

An Ecological Genetic Approach to the Study of Coevolution¹

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SYNOPSIS. Almost forty years ago, Ehrlich and Raven (1964) hypothesized that the great diversity of plants and the herbivores that feed on them arose from a process of coevolution. Plants do possess an amazing diversity of traits that are easily imagined as having arisen from an antagonistic interaction between plants and herbivores. Two basic assumptions lie at the root of most theories of coevolution between plants and their herbivores. First, herbivores are agents of natural selection on plant resistance traits. Second, plants incur a significant fitness cost for possessing these resistance traits. An ecological genetic approach can provide rigorous evidence for these coevolutionary assumptions. In this paper, I present new experimental work on the subject of costs of resistance and review and discuss my own previous work bearing directly on these questions. Using both field experiments on natural populations of the mouse-ear cress (*Arabidopsis thaliana*) and laboratory experiments using genetically modified plants, I demonstrate that herbivores are exerting selection on both a chemical and physical resistance trait and that there are significant fitness costs to possessing these two traits. These results provide direct confirmation that our current models of the evolution of plant defenses are appropriate.

THE PROBLEM

Marlin Perkins, the long-time host of the television show, “Wild Kingdom,” may have attracted more people to the study of biological diversity than Charles Darwin. That tradition continues today with such compelling television naturalists as David Attenborough and the “Crocodile Hunter,” Steve Irwin. A common element of television nature programs is a fascination with the striking characteristics of organisms that seem to protect an individual from being eaten. These “resistance” characters in-

clude such obvious traits as the quills on a porcupine or the puffing of a puffer fish.

Although examples of these traits are most conspicuous among animals, these characters reach great diversity in plants. Far from being passive in the face of attack by predators, plants display an impressive array of characters widely thought to defend them against attack. For example, plants possess a myriad array of chemical resistance—the diverse collection of the so-called “secondary compounds,” such as the alkaloid, caffeine. Plants also possess a great diversity of physical resistance traits, such as spines and thorns.

If you were to learn about the evolution of resistance traits from listening to Marlin Perkins, Steve Irwin or David Attenborough, you would be convinced that evolutionary biologists generally agree about the nature of selection acting on these traits. Clearly, being eaten is bad, and therefore, natural selection has acted on these individuals to evolve these traits that protect them from being eaten. But what evidence do we have that this evolutionary scenario is correct? Are all individuals maximally protected against predation? Are all plants brimming with nasty chemicals and blanketed in sharp thorns?

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³ Although many authors use the terms “resistance” and “defense” interchangeably, I prefer the distinction originally delineated by Rausher (1992a): “a *resistance* trait is any plant character that influences the amount of damage a plant suffers.” Rausher (1992a) reserves the term “defense” for a trait that has evolved *because* of selection by herbivores, and therefore implies something about the “evolutionary *raison d’être*” of the trait. This is exactly parallel to the definitional distinction between a trait that is an adaptation (*e.g.*, defense) and a trait that is adaptive (*e.g.*, resistance) (see Futuyma, 1998, pp. 354–356).

TABLE 1. Analysis of variance for trichome density of *Arabidopsis thaliana* collected from sites in northeastern Georgia in the spring of 1999.

Source of variation	df	Type III sums of squares	F	P
Site	17	9,527	22.8	<0.0001
Family (site) ^{a,b}	160	19,221	4.9	<0.0001
Error	1602	39,378	—	—

^a A number of fruiting females was collected from each site. All offspring from each mother constitute a family. Offspring were grown in the University of Georgia Botany Greenhouses under 12 hour days and were well-watered. Using a dissecting microscope, we estimated trichome density by counting the number of trichomes in a standard area (2.4-mm² area) on the upper surface of two fully developed leaves of equal age from each individual. Ten individuals from each family were used in estimating family means.

^b The family term is nested within the collection site.

In fact, most plants are not absolutely resistant to all herbivores: the mean level of resistance in most plant populations is often at an intermediate value (Simms and Rausher, 1987). One of the most common observations that field biologists have made is that there is considerable variation in resistance traits at both the genotypic and phenotypic levels (Table 1). If herbivores are reducing the fitness of individual plants, one might expect positive Darwinian selection to eliminate variation for resistance and fix the population at a high level of resistance. How do evolutionary biologists explain this contradiction?

