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GENETICS OF *BRASSICA RAPA*. 3. COSTS OF DISEASE RESISTANCE TO THREE FUNGAL PATHOGENS

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Abstract.—Genetic costs of resistance to pathogens may be an important factor maintaining heritable variation for resistance in natural populations. Pleiotropic fitness trade-offs occur when genetic resistance causes reduction in other components of fitness. Although costs of resistance have an important influence on plant-pathogen interactions, few previous studies have detected pleiotropic costs of resistance in the absence of confounding effects of linkage disequilibrium. To avoid this potential problem, we performed artificial selection experiments on resistance to two fungal pathogens, *Leptosphaeria maculans*, and *Peronospora parasitica*, and compared growth rates of resistant and susceptible genotypes of *Brassica rapa* in the absence of pathogens. *Leptosphaeria* resistance had no effect on growth rate, indicating cost-free defense. In contrast, *Peronospora*-resistant genotypes grow 6% slower than *Peronospora*-susceptible genotypes in pathogen-free environments, indicating a significant genetic fitness cost to *Peronospora* resistance. Such genetic trade-offs could maintain genetic variation in the wild. Another factor that might explain heritable variation for resistance is ecological trade-offs, in which genetic resistance to one species causes susceptibility to another. Such ecological trade-offs do not exist for the pathogens studied in this system.

Key words.—*Brassica rapa*, disease resistance, fungal pathogens, *Leptosphaeria maculans*, *Peronospora parasitica*.

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Studies of resistance to insects and pathogens in natural plant populations often reveal significant genetic variation for resistance (Fritz and Price 1988; Karban 1992; Marquis 1990). What genetic and ecological factors may be responsible for these genetic differences?

Persistent genetic variation in natural populations may be due to genetic trade-offs, which can arise in several ways: (1) Fitness trade-offs occur when genetic resistance causes reduction in other components of fitness. This may be due to allocation costs, reflecting physiological costs of plant defense (Simms 1992; Simms and Rausher 1987), or deleterious side effects on other physiological pathways (Lenski 1988a). In practice, these two distinct mechanisms cannot be distinguished without information at the biochemical level; thus, hereafter we discuss them together as fitness costs. (2) Pleiotropy may cause ecological trade-offs if defensive mechanisms affect different species in different ways, causing trade-offs among resistance to different pathogens (Fritz 1992; Maddox and Root 1990; Nakamura et al. 1995; Simms 1992). Alternatively, (3) tightly linked resistance genes, which are known from several plant-pathogen systems (Dickinson et al. 1993; Martin et al. 1993) may cause genetic correlations seen as ecological trade-offs. Moreover, (4) genotype-by-environment interaction can maintain heritable variation if genotypes' levels of resistance change rank between environments (Gillespie and Turelli 1989; Maddox and Cappuccino 1986; Siemens and Mitchell-Olds, unpubl. data).

In addition, the observed levels of heritable variation for resistance may be attributable to patterns of natural selection in the wild: (5) Resistance might be selectively neutral, having no effect on fitness (Nakamura et al. 1995). Alternatively, (6) weak stabilizing selection (Burger and Lande 1994) or (7) disruptive selection (Siemens and Mitchell-Olds, unpubl. data) may permit genetic segregation of resistance in the wild. Finally, (8) genetic variation may represent a transient polymorphism on its way to fixation (Parker 1992).

Here we focus on fitness trade-offs, due to either allocation costs or deleterious side effects. Genes conferring resistance can have pleiotropic effects on other aspects of plant performance (Simms 1992). For example, if resistant plants have reduced growth or fecundity in the absence of pathogen attack, then selection may favor intermediate levels of disease resistance. When allocation to constitutive resistance mechanisms varies among genotypes, then resistant genotypes may experience a fitness benefit in the presence of a pathogen, relative to susceptible plants. However, resistant plants would have reduced relative fitness when pathogen attack is reduced or absent, because resources would be expended on unneeded defensive mechanisms. For constitutive defense pathways, a genetic cost to resistance is indicated by a significant negative genetic correlation between growth or fitness in a pathogen-free environment and resistance measured in the presence of pathogens.

