

VARIATION IN THE DEFENSE STRATEGIES OF PLANTS: ARE RESISTANCE AND TOLERANCE MUTUALLY EXCLUSIVE?

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Abstract. Plants can employ two general strategies to defend themselves against herbivory: they can either reduce the amount of damage they experience (resistance), or they can tolerate herbivore damage. Theoretical considerations suggest that, in many cases, tolerance and resistance are redundant strategies, and may therefore be mutually exclusive adaptations. In this investigation of natural populations of the annual plant *Arabidopsis thaliana* we examine whether the pattern of selection acting on resistance and tolerance favors the evolution of one defense strategy, or the other, but not both. We found that the joint pattern of selection acting on tolerance and two resistance traits, trichome density and total glucosinolate concentration, indicated that there were not alternate peaks in the fitness landscape favoring either resistance or tolerance. Rather, selection favored the retention of both tolerance and resistance. One reason for the absence of mutually exclusive alternative resistance/tolerance strategies is the absence of a negative genetic correlation between resistance and tolerance. An unexpected result is the detection of disruptive selection acting on tolerance, which seems to result from a nonlinear relationship between tolerance and its costs.

Key words: *Arabidopsis thaliana*; compensation; costs; defense; disruptive selection; herbivory; plant–herbivore interactions; resistance; selection; tolerance.

INTRODUCTION

One of the goals of evolutionary ecology is to understand the forces generating and maintaining variation among individuals, populations, and species in characters of ecological importance. A crucial aspect of understanding character variation is to be able to explain patterns of covariation among suites of characters, as well as the implications of covariation for evolution of adaptations. For example, a negative correlation between two characters, both within and across species, is often taken to indicate the existence of trade-offs between those characters (Cheverud 1984, Tilman 1990). Such trade-offs, commonly believed to reflect a negative genetic covariance between the characters, can lead to the evolution of alternate “strategies” or character combinations in different populations or species (Fineblum and Rausher 1995). Genetic constraint is thus a primary explanation for adaptive variation, and has been documented in a variety of organisms (Rose and Charlesworth 1981, Reznick 1983, Beren-

baum et al. 1986). It has also been recognized, however, that character covariation may be the result of patterns of selection that favor certain character combinations but not others (Hartl and Clark 1989:54), though there has been little experimental examination of this hypothesis. Such a pattern of selection could lead to variation in character combinations among populations and species. In this paper, we examine this hypothesis as it applies to defense strategies in plants.

Plants possess an impressive diversity of characters generally thought to defend them against attack by such natural enemies as herbivores and pathogens. For the past 35 yr, most studies directed toward understanding the evolution of plant defenses have focused on examining traits that reduce the amount of damage a plant experiences (Fraenkel 1959, Ehrlich and Raven 1964, Berenbaum et al. 1986). Such “resistance” characters should be beneficial to plants, since it is believed that natural enemies generally reduce the fitness of the plants on which they feed (Morrow and LaMarche 1978, Rausher and Feeny 1980, Marquis 1984).

Several workers, however, have questioned the assumption that herbivore damage always reduces fitness in natural plant populations (Owen and Wiegert 1976, Owen 1980, McNaughton 1983, Paige and Whitham 1987, Mauricio et al. 1993). These studies suggest that some plants have reduced the detrimental effects of

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herbivory by evolving an alternative strategy: tolerance of herbivore damage. Tolerance, or compensation, is the ability of a plant to sustain a fixed amount of herbivore damage without a corresponding reduction in fitness (Painter 1958, Crawley 1983, McNaughton 1983, Paige and Whitham 1987). Unlike resistance, tolerance does not prevent herbivory, but allows the plant to compensate for damage that natural enemies have already inflicted. However, like resistance, tolerance also protects a plant from the detrimental effects of herbivory.

Because both resistance and tolerance serve the same function, a question that naturally arises is whether resistance and tolerance tend to co-occur together in the same plant species, vary independently, or covary negatively, i.e., represent alternative, mutually exclusive, defense "strategies." Several workers have argued theoretically that resistance and tolerance should covary negatively, based on the untested assumption that allocation to these strategies involves a trade-off (van der Meijden et al. 1988, Herms and Mattson 1992, Belsky et al. 1993, but see Rosenthal and Kotanen 1994). Support for this assumption was recently provided by the report of a negative genetic correlation between tolerance of and resistance to apical meristem damage in the morning glory, *Ipomoea purpurea* (Fineblum and Rausher 1995). This report also demonstrated theoretically that such a correlation can produce disruptive selection on both resistance and tolerance that leads to the evolution of either tolerance or resistance, but not both.

Even in the absence of such a trade-off, it is intuitively plausible that the joint pattern of selection on tolerance and resistance could favor the evolution of one, but not both, strategies. In Appendix A, we present a model that shows that fitness peaks at extreme defensive strategies (either complete tolerance and no resistance or complete resistance and no tolerance; see Fig. 1) could result from a combination of less-than-additive benefits of defense and additive costs of defense. Specifically, the valley between the peaks arises because partial tolerance and partial resistance acting together are not as effective at reducing the detrimental effects of herbivores as complete tolerance or complete resistance are alone. The fitness of genotypes possessing complete resistance and complete tolerance is low relative to genotypes exhibiting complete tolerance or complete resistance alone because genotypes with complete resistance and complete tolerance incur twice the cost, while obtaining the same benefit.

The point of this model is not to assert that the assumptions underlying it are necessarily, or even ever, correct. Rather, it is to show that it is possible, under a reasonable set of assumptions, for the pattern of selection on tolerance and resistance to yield alternative evolutionary outcomes. Once the feasibility of such a pattern of selection is demonstrated, it becomes an em-

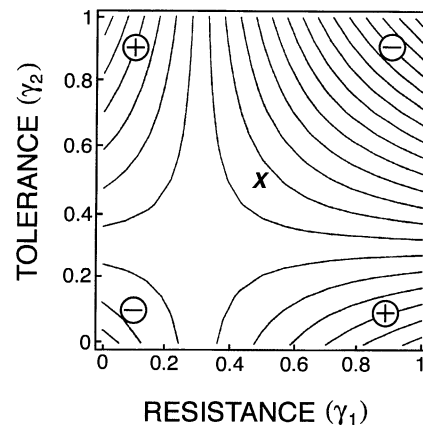


FIG. 1. Hypothetical fitness surface of tolerance and resistance generated from Eqs. A.1 and A.2. The surface shows two peaks, one corresponding to complete tolerance and no resistance, the other corresponding to complete resistance and no tolerance. Values of the parameters are $B_{\max} = 1$ and $C_{\max} = 0.7$. The resistance and tolerance axes correspond to γ_1 and γ_2 in Eq. A.2. The point X corresponds to a genotype with half-maximal resistance and half-maximal tolerance.

pirical issue as to whether, or how frequently, such a pattern arises in nature, i.e., how frequently the pattern of selection on tolerance and resistance tends to cause these traits to covary negatively across populations and species.