In this paper, I use an ecological genetic approach to address two major questions that are at the heart of the study of the coevolution of plants and their herbivores: (1) are herbivores selective agents on plant resistance traits and (2) how do we explain the equilibrium level of resistance (high, low, intermediate) in a natural plant population? By and large, this paper provides a case study demonstrating the use of ecological genetic methods to test specific hypotheses on the coevolution of plants and herbivores. In the first and third parts of the paper, I will provide some insights that I have made from my work on the evolution of plant resistance traits in the annual plant, *Arabidopsis thaliana* and the natural assembly of herbivores that feed on this plant. In the second part of this paper, I present original data from current experiments on the costs of resistance using plants carrying mutations that affect the production of the secondary compounds, glucosinolates, in *Arabidopsis thaliana*.

COEVOLUTIONARY ASSUMPTION 1: SELECTION BY HERBIVORES

Explaining the presence of the considerable variation we see in nature is one of the central questions of evolutionary biology and one of the goals of evolutionary biologists is to try to understand the evolutionary forces that maintain this variation in natural populations. In 1964, Ehrlich and Raven proposed the hypothesis that much of the "secondary" chemical diversity exhibited by plants was due to the coevolution of plants and the herbivores that ate them. At the heart of this coevolution hypothesis are two key assumptions. First, selection imposed by herbivores acts on resistance traits and acts to cause divergence in these resistance traits. Second, selection imposed by plant resistance traits acts on herbivores causing herbivore populations to diverge in traits that allow the herbivore to consume the plant.

There is abundant evidence to support the second assumption (see Moran [1986] for an elegant example). However, there has been a long-standing debate on the role of herbivores in the evolution of plant resistance characters.³ It is generally believed that selection imposed by herbivores has generated the tremendous diversity in plant resistance. Many biologists have interpreted the principal function of plant resistance traits to be the defense of the plant against its enemies (Fraenkel, 1959; Berenbaum, 1983). But other biologists have suggested that selection by herbivores is normally too weak to account for the evolution of these traits and that other evidence suggests that

these traits serve more primary ecological and physiological functions (Muller, 1969; Jermy, 1984; Schmitt *et al.*, 1995).

What exactly is the evidence for the predominant view that herbivores impose selection on plant resistance characters? Numerous ecological studies have demonstrated that attack by herbivores can reduce plant fitness (*e.g.*, Marquis, 1984; Fagan and Bishop, 2000). However, there is a limitation in the inference one can make from this type of study. These studies indicate the *potential* for herbivores to exert selection on their host plants, not that selection actually is exerted by herbivores. The critical piece of information often lacking is that herbivore impact differs among plant genotypes. Although the ecological evidence is consistent with the hypothesis that resistance characters have evolved in response to herbivore selection pressure, it is also consistent with the hypothesis that these characters have evolved in response to some other ecological or physiological selection pressures. Thus, simply demonstrating the potential for selection by herbivores does not definitively indicate that such selection actually occurs.

How does one rigorously demonstrate that herbivores are having an evolutionary impact on plant resistance characters? More generally, how does one show that any presumed selective agent is actually having an evolutionary impact on a particular trait.

I suggest that such a demonstration would have three components. First, one should show that there is genetic variation for the character, since selection acts on variation and that variation must have a genetic basis in order for the population to respond to selection. Second, one must show that there is a relationship between fitness and the character (in other words, that the trait is actually subject to some form of natural selection). And third, and most critically, one must show that experimentally changing the abundance of herbivores changes the pattern of selection acting on resistance. In other words, one must show that the pattern of natural selection changes depending on whether herbivores are present or absent.

Mauricio and Rausher (1997) performed

a study that satisfied all three of these criterion in a single system: natural populations of the predominantly selfing, annual plant, *Arabidopsis thaliana*. In this study, Mauricio and Rausher (1997) found that there was significant genetic variation (among-family variation using 144 families collected in North Carolina) for two traits that had been demonstrated to reduce attack by herbivores in the field: the total concentration of the secondary chemicals, glucosinolates, and the density of leaf trichomes.

In order to determine the relationship between relative fitness (number of fruits) and the two resistance traits, Mauricio and Rausher (1997) used a modification of the partial regression techniques developed by Lande and Arnold (1983) to measure natural selection acting directly on a character. This method, described by Rausher (1992*b*), corrects for bias introduced when there is an environmentally-induced covariance between the character and fitness by measuring selection on breeding values, or in this case, on family means (Mauricio and Mojonier, 1997).