Few published studies provide clear evidence for pleiotropy causing genetic fitness costs. Berenbaum et al. (1986) showed a negative genetic correlation between insect resistance and fecundity in *Pastinaca sativa*. Han and Lincoln (1994) found a significant negative genetic correlation between growth rate and levels of secondary metabolites in *Diplacus aurantiacus*. In *Escherichia coli*, mutations causing resistance to virus T4 carry pleiotropic costs of resistance, which can be ameliorated by epistatic modifiers (Lenski 1988a,b). Several studies from habitually inbreeding species or near-isogenic lines are consistent with the existence of pleiotropic fitness costs (Bergelson 1994; Brinkman and Frey 1977; Burdon and Muller 1987; Chaplin 1970; Frey and Browning 1971; Simons 1979), but linkage disequilibrium and pleiotropy are confounded in these experiments. Additional evidence from ecological and physiological studies are also suggestive of genetic costs of resistance (reviewed in Simms 1992). To date, no studies have examined pleiotropic costs of resistance to microbial pathogens in the absence of

linkage disequilibrium. Here we ask whether there are pleiotropic fitness costs of resistance to three fungal pathogens in the host plant, *Brassica rapa*.

MATERIALS AND METHODS

Mitchell-Olds et al. (1995) selected for multiple disease resistance and susceptibility to three fungal pathogens, and observed rapid genetic changes in levels of resistance. Here we describe the species and results of that experiment, which provided the starting populations for the current research.

White rust (*Albugo candida* Pers ex. Hook., Oomycetes, Albuginaceae) is an obligately biotrophic intercellular parasite that attacks the leaves, stems, and flowers of many crucifers. Strain AC-2 of *A. candida* was grown on *Brassica juncea* (L.) Cosson cultivar Southern Giant Curled Mustard, zoosporangia were collected and stored frozen, and inoculum prepared as described in Williams (1985). Downy mildew (*P. parasitica* Pers ex. Fr., Oomycetes, *Peronosporaceae*) is an obligate biotroph that infects the leaves, stems, roots, and flowers of numerous cultivated and wild species of Cruciferae (Williams 1985). Blackleg disease is caused by *Leptosphaeria maculans* (Desm.) Ces. and de Not. (Loculoascomycetes, Pleosporales), a facultatively saprophytic, necrotrophic pathogen that attacks many crucifers, especially in the genus *Brassica* (Gabrielson 1983). In the selection experiments, levels of disease severity were scored on a 0 to 9 scale for each pathogen, with 0 indicating no disease symptoms on inoculated cotyledons, and 9 indicating large lesions (> 3 mm) with fungal sporulation. Levels of disease were quantitative traits showing continuously variable levels of disease, rather than "all-or-none" resistance or susceptibility. The selection experiments had four treatments: selection to decrease susceptibility to *A. candida*, to *P. parasitica*, or to *L. maculans*, plus a random-selected susceptible control that experienced similar levels of selection and inbreeding.

An open-pollinated, genetically polymorphic population of rapid cycling *B. rapa* (Williams 1985) was used as the host species. *Brassica rapa* plants were maintained in a growth chamber with constant illumination at a density of 500 plants m^{-2} in 30 × 60 cm plastic flats filled with commercial potting soil (Jiffy mix). Seedlings were maintained at 24°C and 90% relative humidity, with continuous illumination at a photon flux density of 250 $\mu E m^{-2} s^{-1}$ from Sylvania cool-white fluorescent bulbs. Five days after sowing, seedlings were wounded in the center of one cotyledon by puncturing with forceps, and inoculated with a 10 μl droplet of distilled water containing 1.0×10^7 *L. maculans* pycnidiospores ml^{-1} , which was placed on the wound site. One day later, the opposite cotyledon was inoculated with 10 μl each of *A. candida* and *P. parasitica* (1.0×10^5 and 1.0×10^4 spores ml^{-1} , respectively). The inoculum was placed in opposite corners of the cotyledon without wounding, flats placed in a dew chamber at 20°C and 100% relative humidity for 24 h, then returned to the growth chamber. Each flat contained highly susceptible positive control plants to verify successful infection by each pathogen (*Brassica oleracea* cultivar Jersey Queen, *B. rapa* cultivar Michihili, and *B. juncea* cultivar Southern Giant Curled Mustard, for *L. maculans*, *P. parasitica*, and *A. can-*