Despite the theoretical arguments we have outlined, there is currently little empirical evidence that addresses the question of whether resistance and tolerance represent alternate, mutually exclusive defensive strategies. Although a few interspecific comparisons of tolerance and resistance have been performed (Bilbrough and Richards 1993, Rosenthal and Welter 1995), these studies involve too few species to yield meaningful estimates of the covariance of these alternate strategies, and to our knowledge, no previous investigations have examined the joint pattern of selection on resistance and tolerance. Consequently, the study reported here was undertaken to determine whether tolerance and resistance are likely to be mutually exclusive defensive adaptations in populations of the annual plant *Arabidopsis thaliana*. Specifically, we sought to address three questions: (1) what is the joint pattern of selection acting on resistance and tolerance? and (2) are tolerance and resistance negatively genetically correlated, so as to produce disruptive selection on both resistance and tolerance? Because the model presented in Appendix A assumes that tolerance, as well as resistance, is costly, and because it is likely that any model that results in alternate fitness peaks associated with resistance and tolerance is also likely to make this assumption, we also asked whether (3) tolerance has fitness costs. Genetic variation in *A. thaliana* for both resistance (Mauricio 1995) and tolerance permits the estimation of the joint pattern of selection on tolerance and resistance,

the genetic correlation between tolerance and resistance, and the genetic correlations that are indicative of costs of defense.

METHODS

Natural history

Arabidopsis thaliana (L.) Heynh. (Brassicaceae) is a winter annual plant typically found in disturbed habitats (Ratcliffe 1961, Baskin and Baskin 1972, Lawrence 1976). In North Carolina, seeds of *A. thaliana* germinate in midautumn. Plants grow vegetatively through the winter, flower in early spring and complete fruiting and senesce by late spring. Although most studies have concluded that outcrossing in natural populations of *A. thaliana* is rare (Jones 1971, Snape and Lawrence 1971, Abbott and Gomes 1987, Trnena et al. 1987), significant levels of genetic variation have been found in natural populations for both allozymes (Gomes and Abbott 1987, Kilian and Maluszynski 1987) and life history characters (Kilian et al. 1985, Mauricio 1995). In the field, we have frequently seen several species of flies (Syrphidae), small bees (Apidae), and skippers (Hesperiidae) actively visit the flowers of *A. thaliana*.

We have observed leaf damage on many individuals of *Arabidopsis thaliana* in the field, as well as insects feeding on the plants, including several species of beetles, lepidopteran larvae, leaf miners, and aphids. The most common herbivores we have collected on *A. thaliana* in Durham County, North Carolina are two species of flea beetles, *Psylliodes convexior* LeConte (Chrysomelidae) and *Phyllotreta zimmermani* Crotch (Chrysomelidae). Although several bacterial and fungal pathogens are reported to occur on natural populations of *A. thaliana* (Morgan 1971, Koch and Slusarenko 1990, Dangel et al. 1992, Tsuji and Somerville 1992), we have not observed any obvious visible symptoms of fungal or bacterial infection in our populations.

Arabidopsis thaliana possesses two resistance characters, one chemical and one morphological, which have been shown to reduce damage by herbivores in the field (Mauricio 1995). First, the leaves and seeds of *A. thaliana* contain glucosinolates, a class of secondary chemicals found in all plants of the Brassicaceae and that have been shown to be toxic or unpalatable to a variety of insect and mammalian herbivores (Vaughan et al. 1976, Blau et al. 1978, Hogge et al. 1988). Second, small hairs, or trichomes, cover the leaves and stems of *Arabidopsis thaliana*.

Experimental design

In the spring of 1992, seeds were collected from 144 plants growing in Durham County, North Carolina. Since *Arabidopsis thaliana* is primarily selfing, seeds from each plant essentially form an inbred line, or family. Differences among these families represent both genetic differences and effects of common (maternal)

environment. To minimize these environmentally derived differences, the families used in this experiment were propagated in a common growth chamber for three generations.

On 7 December 1993, 1728 young plants (144 families, 12 replicates/family) were transplanted to a field site in Durham County, North Carolina, where *Arabidopsis thaliana* grows naturally. The distance between planted individuals, ≈ 10 cm, reflected the density of plants found in the field. Vegetation within the experimental area was not disturbed. Half the plants were exposed to herbivores at natural densities and the other half were sprayed at ≈ 2 wk intervals through the entire field season with a combination of an insecticide (ASANA XL) and two fungicides (Ridomil/Bravo and Benlate) to remove herbivores and fungal pathogens (Mauricio 1995). This spraying regime eliminated virtually all visible leaf damage from the treatment plants (Mauricio 1995). In a separate experiment conducted in a growth chamber, this regime of pesticide application was shown not to affect the seed production of *A. thaliana* (Mauricio 1995). Because of the possibility of contamination of controls by pesticides, plants were arranged in a split plot, with three spatial blocks each consisting of two control and two sprayed subplots. One individual from each of the 144 families was randomly assigned to each treatment subplot.

In mid-March 1994, four entire leaves were collected from each plant in the field. Total glucosinolates, expressed as the amount of glucose (in milligrams) released by enzymatic hydrolysis of glucosinolates (1 glucose = 1 glucosinolate) per milligram leaf (wet mass), were assayed using the microcolumn method of Heaney and Fenwick (1981) with modifications made by I. McGregor (Agriculture Canada) and T. Mitchell-Olds (University of Montana), which allow handling large numbers of samples. Trichome density was estimated as the total number of trichomes within a 2.4 mm² area of the upper central area of the adaxial side of the leaf.

From 9 to 12 April 1994, plants in the field were examined for signs of damage by herbivores. In order to facilitate the measurement of damage in the field, for each plant, we measured rosette diameter (in millimeters), total number of leaf damage holes made by herbivores, the number of leaves in the rosette with damage and the total number of leaves in the rosette. We estimated damage in two ways. First, we calculated the percentage of total leaves damaged by herbivores. Second, we divided the total number of damage holes on the rosette by the total leaf area. Damage holes were rather uniform in size, with an average area of 1.26 mm² and a standard deviation of 1.25. (Average area of a single leaf, by contrast, was 40.5 mm² with a standard deviation of 32.3.) Rosette diameter is a good estimator of total leaf area {total leaf area (mm²) = $2.0 \times$ rosette diameter - 2.6; $r = 0.91$; $N = 76$ plants}.