By adding pesticides that had been shown in a separate greenhouse experiment not to affect fruit number in *Arabidopsis thaliana*, Mauricio and Rausher (1997) were able to eliminate herbivores from experimental field populations of *A. thaliana*. This experimental manipulation caused a significant change in the pattern of selection (as measured by the partial regression method). This result was taken by Mauricio and Rausher (1997) as rigorous evidence that herbivores are agents of natural selection acting on genetic variation for both total glucosinolate concentration and trichome density. Similar studies, on a variety of different resistance traits, by Berenbaum *et al.* (1986) using wild parsnip, *Pastinaca sativa*, Rausher and Simms (1989) using the morning glory, *Ipomoea purpurea*, Juenger and Bergelson (2000) using scarlet gilia, *Ipomopsis aggregata* and Shonle and Bergelson (2000) using jimsonweed, *Datura stramonium*, have all reached similar conclusions: herbivores are commonly exerting selection pressure on plant resistance traits.

COEVOLUTIONARY ASSUMPTION 2: COST OF RESISTANCE

If herbivores are imposing selection on resistance characters, presumably because there is some benefit to having these characters, are all plants maximally protected against predation? In fact, it is a common observation that plants do not contain the maximal amount of resistance commensurate with available resources (Simms and Rausher, 1987), nor are they generally completely unprotected. Most plants possess an intermediate level of resistance (Simms and Rausher, 1987).

Explaining the persistence of intermediate levels of resistance has been the focus of much theoretical work on the evolution of plant defense (Fagerström *et al.*, 1987; Simms and Rausher, 1987). If those plants with higher levels of resistance are better protected against herbivores than those plants with lower levels, and if such resistance is not costly, then there should be directional selection for all plants to have higher levels of resistance and genetic variation for resistance should be eliminated. As this does not seem to be the case, it has been assumed that resistance is costly to plants (Fagerström *et al.*, 1987; Simms and Rausher, 1987). If resistance is costly, there should be some optimal level of resistance that reflects a balance between the benefits of reducing herbivore damage and the costs of diverting resources away from growth and reproduction (Gulmon and Mooney, 1986; Bazzaz *et al.*, 1987; Simms and Rausher, 1987).

Costs of resistance have been envisioned as arising from several paths, but most commonly refer to "pleiotropic" costs, including "allocation" costs (Simms, 1992). Physiologically, an allocation cost is viewed as arising from internal competition for limiting resources. It is generally believed that an organism has some finite access to resources and can partition those resources into three functions: growth, maintenance and reproduction (Bazzaz *et al.*, 1987). According to this view, allocation to defense (maintenance) necessarily reduces the amount of resources that can be allocated to present and future growth and re-

production (but see Bazzaz and Carlson, 1979; Ashman, 1994). Nutrients may be an important factor as a plant limited by nutrients may be under more severe allocational constraints and thus, costs of resistance may be more manifest (Gulmon and Mooney, 1986; Bazzaz *et al.*, 1987; Bergelson, 1994).

Evolutionarily, costs are envisioned as trade-offs between two characters, or antagonistic pleiotropy (hence, "pleiotropic" costs). An evolutionary trade-off exists between two characters if genetic change leading to an increase in the value of one character cannot occur without a decrease in the value of the other character (Reznick, 1985). If both characters are of some benefit to the organism, then this negative genetic correlation will constrain evolutionary change because the benefit gained from selection to increase the value of one character will be counterbalanced by the cost of the correlated decrease in the value of the other character.

This genetic architecture presents a simple ecological genetic approach to experimentally determining if a pleiotropic cost of resistance is present. Such a cost of resistance is present if one can find a significant negative genetic correlation between resistance and fitness in the absence of herbivores. The cost is revealed only in the absence of herbivores because in this environment, there will be no benefit to possessing resistance.

I have investigated such pleiotropic costs of resistance using two approaches. In field experiments, I have looked for the presence of negative genetic correlations between fitness and total glucosinolate concentration and trichome density. Second, I have used strains of *Arabidopsis thaliana* carrying mutations in the glucosinolate biosynthetic pathway to investigate the costs of secondary chemical production.

Costs of resistance traits in natural populations

Mauricio (1998) grew 1,728 individual plants (from 144 families) of *Arabidopsis thaliana* in a field in North Carolina where natural populations of the plant grew. The individuals were placed in the field at a

TABLE 2. Analysis of variance for total glucosinolate concentration (mg glucosinolates/L/mg leaf) of three near isogenic lines of *Arabidopsis thaliana* selected for altered glucosinolate profile and the parental wild-type control from which they were derived (Columbia strain).