didia, respectively). Disease severity was scored 7 (*Peronospora*), 8 (*Albugo*), or 9 (*Leptosphaeria*) d after inoculation.

The experimental design consisted of three selection treatments and a randomly selected control, each replicated three times. Each generation of each replicate contained 200 plants scored for severity of each fungal disease. In each generation, we chose the 20 plants that were most resistant in each replicate, grew them at low density with little opportunity for competitive interactions, and randomly mated them to produce the next generation. In contrast, 20 randomly selected plants were chosen from each of the randomly selected control lines to propagate the next generation. In the next generation, 10 progeny were planted from each maternal plant in randomized positions within each block. Each treatment or control replicate was subject to three cycles of selection and measured for four generations, providing a total of 12 independent replicate lines, or 28,554 measurements of disease severity on 9518 plants. After three cycles of selection, mean population susceptibility declined 25%, 63%, and 70%, for *A. candida*, *P. parasitica*, and *L. maculans*, respectively, relative to the random selected susceptible control populations.

In the current study we tested for a cost to resistance by comparing growth rates (final dry weights in milligrams) in plants from resistant versus susceptible control populations (treatments), grown in the absence of pathogens. We conducted three experiments. First, we compared the four replicated selection treatments. However, this design had limited statistical power because treatment effects were tested over denominator mean squares with few degrees of freedom. Based on findings from experiment 1, we made several changes in subsequent experiments to achieve greater statistical power. Because subsequent experiments were conducted using identical methods in the same controlled environment growth room, FAMILY and TREATMENT × FLAT effects were assumed to be absent. Sample sizes were increased severalfold. Finally, treatment replicates were pooled in subsequent experiments, permitting a simpler experimental design that focuses on the contrast between resistant and susceptible genotypes. For experiment 1, each of 12 lines (4 treatments × 3 replicates/treatment) had been independently derived from the ancestral susceptible population in four generations, selecting parents resistant to a single pathogen (*Albugo*, *Peronospora*, and *Leptosphaeria* treatments), or randomly selected parents in the susceptible control lines (Mitchell-Olds et al. 1995). In experiment 2, we conducted one additional cycle of family-based selection, using disease severity data from the fourth generation of experiment 1 to identify the 10 families with highest levels of resistance to *Peronospora* from each of the three replicate *Peronospora*-resistant lines. Each resistant family contributed five seeds to a pooled resistant population (3 replicates × 10 families × 5 seeds = 150 resistant plants), which was mass pollinated for two generations. To create a pooled *Peronospora*-susceptible population, we used disease severity data from generation 4 of experiment 1 to identify the 10 families with highest levels of susceptibility to *Peronospora* from each of the three replicate random selected control lines. Each susceptible family contributed five seeds to a pooled susceptible population (3 replicates × 10 families × 5 seeds = 150 susceptible plants),

which was mass pollinated for two generations. In a similar manner, we created pooled *Leptosphaeria* resistant and susceptible populations for experiment 3. Thus, three independent replicates of each treatment were pooled and randomized, and we compared growth rates of resistant versus susceptible lines.

