternal parents should not affect the variance component associated with the dam effect but should reduce the variance component of the sire effect, thus reducing the estimated F statistic for the sire effect and making the test conservative. Second, estimates of genetic parameters made using inbred lines as parents are unequal to those made using randomly outcrossed individuals as parents (Cockerham and Weir 1984). However, the use of inbred lines should not invalidate using ANOVA in testing qualitatively for the presence of genetic variation and in determining the sign of genetic covariances, as we do in this study. Last, since inbreeding is expected to cause little change in gene frequencies (Cockerham 1963), allele frequencies among the inbred lines are assumed to be representative of the allele frequencies in the base population.

On June 10, 1996, 40 seeds from each of the paternal half-sib families (approximately 13 seeds from each maternal full-sib family, 1,400 total seeds) were planted into a previously plowed and disked field in Durham County, North Carolina. Seeds were scarified the day before planting and planted into three spatial blocks. Within each block, seeds were planted 70 cm apart within rows, with 100 cm between rows. Weeds were removed from the field weekly during the first 4 wk after planting to minimize competition and assure that experimental plants became well established. Each plant was allowed to twine up a 1-m-tall bamboo stake. Staking mimics *I. purpurea* growth in agricultural fields and allows easy identification of individual plants. All plants emerged within 7 d after planting. Plants began flowering on July 20, and seed capsules began maturing on August 22.

Plants were censused for naturally occurring AMD every 2 d between 7 and 14 d after planting and again 21 d after planting. In order to assure that enough plants of each genotype were damaged to give reliable estimates of tolerance, we cut the meristems of randomly selected plants that had not received natural damage by 19 d after planting. This artificial damage was imposed on enough plants to ensure that approximately 50% of the individuals within each full-sib family experienced natural or artificial damage. The number of individuals within families receiving artificial damage ranged between zero and 14.

Total leaf area on each plant was measured between July 14 and 19 by counting the number of expanded leaves and measuring the length of each leaf. Leaf length was converted to leaf area using the relationship area = $0.67 \times \text{length}^{2.088}$ ($R^2 = 0.97$, from Mojonnier 1996). At the time leaf area was measured, the leaf area missing as a result of feeding by folivores was also recorded. These measurements were made by overlaying each leaf with a clear plastic 0.10-cm^2 grid and recording the number of squares where the leaf was missing (Simms and Rausher 1989). Leaf area missing was converted to proportion damaged by dividing missing leaf area by total leaf area. Total leaf area was also used as a measure of plant size.

All seed capsules were counted and inspected for herbivore damage. A capsule was considered damaged if a hole had been bored into the outer capsule wall. The number of capsules damaged was converted to proportion damaged by dividing the number damaged by the total number of capsules produced by each plant. Usually, if a capsule received any damage, all of the seeds were consumed.

Seeds were collected from each plant during 10 rounds of harvesting lasting from the time the first capsules matured until November 5, when a hard frost killed all plants. Viable seeds were counted, and the number of seeds produced by each plant was used as an estimate of fitness. Only individuals that survived to produce at least one seed were included in the analysis. This resulted in excluding 251 individuals from the analysis because they either failed to emerge or died within 1 wk of emerging; 25 individuals survived past this first week but failed to produce seed. Likelihood ratio tests revealed no significant effect of paternal half-sib family on the likelihood of individuals to survive ($P > .18$) or produce seed ($P > .16$).

Data Analysis: Additive Genetic Variation for Tolerance and Resistance

To test for the presence of additive genetic variation for tolerance, the GLM procedure of the SAS statistical software package (1989) was used to conduct ANOVA (for AMD and capsule consumption) and ANCOVA (for folivory). In each of these analyses, the response variable was relative fitness. For these analyses only, relative fitness was log transformed to achieve a normal distribution of residuals, and plant size was included as a covariate in the analyses for folivory to reduce the error variance. Transformation is appropriate for this analysis because we are not estimating any genetic parameters but want simply to make the most rigorous possible inference about whether genetic variation is present or absent (Mitchell-Olds and Shaw 1987). Relative fitness was calculated by dividing all fitness values by overall mean fitness. Pollen parent (sire)

and seed parent (dam, nested within sire) were treated as random effects. The "test" option under the "random" statement in the GLM procedure was used to obtain proper mean squares for significance testing of random effects. Unless noted otherwise, *F*-tests for the sire × herbivory term were conducted using the dam (nested within sire) × herbivory term as the denominator. Preliminary analysis on untransformed data revealed that fitness was not significantly affected by interactions between different types of herbivory. Therefore, tests for each type of herbivore damage were conducted without the other types of herbivory included in the analysis. The term of interest with regard to tolerance was the interaction between paternal half-sib family and herbivore damage; a significant interaction indicates that herbivory did not affect fitness of all paternal half-sib families equally and is evidence of additive genetic variation for tolerance.

AMD was treated as a class variable. Before analyzing data on AMD, a paired *t*-test, in which paternal half-sib families were paired, was performed to determine if plants responded in a similar manner to natural and artificial AMD. This test was conducted on the residuals of both fitness and plant size after the effects of block had been removed. In addition to testing for genetic variation for tolerance to AMD with fitness as the response variable, a similar analysis was conducted with plant size as the response variable. Relative size was calculated by dividing all individual size measurements by the mean plant size, and for only this analysis it was square root transformed.

To test for additive genetic variation for resistance to AMD, likelihood ratio tests were used to compare the number of individuals with naturally damaged apical meristems to the number of individuals with undamaged meristems. Plants with experimentally damaged meristems were counted as undamaged. ANOVA was used to test for the presence of additive genetic variation for resistance to folivory and capsule damage. For these analyses, proportion herbivory was the response variable, and sire and dam were treated as random effects. These response variables were arcsine–square root transformed before analysis to achieve a normal distribution of residuals for the reason described above.

Estimates of Family Means of Tolerance and Resistance

For individual families, estimates of tolerance to AMD were calculated by subtracting the mean relative fitness of individuals within each half-sib family without AMD from the mean relative fitness of individuals within each half-sib family with AMD. This measure of tolerance equals 0 for complete tolerance (fitness of damaged plants is equal to the fitness of undamaged plants) and is negative for incomplete tolerance. Estimates of tolerance to AMD based on plant size were made in a similar manner. For folivory, which unlike AMD can be treated as a continuous variable, estimates of tolerance for individual families were made by performing a regression of fitness (untransformed) onto proportion damage separately for each paternal half-sib family (Simms and Triplett 1994; Mauricio et al. 1997). This approach is equivalent to defining tolerance as the norm of reaction of plant fitness along an environmental gradient of increasing herbivory (Abrahamson and Weis 1997). Since ANOVA revealed no evidence for nonlinear effects of damage on fitness or evidence that the nonlinear effects of damage differed among families (see below), regressions used to estimate tolerance for individual families included only linear terms. Reported values for estimates of tolerance are from analyses conducted on untransformed values of residual relative fitness after the effects of block had been removed. No covariates were included in the models. For each half-sib family, resistance to AMD was defined as 1 − the proportion of plants damaged, and resistance to foliage damage or seed predation was defined as 1 − the mean proportion of naturally occurring damage (Simms and Triplett 1994; Fineblum and Rausher 1995).