There was a strong positive correlation between the two measures of damage (Pearson correlation coefficient, $\rho = 0.68$; $P = 0.0001$). In this study, we used the first measure since considerable variation was introduced into the second measure by estimation of total leaf area. The percentage of total leaves damaged by herbivores was arcsine transformed for statistical analysis.

Plants were harvested after senescence in mid-May 1994. We used the total number of fruits to estimate fitness. Total fruit number is an excellent predictor of total seed number in field-collected *A. thaliana* (seed number = $40.5 \times$ fruit number - 72.0; $r = 0.98$; $N = 50$ plants). Since *A. thaliana* is a selfing annual, fruit number represents both the total female and male reproductive effort. For analysis, total number of fruits was relativized by dividing by the maximum fruit number. In addition, because of a significant effect of spatial block, we used as our fitness measure the residuals of relative fitness after the effect of block had been removed.

Data were analyzed using procedures in the SAS statistical package (1989, version 6.09). Analyses of variance and regressions were calculated using the GLM procedure and covariances and Pearson correlation coefficients were calculated using the CORR procedure.

Genetic analysis

Our level of genetic analysis is the family (Arnold 1981, Via 1984). A total of 140 families was used in the analysis; four families were eliminated from the analysis due to high mortality. Because each family represents, in a selfing plant like *A. thaliana*, an inbred line, any between-family variance or covariance detected in this experiment represents total, rather than additive, genetic variance or covariance. Estimates of total genetic variance and covariance are more appropriate for this species, however, for two reasons. First, in a population of selfing organisms, natural selection acts on the total genetic variation and not exclusively on the additive genetic variation as in the case of outcrossing species (Roughgarden 1979:55). Second, the crosses that would be used to partition total genetic variation into additive and nonadditive components would generate offspring that would be artificially highly heterozygous, and thus of questionable relevance to evolution in natural populations of *A. thaliana*.

Measurement of tolerance

By definition, tolerance is inversely related to the degree to which fitness is reduced by herbivores. Because an individual plant can not be both damaged and undamaged, it is not possible to estimate tolerance from measurement of a single plant. Instead, one estimates tolerance for a group of related individuals (ideally, individuals of identical genotype, but often members

of a sibship). Tolerance can then be defined as the difference in average fitness between individuals of the group attacked by herbivores and individuals not attacked (Rausher 1992a, Fineblum and Rausher 1995).

This measure of tolerance assumes that all plants attacked experience the same level of damage. However, individual plants may often experience different levels of damage, in large part because the sites in which they grow differ in local herbivore abundance. Therefore, tolerance can be considered a norm of reaction: each level of damage reflects a particular environment in which a plant grows, and the difference in fitness between plants experiencing a particular level of damage and the fitness of plants with no damage is a measure of tolerance for that level of damage. Because plant fitness is expected to decrease monotonically with increasing levels of damage, this norm of reaction can be modeled by a regression of fitness on damage (see Simms and Triplett 1994). In particular, the slope of this regression is an index of tolerance: a small slope indicates low sensitivity to damage (high tolerance), while a large slope indicates high sensitivity to damage (low tolerance). A positive slope indicates overcompensation. In this study, we used this index to estimate the tolerance of each family.

Estimating costs of tolerance

We used two methods to estimate the fitness costs of tolerance. Method 1 is straightforward: it asks whether there is a family-mean (genetic) correlation between tolerance (the slope of the regression of fitness on damage) and fitness in the absence of herbivores. Tolerance was measured in the control treatment, while fitness in the absence of herbivores was measured in the spray treatment. The rationale for this assay follows that used by Simms and Triplett (1994), as well as that commonly used for the measurement of costs of resistance (Simms and Rausher 1987): in the absence of herbivores, the benefits of tolerance are absent and only the costs are manifested. Tolerance of a family is estimated in the control treatment since its measurement is based on assessment of damage. Using this method, we determined both whether there is a linear relationship between fitness and tolerance (linear term in a quadratic regression) and whether there was curvature to the relationship (quadratic term in the regression).

Method 2 of estimating costs has the advantage that it uses only plants in the control treatment, and thus may be used in experiments in which there is no sprayed treatment. In this method, fitness in the absence of herbivores is estimated by the intercept of the regression of fitness on damage. Cost of tolerance is then assayed by a family-mean regression of intercept on slope. One problem with this method is that even in the absence of a cost, slope and intercept are statistically correlated because errors are correlated. In Ap-

pendix B, we describe a correction for this bias that allows the true cost of tolerance to be estimated.

Measurement of selection

To measure selection acting on tolerance, we used the partial regression analysis described by Rausher (1992b). Rausher's (1992b) approach to the measurement of selection on quantitative traits is similar to that of the phenotypic analysis of Lande and Arnold (1983), but accounts for biases due to environmental correlations between characters and fitness (Mitchell-Olds and Shaw 1987) by examining selection acting directly on genotypic values rather than on phenotypic values. Here, we measured selection acting on family means, which should essentially be equivalent to selection on genotypes, since each family represents an inbred line.

In this regression analysis, partial regression coefficients are used to estimate selection gradients, which represent selection that acts directly on a character (Lande and Arnold 1983). An estimate of the directional selection gradient is obtained from the partial regression coefficient of a linear regression of relative fitness on the character (a measure of the slope). The sign of the gradient indicates the direction of evolutionary change expected from selection. An estimate of the stabilizing/disruptive selection gradient is obtained from the second-order coefficient of a quadratic regression of relative fitness on the character (a measure of the curvature). The sign of the gradient indicates whether the fitness function is concave downward (stabilizing selection) or concave upward (disruptive selection).

We employed the constrained-regression method suggested by Mitchell-Olds and Shaw (1987) to test the null hypothesis that a fitness maximum/minimum lies outside the range of family means observed in our experiment (Simms 1990). This technique involves constraining the fitness function to have a maximum/minimum at a value just outside the range of observed family means for a particular character. For that trait, we chose values both just below and above the range of the experimental data ($z_{H_0}^*$; Simms 1990). We then tested the null hypothesis that a family outside the range of the data possesses the maximum or minimum fitness. Rejection of this null hypothesis for both of the extreme values implies that stabilizing/disruptive selection is acting.

RESULTS

Genetic variation for tolerance

We first determined that genetic variation for tolerance existed in our experimental population. To detect genetic variation, we performed an analysis of covariance in which fitness in the control treatment was the dependent variable, family was the independent variable, and the covariate was amount of damage. The family by damage interaction term was significant (Ta-

TABLE 1. Analysis of covariance on residuals of relative fitness after block effects have been removed. The covariate, herbivore damage, is the percentage of leaves with tissue removed by herbivores. Herbivore damage was arcsine transformed.