All experiments were conducted in the laboratory to avoid release of foreign pathogens, which occur in neighboring states but are not yet found in Montana. For measurements of biomass, plants were grown without pathogens in a randomized complete blocks design in a controlled environment chamber with continuous very high output (VHO) fluorescent light, and germination date was recorded for each plant. Individual seed weights were recorded in experiments 2 and 3. Each 30 cm × 60 cm flat contained 96 randomized plants. In experiment 1, 24 maternal parents from each treatment (8 from each replicate) each contributed one seed to each of six flats. Results from experiment 1 indicated that there was no significant variation among families; thus, seeds from experiments 2 and 3 were bulk collected within each genotype. Experiments 2 and 3 used a randomized complete blocks design with eight flats, and 48 resistant and 48 susceptible seeds randomized within each flat. Aboveground biomass was harvested 14 d after planting (experiment 1), or at about 3 wk of age when the first individuals initiated shoot elongation prior to flowering (experiments 2 and 3). Plant height and aboveground biomass show strong positive genetic correlations with total plant biomass and individual fecundity (Mitchell-Olds, unpubl. data). Under these growth conditions, competitive interactions are minimal during this phase of the life cycle. Plants were dried in a forced air drying oven and individually weighed to the nearest milligram. Shoot biomass is a good predictor of fitness in many annual plant species (Mitchell-Olds 1992). For example, a quadratic model incorporating stem height and stem diameter of 1-month juveniles in *B. rapa* explained 58% of final adult fecundity in the wild (Mitchell-Olds, unpublished analyses of data from Nakamura et al. 1995). In addition, by measuring growth rate prior to flowering we avoid the difficulties in interpretation that may result from genetic variation in flowering time and subsequent effects on fecundity (Agren and Schemske 1994). Although growth rate and flowering date are both important components of fitness, we do not wish to confound the effects of functionally separate loci (Mitchell-Olds 1995).

In experiment 1, mixed-model randomized complete blocks ANOVA was used to compare growth rate in replicated resistant lines versus control lines, with TREATMENT as fixed effect and FLAT, REPLICATE within TREATMENT, and FAMILY within REPLICATE as random effects. We did not test for FLAT × FAMILY interactions because families were unreplicated within flats. Tests for FLAT, TREATMENT, FLAT × TREATMENT, and REPLICATE employed Satterthwaite approximate F-ratios (Shaw and Mitchell-Olds 1993) in SAS (SAS Institute Inc. 1989). Experiments 2 and 3 were designed and conducted after completion of experiment 1, using a simpler design with greater statistical power and larger sample sizes. Because subsequent experiments were conducted using identical methods in the same controlled environment growth room, FAMILY and TREATMENT × FLAT effects were assumed to be absent (see Dis-

TABLE 1. ANOVA comparing dry weight of replicated resistant and susceptible populations. ANOVA contrasting growth rate in replicated resistant selection lines versus control lines (experiment 1). $N = 507$, $R^2 = 33.6\%$.

Source	df	Mean-square	F-ratio	P
FLAT	5	182.8	1.14	0.379
TREATMENT	3	474.6	1.89	0.268
FLAT × TRT	15	159.6	0.71	0.755
REP (TRT)	8	314.5	1.47	0.210
FAM (REP)	84	156.2	0.94	0.622
FLAT × REP (TRT)	40	223.9	1.34	0.083
Error	351	165.9		

cussion). In experiments 2 and 3, we controlled for individual variation in seed weight and germination date by ANCOVA, which provides great statistical power to detect differences between resistant and susceptible genotypes. All factors and covariates were tested over the error mean squares in experiments 2 and 3 using SYSTAT.