Costs of Tolerance

Three types of costs were assessed: a trade-off between tolerance and resistance for each type of herbivory, a trade-off between tolerance to different types of herbivory, and a trade-off between tolerance and fitness in the absence of herbivory. The existence of costs was initially tested by determining whether paternal half-sib family-mean correlations between these traits differ from 0, which would imply the genetic covariance differs from 0. Genetic correlations calculated in this manner are potentially biased because of a within-family component included in each of the variance and covariance components of the correlation (Arnold 1981). Nevertheless, these approximations should approach the true correlation as the number of individuals within each genetic family increases, and simulation studies have found little bias with families comprised of 20 or more individuals (Roff and Preziosi 1994). In this study, half-sib family sizes ranged from 27 to 38 individuals. Tests for nonlinear relationships between tolerance and fitness in the absence of damage were made by assessing the significance of the quadratic term in a quadratic regression of fitness in the absence of herbivory on tolerance. These analyses were conducted with untransformed values of relative fitness.

Since not all individuals within any family experienced AMD, fitness in the absence of damage was estimated directly. In contrast, all plants received some foliage and capsule damage, so we did not have direct estimates of

fitness in the absence of damage. Therefore, the *Y*-intercept value from the regression of fitness on damage was used as an estimate of fitness in the absence of herbivory.

Using the same set of data to estimate both the slope (tolerance) and the *Y*-intercept value results in an artifactual covariance and, hence, elevated genetic correlation between tolerance to folivory and fitness in the absence of damage. This artifactual covariance is equal to

$$E_j\left[\sigma_j^2\left(\frac{-\overline{X}_j}{\sum (X_{ij} - \overline{X}_j)^2}\right)\right],$$

where E_j denotes the mean taken across the j families, σ_j^2 is the error variance from the regression of fitness on damage for each family j, $\overline{X}_j$ is the mean damage of individuals within each family j, and X_{ij} is the damage experienced by each individual i within each family j (Mauricio et al. 1997). This artifactual covariance was subtracted from the calculated covariances to give an unbiased estimate of the relationship. Since we are only interested in testing for a negative relationship between tolerance and fitness of undamaged plants, a one-tailed t statistic was used to calculate confidence intervals.

A similar artifactual covariance exists in estimating the relationship between tolerance to AMD and fitness of undamaged plants. This artifact is equal to

$$E_j\left(\frac{-\sigma_{0j}^2}{n_j}\right),$$

where, once again, E_j denotes the mean across families, and σ_{0j}^2 is the variance of undamaged individuals within family j divided by n_j, the number of undamaged individuals within family j (see app. B for derivation). Once again, this artifact was subtracted from the calculated covariance to obtain an unbiased estimate of the relationship. Standard errors of these corrected covariances were made by jackknifing paternal half-sib family estimates (Cohen 1969; Gray and Schucany 1972), and a one-tailed t statistic was used to calculate a confidence interval.

Selection Acting on Tolerance

The partial regression analysis described by Rausher (1992) was used to characterize the type and magnitude of selection acting on tolerance and resistance to AMD and folivory. This analysis is similar to that described by Lande and Arnold (1983) except that it is based on genotypic or breeding values rather than the phenotypic values. Since tolerance cannot be assessed on a single individual, the Lande and Arnold procedure is inappropriate for this trait. All values of tolerance, resistance, and fitness used in the selection analyses are family means. Before conducting these analyses, all predictor variables (tolerance to folivory, resistance to folivory, tolerance to AMD, resistance to AMD) were standardized to a mean of 0 and a standard deviation equal to 1. The response variable was the residual of relative fitness after the effects of block had been removed. Directional selection gradients were estimated from a regression model that included only linear terms for each of the four traits, while stabilizing/disruptive and bivariate nonlinear selection gradients (correlational selection) were estimated from the full model, including linear, quadratic, and interaction terms (Lande and Arnold 1983; Brodie et al. 1995). Contour plots of selection surfaces were drawn using the "G3grid" and "Gcontour" procedures in SAS (1989) with a smoothing parameter equal to 0.3. The cubic spline has been shown to produce a more accurate representation of selective surfaces than parametric graphic procedures (Schluter 1988; Schluter and Nychka 1994).

Because AMD was in part experimentally imposed, the plants in this experiment experienced levels of AMD two to three times higher than under natural conditions. Because of the possibility that elevated levels of AMD altered the pattern of selection acting on resistance to AMD and/or tolerance, we conducted the selection analysis a second time using an adjusted measure of relative fitness. Adjusted relative fitness for each half-sib family was calculated as

$$\rho D \times W_D + (1 - \rho D) \times W_0,$$

where ρD is equal to the proportion of individuals within each family that had meristems damaged by insects, W_D is equal to the fitness of damaged plants, and W_0 is equal to the fitness of undamaged plants. This adjustment assigns a mean fitness to each family that represents the expected mean fitness with natural levels of meristem damage.

Results

Genetic Variation for Tolerance

ANCOVA revealed a significant interaction between sire and proportion leaf area eaten, indicating significant additive genetic variation for tolerance to folivory (table 1; fig. 1). Because the interaction between plant size and sire and the interaction between plant size and dam in this analysis were also significant, including size in the model may have not only reduced the error term but also altered estimates of tolerance (Sokal and Rohlf 1981, p. 510). To

test this possibility, two reduced analyses were also run: without plant size as a covariate, and with plant size as a covariate but without the size by proportion damage interaction. The relative rank of tolerance of the families in these three analyses was then compared by computing a Spearman rank correlation. These correlations were all highly significant and positive (the lowest of the three pairwise correlation coefficients was 0.803, $P < .0001$), indicating that the primary effect of including the covariate was to reduce error variance and not to alter estimates of tolerance.

ANOVA conducted with untransformed values of relative fitness revealed no evidence for nonlinear effects of herbivory on fitness (proportion damage^2 term from the ANOVAs was not significant: $F = 0.99$, df = 1, 796, $P > .30$) or evidence that nonlinear effects of herbivory differed among families (the sire × proportion damage^2 term from the ANOVA was not significant: $F = 0.90$, df = 34, 796, $P > .60$). The absence of nonlinear effects indicates that using a liner function to describe tolerance is appropriate for this study. Including plant size in these analyses as a covariate did not change the significance of the results.