Source of variation	df	Type III ss	F	P
Family	140	1.1648	1.05	0.3605
Herbivore damage	1	0.2551	32.05	0.0001
Family \times Herbivore damage	140	1.7108	1.54	0.0004
Error	540	4.2984		

ble 1), indicating that families differed in the slope of the regression of fitness on damage. In other words, families differed for tolerance, indicating the presence of genetic variation for tolerance in our experimental population.

Joint pattern of selection on tolerance and resistance

The joint pattern of selection acting on tolerance and the two resistance characters is reflected in the coefficients of the multiple family-mean quadratic regressions of fitness in the control treatment on each of the characters. A regression involving only linear terms indicated significant negative directional selection acting on trichome density and glucosinolate concentration, but no significant directional selection on tolerance (Table 2). By contrast, in a regression including the quadratic terms, the only detectable effect was disruptive selection on tolerance (Tables 3 and 4). Because there is no detectable disruptive selection on either glucosinolate concentration or trichome density and no significant two-way interactions (reflecting correlational selection) involving tolerance and these resistance characters (Table 3), the fitness surface cannot have multiple peaks, with each peak corresponding to either tolerance alone or resistance alone. In other words, the joint pattern of selection detected in this experiment does not appear to be of the type portrayed in Fig. 1.

Nevertheless, there was significant disruptive selection acting on tolerance, as indicated by the positive value of the stabilizing/disruptive selection gradient for this character (Tables 3 and 4). Moreover, a constrained regression analysis indicated that no family outside the

TABLE 2. Analysis of variance for relative fitness of control plants grown in the field. Only linear terms are included because adding quadratic terms results in biased estimates of directional selection gradients. All effects are considered fixed.

Source of variation	df	Type III ss	F	P
Tolerance	1	0.00110	0.60	0.4394
Glucosinolate concentration	1	0.00752	4.12	0.0444
Trichome density	1	0.00470	14.07	0.0003
Error	136	0.24855		

TABLE 3. Analysis of variance for relative fitness of control plants grown in the field. Both linear and quadratic terms are included. All effects are considered fixed.

Source of variation	df	Type III ss	F	P
Tolerance	1	0.002079	1.20	0.2748
Glucosinolates	1	0.000881	0.51	0.4767
Trichome density	1	0.000544	0.31	0.5759
(Tolerance) ²	1	0.016547	9.57	0.0024
(Glucosinolates) ²	1	0.000605	0.35	0.5552
(Trichome density) ²	1	0.000007	0.00	0.9480
Tolerance × Glucosinolates	1	0.000294	0.17	0.6807
Tolerance × Trichome density	1	0.000769	0.44	0.5060
Glucosinolates × Trichome density	1	0.000318	0.18	0.6686
Error	130	0.224734		

range of tolerance in our experiment possessed a minimum fitness. In this analysis we set $z_{H_0}^* = -0.3$ to represent a family with tolerance below the minimum observed, and $z_{H_0}^* = 0.6$ to represent a family just above the maximum observed. The probabilities that these extremes represented a minimum for fitness were $P = 0.004$ and $P = 0.002$, respectively. Thus, there is an intermediate minimum fitness, indicating true disruptive selection on tolerance.

Fitness costs of tolerance

We found no evidence for a linear cost of tolerance. Using Method 1, the linear term of a family mean regression of fitness in the spray treatment on tolerance was not significant (Table 5). Using Method 2, the slope and intercept of a regression of fitness in the control treatment on tolerance were significantly negatively correlated ($\rho = -0.73$; $P = 0.0001$), and the calculated covariance between tolerance and fitness was -0.00674 . Although, on the surface, this might be interpreted as indicating a fitness cost of tolerance, application of the correction for the bias in the relationship described in Appendix B yielded an unbiased estimate for the covariance of 0.00425. Using the jackknife, we calculated the 95% confidence intervals of this covariance to be 0.00495. Because this interval overlaps zero (and is a positive value), there is no indication that there is a significant negative covariance

between tolerance and fitness in the absence of herbivores and thus, no evidence that there are fitness costs of tolerance as measured by this method. In addition, there is no significant difference between the estimates of the covariance provided by the two methods ($z = 1.3$, $P = 0.09$).

Although there is no apparent linear relationship between tolerance and fitness in the absence of herbivores, there is a highly significant quadratic relationship between tolerance and the fitness of sprayed plants (Table 5). The fitness minimum, as calculated from the coefficients for the selective surface (Table 4), corresponds to a tolerance value of ≈ 0.17 . Hence, for values of tolerance < 0.17 , fitness decreases with increasing tolerance, suggesting a cost of tolerance in this range. However, for values of tolerance > 0.17 , fitness increases with increasing tolerance. It is unclear what causes this latter pattern.

Correlation between tolerance and resistance

We found no evidence of a significant genetic correlation between tolerance and either of the two resistance characters in *Arabidopsis*. The family-mean correlation between tolerance and trichome density was $\rho = 0.04$ ($P = 0.67$), while the family-mean correlation between tolerance and glucosinolate concentration was $\rho = -0.08$ ($P = 0.33$).

TABLE 4. Directional (β_i) and stabilizing/disruptive (γ_{ij}) selection gradients from selection analysis (Rausher 1992b). Standard errors are in parentheses. The model and error sums of squares (and degrees of freedom) for the linear model were 0.04908 (3) and 0.24855 (136), respectively. The model and error sums of squares (and degrees of freedom) for the quadratic model were 0.07290 (9) and 0.22743 (130), respectively.

Character	β	γ		
		Tolerance	Trichome density	Glucosinolate concentration
Tolerance	0.02133 (0.02751)	0.37490** (0.12118)	0.00361 (0.00541)	0.02590 (0.06278)
Trichome density	-0.00296** (0.00079)		0.000009 (0.00013)	-0.00060 (0.00130)
Glucosinolate concentration	-0.01086* (0.00535)			-0.00289 (0.00489)

* $P \leq 0.05$; ** $P \leq 0.01$.

TABLE 5. Multiple family-mean regression of relative fitness of sprayed plants on tolerance.

Source of variation	df	Type III ss	F	P
Tolerance	1	0.00019	0.05	0.8290
(Tolerance) ²	1	0.01581	3.98	0.0479
Error	137	0.24855		

DISCUSSION

Joint pattern of selection on tolerance and resistance

The primary objective of this investigation was to ascertain whether tolerance and resistance represent mutually exclusive adaptations to attack by herbivores, i.e., whether selection favors either tolerance or resistance, but not both. A priori, there are reasons to suspect such mutual exclusivity, since complete resistance and complete tolerance are redundant, and having both presumably incurs a total cost greater than having just one. Moreover, at least one study suggests that redundant resistance characters are mutually exclusive: Rehr et al. (1973) found that *Acacia* species possessed either cyanogenic glycosides or symbiotic ant-based defenses, but not both.