RESULTS

In experiment 1, the replicated pathogen resistant and susceptible treatments did not differ significantly for dry weight ($F = 1.89$, $P = 0.27$; Table 1). However, due to the limited power of this experimental design, we place little weight on the significance levels from this experiment. Compared to the random selected susceptible control lines, the resistant populations grew 14% and 11% smaller for *Leptosphaeria* and *Peronospora*, and 3% larger for *Albugo*. Mean dry weight in milligrams for these populations (least-square mean ± standard errors) were 23.09 ± 1.16 , 25.27 ± 1.13 , 28.42 ± 1.13 , and 27.58 ± 1.13 for the susceptible control population. No significant variation was found among families. Such low heritabilities are expected after several generations of strong selection. All flats were grown together and randomized in a small area, about 1.3 m on a side. Consequently, we found no significant FLAT × TREATMENT interactions, and these interactions were assumed to be absent in subsequent experiments 2 and 3. These experiments focused on *Peronospora* and *Leptosphaeria* resistance, which showed potential fitness costs. In summary, the primary useful result from experiment 1 was justification for the more powerful experimental design used in experiments 2 and 3, which were conducted under identical conditions in a controlled environment growth room.

Experiment 2 compared pooled populations with genetic resistance and susceptibility to *Peronospora*. Lines did not differ for seed weight ($F = 2.02$; $df = 1, 663$; $P = 0.156$; not shown). The *Peronospora*-susceptible line germinated a few hours earlier than the resistant population (mean time to germination 1.58 versus 1.72 d; $F = 5.55$; $df = 1, 635$; $P = 0.019$; not shown). Controlling for seed weight and germination date by ANCOVA, the *Peronospora*-susceptible line grew 6% larger than the resistant line (23.38 ± 0.38 versus 22.10 ± 0.38 mg; $p = 0.018$; Table 2; Fig. 1). Slope of dry weight onto covariates was -3.283 and 8.090 for germination date and seed weight, respectively, showing that early ger-

TABLE 2. ANCOVA comparing dry weight of *Peronospora* resistant and susceptible genotypes in pooled populations. ANCOVA contrasting growth rate in pooled *Peronospora* resistant and susceptible populations (experiment 2). $N = 627$, $R^2 = 27.7\%$.

Source	df	Mean square	F-ratio	P
FLAT	7	464.710	10.265	0.001
GENOTYPE	1	256.881	5.674	0.018
GERM DATE	1	3456.641	76.354	0.001
SEED WEIGHT	1	3589.870	79.297	0.001
ERROR	616	45.271		

mination and large seeds resulted in significantly larger plants.

Experiment 3 compared pooled populations with genetic resistance and susceptibility to *Leptosphaeria*. Populations did not differ for germination date ($F = 1.63$; $df = 1, 614$; $P = 0.203$) or seed weight ($F = 0.036$; $df = 1, 663$; $P = 0.849$), respectively (not shown). The susceptible population grew 2.4% faster than the resistant population, but this difference was not significantly different from zero (15.05 ± 0.26 versus 14.67 ± 0.26 mg; $p = 0.292$; Table 3; Fig. 1). Slope of dry weight onto covariates was -1.811 and 6.334 for germination date and seed weight, respectively, showing that early germination and large seeds resulted in significantly larger plants.

How consistent were the results of these three experiments? Experiment 1 did not find significant differences in growth rate among the four replicated treatments ($p = 0.27$, Table 1). However, this experiment provided essential information to redesign our experimental approach for subsequent experiments. Experiments 2 and 3 examined potential costs of resistance using statistically powerful designs where treatment effects were tested over the error mean square. These experiments focused on particular contrasts with larger sample sizes: $2.5 \times$ and $2.3 \times$ larger for the *Peronospora* and *Leptosphaeria* comparisons, respectively (Tables 2, 3). We found a cost of resistance (reduced growth rate) for *Peronospora* but not for *Leptosphaeria*. Even if we had chosen to conduct a one-tailed significance test for slower growth rate in *Leptosphaeria* resistant genotypes, experiment 3 would not indicate significant costs of *Leptosphaeria* resistance. Due to the larger sample sizes and more efficient experimental designs in experiments 2 and 3, results from these latter experiments provide the best estimates of costs of resistance.

