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EVOLUTIONARY GENETICS OF RESISTANCE AND TOLERANCE TO NATURAL HERBIVORY IN *ARABIDOPSIS THALIANA*

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Abstract.—Resistance and tolerance are widely viewed as two alternative adaptive responses to herbivory. However, the traits underlying resistance and tolerance remain largely unknown, as does the genetic architecture of herbivory responses and the prevalence of genetic trade-offs. To address these issues, we measured resistance and tolerance to natural apical meristem damage (AMD) by rabbits in a large field experiment with recombinant inbred lines (RILs) of *Arabidopsis thaliana* (developed from a cross between the Columbia × Landsberg *erecta* ecotypes). We also measured phenological and morphological traits hypothesized to underlie resistance and tolerance to AMD. Recombinant inbred lines differed significantly in resistance (the proportion of replicates within an RIL that resisted herbivory), and early flowering plants with tall apical inflorescences were more likely to experience damage. Tolerance (the difference in fitness between the damaged and undamaged states), also differed significantly among RILs, with some lines overcompensating for damage and producing more fruit in the damaged than undamaged state. Plastic increases in basal branch number, basal branch height, and senescence date in response to damage were all associated with greater tolerance. There was no evidence for a genetic trade-off between resistance and tolerance, an observation consistent with the underlying differences in associated morphological and phenological characters. Selection gradient analysis detected no evidence for direct selection on either resistance or tolerance in this experiment. However, a statistical model indicates that the pattern of selection on resistance depends strongly on the mean level of tolerance, and selection on tolerance depends strongly on the mean level of resistance. These observations are consistent with the hypothesis that selection may act to maintain resistance and tolerance at intermediate levels in spatially or temporally varying environments or those with varying herbivore populations.

Key words.—Apical meristem damage, *Arabidopsis thaliana*, herbivory, overcompensation, resistance, tolerance.

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Plants have evolved numerous traits in response to ubiquitous attacks by herbivores, pathogens, and other consumers. These traits broadly fall into two categories—resistance and tolerance. These classes of traits are thought to reduce the detrimental effects of consumer damage on fitness by distinct mechanisms: either by reducing the amount of damage suffered (resistance) or by reducing the fitness consequences of damage (tolerance). Intuitively, it would appear that plants would be either resistant or tolerant, but not both (van der Meijden et al. 1988; Herms and Mattson 1992; Mauricio 2000; Roy and Kirchner 2000): a plant that is maximally resistant would not benefit from tolerance. In like fashion, a plant or genotype that is maximally tolerant would seemingly never benefit from being resistant. In quantitative genetic terms, these arguments lead to two readily testable predictions: first, a negative genetic correlation should exist between resistance and tolerance, and second, the pattern of natural selection on resistance and tolerance should favor the maintenance of one, but not both, of these traits (Fineblum and Rausher 1995; Mauricio et al. 1997).

Despite the logical appeal of the argument that resistance and tolerance should be mutually exclusive characters, several investigations have not detected significant negative genetic correlations between them (e.g., Simms and Triplett 1994; Mauricio et al. 1997; Tiffin and Rausher 1999; Stinchcombe and Rausher 2002, but see Fineblum and Rausher 1995; Stowe 1998; Pilson 2000). Moreover, the evidence available to date suggests that correlational selection rarely acts to create negative genetic correlations between tolerance and resistance (Mauricio et al. 1997; Tiffin and Rausher 1999, but see Pilson 2000). Assuming that these studies are representative, the failure to detect pervasive genetic trade-offs between resistance and tolerance suggests that other evolutionary mechanisms must be important for maintaining both tolerance and resistance at the intermediate levels frequently observed in natural populations (Mauricio et al. 1997; Tiffin and Rausher 1999; Mauricio 2000; Pilson 2000; Stinchcombe 2002). Explaining the maintenance of intermediate levels of tolerance has been especially challenging, because theoretical models predict that tolerance should sweep to fixation if it is able to invade a population (Roy and Kirchner 2000; Tiffin 2000a), further suggesting that additional evolutionary mechanisms constrain the evolution of tolerance.

Costs of resistance (e.g., Bergelson and Purrington 1996; Mauricio 1998) and tolerance (Simms and Triplett 1994; Tiffin and Rausher 1999; Stinchcombe 2002) may be one such mechanism. Significant costs of resistance and tolerance traits may contribute to the maintenance of these traits at intermediate levels in plant populations via stabilizing selection (Simms and Rausher 1987; Tiffin and Rausher 1999). In addition, costs could create temporally or spatially fluctuating selection on resistance and tolerance, depending on the abundance of herbivore populations or other environmental factors that alter the relative costs and benefits of resistance and tolerance (Bergelson 1994; Abrahamson and Weis 1997; Tiffin and Rausher 1999; Stinchcombe 2002). Regardless of cost, tolerance traits may also be maintained by selection for functions unrelated to herbivory, such as resource competition or tolerance of other forms of meristem damage(Aarssen 1995; Aarssen and Irwin 1991; Tiffin 2002). For instance, developmental responses evolving in response to seasonally

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variable frost damage may also confer tolerance to herbivory (Aarssen and Irwin 1991).

The expression of resistance and tolerance involves variation in a range of developmental, morphological, and life-history traits. For instance, in rosette plants, apical dominance suppresses meristems in the rosette leaf axils, thereby ensuring a pool of quiescent meristems from which additional inflorescences can potentially be differentiated following damage. The number and production rate of axillary inflorescences is likely to strongly influence fitness (e.g., Juenger and Bergelson 2000; Juenger et al. 2000). Greater height of axillary inflorescences may also enhance fitness, if fecundity increases with inflorescence size or if developing fruits are actively photosynthetic and exposure of fruits to sunlight defrays the carbon cost of seed production (e.g., Bazzaz et al. 1979; Galen et al. 1993). In addition, delayed senescence would ensure that fruits on later-developing axillary inflorescences have time to mature. Understanding the mechanisms of tolerance and resistance, as well as additional functions of these traits, will clarify the evolutionary dynamics of responses to herbivory (Tiffin 2000b).