By contrast, a common pattern in nature is that plant species have often evolved several distinct types of resistance characters (Berenbaum 1985). For example, some plants produce two or more different classes of secondary compounds (Hugentobler and Renwick 1995), or both chemical and physical defenses (e.g., *Arabidopsis thaliana*). Although this pattern suggests that different types of resistance are not necessarily mutually exclusive (Steward and Keeler 1988), it is not known to what extent different types of resistance may have evolved in response to different sets of herbivores. Mutual exclusivity is only likely when two resistance factors are redundant, i.e., directed toward the same set of enemies.

To the extent that tolerance of herbivory is nonspecific, i.e., that mechanisms that produce tolerance allow plants to compensate for damage inflicted by a wide variety of herbivores, it is likely that resistance and tolerance are largely redundant. In *Arabidopsis thaliana*, redundancy appears to be the case, since tolerance appears to alleviate the adverse effects of the same type of leaf damage that trichomes and glucosinolates reduce. Nevertheless, we failed to detect a joint pattern of selection on resistance and tolerance that would produce mutual exclusivity. Moreover, disruptive selection on tolerance indicates that high levels of tolerance are favored, as long as the initial level of tolerance is above a threshold value. And despite the negative directional selection gradient acting on glucosinolates, Mauricio (1995) has shown that the overall pattern of selection on glucosinolates favors maintenance of intermediate levels of these secondary compounds. The pattern of

selection on these characters thus seems to favor the presence of both resistance and tolerance. Consequently, to the extent that results from this one system can be generalized, we would predict that there may be many plant species that possess resistance and tolerance to the same herbivores simultaneously. In particular, in agreement with Rosenthal and Kotanen (1994), we predict that there will not be negative covariance across plant species between resistance and tolerance.

Two explanations may be offered for the absence of multiple resistance/tolerance peaks in the resistance-tolerance selective surface in *Arabidopsis thaliana*. First, unlike in *Ipomoea purpurea* (Fineblum and Rausher 1995), tolerance is not genetically correlated with resistance in *A. thaliana*. Therefore, the negative correlations that might generate a pattern of selection leading to mutual exclusivity (Fineblum and Rausher 1995) are not present. Second, we obtained no evidence that resistance and tolerance combine less than additively in their effects on fitness (i.e., the interaction terms involving tolerance in Table 3 were not significant). Yet some sort of less-than-additive combining would seem to be a requirement for the existence of a valley separating a resistance peak from a tolerance peak in the resistance/tolerance selective surface (e.g., Simms and Rausher 1993). By contrast, the absence of a cost of tolerance does not constitute an explanation for mutual exclusivity of tolerance and resistance in *A. thaliana*, since over the range of incomplete compensation (tolerance between -0.25 and 0.0), fitness declines in the absence of herbivores.

Disruptive selection on tolerance

Although resistance and tolerance do not appear to be mutually exclusive defensive adaptations in *Arabidopsis thaliana*, we did detect disruptive selection acting on tolerance. It thus appears that there are two alternative stable evolutionary states: (1) absence of tolerance and (2) complete tolerance or overcompensation. Even in the absence of environmental variation between sites, the equilibrium state of a population will depend upon its initial level of tolerance. We can easily envision that, in a weed like *A. thaliana*, new populations may be repeatedly founded by a mixture of propagules from populations that differ in their levels of tolerance. Some populations may, by chance, be founded primarily by propagules with mostly low levels of tolerance. Such populations would then evolve toward the absence of tolerance. Other populations may, by chance, be founded primarily by propagules from populations with high levels of tolerance and would evolve toward complete tolerance or even overcompensation. It thus seems possible that *A. thaliana* metapopulations may be composed of a shifting mosaic of populations that differ radically in their level of tolerance.

It appears that disruptive selection on tolerance aris-

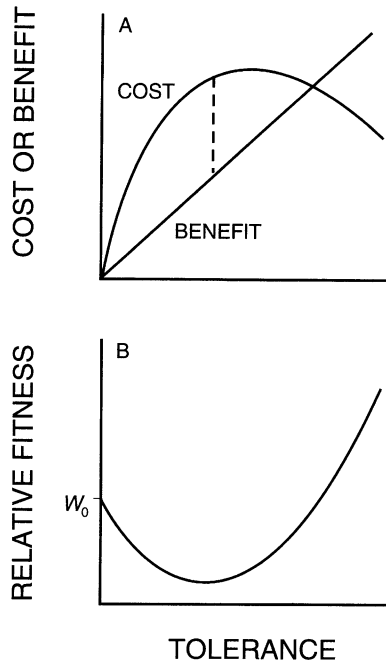


FIG. 2. Graphical model of the net effects of costs and benefits of tolerance. (A) Cost increases, then decreases as a function of tolerance, while benefit increases linearly. The broken line indicates the maximal difference between cost and benefit and thus corresponds to the tolerance level with minimum fitness. (B) The net effect of costs and benefits, as determined by the equation, is relative fitness = W_0 + benefit - cost, where W_0 is the fitness of a genotype lacking tolerance.

es primarily from the nonlinear form of the cost of tolerance. Because fitness in the absence of herbivores first decreases, then increases as tolerance increases, the "cost" of tolerance first increases, then decreases. By contrast, the benefit of tolerance is by nature linear, since a unit increase in tolerance constitutes a unit increase in fitness. These two effects together yield disruptive selection on tolerance (Fig. 2), provided that the initial rate of increase in cost with tolerance is greater than the rate of increase of the benefit of tolerance.

Although the initial increase in cost of tolerance is consistent with the notion that the mechanism(s) producing tolerance divert limiting resources away from other functions that enhance growth and reproduction (allocation costs; see Simms 1992, Simms and Triplett 1994), our study sheds no light on why at higher levels of tolerance, costs actually decrease. One complex possibility is that the net pattern of costs that we measured actually reflects the superposition of two distinct processes. One of these processes would entail a true allocation cost, but one in which fitness asymptotes as tolerance increases. The other process could entail a pleiotropic benefit associated with the alleles that confer tolerance. For example, if some of the loci influ-

encing tolerance affect general vigor, alleles at the loci that confer tolerance may also contribute to enhanced fitness. And if vigor increased more than linearly with the number of loci at which the tolerance alleles were present, this process would yield a relationship between fitness and tolerance that increased slowly at first, then at an ever-increasing rate (i.e., a concave-upward curved relationship). The combined (additive) effect of these two processes would be to produce an overall cost of the type observed. Whether this explanation, or some other, is correct will probably not be determined until the mechanisms conferring tolerance have been characterized at the physiological, genetic, and molecular levels.