Preliminary analysis found no difference between the effects of artificial AMD and natural AMD on either plant size or fitness ($T = 0.62$, $P > .6$, $T = 0.37$, $P > .3$, respectively). Natural and artificial AMD were thus combined in subsequent analyses. ANOVA revealed no significant sire × AMD interaction, indicating no significant genetic variation for tolerance to AMD (table 2) when fitness was used as the response variable. Moreover, this analysis indicated that AMD did not have a significant overall effect on plant fitness (table 2). By contrast, a similar analysis using plant size rather than plant fitness as the response variable revealed significant genetic variation for tolerance to AMD; that is, families differed in the magnitude of the effect of AMD on plant size (table 2). Furthermore, tolerance to AMD assayed by fitness and tolerance to AMD assayed by plant size were highly correlated among the half-sib families ($r = 0.807$, $P < .0001$), suggesting that our failure to detect variation in tolerance assayed by fitness may result from insufficient statistical power rather than true absence of variation. This inference is further strengthened by the existence of a cost of tolerance to AMD and the significant genetic covariance between tolerance to folivory and tolerance to AMD (see below). No additive

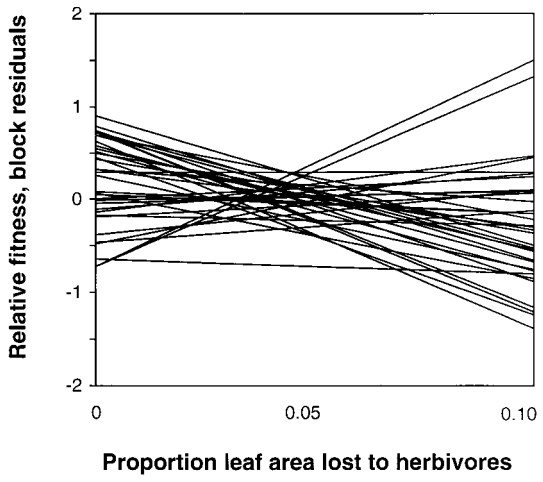

Figure 1: Relationship between relative fitness and proportion of leaf area lost to herbivores for the 35 paternal half-sib families. Slopes of the lines represent tolerance.

Table 1: ANCOVA for tolerance to folivory

Source of variation	df	Type III SS	*F* value	Pr > *F*
Block	2	6.565	6.59	.0015
Proportion damage	1	.340	.68	.4091
Size	1	247.594	496.71	.0001
Sire	34	49.571	1.81[a]	.0158
Dam(sire)	69	58.511	1.70	.0005
Proportion damage × size	1	1.159	2.33	.1277
Size × sire	34	44.923	2.65	.0001
Size × dam(sire)	69	78.846	2.29	.0001
Proportion damage × sire	34	27.263	1.61[b]	.0162
Proportion damage × dam(sire)	69	38.165	1.11	.2599
Error	797	397.280		

Note: Relative fitness, the response variable, was log transformed. Significant interaction between proportion damage and sire indicates significant additive genetic variation for tolerance to folivory. Sire and dam nested within sire (dam(sire)) are considered random effects; all other sources of variation are considered fixed.

[a] *F* values are Satterthwaite approximations, df = 34, 80.45.

[b] Proportion damage × dam (sire) term was not significant, and therefore the proportion damage × sire term was tested over error mean square.

Table 2: ANOVA for detecting tolerance to AMD

		Relative fitness			Relative size		
Source of variation	df	Type III SS	*F* value	Pr > *F*	Type III SS	*F* value	Pr > *F*
Block	2	215.054	98.86	.0001	28.889	119.92	.0001
AMD	1	.001	.12	.9757	.099	.82	.3651
Sire	34	65.665	1.51[a]	.0719	4.986	.86[a]	.6761
Dam(sire)	70	87.204	1.04	.3894	12.012	1.42	.0150
AMD × sire	34	46.457	1.15	.2564[b]	6.253	1.53	.0284[b]
AMD × dam(sire)	69	73.153	.90	.7030	8.363	1.01	.4658
Error	913	1,096.554			109.127		

Note: Response variables are relative fitness and relative plant size. Interactions between AMD and sire test for additive genetic variation for tolerance. Sire and dam nested within sire (dam(sire)) are considered random effects; all other sources of variation are considered fixed. AMD = apical meristem damage.

[a] *F* values are Satterthwaite approximations, df = 34, 75.35.

[b] AMD × dam(sire) term was not significant, and therefore the AMD × sire term was tested over error mean square.

genetic variation was detected for either linear (table 3) or nonlinear effects of capsule damage on fitness (the sire × proportion damage^2 term from the ANOVA was not significant, $F = 0.48$, df = 34, 695, $P > .95$), regardless of whether covariates were included in the analysis.

Genetic Variation for Resistance

ANOVA indicated significant additive genetic variation for resistance to folivory (table 4). The mean proportion of leaf area eaten ranged from 0.018 to 0.063 among half-sib families, with an overall mean of 0.034. Likelihood ratio tests revealed nearly significant genetic variation ($P = .098$) for resistance to AMD. The proportion of individuals within each paternal half-sib family that incurred natural AMD ranged from 0.06 to 0.39, with an overall mean of 0.23. Finally, there was no indication of genetic variation for resistance to capsule damage (table 4). The proportion of capsules damaged ranged from 0.10 to 0.25 for half-sib families, with an overall mean of 0.17.

Costs of Tolerance

Tolerance to folivory is costly, as revealed by a negative genetic correlation between tolerance and relative fitness in the absence of damage (fig. 2*A*), where fitness in the absence of folivore damage was estimated using the *Y*-intercept of a regression of fitness on damage. The corrected covariance between tolerance to folivory (slope) and estimated fitness of undamaged plants (intercept) was equal to −0.381, with a 95% confidence interval of 0.384. This confidence interval just barely includes 0, with the corrected covariance significantly different from 0 at $P = .052$. There was no evidence for a nonlinear relationship between tolerance and fitness in the absence of herbivory (the tolerance^2 term from a regression of fitness in the absence of herbivory on tolerance was not significant, $F = 1.63$, df = 1, 32, $P > .20$). Tolerance to AMD, with tolerance measured by effects of damage on plant fitness, was also found to be costly (fig. 3*A*), with a corrected covariance between tolerance and the relative fitness of undamaged plants equal to −0.0258, which was significantly <0 ($P < .01$).

Genetic Correlations Involving Tolerance and Resistance

Genetic correlations between tolerance and resistance to either folivory or AMD (table 5) were low and not significantly different from 0. Furthermore, the cross correlations between tolerance to folivory and resistance to AMD and between tolerance to AMD and resistance to folivory were also not significant (table 5). By contrast, tolerance to folivory and tolerance to AMD assayed by fitness were positively correlated with one another (fig. 4*A*), as were resistance to folivory and resistance to AMD (fig. 4*B*).