DISCUSSION

Costs of resistance can have an important influence on ecological and evolutionary interactions between plants and insects or pathogens and may be an important factor maintaining genetic variation for resistance in natural populations. Equilibrium levels of resistance are strongly influenced by allocation costs in evolutionary models (Gillespie 1975; Gould 1988; Leonard and Czochoz 1980; Simms and Rausher 1987). Here we report that genetic resistance to *Leptosphaeria* is cost free, whereas resistance to *Peronospora* is costly, with a negative genetic correlation between disease resistance and growth rate. Thus, resistance to *Leptosphaeria* can evolve rapidly, while the evolution of resistance to *Peronospora* is constrained by genetic trade-offs.

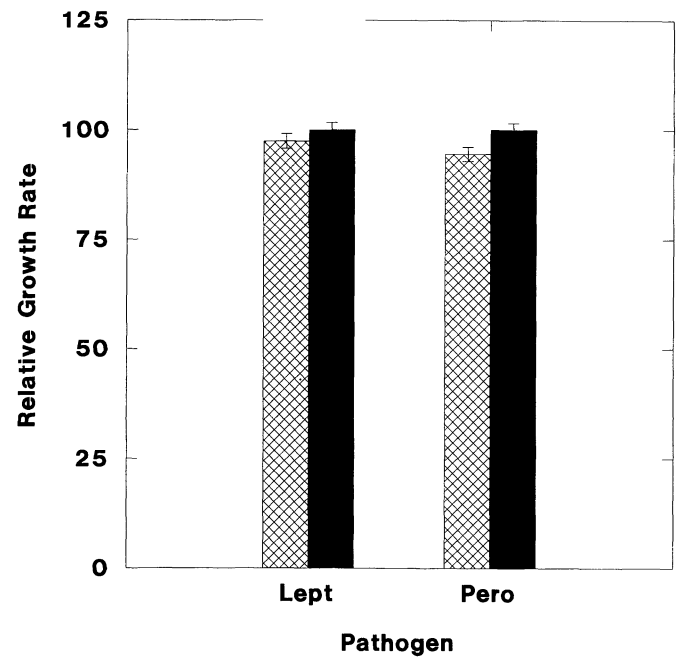


FIG. 1. Relative growth rates in experiments 2 and 3: Bars indicate least-square means and standard errors for biomass accumulation during vegetative growth of resistant (gray bar) and susceptible (black bar) genotypes from the *Leptosphaeria* and *Peronospora* experiments. *Leptosphaeria* resistance had no significant effect on growth rate, indicating cost-free defense. In contrast, *Peronospora*-resistant genotypes grow 6% slower than *Peronospora*-susceptible genotypes in pathogen-free environments, indicating a significant genetic fitness cost to *Peronospora* resistance.

Although genetic trade-offs between resistance and fitness are undetectable in several large, empirically sufficient studies (Agren and Schemske 1994; Nunez-Farfan and Dirzo 1994; Parker 1990; Simms and Rausher 1989), a few studies have found pleiotropic costs of resistance in natural plant populations. Berenbaum et al. (1986) found negative genetic correlations between levels of defensive compounds and estimators of individual fecundity. Han and Lincoln (1994) found a significant negative genetic correlation between growth rate and levels of secondary metabolites in *Diplacus aurantiacus*. Many studies, including our own, have measured fitness components in controlled environments, where environmental variation may be reduced, or gene expression may be altered by novel environments. In contrast, Siemens and Mitchell-Olds (unpubl. data) found significant negative genetic correlations in *B. rapa* between flea beetle resistance and fecundity measured in the wild.

TABLE 3. ANCOVA comparing dry weight of *Leptosphaeria* resistant and susceptible genotypes in pooled populations. ANCOVA contrasting growth rate in pooled *Leptosphaeria* resistant and susceptible populations (experiment 3). $N = 589$, $R^2 = 33.2\%$.