CONCLUSIONS

When one recognizes the possibility that defensive adaptations of plants may be redundant, it becomes evident that two strikingly different patterns of variation are possible within and among species. On the one hand, the redundant adaptations may be mutually exclusive because selection favors retaining one, but not both. In this case, some species (or populations) are expected to exhibit one adaptation, while others are expected to exhibit the other, but few, if any, species are expected to exhibit both. On the other hand, if redundant adaptations are not mutually exclusive, many species (or populations) are expected to exhibit both types of adaptation. This study has demonstrated that one species, *Arabidopsis thaliana*, exhibits genetic variation for both resistance and tolerance. The pattern of selection acting on this variation suggests that both tolerance and resistance may coexist stably in populations of this species, and thus calls into question the likelihood of mutual exclusivity for tolerance and resistance suggested by previous authors.

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LITERATURE CITED

- Abbott, R. J., and M. F. Gomes. 1987. Outcrossing rates in natural populations of *Arabidopsis thaliana*. *Arabidopsis Information Service* 25:85-88.
- Arnold, S. J. 1981. Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations

- between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. *Evolution* **35**:489–509.
- Baskin, J. M., and C. C. Baskin. 1972. Ecological life cycle and physiological ecology of seed germination of *Arabidopsis thaliana*. *Canadian Journal of Botany* **50**:353–360.
- Belsky, A. J., W. P. Carson, C. L. Jensen, and G. A. Fox. 1993. Overcompensation by plants: herbivore optimization or red herring? *Evolutionary Ecology* **7**:109–121.
- Berenbaum, M. R. 1985. Brementown revisited: interactions among allelochemicals in plants. *Recent Advances in Phytochemistry* **19**:139–169.
- Berenbaum, M. R., A. R. Zangerl, and J. K. Nitao. 1986. Constraints on chemical coevolution: wild parsnips and the parsnip webworm. *Evolution* **40**:1215–1228.
- Bilbrough, C. J., and J. H. Richards. 1993. Growth of sagebrush and bitterbrush following simulated winter browsing: mechanisms of tolerance. *Ecology* **74**: 481–492.
- Blau, P. A., P. Feeny, L. Contardo, and D. S. Robson. 1978. Allylglucosinolate and herbivorous caterpillars: a contrast in toxicity and tolerance. *Science* **200**:1296–1298.
- Cheverud, J. M. 1984. Quantitative genetics and developmental constraints on evolution by selection. *Journal of Theoretical Biology* **110**:155–171.
- Crawley, M. J. 1983. *Herbivory: the dynamics of animal-plant interactions*. University of California Press, Berkeley, California, USA.
- Dangl, J. L., E. B. Holub, T. Debener, H. Lehnackers, C. Ritter, and I. R. Crute. 1992. Genetic definition of loci involved in *Arabidopsis*-pathogen interactions. Pages 393–418 in C. Kocz, N.-H. Chua, and J. Schell, editors. *Methods in Arabidopsis research*. World Scientific, Singapore, Malaysia.
- Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**:586–608.
- Fagerström, T., S. Larsson, and O. Tenow. 1987. On optimal defence in plants. *Functional Ecology* **1**:73–81.
- Fineblum, W. L., and M. D. Rausher. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. *Nature* **377**:517–520.
- Fraenkel, G. S. 1959. The *raison d'être* of secondary plant substances. *Science* **129**:1466–1470.
- Gomes, M. F., and R. J. Abbott. 1987. Genotypic diversity in British populations of *Arabidopsis thaliana* based on a survey of isozyme variation. *Arabidopsis Information Service* **23**:25–30.
- Hartl, D. L., and A. G. Clark. 1989. *Principles of population genetics*, Second edition. Sinauer, Sunderland, Massachusetts, USA.
- Heaney, R. K., and G. R. Fenwick. 1981. A micro-column method for the rapid determination of total glucosinolate content of cruciferous material. *Zeitschrift für Pflanzenzüchtung* **87**:89–95.
- Hermes, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**:283–335.
- Hogge, L. R., D. W. Reed, E. W. Underhill, and G. W. Haughn. 1988. HPLC separation of glucosinolates from leaves and seeds of *Arabidopsis thaliana* and their identification using thermospray liquid chromatography/mass spectrometry. *Journal of Chromatographic Science* **26**:551–556.
- Hugentobler, U., and J. A. A. Renwick. 1995. Effects of plant nutrition on the balance of insect relevant cardenolides and glucosinolates in *Erysimum cheiranthoides*. *Oecologia* **102**:95–101.
- Jones, M. E. 1971. The population genetics of *Arabidopsis thaliana*. I. The breeding system. *Heredity* **27**:39–50.
- Kilian, A., and M. Maluszynski. 1987. Genetic variability of *Arabidopsis thaliana* populations from regions of different pollution level. *Arabidopsis Information Service* **25**: 57–66.
- Kilian, A., A. Rostanski, and M. Maluszynski. 1985. Analysis of the variability of *Arabidopsis thaliana* natural populations in Poland. *Arabidopsis Information Service* **22**: 77–86.
- Koch, E., and A. Slusarenko. 1990. *Arabidopsis* is susceptible to infection by a downy mildew fungus. *Plant Cell* **2**: 437–445.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* **37**:1210–1226.
- Lawrence, M. J. 1976. Variation in natural populations of *Arabidopsis thaliana* (L.) Heynh. Pages 167–190 in J. G. Vaughan, A. J. MacLeod, and B. M. G. Jones, editors. *The biology and chemistry of the Cruciferae*. Academic Press, London, UK.
- Marquis, R. J. 1984. Leaf herbivores decrease fitness of a tropical plant. *Science* **226**:537–539.
- Mauricio, R. 1995. The evolution of resistance to natural enemies in field populations of the annual plant, *Arabidopsis thaliana*. Dissertation. Duke University, Durham, North Carolina, USA.
- Mauricio, R., M. D. Bowers, and F. A. Bazzaz. 1993. Pattern of leaf damage affects fitness of the annual plant *Raphanus sativus* (Brassicaceae). *Ecology* **74**:2066–2071.
- McNaughton, S. J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* **40**:329–336.
- Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**:1149–1161.
- Morgan, O. D. 1971. A study of four weed hosts of *Sclerotinia* species in alfalfa fields. *Plant Disease Reporter* **55**: 1087–1089.
- Morrow, P. A., and V. C. LaMarche, Jr. 1978. Tree ring evidence for chronic insect suppression of productivity in subalpine *Eucalyptus*. *Science* **201**:1244–1246.
- Owen, D. F. 1980. How plants may benefit from the animals that eat them. *Oikos* **35**:230–235.
- Owen, D. F., and R. G. Wiegert. 1976. Do consumers maximize plant fitness? *Oikos* **27**:488–492.
- Paige, K. N., and T. G. Whitham. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. *American Naturalist* **129**:407–416.
- Painter, R. H. 1958. Resistance of plants to insects. *Annual Review of Entomology* **3**:267–290.
- Ratcliffe, D. 1961. Adaptation to habitat in a group of annual plants. *Journal of Ecology* **49**:187–203.
- Rausher, M. D. 1992a. Natural selection and the evolution of plant-insect interactions. Pages 20–88 in B. D. Roitberg and M. S. Isman, editors. *Insect chemical ecology: an evolutionary approach*. Routledge, Chapman and Hall, New York, New York, USA.
- . 1992b. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* **46**:616–626.
- Rausher, M. D., and P. Feeny. 1980. Herbivory, plant density, and plant reproductive success: the effect of *Battus philenor* on *Aristolochia reticulata*. *Ecology* **61**:905–917.
- Rehr, S. S., P. Feeny, and D. H. Janzen. 1973. Chemical defence in Central American non-ant-acacias. *Journal of Animal Ecology* **42**:405–416.
- Reznick, D. 1983. The structure of guppy life histories: the tradeoff between growth and reproduction. *Ecology* **64**: 862–873.
- Rose, M. R., and B. Charlesworth. 1981. Genetics of life history in *Drosophila melanogaster*. I. Sib analysis of adult females. *Genetics* **97**:187–196.
- Rosenthal, J. P., and P. M. Kotanen. 1994. Terrestrial plant tolerance to herbivory. *Trends in Ecology and Evolution* **9**:145–148.
- Rosenthal, J. P., and S. C. Welter. 1995. Tolerance to her-