Selection Acting on Tolerance and Resistance

At the levels of herbivory experienced, there was no evidence for either directional or stabilizing (disruptive) selection acting on tolerance to folivory (table 6; fig. 2*B*); however, the pattern of selection acting on tolerance is expected to vary with the average level of herbivory (app. A). To estimate how selection would act on tolerance at levels of folivory higher and lower than those observed during this experiment, we used the regression equations of fitness on proportion leaf area eaten calculated for each half-sib family (W = fitness in the absence of damage + tolerance × herbivory) to estimate the mean relative fitness of each paternal half-sib family with either no folivory or two times the mean level of measured folivory. With

Table 3: ANOVA for detecting tolerance to proportion seed capsules consumed

Source of variation	df	Type III SS	*F* value	Pr > *F*
Block	2	207.649	101.13	.0001
Proportion damage	1	12.563	12.24	.0005
Sire	34	50.008	1.43[a]	.0533
Dam(sire)	70	95.485	1.35	.0349
Proportion damage × sire	34	43.490	1.25	.1597[b]
Proportion damage × dam(sire)	69	76.097	1.07	.3229
Error	913	936.339		

Note: Relative fitness is the response variable. Interaction between proportion damage and sire test for additive genetic variation for tolerance. Sire and dam nested within sire (dam(sire)) are considered random effects, all other sources of variation are considered fixed effects.

[a] *F* values are Satterthwaite approximations df = 34,75.93.

[b] Proportion damage × dam(sire) term was not significant, and therefore the proportion damage × sire term was tested over error mean square.

no folivory (fig. 2*A*), there was a negative relationship between tolerance and fitness, reflecting the cost of tolerance and indicating that directional selection is expected to favor reduced levels of tolerance. By contrast, fitness estimates at elevated levels of herbivory indicate that directional selection is expected to favor higher levels of tolerance (fig. 2*C*). Although the elevated level of damage at which we estimated fitness was well within the range of damage experienced by individual plants, it is possible that at this level of damage the functions describing tolerance may no longer be linear. The magnitude, and perhaps even the shape, of the estimated selection gradient at the elevated level of damage should thus be viewed with some caution.

Selection acting on tolerance to AMD is also dependent on levels of AMD damage. There was no evidence for selection acting on tolerance to AMD either when half of all individuals within each half-sib family experienced meristem damage (fig. 3*B*) or at natural levels of meristem damage (covariance between adjusted relative fitness and tolerance was not significantly different from 0, $P > .50$). At lower levels of damage, selection is expected to act against tolerance to AMD, as demonstrated by the negative correlation between tolerance to AMD and fitness of undamaged plants. We estimated that, when fewer than 0.17 of all individuals within each family experience meristem damage, the covariance between fitness and tolerance to AMD was <0 ($P < .05$), and thus negative directional selection would be expected to act against tolerance to AMD. In making this estimate we made two assumptions: that the covariance between tolerance to AMD and fitness increases linearly from 0% to 50% damage, and that the confidence interval calculated for the covariance between tolerance and the fitness of undamaged plants was an appropriate confidence interval to use for all levels of damage. Although this threshold level of damage was below the mean level experienced this year, it was within the range of damage experienced by half-sib families and thus suggests that at least in some years selection may act against tolerance to AMD. We detected no evidence for directional selection acting on resistance to either folivory or AMD and no stabilizing/disruptive selection acting on any of the traits (table 6).

In contrast to the general lack of significant selection gradients acting directly on tolerance and resistance, several of the bivariate selection gradients were significantly different from 0 (table 6), indicating that selection was acting to favor combinations of traits (Phillips and Arnold 1989; Brodie et al. 1995). In particular, selection favored genotypes with high values of resistance and either high or low values of tolerance to folivory (table 6; fig. 5*A*) and genotypes with low values of tolerance and resistance to AMD (table 6; fig. 5*B*). In contrast, the significant negative selection gradient acting on correlations between tolerance to one type of damage and resistance to the other type of damage (table 6; fig. 5*C*, 5*D*) indicates selection favored genotypes for which these traits were negatively correlated. Moreover, the selective surfaces clearly indicate that any quadratic selection gradient acting on the traits is likely to be disruptive rather than stabilizing. Selection analyses conducted on fitness at natural levels of meristem damage and at the experimentally elevated levels of meristem damage were similar (table 6), indicating that these selection gradients are not a result of biases associated with our operational definition of tolerance to AMD. The one exception was selection acting on tolerance to AMD, which is heavily biased by the artifactual negative covariance between estimates of tolerance and fitness in the absence of damage.

Discussion

Costs of Tolerance

At least three different types of cost are potentially associated with tolerance to herbivory: allocation, or physiological, costs measured as a trade-off between tolerance and fitness in the absence of damage; trade-offs between tolerance and resistance; and genetic correlations among tolerances to different types of damage (van der Meijden et al. 1988; Herms and Mattson 1992; Belsky et al. 1993; Simms and Triplett 1994; Fineblum and Rausher 1995; Abrahamson and Weis 1997; Stowe 1998). Our results indicate that, in *Ipomoea purpurea*, two of these three types of costs exist. Allocation costs, reflected in negative correlations between tolerance and fitness in the absence of damage, were detected for tolerance to both folivory and AMD. In addition, we detected a positive genetic correlation between the two types of tolerance. This correlation may function as a cost, and hence as a constraint on the evolution of tolerance, if the balance between the benefits and physiological costs favors very different levels of tolerance to folivory and to AMD, that is, if selection favors a simultaneous increase in one type of tolerance and a decrease in the other type (Lande 1979; Lande and Arnold 1983).

The absence of any detectable trade-offs between tolerance and resistance is surprising for two reasons. First, a trade-off between these traits has been predicted on theoretical grounds because of the potential redundancy in the benefits of tolerance and resistance (van der Meijden et al. 1988; Herms and Mattson 1992; Fineblum and Rausher 1995; Abrahamson and Weis 1997; Stowe 1998). In particular, highly tolerant genotypes are expected to experience no benefit from increased resistance, while highly resistant genotypes are expected to experience no benefit from increased tolerance. If there are physiological or other costs associated with both tolerance and resistance, then selection will act against genotypes that are both tolerant and resistant, compared to genotypes that are either tolerant or resistant but not both. In addition, selection is expected to act against genotypes that lack both tolerance and resistance. This pattern of selection would act to create a negative genetic correlation between tolerance and resistance. As our selection analysis showed, however, we found no evidence for selection favoring genotypes that were highly tolerant or highly resistant but not both. Rather, our analysis indicated that for folivory, selection favored genotypes that had a high level of both