Source of variation	df	Type III sums of squares	F	P
Line	3	0.22	2.95	0.0397
Nutrient level*	1	0.07	2.67	0.1072
Block	3	0.51	7.51	0.0002
Line * Nutrient level	3	0.08	0.98	0.4067
Line * Block	9	0.16	0.70	0.7024
Nutrient level * Block	3	0.07	0.96	0.4172
Line * Nutrient level * block	9	0.36	1.57	0.1455
Error	61	1.54	—	—

* Half the plants were watered with a fertilizer solution every other day and the other half were watered with a weak fertilizer solution every other day.

very early stage of development—when each plant had only 4 small leaves—and at a time when the natural populations in the same field were at the same developmental stage (mid-autumn for this winter annual plant species). Half the plants were exposed to the natural assemblage of herbivores that are normally found on *A. thaliana* (mainly two species of flea beetles) and the other half of the plants were sprayed with pesticides which had previously been shown not to affect plant fitness.

During the experiment, Mauricio (1998) sampled each of the plants and measured the total glucosinolate concentration and trichome density (see Tables 1 and 2 for details on measurement of the traits). At the end of the experiment, fitness was estimated by counting the total number of fruits produced.

Mauricio (1998) demonstrated that there was very little herbivore damage on the plants that had been sprayed with pesticides. These plants, therefore, had escaped herbivores and, if an allocation cost of resistance was present, there should have been a negative genetic correlation between the resistance trait and fitness. Mauricio (1998) found strong negative genetic correlations between both total glucosinolate concentration and fitness and between trichome density and fitness. Therefore, significant costs to producing glucosinolates and trichomes exist in these populations. A growing number of studies using similar or related (selection experiments) approaches

have documented significant costs of resistance, including work by Berenbaum *et al.* (1986), Bergelson *et al.* (1996), Zangerl and Berenbaum (1997), and Siemens and Mitchell-Olds (1998).

Use of mutants to study costs of resistance

An alternative approach to studying costs of resistance is the use of mutants that genetically and phenotypically differ in the levels of resistance expressed. Studies in natural populations take advantage of the great amount of genetic variation normally found in nature. However, laboratory experiments using genetically characterized mutant lines have some advantages. First, environmental conditions within the laboratory (growth chambers or greenhouses) are generally less variable than in the field, thus allowing more sensitivity in detecting effects. Second, the laboratory allows for more careful control of environmental manipulations. Third, mutant lines are generally well characterized with regards to the specific genetic lesion conferring the phenotype. Therefore, one can make some inferences about the effect of specific genetic changes.

In this section, I describe a laboratory experiment that tests for a fitness cost of a specific class of secondary compounds, the glucosinolates, in three nearly isogenic lines of *Arabidopsis thaliana* differing in levels of glucosinolates. Because resource availability has been suggested to play an important role in the manifestation of costs

of resistance (Gulmon and Mooney, 1986; Bazzaz *et al.*, 1987), I manipulated the nutrients available to plants in this experiment. Since these data have not previously been published, the description of the experiments and results in this section are appropriately more detailed than in other sections.

The predominant secondary compounds found in the plant family Brassicaceae are the glucosinolates (Vaughn *et al.*, 1976). Hozge *et al.* (1988) separated and identified twenty-three different glucosinolates in the leaves and fruits of *A. thaliana*. Haughn *et al.* (1991) isolated and described several mutant lines of *A. thaliana* that differ in glucosinolate production compared to the wild-type strain (the Columbia strain). In this experiment, I used three of these mutant lines, obtained from the Arabidopsis Biological Resource Center at Ohio State University, designated by Haughn *et al.* (1991) as TU1 (Stock Center #CS2226), TU3 (CS2228) and TU7 (CS2229).

All mutant lines were isolated by screening a pool of seeds mutagenized with ethyl methane sulfonate (Haughn *et al.*, 1991). The genetic lesion in line TU1 was genetically characterized and found to be due to a recessive allele (designated *gsm1-1*) of a single nuclear gene. Line TU1 was backcrossed to the parental wild-type line (the Columbia strain) seven times and lines TU3 and TU7 were backcrossed to the Columbia strain three times each. Repeated backcrossing to the parental strain (with selection for the mutant phenotype in the offspring) tends to make all other loci in the offspring identical to the parent (heterozygosity at loci unlinked to the selected locus will approximately halve each generation).