In the present paper, we investigate the joint evolution of resistance and tolerance to rabbit herbivory in the annual plant *Arabidopsis thaliana*. In particular, we sought to address several related questions: (1) Does a genetic trade-off exist between resistance and tolerance to rabbit herbivory in *A. thaliana*? (2) What are the ecological and developmental mechanisms underlying resistance and tolerance to rabbit herbivory? (3) Is tolerance to rabbit herbivory costly in the absence of damage? (4) What is the pattern of natural selection acting on resistance and tolerance in this species, and how sensitive are those patterns to the mean level of resistance and tolerance exhibited by the population? Our findings suggest that resistance and tolerance to rabbit herbivory are not mutually exclusive characters in *Arabidopsis thaliana*, and that these traits are complex characters related to a variety of size, phenological, and architectural traits. Although selection was not actively maintaining either trait in our study, a simple model suggests that selection is likely to act for or against resistance depending on the mean level of tolerance in the population, and for or against tolerance depending on the mean level of resistance in the population. In spatially or temporally varying environments or those with varying herbivore populations, selection similar to that observed here may act to maintain resistance and tolerance in *Arabidopsis* at intermediate levels.

Materials and Methods

Study Organism and Experimental Plantings

Arabidopsis thaliana (L.) Heynh. (Brassicaceae) is an annual plant originally native to Eurasia and now widely occurring in disturbed habitats in the United States (Baskin and Baskin 1983; Westerman and Lawrence 1970). Plants initially grow as a vegetative rosette, then bolt and produce a flowering inflorescence from the apical meristem. Branches may be differentiated both from meristems in the axils of cauline leaves on inflorescences and in the axils of rosette leaves. At bolting, the production of rosette leaves ceases, thereby setting the upper limit for the number of inflorescences that may be differentiated from the rosette. To distinguish branches initiated from different meristems, we refer to the inflorescence differentiated from the apical meristem as the ''apical inflorescence'' and to axillary branches on the main inflorescence as ''inflorescence branches.'' We refer to branches differentiated from axillary meristems in the rosette as ''basal branches.'' It is worth noting that *A. thaliana*'s architecture resembles that of the monocarpic rosette plants, *Gentianella* and *Ipomopsis*, the subjects of many previous studies on plant responses to herbivory and apical meristem damage (e.g., Paige and Whitham 1987; Lennartson et al. 1997; Juenger and Bergelson 2000). The similarity of architecture among these three species suggests that similar developmental and physiological mechanisms may underlie responses to damage, even if the evolutionary dynamics of these tolerance traits may vary between species with different life histories (i.e., annuals vs. perennials or herbaceous vs. woody plants; e.g., Haukioja and Koricheva 2000).

In the current study, we used recombinant inbred lines (RILs) of *A. thaliana* developed from a cross between the Landsberg *erecta* and Columbia ecotypes and advanced through single-seed descent to the F8 (Lister and Dean 1993). Several studies suggest that *A. thaliana* reproduces primarily through selfing in natural populations (Abbott and Gomes 1989). As a colonizing species, the genetic structure of natural populations is likely shaped by many successive generations of selfing in founder individuals punctuated by rare outcrossing events. Thus, the genetic structure of natural populations may well resemble those of recombinant inbred lines, such as those used here.

As part of a larger experiment mapping QTL for fitness in and phenotypic responses to different seasonal environments (Weinig et al. 2002; 2003a), we planted seedlings of these RILs into a plowed field at Brown University's Haffenreffer Reserve, Bristol, Rhode Island. Analyses of QTL and full details of the experimental design are reported elsewhere (Weinig et al. 2002). In brief, replicate seeds of 98 RILs were planted into each of thirty 98-cell plug trays. Seeds were sown on three consecutive days between the 3–5 of March 2000, and were then dark stratified at 4°C for 14 days to mimic the effects of overwintering. Following the stratification treatment, seeds were germinated in the Brown University greenhouses, and seedlings from each tray were transplanted into one of thirty randomized blocks at the field site in the order of sowing between April 5–7 ($n = 2940$). The timing of planting coincided with the developmental stage of plants growing in local populations. We measured both morphological and life-history traits, including rosette diameter at bolting, timing of reproduction (estimated as the number of days from planting to flowering), final height of the apical inflorescence at harvest (''apical inflorescence height''), number of basal branches, height of the tallest basal branch, and senescence date (defined as the date when the last flower senesced). Fruit number was used to estimate fitness. Because *Arabidopsis* reproduces primarily via self-fertilization, fruit production includes the fitness effects of male and female function. In addition, prior studies have shown that this character is highly correlated with seed number (Mauricio et al. 1997). We calculated relative fitness by dividing individual fruit set by the mean fruit set of the pop-

ulation. Plants that died from transplant shock (within seven days of transplanting) were scored as missing data; plants that survived transplanting and subsequently died before setting fruit were assigned a fitness value of zero and included in analyses. Initial analyses of fruit production show significant genetic variation among RILs in fitness (Weinig et al. 2003b).

Plants in the spring seasonal cohort began flowering on May 1. Within days after flowering, many plants experienced natural damage by herbivores, which removed some portion of the apical inflorescence. Based on frequent sightings of rabbits at the site, the presence of rabbit feces in the field, the observed rapid consumption of the entire inflorescence (which suggests insect damage was unlikely), and the absence of tracks that might indicate deer herbivory, we attribute this apical meristem damage (AMD) to rabbits. The damage occurred within a two-week period within the season and was recorded following this interval. Within the spring seasonal cohort, an average of 5 replicates within an RIL experienced damage, while 14 avoided damage. The difference between the average number of damaged and undamaged replicates within an RIL ($n = 19$) and the number originally planted ($n = 30$) results from transplant mortality and exclusion of lines whose damage status due to herbivory was unclear (e.g., were damaged before bolting).