Source	df	Mean square	F-ratio	P
FLAT	7	195.963	10.214	0.001
GENOTYPE	1	21.360	1.113	0.292
GERM DATE	1	955.484	49.800	0.001
SEED WEIGHT	1	3139.808	163.647	0.001
ERROR	578	19.186		

Recent studies have shown little evidence for genetic fitness costs of plant defense (Agren and Schemske 1994; Parker 1990; 1992; Simms and Rausher 1989). Simms (1992) reviews numerous papers on physiological costs of resistance, but genetic studies are rare. Although some data have shown a fitness or yield cost in resistant genotypes, most have involved wide crosses or inbred lines where other loci may be in disequilibrium with resistance genes (Bergelson 1994; Brinkman and Frey 1977; Burdon and Muller 1987; Chaplin 1970; Frey and Browning 1971; Simons 1979). In one highly inbred species, Parker (1991) found nonadaptive evolution of disease resistance attributable to linkage disequilibrium. Estimates of pleiotropy from near-isogenic lines can be confounded by linkage drag, when large chromosome segments are introgressed along with the gene of interest. For example, Young and Tanksley (1989) found an introgressed chromosome segment more than 50 centiMorgans long following 11 generations of backcrossing. Thus, near-isogenic lines may differ at thousands of loci. Quantitative genetic studies from habitually inbreeding species and near-isogenic lines may provide suggestive evidence regarding the existence of genetic fitness costs, but they are empirically insufficient to prove that these costs are due to pleiotropy. Fortunately, data from molecular markers can greatly reduce this problem of linkage drag (Mitchell-Olds 1995; Young and Tanksley 1989).

Other studies have focused on fitness costs in habitual outcrossers. In these studies, linkage disequilibrium is unlikely to exist prior to the onset of selection (Crow and Kimura 1970); thus, correlated response to selection is a strong indicator of pleiotropy. Simms and Rausher (1989) found no cost of insect resistance in *Ipomea purpurea*. Agren and Schemske (1994) found no evidence for a fitness cost of trichome production in the field. Simms (1992) reviews a number of studies suggesting a fitness cost to cyanogenesis in *Trifolium*. However, these studies may need to be reevaluated, because molecular analysis of the hydrolytic enzyme linamarase reveals that genetic control of this system is considerably more complicated than the classic two locus model (Oxtoby et al. 1991). Berenbaum et al. (1986) found a genetic fitness cost of resistance to insect herbivores. To our knowledge, the present study is the first to show genetic costs for resistance to a microbial pathogen using an outbreeding species in which the confounding effects of linkage disequilibrium are expected to be absent (Crow and Kimura 1970).

In comparison to family-structured quantitative genetic analyses, selection experiments have many advantages for study of genetic correlations (Mitchell-Olds and Rutledge 1986; Rose and Charlesworth 1981; Dorn and Mitchell-Olds 1991; Fry 1993; Bennet et al. 1992; Agren and Schemske 1994). Although statistical power to detect genetic correlations is very poor in most family-structured analyses, genetic correlations can be detected after selection experiments by simple comparison of group means. This occurs because response to selection is predicted by $\Delta\bar{z} = \mathbf{G}\beta$, where $\Delta\bar{z}$ is a column vector of genetic changes in trait means, \mathbf{G} is the additive genetic variance-covariance matrix, and β is a column vector of selection gradients (Lande and Arnold 1983). Element β_i gives the derivative of relative fitness with respect to the i th trait, controlling for correlated changes in other