Although each of the mutant lines differed significantly from the wild-type in glucosinolate content (see Fig. 2 of Haughn *et al.*, 1991), only TU1 was studied in detail (see Table II of Haughn *et al.*, 1991). Haughn *et al.* (1991) found that TU1 differed in both the total amount of glucosinolates present in leaves and seeds and in the types of the individual types of glucosinolates present. Specifically, Haughn *et al.* (1991) found that the *gsm1* allele resulted in blocks in the biosynthetic pathway of

glucosinolate production which resulted in a depletion of key amino acid substrates. This mutation greatly reduced amounts of eight aliphatic glucosinolates (but higher levels of three aliphatic glucosinolates). The TU3 mutant had lower levels of two glucosinolates and the TU7 mutant had lower levels of all the aliphatic glucosinolates.

In February of 1999, I randomly assigned individuals from each of the four lines (wild-type parent, TU1, TU3, TU7) to one of two treatments: "high nutrients" where plants were watered every other day with 1/4 strength fertilizer solution and "low nutrients" where plants were watered every other day with 1/16 strength fertilizer solution. On alternate days, plants were watered with deionized water. Plants were grown under 12 hr of light and were planted in approximately 3 cm², freely draining plastic pots, which were filled with sand that had been washed with distilled water. The individual plants were arranged in a completely randomized block design and were allowed to grow until senescence. With two nutrient treatments, four lines, four spatial blocks and nine replicates, the total number of plants in the experiment was 288.

Three weeks after the start of the experiment, I collected 2 leaves from a subsample of plants for chemical analysis (statistical analysis showed that these damaged plants did not differ in fitness compared to undamaged plants). Plants were approximately the same size and at the same phenological stage. I measured the wet leaf weight of the sampled leaves and measured the total glucosinolate content of the sample using the micro-column method (Heaney and Fenwick, 1981) as described by Siemens and Mitchell-Olds 1998. Total glucosinolate concentration was determined by dividing the total glucosinolate content by the wet weight of the sample. There was no indication of attack by any herbivores in the growth chamber. Although glucosinolates have been shown to increase in damaged tissue in other species in the family Brassicaceae (Bodnaryk, 1992; Agrawal, 1999), Mauricio (1998) found no evidence for induction of glucosinolates in *A. thaliana*. When all plants had finished flowering, I

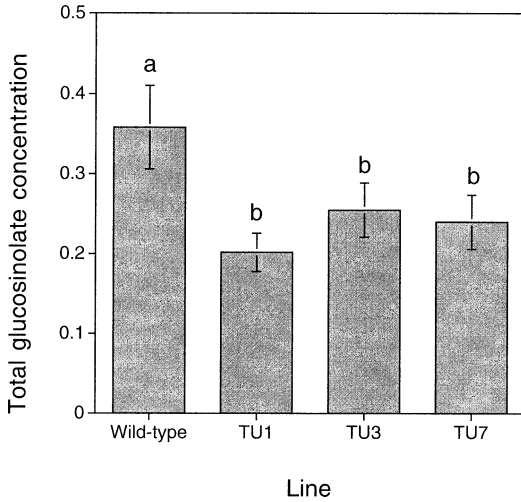


FIG. 1. Total glucosinolate concentration (mg glucosinolates/liter/mg leaf) of three near-isogenic lines selected for altered glucosinolate profile and the parental wild-type control from which they were derived (Columbia strain). Bars with different letters are significantly different (All pairwise comparisons were performed using the Bonferroni procedure with an overall significance level of 0.05). The error bars are standard errors ($n = 72$ for each bar).

counted the total number of fruits as a measure of fitness. Total fruit number is an excellent predictor of total seed number in *Arabidopsis thaliana* and is assumed to be highly correlated to fitness in a highly selfing plant (Mauricio and Rausher, 1997). All statistical analyses were done using the JMP 3.2.6 statistical package (SAS Institute).

The analysis of total glucosinolate concentration revealed significant differences

among the lines (Table 2). Post-hoc statistical tests (correcting for multiple comparisons) showed that the wild-type parental control had a significantly higher total glucosinolate concentration than the mutant lines (Fig. 1). On average, the parent had approximately 50% more glucosinolates than the mutant lines. There were no significant differences in total glucosinolate concentration among the mutant lines (Fig. 1). There was no evidence for any effect (main effect or interaction effect) of nutrient treatment on the total glucosinolate concentration (Table 2).