Statistical Methods

Effects of herbivore damage on phenotypic traits.—We used PROC GLM of SAS (SAS 1999) to perform mixed model analysis of variance (ANOVA) with RIL as a random effects and damage class (presence or absence of AMD) and spatial block as fixed effects to evaluate the effect of AMD resulting on basal branch production, basal branch height, and senescence timing.

Genetic variation for resistance and tolerance.—To determine whether the experimental population exhibited genetic variation for resistance and tolerance to apical meristem damage (AMD), we used logit modeling and mixed-model ANOVA. For the logit modeling, we considered the presence or absence of AMD as a binary response variable (0 = no AMD, 1 = AMD) and spatial block and RIL as independent categorical variables. In these analyses, we interpreted a significant χ^2 statistic for RIL to indicate that the RILs differed in the proportion of individuals that suffered AMD—in other words, that there was significant genetic variation for resistance to AMD. We implemented this analysis using the PROBIT procedure in SAS (1999) with the ''D=LOGISTIC'' option.

To determine if there was genetic variation for tolerance to AMD, we used mixed-model ANOVA. In this ANOVA, we evaluated the effects on relative fitness of spatial block, RIL, AMD, and the AMD $\times$ RIL interaction, with RIL and AMD $\times$ RIL considered random effects and all other effects fixed. For this analysis, a significant AMD $\times$ RIL interaction indicates genetic variation for tolerance to AMD, that is, the effects of AMD on fitness differ by RIL. We implemented this analysis with the GLM procedure of SAS, using the RANDOM and TEST options to obtain appropriate expected mean squares for hypothesis testing.

Operational definitions of resistance and tolerance.—For each RIL, we operationally defined resistance to AMD as $(1 - p)$, where p is the proportion of individuals that suffered AMD (Simms and Triplett 1994; Fineblum and Rausher 1995; Tiffin and Rausher 1999). We operationally defined tolerance for each RIL as $(W_D - W_U)$, the difference between mean relative fitness of plants with AMD and those without AMD (Simms and Triplett 1994; Tiffin and Rausher 1999). Although some researchers (e.g., Fineblum and Rausher 1995; Juenger and Bergelson 2000; Agrawal 1999; Strauss and Agrawal 1999) have suggested defining tolerance as a ratio of relative fitness of damaged plants to undamaged plants (W_D/W_U), we chose to define tolerance as the difference in relative fitness ($W_D - W_U$) for several reasons. First, ($W_D - W_U$) is linear and defined over its entire range of possible values, while W_D/W_U can be both nonlinear and undefined. Second, this approach is conceptually similar to using a regression coefficient of fitness on damage for continuous measures of damage (e.g., Simms and Triplett 1994; Mauricio et al. 1997; Tiffin and Rausher 1999) because the difference between two means is equal to a slope if the difference between the two categories is 1 unit (Strauss and Agrawal 1999). Thus, categorical types of damage such as AMD are simply special cases of continuous forms of damage (Tiffin and Inouye 2000). Finally, because tolerance is simply plasticity in fitness in response to natural enemy damage (Abrahamson and Weis 1997), defining tolerance as $W_D - W_U$ is conceptually and computationally consistent with studies of phenotypic plasticity that often calculate plasticity as the difference between inbred line or family means between two treatment groups (Dudley and Schmitt 1996; Dorn et al. 2000).

Estimates of tolerance, calculated by either method, may be biased in studies using natural levels of damage if families differ in their degree of resistance to damage, and resistance and tolerance are genetically correlated (e.g., Fineblum and Rausher 1995; Stowe 1998). Our estimates of tolerance are unlikely to be biased for this reason, because as described below, resistance and tolerance to AMD were genetically uncorrelated in this experiment. In addition, taking advantage of naturally occurring herbivory allows for greater power in detecting costs of tolerance, genetic correlations between tolerance and other traits, and the pattern of natural selection acting on tolerance (Tiffin and Inouye 2000).

Genetic correlations.—After confirming that the experimental population exhibited significant genetic variation for resistance and tolerance, we evaluated whether resistance and tolerance were genetically correlated with each other, and with other traits that we hypothesized might be mechanistically related to our operationally defined measures of resistance and tolerance. For resistance, we evaluated the significance of RIL mean correlations between resistance and two measures of plant size, rosette diameter and apical inflorescence height, and one measure of phenology, flowering date. Because herbivory eliminated the apical inflorescence, we estimated the apical inflorescence height only from the plants in each RIL that escaped herbivory.

To determine whether operationally defined tolerance was genetically correlated with putative tolerance traits we utilized a slightly different approach. Because tolerance is the

plastic response of fitness to herbivory (Abrahamson and Weis 1997), we evaluated whether operationally defined tolerance was genetically correlated with the plastic responses of other traits in response to herbivory. Based upon field observations, we hypothesized that the plastic response of basal branch production, basal branch height, and senescence date to herbivory might contribute to an RIL's ability to tolerate AMD. We calculated plasticity to herbivory for these traits as the mean values of damaged individuals minus the mean values of these traits in the undamaged individuals for each RIL. We then evaluated the correlation between genotypic tolerance and plasticity of basal branch production, basal branch height, and senescence date.

Correlations between tolerance and resistance and fitness in the absence of damage.—Because we did not have an herbivore exclusion treatment, we did not formally evaluate whether resistance was costly (e.g., Bergelson and Purrington 1996; Mauricio 1998). However, because this limitation does not apply to analyses of costs of tolerance (e.g., Mauricio et al. 1997; Tiffin and Rausher 1999; Mauricio 2000), we evaluated two alternative hypotheses about the relationship between tolerance and fitness in the absence of damage. On the one hand, it is possible that tolerance is costly (e.g., Simms and Triplett 1994; Tiffin and Rausher 1999; Stinchcombe 2002). We tested this hypothesis by evaluating the RIL-mean correlation between tolerance and fitness of undamaged plants. The logic behind this test is simple: in the absence of herbivory, only the costs of tolerance will be manifested, but not the benefits. Therefore, if tolerance is costly, a negative genetic covariance should exist between these traits and fitness in the absence of damage. On the other hand, it is possible that tolerance is a function of general vigor (Futuyma and Phillipi 1987; Weis et al. 2000). If this were the case, one would predict that RILs that had higher fitness in the absence of damage would also be more tolerant, and thus a positive genetic covariance would exist between tolerance and fitness in the absence of damage.