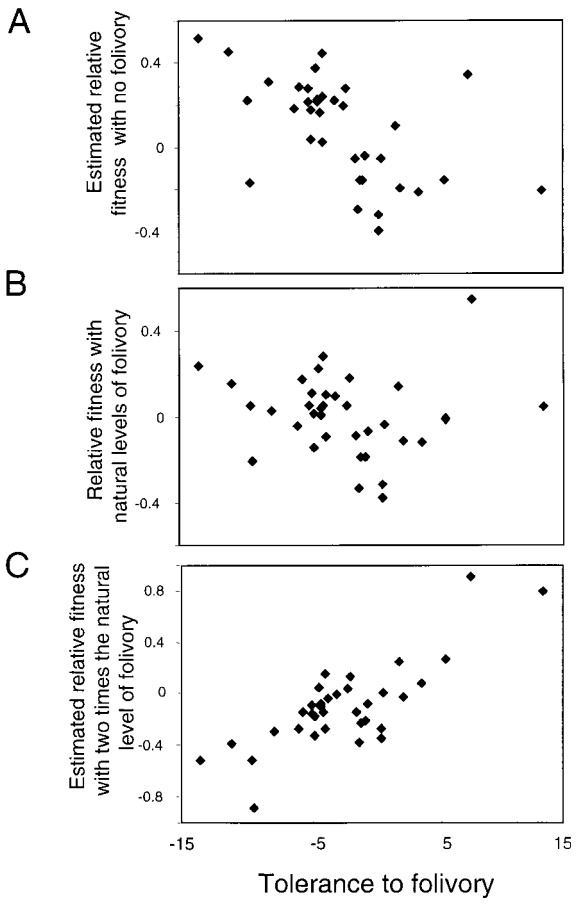

Figure 2: Paternal half-sib family means of relative fitness versus tolerance to folivory at no herbivory (*A*), measured levels of herbivory (*B*), and elevated herbivory (*C*). Relative fitness values are residuals after removing block effects. Tolerance was defined as the slope of a regression of fitness on folivory; more positive values indicate higher tolerance.

Table 4: ANOVA for resistance to two types of herbivory: folivory and capsule consumption

		Folivory			Capsule consumption		
Source of variation	df	Type III SS	*F* value	Pr > *F*	Type III SS	*F* value	Pr > *F*
Block	2	.510	17.94	.0001	2.058	14.79	.0001
Sire	34	.803	1.66[a]	.0340	2.724	.99[a]	.5050
Dam(sire)	70	.991	.99	.4916	5.717	1.17	.1613
Error	1,008	14.343			70.620		

Note: Sire and dam nested within sire (dam(sire)) are treated as random variables.

[a] *F*-tests are Satterthwaite approximations. df = 34, 74.90 for folivory, and df = 34, 74.16 for capsule consumption.

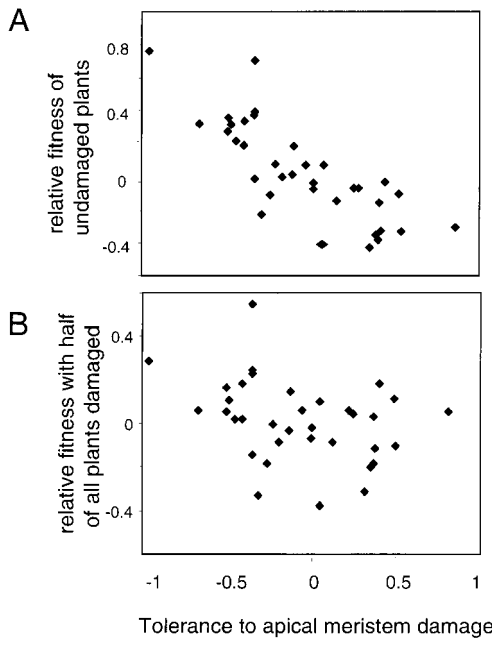

Figure 3: Paternal half-sib family means of relative fitness versus tolerance to AMD at natural levels of damage (*A*); half of all plants within each half-sib family damaged (*B*). Relative fitness values are residuals after removing block effects. Tolerance was defined as the fitness of damaged plants minus the fitness of undamaged plants; more positive values indicate higher tolerance.

tolerance and resistance, perhaps because even for extreme genotypes, neither tolerance nor resistance is complete. In contrast, for AMD we detected a selective optimum of low tolerance and low resistance, suggesting that costs associated with both tolerance and resistance were too high to be offset by the benefits of these traits. Thus, absence of the type of selection that would generate a negative correlation between tolerance and resistance can explain our failure to detect such a correlation.

A second reason for expecting a trade-off between tolerance and resistance, at least to AMD, is that such a trade-off was reported previously in *I. purpurea* (Fineblum and Rausher 1995). Our results may differ from those of that experiment because we assayed both tolerance and resistance under common field conditions, whereas Fineblum and Rausher assayed tolerance in the greenhouse. A correlation between resistance and tolerance expressed in the greenhouse may not accurately reflect the analogous correlation expressed in the field because the novel environment of the greenhouse may have resulted in the expression of genetic correlations that are not expressed in the field (e.g., Service and Rose 1985). Alternatively, our results may differ from those of Fineblum and Rausher because the experimental population we used was sampled from a different field population with a different correlational structure.

Costs and Expected Patterns of Selection

Both of the types of costs we detected have the potential to generate patterns of selection that would act to maintain tolerance to folivory and to AMD at intermediate levels. For example, as shown in appendix A, the interaction of benefits and physiological costs may generate stabilizing selection on tolerance, provided that costs are a nonlinear function of tolerance. Similarly, if, as described above, the positive correlation between tolerance to folivory and tolerance to AMD functions as a constraint, net selection on both tolerances may be greatly reduced or eliminated as direct selection on each is opposed by indirect selection acting through the correlation. The correlation may thus render variation in each type of tolerance effectively neutral, eliminating any selection pressure to move either type of tolerance away from intermediate levels.

Despite this potential, the form and magnitude of the costs detected seem unlikely to generate these patterns of selection. For folivory, physiological costs appear to be a linear function of tolerance, while the relationship between damage and fitness is also not detectably nonlinear. This linearity is expected to yield only directional selection on tolerance, not stabilizing selection (Abrahamson and Weis 1997; app. A). Similarly, the genetic correlation between tolerances is only about 0.5, probably not strong enough to significantly constrain response to directional selection (Via and Lande 1985). Our expectation of the pattern of selection on tolerances, based on the form and magnitude of the costs, is therefore that it should be neither stabilizing nor absent, that is, that there should be detectable directional selection on tolerance to both folivory and AMD.

Observed Patterns of Selection and Maintenance of Intermediate Levels of Tolerance

In contrast to this expectation, we detected no selection acting on either tolerance to folivory or tolerance to AMD. There are three possible interpretations of this result relevant to understanding the maintenance of intermediate levels of tolerance: weak stabilizing selection was acting on tolerance, but we were not able to detect it; weak directional selection was acting on tolerance, but we were not able to detect it; or variation in tolerance was effectively neutral under the conditions of our experiment. We favor the second and third explanations for two reasons. First, while we cannot statistically rule out the possibility of stabilizing selection (the confidence intervals for the quadratic selection gradients overlap 0, although just barely so), the point estimates of the quadratic selection gradients are

positive for both types of tolerance. Second, several of the correlational selection gradients were statistically significant, and the selective surfaces corresponding to these gradients all indicate that selection on both tolerance and resistance is disruptive. This pattern is consistent with the apparent pattern of weak disruptive selection indicated by the quadratic gradients. It thus seems unlikely that stabilizing selection is acting on tolerance to either folivory or AMD. Rather, any nonlinear selection acting is likely to be weakly disruptive, which should not contribute to maintenance of intermediate levels of tolerance.