- bivory by a stem-boring caterpillar in architecturally distinct maize and wild relative. *Oecologia* **102**:146–155.
- Roughgarden, J. 1979. Theory of population genetics and evolutionary ecology: an introduction. MacMillan, New York, New York, USA.
- SAS. 1989. SAS/STAT user's guide. Release 6.03 edition. SAS Institute, Cary, North Carolina, USA.
- Simms, E. L. 1990. Examining selection on the multivariate phenotype: plant resistance to herbivores. *Evolution* **44**: 1177–1188.
- . 1992. Costs of plant resistance to herbivory. Pages 392–425 in R. S. Fritz and E. L. Simms, editors. Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. University of Chicago Press, Chicago, Illinois, USA.
- Simms, E. L., and M. D. Rausher. 1987. Costs and benefits of plant resistance to herbivory. *American Naturalist* **130**: 570–581.
- Simms, E. L., and M. D. Rausher. 1993. Patterns of selection on phytophage resistance in *Ipomoea purpurea*. *Evolution* **47**:970–976.
- Simms, E. L., and J. Triplett. 1994. Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution* **48**:1973–1985.
- Snape, J. W., and M. J. Lawrence. 1971. The breeding system of *Arabidopsis thaliana*. *Heredity* **27**:299–302.
- Steward, J. L., and K. H. Keeler. 1988. Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae). *Oikos* **53**:79–86.
- Tilman, D. 1990. Constraints and trade-offs toward a predictive theory of competition and succession. *Oikos* **58**:3–15.
- Trnena, L., M. Skárova, J. Relichová, and I. Cetl. 1987. High outcrossing rates in a partially fertile line and their significance in the genetic structure of populations in *Arabidopsis thaliana* (L.) Heynh. *Arabidopsis Information Service* **23**: 31–40.
- Tsuji, J., and S. C. Somerville. 1992. First report of natural infection of *Arabidopsis thaliana* by *Xanthomonas campestris* pv. *campestris*. *Plant Disease* **76**:539.
- van der Meijden, E., M. Wijn, and H. J. Verkaar. 1988. Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos* **51**:355–363.
- Vaughan, J. G., A. J. Macleod, and B. M. G. Jones. 1976. The biology and chemistry of the Cruciferae. Academic Press, London, UK.
- Via, S. 1984. The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. *Evolution* **38**:896–905.

APPENDIX A

In this appendix, we describe a simple model of the relationship between benefits and costs of tolerance and resistance that yields a selective surface with two fitness peaks, one corresponding to complete tolerance and no resistance, the other corresponding to complete resistance and no tolerance. This model is similar in spirit to previous models of the evolution of resistance (Simms and Rausher 1987). In this model, plant fitness reflects a balance between, on the one hand, benefits (reduction in herbivory) due to resistance or tolerance or both, and, on the other hand, costs of resistance and tolerance: Let plant fitness, W , be represented by the equation

$$W = W_0 + B(R, T) - C(R, T). \quad (\text{A.1})$$

Here, W_0 is the fitness of plants with no tolerance and no resistance, $B(R, T)$ is the incremental increase in fitness due to reduction in herbivory as a function of allocation to resistance, R , and to tolerance, T , and $C(R, T)$ is the incremental decrease in fitness due to the costs of resistance and tolerance. Finally, let R_{\max} and T_{\max} be the allocations to resistance and tolerance that yield complete resistance or complete tolerance, respectively, and thus that yield maximum benefit, B_{\max} . Then R and T can be expressed in terms of R_{\max} and T_{\max} as $R = \gamma_1 R_{\max}$ and $T = \gamma_2 T_{\max}$, when $0 \leq \gamma_1 \leq 1$ and $0 \leq \gamma_2 \leq 1$.

In this model, we make the following assumptions about costs and benefits of resistance and tolerance.

1) Costs increase linearly with allocation to resistance or tolerance. Although little is known about the relationship between costs and allocation, this relationship is commonly used in models of the evolution of resistance (Fagerström et al. 1987, Simms and Rausher 1987). In symbols, this assumption can be stated as

$$C(\gamma_1 R_{\max}, 0) = \gamma_1 C(R_{\max}, 0)$$

and

$$C(0, \gamma_2 T_{\max}) = \gamma_2 C(0, T_{\max}).$$

2) Cost of resistance and tolerance are additive, i.e.,

$$C(R, T) = C(R, 0) + C(0, T).$$

3) The benefit of resistance or tolerance alone increases linearly with allocation to tolerance or resistance, i.e.,

$$B(\gamma_1 R_{\max}, 0) = \gamma_1 B(R_{\max}, 0)$$

and

$$B(0, \gamma_2 T_{\max}) = \gamma_2 B(0, T_{\max}).$$

This assumption is made simply for illustrative purposes. It appears to differ from the usual assumption that benefit is an asymptotic function of allocation to resistance or tolerance (Fagerström et al. 1987, Simms and Rausher 1987). However, this assumption actually produces a linear increase in benefit to R_{\max} or T_{\max} , after which there is no further increase in benefit. Thus, this model represents a limiting case of plateauing. A similar model can be constructed in which the benefit is a nonlinear, asymptotic function of allocation. Such a model, with appropriate, reasonable assumptions about how benefits of tolerance and resistance combine, produces a qualitatively similar fitness surface.