Because tolerance is defined as the fitness of damaged plants minus the fitness of undamaged plants, a straightforward analysis of the correlation between tolerance and fitness in the absence of damage will be biased. The source of this bias is an artifactual covariance introduced by using the same data to estimate tolerance and fitness of undamaged plants (Tiffin and Rausher 1999). We calculated this artifactual covariance according to the methods described by Tiffin and Rausher (1999) and subtracted it from the estimated covariance to obtain a corrected, bias-free estimate of the covariance between tolerance and fitness in the absence of damage. We then used standard jackknifing techniques and a two-tailed *t*-statistic to determine if the 95% confidence limit for the corrected covariance included zero.

Selection analyses.—To evaluate the pattern of selection acting on resistance and tolerance in the experimental population, we used Rausher's (1992) genotypic selection analysis, following standard methods (e.g., Mauricio et al. 1997; Tiffin and Rausher 1999; Stinchcombe and Rausher 2002). This procedure consists of regressing mean relative fitness for an inbred line or family on the line or family mean of the trait of interest. Selection analyses using this approach have two distinct advantages over the typical Lande and Arnold (1983) approach, which uses phenotypic values: first, the estimated selection gradients are unbiased by environmental covariances between traits and fitness (Rausher 1992; Mauricio and Mojonnier 1997; Stinchcombe et al. 2002), and second, it is applicable for traits such as tolerance or plasticities that cannot be measured on a single individual (Stinchcombe et al. 2002).

We performed a joint analysis of selection acting on resistance and tolerance, and the genetically correlated traits we identified. This selection model regressed relative fitness on seven traits: resistance to AMD, tolerance to AMD, apical inflorescence height, flowering date, and plasticity of basal branch production, basal branch height, and senescence timing in response to herbivory. Apical inflorescence height and flowering date were included in the analysis because they were genetically correlated with resistance to AMD, while plasticity in basal branch production, basal branch height, and senescence date in response to herbivory were included because they were correlated with tolerance to AMD (see below). All independent variables were standardized to a mean of zero and a variance of one. We detected no evidence for stabilizing, disruptive, or correlational selection, and therefore only present results of analyses of directional selection. Finally, the L*er* parent segregates for an induced mutation (*ERECTA*) that segregates only in laboratory strains. To control for the possible fitness effects of this locus, we performed an ANCOVA that included *ERECTA* as a main effect and all quantitative traits originally included in the genotypic selection model as covariates. We failed to detect a main effect of *ERECTA* ($P = 0.23$) or any difference in the estimates of selection on the quantitative traits between the two models (data not shown); these results are supported by QTL mapping results in which the ERECTA had no effect on fitness (Weinig et al. 2003b). Accordingly, we present only the results of the genotypic selection analysis without the *ERECTA* main effect (see Results below).

Selection functions for resistance and tolerance.—In many cases it is of interest to determine how selection would act on the observed variation in tolerance over a broad range of mean levels of resistance, and how selection would act on the observed variation in resistance over a broad range of mean levels of tolerance. For example, if the herbivore population had been larger, the mean level of damage experienced by the experimental population might have been higher and, in similar fashion, the mean level of tolerance exhibited by the population might have been higher had the plants been grown in a more nutrient-rich environment. To assess the implications of these effects on our results and to evaluate how selection would act on resistance and tolerance under alternative hypothetical scenarios, we implemented the following statistical modeling approach. In doing so, we assume that resistance and tolerance are genetically uncorrelated (see below). First, consider that the mean fitness of any inbred line can be represented by equation 1 (Tiffin and Rausher 1999): $\bar{w} = (p) \times (W_D) + (1 - p) \times (W_U)$, where $\bar{w}$ is mean fitness of a line, W_D is mean fitness when damaged, W_U is mean fitness when undamaged, p is the proportion of individuals damaged, and $1 - p$ is the proportion of undamaged individuals in that line. To evaluate how selection would act on observed levels of tolerance for varying levels of resis-

TABLE 1. Mixed-model ANOVA for fitness (log-transformed) indicating significant genetic variation for tolerance to AMD. RIL and RIL × AMD are random effects, all other effects are fixed.

Source	df	Type III SS	*F*	*P*
Block[a]	29	815.99	65.12	<0.0001
RIL[b]	95	98.44	1.64	0.0085
AMD[b]	1	0.23	0.41	0.52
RIL × AMD[a]	95	59.92	1.46	0.003
Error	1978	854.65		

[a] Tested over mean square error.
[b] Tested over synthetic denominator including RIL × AMD and mean square error.

tance, we simply calculated adjusted values of fitness by assigning all RILs a new value for p but not altering our empirical estimates of W_D and W_U for each RIL. We then calculated selection gradients on tolerance for each of these adjusted relative fitness values to determine how selection would act on tolerance if the population had been fixed at resistance levels we had chosen. We then repeated these analyses, using adjusted relative fitness values calculated from equation 1 by varying p from zero to 1 in units of 0.04. Although zero and 1 are beyond the lower and upper limits for our empirical estimates of the RIL means for p, they are within the range of observed phenotypic values and represent biologically important cases of complete resistance and complete susceptibility. In the selection analyses using adjusted relative fitness values, we also included other traits that we had determined were genetically correlated with tolerance. To analyze the pattern of selection on resistance for various levels of tolerance, we used a similar approach. Because tolerance is defined as $W_D - W_U$, one can substitute (W_U + Tolerance) for W_D in equation (1), and after some simplification, solve for equation 2: $\bar{w} = W_U + (p) \times (Tolerance)$, where $\bar{w}$ is mean fitness of the entire line, W_U is mean fitness in the absence of damage, p is the proportion of individuals damaged in a family, and *Tolerance* is calculated as described above. In this case, we calculated adjusted relative fitness values by choosing a fixed value for the *Tolerance* term, but not altering our empirical estimates of W_U and p for each RIL. We then calculated selection gradients on resistance for each of these adjusted relative fitness values to determine how the pattern of selection on resistance would change as a function of fixed values of tolerance. Once again, we repeated these analyses, using adjusted relative fitness values calculated from equation 2 by varying the *Tolerance* term from −2 to 2 in units of 0.04; −2 and 2 are slightly beyond the observed lower and upper limits for our empirical estimates of tolerance, and were chosen to represent scenarios in which a population was fixed at either extreme of the distribution of tolerance values. As before, in the selection analyses using the adjusted relative fitness values we also included other traits that we had determined were genetically correlated with resistance.