We believe it more likely that for both types of tolerance the detected allocation costs approximately or exactly balanced the benefits of tolerance to either produce very weak and undetectable directional selection (second explanation) or render variation in tolerance effectively neutral (third explanation). As our analyses indicated, however, such a balance depends critically on the average amount of damage plants experience and, hence, on herbivore abundance. In particular, our analyses indicate that, if herbivores had been less abundant, strong directional selection would likely have favored reduced tolerance to folivory; whereas if herbivores had been more abundant, strong directional selection would likely have favored increased tolerance to folivory. Given the observation that most herbivorous insect populations fluctuate over time and space (reviewed in Denno and McClure 1983; Strong et al. 1984; Cappuccino and Price 1995), it seems unlikely that this critical abundance would occur every year, or even most years, at most localities. Consequently, it also seems unlikely that in most times and at most places there would be an exact balancing of costs and benefits of tolerance; rather, it seems more likely that selection on tolerance may fluctuate and that such fluctuation may contribute to maintaining tolerance to folivory at intermediate levels.

The situation is somewhat different for tolerance to AMD, for which we suspect the experimentally elevated levels of herbivory are as high or higher than even extreme natural levels. If this suspicion is true, then herbivore fluctuations are seldom likely to result in damage levels higher than that in our experiment. Since in our experiment benefits just balanced costs, we would expect in most years that costs would exceed benefits, resulting in directional selection to reduce tolerance to AMD. If this interpretation is correct, some mechanism other than fluctuating selection acting directly on tolerance may be necessary to explain why tolerance to AMD is found at intermediate lev-

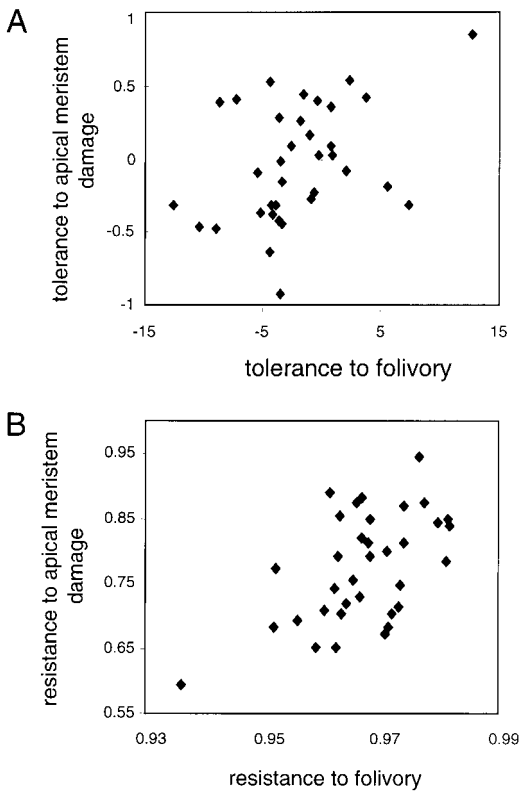

Figure 4: Relationship between paternal half-sib family values for tolerance to AMD and folivory (*A*) and resistance to AMD and folivory (*B*). Values of tolerance to AMD are based on fitness. The effects of plant size were not removed when calculating the values for tolerance to folivory. The correlations were significant: $r = 0.48$, $P < .02$, for tolerance; $r = 0.51$, $P < .01$, for resistance.

Table 5: Genetic variance and covariance matrix

	Tolerance to folivory	Tolerance to AMD	Resistance to folivory	Resistance to AMD
Tolerance to folivory	1.0	.418[a]	.065	.068
Tolerance to AMD		1.0	−.121	−.151
Resistance to folivory			1.0	.454[a]
Resistance to AMD				1.0

Note: All trait values were standardized to $\bar{X} = 0$ and SD = 1.

[a] Correlations between traits were significantly different from 0 at $P < .02$ for tolerance values and $P < .01$ for resistance values.

Table 6: ANOVAs showing the linear, quadratic, and correlational selection gradients acting on tolerance to folivory, resistance to folivory, tolerance to AMD, and resistance to AMD

		Relative fitness				Adjusted relative fitness			
Source of variation	df	Estimate	Type III SS	*F*	*P*	Estimate	Type III SS	*F*	*P*
Tolerance to folivory	1	.010	.003	.09	.77	−.123	.427	3.38	.076
Tolerance to AMD	1	−.070	.136	4.16	.05	.200	1.114	8.82	.006
Resistance to folivory	1	−.033	.027	.82	.37	−.524	.065	.52	.478
Resistance to AMD	1	−.055	.073	2.23	.15	−.233	.013	.10	.751
Tolerance to folivory^2	1	.001	.039	1.72	.20	.001	.038	1.67	.211
Tolerance to AMD^2	1	.054	.069	2.19	.15	.061	.085	3.77	.167
Resistance to folivory^2	1	−.079	.050	2.99	.10	−.077	.047	2.06	.067
Resistance to AMD^2	1	−.014	.003	.13	.73	−.017	.005	.21	.651
Tolerance to folivory × resistance to folivory	1	.173	.117	5.11	.035	.172	.116	5.08	.036
Tolerance to folivory × tolerance to AMD	1	.014	.002	.10	.757	.006	.001	.02	.890
Tolerance to folivory × resistance to AMD	1	−.182	.205	8.96	.007	−.186	.215	9.43	.006
Resistance to folivory × tolerance to AMD	1	−.186	.151	6.62	.018	−.180	.141	6.19	.022
Resistance to folivory × resistance to AMD	1	.112	.056	2.45	.134	.114	.058	2.54	.127
Tolerance to AMD × resistance to AMD	1	.100	.108	4.74	.042	−.282	.856	37.50	.001
Error[a]	20		.456				.456		

Note: AMD = apical meristem damage. Analyses were conducted using both relative fitness and relative fitness adjusted to natural levels of AMD as response variables. Paternal half-sib family values for tolerance and resistance were standardized to $\bar{X} = 0$ and variance equal to 1 before analysis. Reported linear terms are from a model with no quadratic or interaction terms included. Tolerance to AMD is based on fitness.