In the absence of herbivores, there were significant differences among the lines in the total number of fruits, the measure of fitness (Table 3). There was no significant effect of nutrient treatment on fruit number (Table 3). Post-hoc statistical tests (correcting for multiple comparisons) showed that line TU3 had significantly more fruits than the parent from which it was derived (Fig. 2). TU3 plants produced approximately 50% more fruits than the parental control (Fig. 2). Although the mean fruit numbers of the TU1 and TU7 lines were higher than the wild-type parent's, they were not statistically significantly higher (Fig. 2).

If we consider the relationship between the parental line and TU3, there is evidence for a significant cost of resistance. TU3 plants produced about 40% less glucosinolates than the wild-type parent and, in the absence of herbivores (and presumably any benefit to possessing these chemicals) had approximately a 50% increase in fitness

TABLE 3. Analysis of variance for fitness (total fruit number) of three near isogenic lines of *Arabidopsis thaliana* selected for altered glucosinolate profile and the parental wild-type control from which they were derived (Columbia strain).

Source of variation	df	Type III sums of squares	F	P
Line	3	23,116	3.08	0.0282
Nutrient level*	1	3,677	1.47	0.2268
Block	3	144,591	19.24	<0.0001
Line * Nutrient level	3	11,406	1.52	0.2103
Line * Block	9	19,339	0.86	0.5635
Nutrient level * Block	3	8,648	1.15	0.3291
Line * Nutrient level * Block	9	30,732	1.36	0.2051
Error	256	641,159	—	—

* Half the plants were watered with a fertilizer solution every other day and the other half were watered with a weak fertilizer solution every other day.

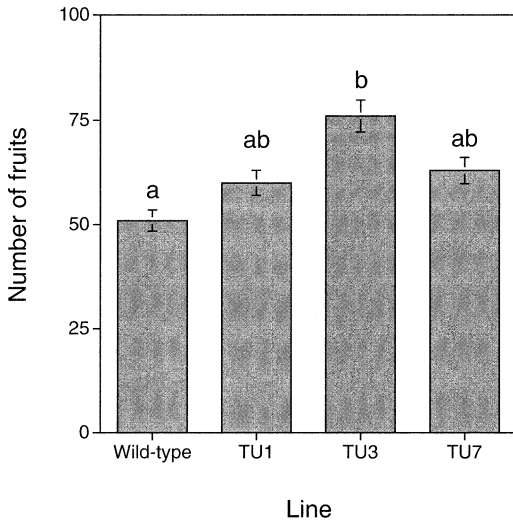


FIG. 2. Fitness of three near-isogenic lines selected for altered glucosinolate profile and the parental wild-type control from which they were derived (Columbia strain). Bars with different letters are significantly different (All pairwise comparisons were performed using the Bonferroni procedure with an overall significance level of 0.05). The error bars are standard errors ($n = 72$ for each bar).

compared to the parent. However, there was no evidence for a cost of resistance when comparing the fitness of the TU1 and TU7 mutant lines. In those cases, a decreased total concentration of glucosinolates did not lead to an increase in fitness in the absence of herbivores.

These results suggest that costs may be dependent on the actual individual glucosinolates changing in these mutant lines since the documented reduction in total glucosinolates did not lead to increased fitness in all the reduced glucosinolate lines. Since the three lines do differ in the individual glucosinolates produced, these result may indicate that the fitness costs of producing different chemical compounds, even those produced by the same biosynthetic pathway, can be different. At this time, we have no detailed information about specific mutations and their effects in the biosynthetic pathway that are carried in lines TU3 and TU7.

One might expect, for example, that mutations far upstream in the pathway might not show significant costs, since the precursors that might build up could be used

for other purposes. On the other hand, downstream mutations could still incur significant costs since resources might be tied up in the pathway. In both scenarios, the final phenotype, total glucosinolate concentration might be similar, but such a scenario might explain the differences in fitness shown in this experiment. In addition, certain precursors may be especially toxic to the plant. Mutations that led to an accumulation of such intermediates might exact a higher cost than more benign intermediate metabolites. Further genetic and biochemical characterization of the mutations would be invaluable in determining the mechanism of costs of glucosinolate production in this system (Mitchell-Olds and Pedersen, 1998).