RESULTS

Effects of AMD on phenotypic traits.—Apical meristem damage significantly increased basal branch production, the average height of basal branches, and the time to senescence.

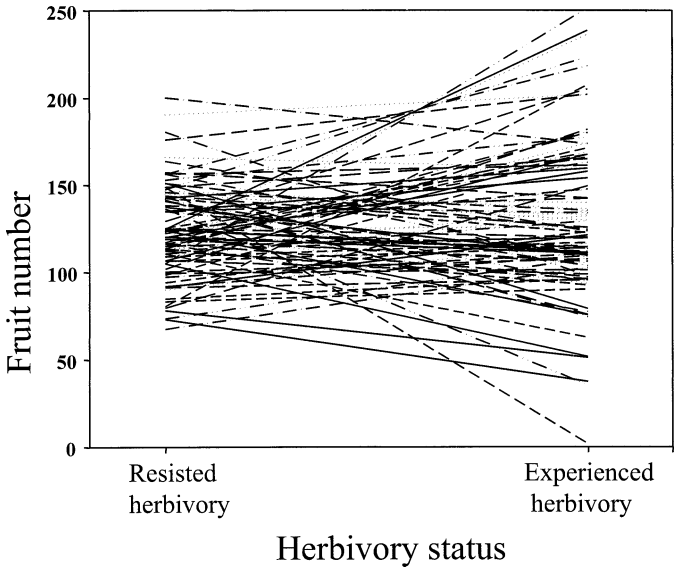

FIG. 1. Effect of herbivory on fruit production. Different lines denote RIL means within the damaged and undamaged states.

On average, plants with AMD had 3.1 (±0.09, 1 SE) basal branches compared with 1.3 (±0.05) inflorescences in plants resisting AMD ($F_{1,2080} = 235.96$, $P < 0.0001$). The height of basal branches averaged 13.38 (±0.36) cm in damaged plants, but only 10.67 cm (±0.26) in undamaged plants ($F_{1,939} = 38.49$, $P < 0.0001$). Senescence was also delayed by 2.6 days on average in plants with AMD relative to those that resisted AMD (172.8 ± 0.47 vs. 170.2 ± 0.23 days; $F_{1,2081} = 21.89$, $P < 0.0001$).

Genetic variation for resistance and tolerance to damage.—The experimental population exhibited significant genetic variation for resistance to AMD. The logit analysis indicated a significant effect of RIL on whether or not a plant received AMD in the Rhode Island spring cohort ($\chi^2 = 195.03$, df = 95, $P < 0.0001$). The RILs also exhibited significant genetic variation for tolerance, or the effects of AMD on fitness, as indicated by a significant AMD × RIL interaction for fitness ($F_{95, 1978} = 1.46$, $P = 0.003$; Table 1; Fig. 1). In addition, the ANOVA results indicate that a positive genetic correlation between fitness in the undamaged and damaged states existed in our experimental population (i.e., significance of RIL when tested over RIL × AMD in Table 1; (Fry 1992).

Resistance and tolerance.—Resistance to AMD varied from 0.35 to 0.96 for the RILs, with a mean resistance of 0.78. Operationally defined tolerance for the RILs ranged from incomplete tolerance (damaged plants having lower relative fitness than undamaged plants) to overcompensation (damaged plants having higher relative fitness than undamaged plants) (Fig. 1). Mean tolerance for the experimental population (i.e., the genotypic difference in fruit production between plants experiencing and resisting herbivory) was 0.36, indicating slight overcompensation overall.

Genetic correlations.—Although resistance and tolerance to AMD were not significantly genetically correlated with each other ($r = -0.13$, $P = 0.22$; jackknifed 95% confidence limits = −0.31, 0.05), as estimated by the correlation of RIL means, each trait was correlated with a variety of other size,

TABLE 2. Regression analysis of selection on resistance and tolerance to AMD and correlated traits. Traits were standardized to a mean of zero and variance of one prior to analysis. Estimates from joint analysis are from a single, multiple regression that included all terms; significant effects shown in bold.

Trait	β′	Standard error	*P*-value
Tolerance	0.057	0.035	0.11
Resistance	0.024	0.044	0.59
Flowering date	0.028	0.025	0.27
Apical inflorescence height	**0.083**	**0.025**	**0.0015**
Plasticity of Basal Branch Production	−0.023	0.031	0.46
Plasticity of Basal Branch Height	−0.046	0.028	0.11
Plasticity of Senescence Date	−0.007	0.009	0.44

phenological, or plastic traits in response to herbivory. For instance, resistance to AMD was genetically correlated with both apical inflorescence height of the undamaged plants ($r = -0.34$, $P = 0.002$; 95% CL = −0.52, −0.15) and with flowering date ($r = 0.54$. $P < 0.0001$; 95% CL = 0.37, 0.72), but not with rosette diameter ($r = -0.07$, $P = 0.47$; 95%CL = −0.25, 0.11). These data suggest that early flowering, tall RILs were more susceptible to AMD, and that herbivores did not select plants on the basis of rosette size.