[a] Error sums of squares for linear terms; df = 0.977, 30 for relative fitness, and df = 3.78, 30 for adjusted relative fitness.

els. One possibility is that tolerance to AMD is correlated with tolerance to other environmental stresses, such as shading or competition (Coughenour 1985; Aarssen 1995). Under this hypothesis, the pattern of selection will be environmentally dependent: selection will act indirectly to increase tolerance when plants experience stress and to decrease tolerance when plants grow under relatively stress-free conditions. One caveat to bear in mind is that a direct analysis of the effects of damage on plant fitness revealed no significant genetic variation for tolerance to AMD; whereas a similar analysis of the effects of damage on plant size revealed substantial genetic variation for tolerance to AMD. Nevertheless, because genetic and phenotypic correlations between size and fitness were highly significant, and a correlation between tolerance to folivory and tolerance to AMD (measured using plant fitness) was highly significant, the preponderance of evidence indicates that tolerance to AMD was genetically variable in our experimental population.

Conclusion

In the absence of costs, tolerance to herbivory should be beneficial whenever plants experience herbivore damage, and tolerance would be expected to evolve to maximal levels. However, results from this and other studies (Simms and Triplett 1994; Fineblum and Rausher 1995; Mauricio et al. 1997; Stowe 1998) indicate that substantial genetic variation often exists for tolerance and that, therefore, tolerance levels are less than maximal. One explanation for these results is that tolerance is costly and that the interaction between costs and benefits generates stabilizing selection that maintains intermediate levels of tolerance. Although in *I. purpurea* tolerance to folivory and tolerance to AMD incur substantial allocation costs, we could detect no evidence of stabilizing selection acting on either of these characters; if anything, selection acting on tolerance in the year of our study appeared to be weakly disruptive. Moreover, at least for tolerance to folivory, the linearity of costs and benefits of tolerance is consistent with the observation

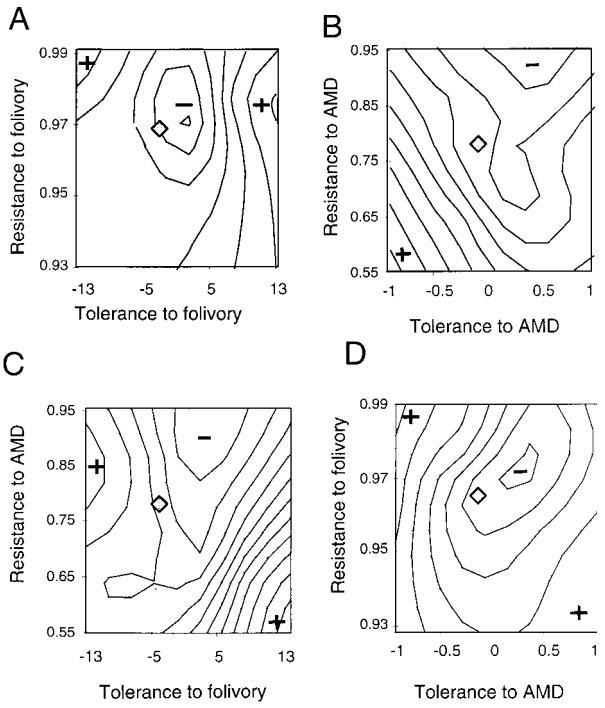

Figure 5: Selective surfaces acting on tolerance and resistance to folivory (*A*), tolerance and resistance to AMD (*B*), tolerance to folivory and resistance to AMD (*C*), and tolerance to AMD and resistance to folivory (*D*). Population mean is indicated by the diamond. The range of values of tolerance to folivory was from −12.9 to 12.4; resistance to folivory, from 0.935 to 0.981; tolerance to AMD, from −0.947 to 0.829; and resistance to AMD, from 0.589 to 0.941.

of absence of stabilizing selection. However, because our analyses indicated that strong directional selection on tolerance would occur at levels of herbivory different from those we observed, we suspect spatial and temporal fluctuations in damage, mediated by spatial and temporal fluctuation in herbivore abundance, are largely responsible for maintaining tolerance to folivory at intermediate levels. This potential role of fluctuating selection suggests that further attempts to understand the evolution of tolerance in *I. purpurea*, and perhaps in other species, should examine the nature and consequences of temporal and spatial variation in herbivory.

Acknowledgments

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APPENDIX A

Model for the Evolution of Tolerance

In this appendix we present and analyze a model for the evolution of tolerance in order to clarify the conditions under which costs are expected to give rise to directional, stabilizing, and disruptive selection. Previous models of the evolution of tolerance that assume no trade-off between tolerance and resistance, but assume that tolerance itself is costly (Abrahamson and Weis 1997; Mauricio et al. 1997), predict that selection on tolerance is always directional, with selection favoring either an increase or decrease in tolerance, depending on levels of herbivory. This result, however, is crucially dependent on the assumption that costs of tolerance are linearly related to level of tolerance. To demonstrate this contention, we present the following model in which the assumption of linear costs is relaxed.

The expected fitness, W_{Hi}, of an individual i, with tolerance T, under herbivory level H is given by

$$W_{Hi} = W_m - C(T_i) - HB + HT_i, \qquad \text{(A1)}$$

where W_m is the expected fitness of an individual with no tolerance in the absence of herbivory, C is a function of T_i describing the cost of tolerance, and B is the fitness detriment per unit of herbivory. The term HB represents the detrimental effects of herbivory, while the term HT_i represents the benefit of tolerance. If overcompensation is not possible then $0 < T_i < B$.

Using this equation to generate a fitness surface as a function of both herbivory level and tolerance level, we consider the pattern of selection corresponding to three general cases: costs increase linearly with increasing tolerance, that is, $C = kT_i$, where k is a constant >0; costs increase more than linearly with tolerance (the relationship between cost and tolerance is concave upward), that is, $C = aT_i^m$, where a and m are constants >0 and >1, respectively; and costs increase less than linearly with tolerance (the relationship between cost and tolerance is convex upward), that is, $C = aT_i^n$, where $a > 0$ and $0 < n < 1$.

For a given cost function, the pattern of selection on tolerance is given by the selection gradient, β, which is

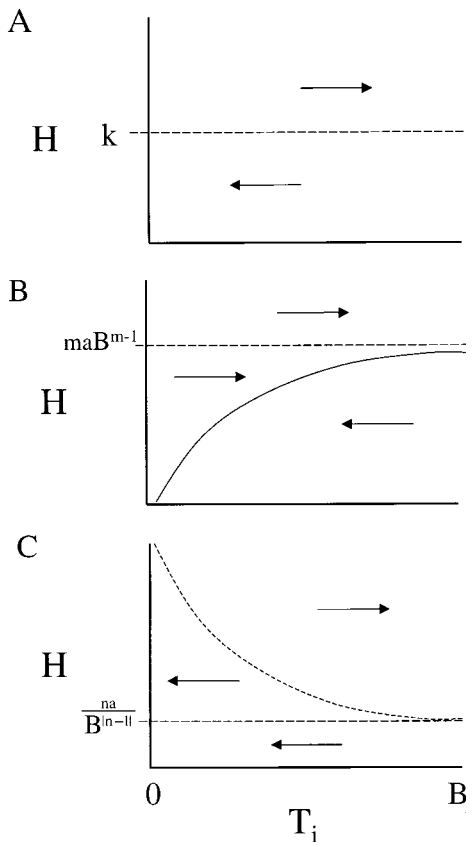

Figure A1: Phase diagrams showing the expected direction of selection acting on tolerance (T) as a function of herbivory (H) and tolerance when costs increase linearly with tolerance ($c = kT_i$, k is a constant >1) (*A*); costs increase more than linearly with tolerance ($c = aT_i^m$, a and m are constants >0 and >1, respectively) (*B*); and costs increase less than linearly with tolerance ($c = aT_i^n$, a is a constant >0, and n is a constant $0 < n < 1$) (*C*). Arrows indicate the expected direction of change in tolerance due to selection, panel *B* represents the maximum possible value of tolerance. Solid line in panel *B* represents a line of stable equilibria.