traits. Even if inadvertent selection occurred in these experiments, such effects cannot explain differences between selection treatments, because experimental treatments differ only with respect to β_i , the selection gradient quantifying artificial selection on trait i . When selection treatments cause genetic differences in another trait, j , which is not subject to direct selection, this can result only from genetic correlation between traits i and j . This follows because β_j (the selection gradient for unselected trait j , controlling for artificial selection on trait i) is identical among all treatments. In some quantitative genetic studies, failure to find significant evidence for antagonistic pleiotropy may be attributable to low statistical power due to modest sample size. In contrast, the lack of fitness costs of trichome production in the study by Agren and Schemske (1994) provides strong evidence that these defensive structures do not reduce fitness in this field environment. Moreover, correlated responses to selection indicate the existence of additive genetic covariance unconfounded by dominance or maternal effects (Falconer 1989). Another major advantage of artificial selection experiments is that theoretical difficulties arising from unmeasured traits (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987) are unlikely to cause problems in artificial selection experiments. Interpretation of direct measurements of correlated response to selection requires fewer assumptions than predictions of multivariate response to selection from quantitative genetic theory. In addition, selection experiments can avoid the problems of linkage drag that plague studies of near-isogenic lines (Young and Tanksley 1989), because linked loci are expected to be in linkage equilibrium prior to the onset of selection. For these reasons, artificial selection experiments have great potential to address evolutionary questions regarding genetic trade-offs and constraints. However, selection experiments are primarily useful for focused analysis of a small number of traits in outcrossing species.

Another factor that may explain heritable variation for resistance in natural plant populations is ecological trade-offs, in which genetic resistance to one species causes susceptibility to another (Fritz 1992; Simms 1992). Such ecological trade-offs do not exist among the pathogens studied in this system. Instead, resistance to *Leptosphaeria* and *Peronospora* displayed significant positive genetic correlations (Mitchell-Olds et al. 1995), despite the distant genetic relationship between *Leptosphaeria* and *Peronospora* (Bruns et al. 1991). Conceivably, such broad-based multiple disease resistance could contribute to a durable defense that might not be easily circumvented by rapidly evolving microbial pathogens. On the other hand, resistance to *Albugo* was genetically independent of the other pathogens. In *Solidago*, Maddox and Root (1990) found predominately positive genetic correlations among levels of resistance to 17 species of insect herbivores, but negative genetic correlations were also observed between some species pairs. In cucumber, cucurbitacin attracts cucumber beetles but provides resistance to generalist mites (DaCosta and Jones 1971). We have also found significant negative correlations in *B. rapa* between resistance to a fungal pathogen and an insect seed predator in the field (Nakamura et al. 1995). Although these correlations did not have a genetic basis in the two populations studied, ecological trade-offs may be important in other instances.

After completing experiment 1, we faced several choices for the design of subsequent experiments. In experiments 2 and 3, we could have employed a statistical model beginning with significance tests for TREATMENT \times FLAT interactions. Had we pursued that strategy, we would have found no trace of such interactions, with *p*-values exceeding 0.3 and 0.8 (not shown). However, that approach can pay a high cost in statistical power, because a posteriori pooling rules are quite conservative (Sokal and Rohlf 1981). The decision to pool FLAT \times TREATMENT interactions was an a priori decision justified by previous experimentation under controlled conditions in a homogeneous environment. In many circumstances, power to detect statistical differences is strongly influenced by the analytical decisions made before the start of an experiment. There is an important trade-off between generality and power. If we can control experimental conditions and focus our question, we can learn more from a given experiment, provided that we have not ignored something important. Under laboratory conditions, this sequential experimental approach can result in important gains in power to detect biological differences.

Explicit consideration of physiological mechanisms of plant defense may also contribute to understanding of resistance to multiple pest species. For example, glucosinolates can serve as feeding stimulants for diamondback moths (Reed et al. 1989), but have deleterious effects on generalist herbivores (Chew 1988). Glucosinolate concentration can evolve rapidly in wild populations of *B. rapa*, causing genetic changes in resistance to several insect herbivores (Siemens and Mitchell-Olds, unpubl. data). Similarly, the enzyme chitinase hydrolyzes chitin, a constituent of cell walls in many fungi (Broglie et al. 1991). In these populations of *B. rapa*, high levels of chitinase activity reduce blackleg disease caused by *Leptosphaeria*, but have no effect on resistance to *Peronospora* (Mitchell-Olds, unpubl. data), which does not have chitin in its cell wall (Bartnicki-Garcia 1968). Clearly, evolutionary studies of molecular physiological ecology of plant defense can improve our understanding of the causes and consequences of genetic variation in resistance.

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