Tolerance to AMD, or plasticity in fitness to AMD, was also significantly correlated with plasticity of several other characters to AMD: basal branch production ($r = 0.56$, $P < 0.0001$; 95% CL = 0.41, 0.72), basal branch height ($r = 0.64$, $P < 0.0001$; 95% CL = 0.50, 0.78), senescence date ($r = 0.31$, $P = 0.002$; 95% CL = 0.06, 0.58). These data suggest that tolerance to AMD was, in part, associated with the activation and production of additional inflorescence branches, taller inflorescences, and an extension of lifespan (i.e., delayed senescence).

Correlations between tolerance and fitness in the absence of damage.—Our analyses of the correlations between tolerance to AMD and fitness in the absence of damage provide little evidence of either a fitness cost to tolerance or for the hypothesis that general vigor underlies these traits. The corrected covariance between tolerance to AMD and fitness in the absence of damage was less than zero suggesting a cost of tolerance (corrected covariance = −0.019. However, the 95% confidence limits of this corrected covariance (−0.043, 0.006) barely included zero. Thus, these data provide only equivocal support for the hypothesis that tolerance is costly in the absence of damage, but do reject the hypothesis that tolerance is simply a function of general vigor (which predicts a positive correlation between tolerance and fitness).

Selection analyses.—The joint analysis of selection indicated significant directional selection to increase apical meristem height (Table 2). In this analysis, we also detected a nonsignificant trend for selection to increase tolerance to AMD and plasticity in basal branch height ($P = 0.11$ for each), but no detectable selection on resistance, flowering date, and plasticity of basal branches or senescence date ($P > 0.27$ for each term).

Selection functions for resistance and tolerance.—Selection functions for resistance and tolerance are presented in Figure 2a and b. In general, our results largely agree with the two locus-two allele model presented by Abrahamson and Weis (1997): as either resistance or tolerance evolves towards higher levels, the strength of selection on the other trait declines. However, in contrast to Abrahamson and Weis's (1997) model, our results suggest that at high levels of tolerance selection acts against resistance and at high levels of resistance selection acts against tolerance. It is likely that the pattern of selection against resistance at high levels of tolerance is due to overcompensation, and that selection against tolerance at high levels of resistance is due to costs of tolerance.

Selection on resistance is clearly a function of the mean value of tolerance in the population. Significant directional selection acts to increase resistance for all values of incomplete tolerance (i.e., for $W_D < W_U$; all points to the left of zero on the x-axis in Fig. 2b). As the mean tolerance declines, the strength of selection on resistance increases, that is, the more detrimental AMD is for fitness, the stronger the selection to increase resistance to AMD. Selection also acts to significantly increase resistance at complete tolerance (zero on the x-axis in Fig. 2b), and is positive but nonsignificant for values of slight overcompensation. As tolerance values increase, selection on resistance becomes negative, although nonsignificant. When overcompensation becomes more pronounced and extreme (more than three times the population mean tolerance of our study population), selection acts to significantly decrease resistance: when damage appreciably enhances fitness, selection acts against traits that reduce the likelihood of damage.

In like fashion, selection on tolerance is a function of the mean value of resistance in the population (Fig. 2a). For mean resistance values of less than approximately 0.74, or when 26% or more of the individuals are damaged by herbivores, selection acts to increase tolerance, thus reducing the fitness effects of herbivory. For a range of resistance values (between 0.74–0.94), there is no significant selection on tolerance to AMD, although in this range of resistance values selection on tolerance shifts from positive to negative. Finally, as resistance values increase to the point where few plants are receiving damage (6% or fewer), selection acts to significantly decrease tolerance to AMD, presumably because of costs of tolerance. These findings suggest that the marginally significant cost of tolerance detected by the jackknifing method was in fact biologically significant.

The observed means for resistance and tolerance are also presented on Fig. 2a and 2b. Both of these means fall in the range of values where selection acting on resistance and tolerance is not significant. Thus, the RILs are resistant enough on average that there is no selection for increased tolerance, but not so resistant that selection would act against tolerance. Similarly, the lines are on average tolerant enough of her-