4) For simplicity of portrayal, we assume that benefits and costs per unit allocation are similar for tolerance and resistance, i.e.,

$$T_{\max} = R_{\max},$$

$$B_{\max} = B(R_{\max}, 0) = B(0, T_{\max}),$$

and

$$C_{\max} = C(R_{\max}, 0) = C(0, T_{\max}).$$

5) Finally, we assume that benefits are less than additive, i.e.,

$$B(R, T) < B(R, 0) + B(0, T).$$

This assumption would be satisfied, for example, if the benefits of tolerance and resistance are multiplicative, i.e.,

$$\begin{aligned} B(R, T) &= B(\gamma_1 R_{\max}, \gamma_2 T_{\max}) \\ &= B_{\max} [1 - (1 - \gamma_1)(1 - \gamma_2)]. \end{aligned} \quad (\text{A.2})$$

The effect of this specific assumption is most easily seen by considering an individual in which allocation to both resis-

tance and tolerance is half maximal, i.e., $R = 1/2R_{\max}$ and $T = 1/2T_{\max}$, corresponding to $\gamma_1 = \gamma_2 = 1/2$. This allocation corresponds to the point X in Fig. 1., which is midway on the line connecting the upper left and lower right corners. This line represents a collection of points with equal cost, and the extremes of this line segment have a benefit of B_{\max} . A plant with half-maximum resistance would experience half the damage of a plant with maximum resistance, and therefore the benefit it receives from resistance is half the maximal

benefit. This 50% reduction in benefit is in turn reduced by one-half since the plant is not completely intolerant, but is half maximally tolerant. The reduction from the maximum benefit is thus one-quarter, so that the overall benefit is $0.75 B_{\max}$, i.e., $[1 - (1 - 1/2)(1 - 1/2)]B_{\max}$.

Incorporating these assumptions and using Eq. A.1 to generate a selective surface produces the contours portrayed in Fig. 1.

APPENDIX B

In this appendix, we demonstrate that Method 2 for estimating the cost of tolerance yields a biased estimate of the cost. We also describe a correction factor that can be used to produce an unbiased estimate of the cost.

Method 2 estimates the fitness of a family in the absence of herbivores by the intercept of a regression of fitness on damage for that family. Tolerance for that family is estimated by the slope of that regression. A natural measure of the cost of resistance is then the coefficient of a family-mean regression of intercepts on slopes, i.e., of fitness in the absence of herbivores on tolerance. In particular, a fitness cost of tolerance occurs when the covariance over families between tolerance (slope) and fitness in the absence of herbivores (intercept) is negative. This covariance is given by

$$E[(\beta_{0j} - \beta_0)(\beta_{1j} - \beta_1)], \tag{B.1}$$

where β_{0j} and β_{1j} are, respectively, the true intercept and slope for the j^{th} family, and β_0 and β_1 are the respective averages of these quantities over the population of families.

If we knew β_{0j} and β_{1j} for each family in the study, we could estimate Eq. B.1 directly from the sample covariance of these quantities. Instead, we have estimates $\hat{\beta}_{0j}$ and $\hat{\beta}_{1j}$ obtained from linear regressions of fitness on damage for samples of individuals taken from each of the families. The random variation produced by sampling of individuals from families creates an artifactual covariance between the slope and the intercept, as the following argument will demonstrate.

The sample covariance of the estimates $\hat{\beta}_{0j}$ and $\hat{\beta}_{1j}$ is an unbiased estimator of

$$E[(\hat{\beta}_{0j} - \beta_0)(\hat{\beta}_{1j} - \beta_1)], \tag{B.2}$$

which differs from Eq. B.1 because the true intercept and slope for the j^{th} family has been replaced by the estimates $\hat{\beta}_{0j}$ and $\hat{\beta}_{1j}$. The expectation E in Eq. B.2 represents an average over individuals in a family and families in the population. If we separate the averaging process into two stages, we can rewrite Eq. B.2 as

$$E_j E_{ij} [(\hat{\beta}_{0j} - \beta_{0j} + \beta_{0j} - \beta_0)(\hat{\beta}_{1j} - \beta_{1j} + \beta_{1j} - \beta_1)], \tag{B.3}$$

where E_j denotes an average over all families in the population and E_{ij} denotes an average over all individuals, observed and unobserved, within a family. If we expand Eq. B.3 and use the fact that $(\hat{\beta}_{0j} - \beta_{0j})$ and $(\hat{\beta}_{1j} - \beta_{1j})$ average to zero within families, we find that the cross products vanish and Eq. B.3 becomes

$$E_j E_{ij} [(\hat{\beta}_{0j} - \beta_{0j})(\hat{\beta}_{1j} - \beta_{1j})] + E_j [(\beta_{0j} - \beta_0)(\beta_{1j} - \beta_1)] \tag{B.4}$$

The second term of Eq. B.4 is identical to Eq. B.1. The first term is therefore the source of the artifact.

If we can estimate the first term, we can subtract that estimate from the sample covariance of the family slopes and intercepts to obtain an estimate of the true covariance. In deriving an estimate for the first term of Eq. B.4, we note that

$$E_{ij} [(\hat{\beta}_{0j} - \beta_{0j})(\hat{\beta}_{1j} - \beta_{1j})] = \sigma_j^2 \left[\frac{-\bar{x}_j}{\sum_i (x_{ij} - \bar{x}_j)^2} \right], \tag{B.5}$$

where x_{ij} is the damage for the i^{th} individual in the j^{th} family, \bar{x}_j is the mean of the x_{ij} for the j^{th} family, and σ_j^2 is the error variance from the regression of fitness on damage for the individuals from the j^{th} family. Using the error mean square from that regression to estimate σ_j^2 , we can obtain an estimate of Eq. B.5. We can then estimate the artifactual covariance

$$E_j \left\{ \sigma_j^2 \left[\frac{-\bar{x}_j}{\sum_i (x_{ij} - \bar{x}_j)^2} \right] \right\} \tag{B.6}$$

by averaging the estimates of Eq. B.5 over families. Subtracting Eq. B.6 from the sample covariance of the estimates $\hat{\beta}_{0j}$ and $\hat{\beta}_{1j}$ gives the desired estimate of the true covariance. Standard errors of the estimates can be obtained using standard jackknife or bootstrap techniques.