proportional to the derivative of fitness with respect to tolerance:

$$\beta \propto \frac{dW}{dT_i} = H - \frac{dC}{dT_i}. \tag{A2}$$

Assuming that H is equal for all genotypes, equation (A2) is independent of the shape of the function used to describe tolerance. Therefore, although tolerance has generally been described as a linear function between fitness and damage (Simms and Triplett 1994; Mauricio et al. 1997; Stowe 1998), the results of this model are robust to departures from a linear definition.

Costs Increase Linearly with Tolerance. In this case, $dC/dT_i = k$ is a constant, and substituting this into equation (A2) produces

$$\frac{dW}{dT_i} = H - k. \tag{A3}$$

Because k is constant, the pattern of selection depends only on herbivore load, H. When $H = k$, the level of tolerance is at an unstable equilibrium. By contrast, when $H < k$, dW/dT_i is negative and selection will act to reduce levels of tolerance, while when $H > k$, dW/dT_i is positive and selection will tend to increase tolerance (fig. A1*A*). This result is simply a restatement of the analyses presented by Abrahamson and Weis (1997).

Costs Increase More than Linearly with Tolerance. In this case, $dC/dT_i = maT_i^{(m-1)}$, and substituting this into equation (A2) yields

$$\frac{dW}{dT_i} = H - maT_i^{(m-1)}. \tag{A4}$$

This equation produces a phase diagram in the variables H and T_i that is portrayed in figure A1*B*. When herbivore levels are low ($H < maB^{(m-1)}$), an intermediate level of tolerance, corresponding to a line of stable equilibria, is maintained by stabilizing selection. Only if levels of herbivory are high, so that $H > maB^{(m-1)}$, will selection consistently favor complete tolerance. Thus, over a broad range of herbivory levels, a cost of tolerance of this form will contribute to maintenance of intermediate levels of tolerance.

Costs Increase Less than Linearly with Tolerance. In this case, $dC/dT_i = naT_i^{(n-1)}$, and substituting this into equation (A2) yields

$$\frac{dW}{dT_i} = H - naT_i^{(n-1)} = H - \frac{na}{T_i^{|n-1|}}. \quad \text{(A5)}$$

This equation produces the phase diagram portrayed in figure A1*C*. When herbivore levels remain high ($H > na/B^{|n-1|}$), selection on tolerance is largely disruptive, leading to complete tolerance or lack thereof. When herbivore levels remain low ($H < na/B^{|n-1|}$), directional selection will act to eliminate tolerance. From this analysis we infer that nonlinear costs of tolerance can interact with benefits to produce stabilizing selection on tolerance.

APPENDIX B

Statistical Correction for Calculating the Relationship between Tolerance to AMD and the Fitness of Undamaged Plants

In this appendix we show that calculating the relationship between tolerance to AMD and fitness of undamaged individuals using the same set of data results in a biased estimate of the covariance. The source of this bias and a correction factor used to remove it are presented. The method is of general use whenever one is interested in calculating the relationship between the difference of the mean of two character states and the mean value of one of the character states used in calculating that difference.

A negative covariance between tolerance and fitness of undamaged plants is a cost of tolerance. This covariance is equal to

$$E_j[(\Delta_j - \delta)(M_{0j} - \mu_0)], \quad \text{(B1)}$$

where Δ_j is the true tolerance of family j, equal to $M_{1j} - M_{0j}$; δ is the true value of tolerance averaged across all j families; M_{0j} is the true mean fitness of undamaged plants within family j; M_{1j} is the mean fitness of damaged plants within family j; and μ_0 is the average of the M_{0j} across all j families.

However, equation (B1) cannot be calculated directly since experimental data provides estimates $\hat{\Delta}_j$ and $\hat{M}_{0j}$ rather than true values of tolerance and the fitness of undamaged plants, respectively. Given estimates rather than true values, the covariance between the estimates of tolerance and fitness of undamaged individuals is equivalent to

$$E_j E_{k|j}[(\hat{\Delta}_j - \delta)(\hat{M}_{0j} - \mu_0)], \quad \text{(B2)}$$

where E_j denotes the expectation over all families in the population, and $E_{k|j}$ denotes an average over all k individuals within each j family. Equation (B2) can be rewritten as

$$E_j E_{k|j}[(\hat{\Delta}_j - \Delta_j + \Delta_j - \delta)(\hat{M}_{0j} - M_{0j} + M_{0j} - \mu_0)]. \quad \text{(B3)}$$

Expanding equation (B3) and using the fact that $(\hat{\Delta}_j - \Delta_j)(M_{0j} - \mu_0)$ and $(\Delta_j - \delta)(\hat{M}_{0j} - M_{0j})$ average to 0 within each family, equation (B3) becomes

$$E_j E_{k|j}[(\hat{\Delta}_j - \Delta_j)(\hat{M}_{0j} - M_{0j})] + E_j[(\Delta_j - \delta)(M_{0j} - \mu_0)]. \quad \text{(B4)}$$

The second term is equivalent to equation (B1). Therefore, the first term is the source of the artifact and must be removed in order to obtain an unbiased estimate of the covariance. The first term reduces to

$$E_j E_{k|j}[(\hat{M}_{1j} - M_{1j})(\hat{M}_{0j} - M_{0j}) - (\hat{M}_{0j} - M_{0j})^2]. \quad \text{(B5)}$$

The second term of this expression averages to 0 within each family, therefore equation (B5) can be rewritten as

$$E_j E_{k|j}[-(\hat{M}_{0j} - M_{0j})^2]. \quad \text{(B6)}$$

This term can be calculated by calculating the variance of undamaged individuals within each family, dividing that variance by the number of individuals used to calculated that variance, then taking the mean of these values across all j families. Calculating this mean and subtracting it from the calculated covariance between tolerance and fitness of undamaged plants results in an unbiased estimate of covariance. Jackknifing or bootstrapping techniques can be used to obtain a standard error around this unbiased estimate.

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