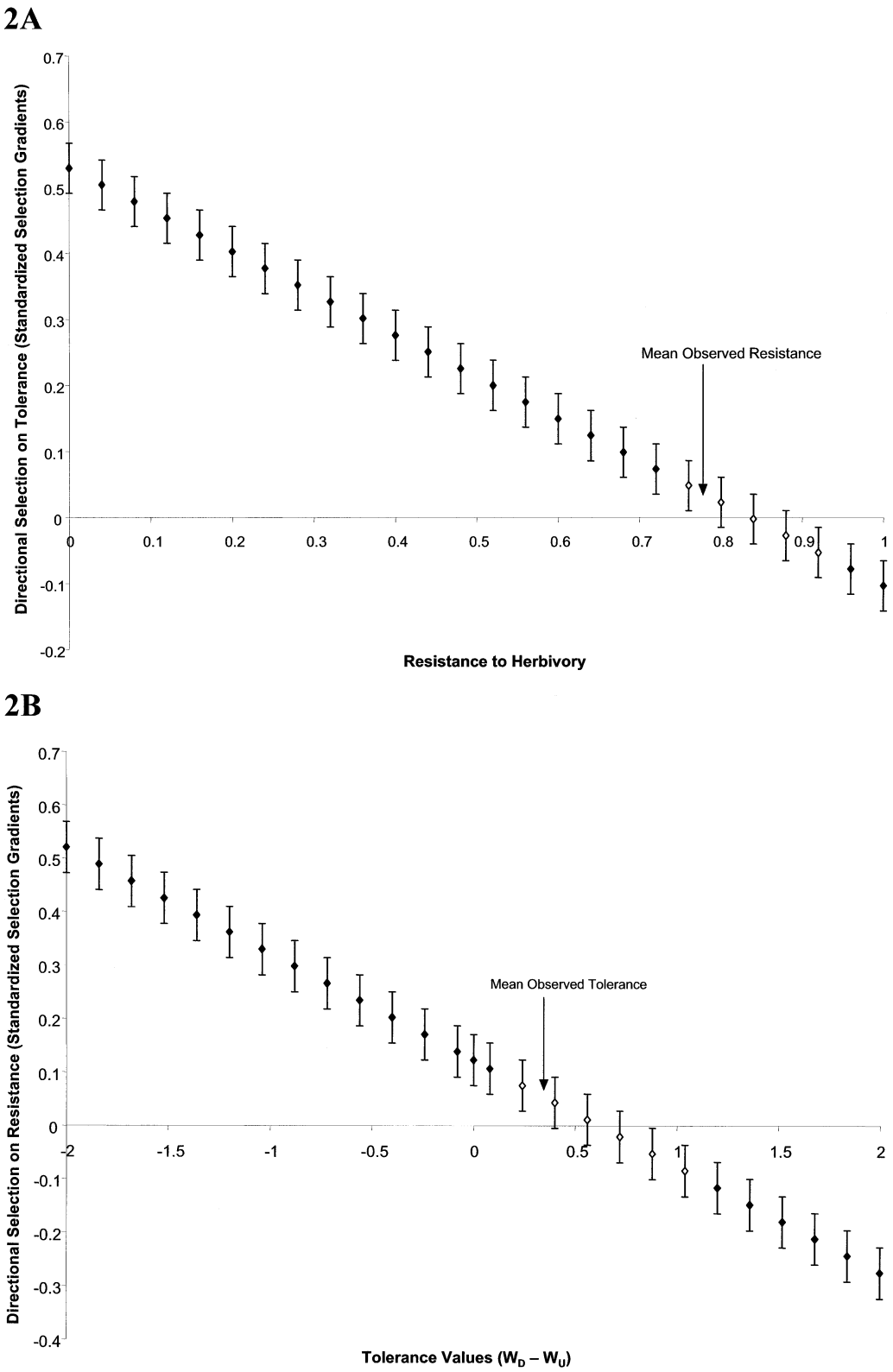

FIG. 2. (A) The strength of directional selection on empirically observed tolerance as a function of fixed values of resistance. Adjusted relative fitness values for selection analyses were calculated according to equation (1). (B) The strength of directional selection on empirically observed resistance as a function of fixed values of tolerance. Adjusted relative fitness values for selection analyses were calculated according to equation (2). Filled diamonds indicate statistically significant selection gradients, bars represent ± 1 SE of the estimated selection gradient.

bivory that there is no selection to increase resistance, but not overcompensating for herbivory enough to create selection to decrease resistance. However, there were many RILs with resistance and tolerance values that were within the ranges that would have produced significant directional selection on both resistance and tolerance.

Discussion

Maintenance of Tolerance and Resistance at Intermediate Levels

Despite the intuitive nature of the prediction that plants should be either resistant or tolerant, but not both, we find no evidence in support of this intuition. For instance, we failed to detect significant trade-offs between resistance and tolerance to herbivore damage, an observation consistent with other field studies (e.g., Simms and Triplett 1994; Mauricio et al. 1997; Tiffin and Rausher 1999; Stinchcombe and Rausher 2002). In addition, we detected no evidence of ''disruptive'' correlational selection on resistance and tolerance that would maintain only one of these traits. Our data support the conclusion that tolerance and resistance are alternative, but not mutually exclusive, strategies by which plants respond to damage by herbivores (e.g., Rosenthal and Kotanen 1994; Mauricio et al. 1997).

Our results also support a plurality of potential mechanisms that could lead to the maintenance of both tolerance and resistance at intermediate levels. First, both resistance and tolerance were genetically correlated with a number of quantitative traits (or their plasticities in the case of tolerance). Natural selection on any of these correlated traits could lead to the maintenance of resistance and tolerance at intermediate levels. For example, the observed positive directional selection on apical inflorescence height could potentially lead to a correlated response that would reduce resistance to AMD. In contrast, any selection to favor later flowering (i.e., at a larger size), would potentially lead to a correlated response that would increase resistance to AMD. Furthermore, as described below, the traits underlying resistance and tolerance to rabbit herbivory in *A. thaliana* may themselves be under selection for different ecological functions. Second, we detected weak but suggestive evidence for a physiological cost of tolerance. Although our evidence for physiological costs of tolerance is tentative, such costs of tolerance, when combined with spatial and temporal fluctuations in herbivore populations, could interact to produce a fluctuating pattern of selection for and against tolerance to herbivory (Tiffin and Rausher 1999). Finally, our statistical model illustrates how the pattern of natural selection on resistance and tolerance can shift between positive directional selection, nonsignificant selection, and negative directional selection depending on the mean value of the other trait.

Mechanisms of Resistance

Resistance is commonly defined operationally, that is, plants with low levels of herbivory are more resistant than plants with higher levels of herbivory (Simms and Rausher 1987). Mechanisms underlying resistance include traits that reduce the feeding rates or performance of herbivores, including morphological traits such as trichomes (Mauricio et al. 1997) or biochemical attributes such as concentration of tannins (Feeny et al. 1970), furanocoumarins (e.g., Berenbaum 1983), or glucosinolates (e.g., Mauricio et al. 1997; Mauricio 1998). We found that operationally defined resistance to AMD in *Arabidopsis* was positively genetically correlated with both flowering time and apical inflorescence height. It is likely that these traits reduced the likelihood that an herbivore would feed on a plant, rather than being traits that directly reduced the amount of tissue consumed by an herbivore once it had begun feeding (i.e., avoidance rather than antibiosis resistance (Tiffin 2000b). For a species like *A. thaliana*, visibility or apparency (Feeny 1976) may be one of the most important determinants of herbivory, because *A. thaliana* is one of the earliest species to flower and elongate inflorescences in the disturbed sites it inhabits (C. Weinig, pers. obs.). Given that these traits affect herbivory, exhibit genetic variation, and can potentially respond to selection imposed by herbivores, it seems reasonable to consider them as resistance traits. Nevertheless, it is likely that both flowering time and apical inflorescence height in *A. thaliana* are under selection for reasons other than resistance to herbivory.

Mechanisms of Tolerance and Overcompensation

An initial observation of overcompensation was made over a decade ago (Paige and Whitham 1987), prompting considerable debate regarding both methods of evaluating responses to herbivory and whether overcompensation is indicative of plant-herbivore mutualisms (e.g., Bergelson and Crawley 1992; Belsky et al. 1993; Paige 1994, 1999; Bergelson et al. 1996; Agrawal 2000). Although it is beyond the scope of this study to review the entire overcompensation controversy, our data raise several issues bearing on this debate.

Our results are consistent with reports of overcompensation from field studies of three other plant species (*Ipomopsis aggregata* [Paige and Whitham 1987], *Gentianella campestris* [Lennartsson et al. 2000], and *Erysimum strictum* [Huhta et al. 2000]), from three families (Polemoniaceae, Gentianaceae, and Brassicaceae, respectively). These observations collectively suggest a consistent pattern: overcompensation appears to be triggered by real or simulated mammalian herbivory and release from apical dominance. All of these species are monocarps; all form basal rosettes and then activate a single, apically dominant inflorescence. In each case, overcompensation appears related to the activation of axillary meristems after the loss of apical dominance. This mechanism may often be involved in responses to apical damage in other plant species with similar life histories.

In our study, tolerance of *A. thaliana* to rabbit herbivory was related to plasticity of basal branch production in response to apical meristem damage (that is, release of apical dominance). Such tolerance mechanisms may evolve in response to predictable selective agents other than herbivory (e.g., frost or fire damage; Rosenthal and Kotanen 1994; Aarssen 1995). In a related experiment with the same RILs planted in November 1999 in North Carolina (Weinig et al. 2002; 2003b), we observed little herbivore damage, but a large number of plants lost their apical inflorescence to frost during an atypical winter snow storm. Frost AMD had the

same phenotypic effects as that produced by rabbits in the present study: frost-damaged plants produced more basal branches, taller basal branches, lived longer, and produced more fruits (C. Weinig and J. Schmitt, unpubl. data). These parallel responses to herbivory and frost damage are consistent with the hypothesis that tolerance to apical meristem damage may evolve as a generalized response to inflorescence damage in *A. thaliana*. We note, however, that tolerance to damage to different organs could potentially have to have different developmental, physiological, and genetic mechanisms even in the same species (e.g., tolerance to leaf damage in *Arabidopsis*; Mauricio et al. 1997).

The hypothesis that apical dominance is the mechanism underlying tolerance suggests some predictions about the ecological constraints on expression of overcompensation. First, tolerance to AMD and the potential for overcompensation will be reduced in low resource environments (e.g., Maschinski and Whitham 1989; Huhta et al. 2000) or in environments with short growing seasons where fecundity is not meristem limited (e.g., Geber 1990). For example, if resources are not available for branch production or if a killing frost prevents delayed senescence, release of apical dominance may not translate into increased fecundity. Second, tolerance and overcompensation may be less likely in environments where the potential benefit of apical dominance is high, especially in the absence of herbivory; for example, in highly competitive environments where apical dominance and greater stem elongation contribute to shade avoidance (e.g., Schmitt et al. 1995; Dudley and Schmitt 1996; Weinig 2000), or when fecundity is pollinator limited and pollinator visitation is an increasing function of plant height (e.g., Donelly et al. 1998). Thus, identifying the developmental mechanisms for tolerance and overcompensation to herbivory can clarify ecological constraints on their expression and evolution.

As the RILs used in our experiment were produced from a cross between individuals from two geographically distinct populations, our finding of overcompensation should be viewed with some caution. For instance, we may have observed overcompensation because the RILs segregated more genetic variation for fitness than do typical natural populations of *A. thaliana*. In any case, the variation in our RILs is only a small sample of the range of natural variation expressed by accessions from natural populations (Botto and Smith 2002; Nordborg and Bergelson 1999), and the observation of overcompensation in this study demonstrates the genetic and physiological potential for this response in *A. thaliana*.

Theoretical models usually require a trade-off between fitness in the undamaged and damaged states for overcompensation to evolve (e.g., Vail 1992, 1994; Matthews 1994; Nilsson et al. 1996), a condition supported in part by empirical data (e.g., Simons and Johnston 1999; Juenger et al. 2000). As such, overcompensation has been viewed as an adaptation to a predictable and reliable occurrence of herbivore damage that imposes a fitness cost in the undamaged state (Juenger et al. 2000). Our data, however, do not support this hypothesis: fitness in the undamaged state was positively correlated with fitness in the damaged state (cf. RIL term in Table 1). This discrepancy might exist, in part, because of the restrictive nature of the theoretical models. In these models, allocation to reproduction falls into two discrete categories: either before or after damage, and meristems or buds allocated to postdamage reproduction are unavailable or unusable in the undamaged state. In some species, including *A. thaliana*, it is likely that each plant has a finite number of meristems, some of which will be activated to produce basal branches even in the absence of AMD. In this scenario, AMD probably serves to accelerate the activation of these meristems, increasing the chances that fruit and seeds will be set on these branches before the end of the growing season.

Conclusions

In this study we identified several traits that underlie either the expression of both resistance and tolerance to inflorescence damage in *A. thaliana*. The dissimilarity of these underlying traits (e.g., flowering time vs. basal branch number) is consistent with the hypothesis that resistance and tolerance are distinct, but not mutually exclusive, mechanisms of response to herbivory. The absence of significant, negative genetic correlations between these two traits further suggests that these two traits may evolve independently (e.g., Mauricio 1997). However, the evolution of resistance and tolerance may depend on the frequency and predictability of herbivory, as well as the genetic make-up and expression of resistance or tolerance in the focal plant population. An interesting avenue for further investigation is how selection resulting from herbivory operates in the face of secondary selective agents targeting resistance and tolerance traits.

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