Surprisingly, there was no main effect of nutrient addition on glucosinolate production (Table 2) or fitness (Table 3). In addition, there were no significant interaction effects involving nutrient treatment. This result suggests that these experimental plants were not limited for nutrients (see Bergelson [1994] and Siemsen and Mitchell-Olds [1998] for examples where nutrients were important in detecting costs). Again, however, there could have been subtle shifts in the amounts of individual glucosinolates produced that were not reflected in the measure of total glucosinolates.

Despite the advantages to using near isogenic lines in evolutionary studies, there are significant drawbacks to the use of such mutant lines. Even though repeated backcrossing to the parent with selection for the mutant phenotype will theoretically homogenize the genetic background for all loci other than the selected gene, empirical studies have demonstrated that this may take many generations, particularly in regions linked to the selected locus. Young and Tanksley (1989) showed that such "linkage drag" remained for tens of generations in their study of the *TM-2* locus in tomato. In this experiment, lines TU3 and TU7 were backcrossed to the parent line three times (the genetic background of these lines should be close to 90% identical to the parent). Thus, there may be many genetic differences between the parent line and the mutant line in addition to the differences in

glucosinolate metabolism. This may also be true for line TU1, although the genetic background of this line and its parent should be greater than 99% identical.

There are several alternatives to the experiment described here that can take advantage of knowledge of specific mutations. Bergelson *et al.* (1996) used well-controlled transgenic technology to create lines that were assumed to differ only in the gene of interest (an herbicide resistance gene). They found significant costs of herbicide resistance by comparing, in the absence of herbicide, the transgenic lines (with the resistance gene) to control lines (without the resistance gene). However, they performed a parallel experiment using a mutant line (isolated using EMS mutagenesis, like the glucosinolate mutants) that had been backcrossed to the wild-type parent for 6 generations (98% identical). They also found a significant cost of resistance using this line. However, the fitness cost detected using the mutant line did not differ at all from the cost detected using the much better controlled transgenic lines. In this case, it seems, the use of a mutant was a significant shortcut to finding a cost than the technically challenging creation of transgenic lines. An advantage of the mutant line approach in comparison to the transgenic approach is that we often know the location and nature of the mutation. In most transgenic studies in plants, the transgene is randomly inserted into the genome. Therefore, it is difficult to separate the effect of transgene expression from the effects of disrupting the genome.

Another alternative to a transgenic approach would be to cross the mutant line to many different parents from a natural population and selecting for the mutant phenotype in the offspring. After several generations of backcrossing to each parent, one would have numerous lines differing in glucosinolate concentration, but in a variety of naturally occurring genetic backgrounds.

THE EVOLUTIONARY IMPACT OF HERBIVORES

In natural populations of *Arabidopsis thaliana*, herbivores are agents of selection on two traits commonly believed to have evolved in response to herbivores: glucos-

inolates and trichomes. In addition, I have provided evidence from two experimental approaches that resistance can incur fitness costs on plants. Together, what do these two findings tell us about the evolutionary impact of herbivores on plant defense traits?

Selection by herbivores on these traits implies that the plants accrue some benefit to being defended. And, we know that there can be a significant cost to possessing these resistance traits. Mathematical models have demonstrated that trade-offs between costs and benefits of resistance can stabilize resistance at levels less than what is genetically achievable in a population (Fagerström *et al.*, 1987; Simms and Rausher, 1987). These models, therefore, potentially address the contradiction first introduced in this paper: the presence of genetic variation for resistance (costs of resistance in fluctuating environments of herbivores) and the fact that most plants are not maximally protected against herbivory (balance between costs and benefits).

Mauricio and Rausher (1997) provided a graphical approach to viewing the overall impact of herbivores on the defensive traits of *Arabidopsis thaliana*. Mauricio and Rausher (1997) generated "selective surfaces" that portray the three-dimensional relationship between relative fitness, total glucosinolate concentration and trichome density. Because they measured relative fitness in both the presence and absence of herbivores, Mauricio and Rausher (1997) were able to decompose the overall fitness surface into component surfaces reflecting costs and benefits.

One surface (Fig. 1E, F in Mauricio and Rausher, 1997) represented the pattern of selection exerted by herbivores alone on these characters. This surface was generated by subtracting the selective surface for the sprayed treatment from that of the control treatment. This surface revealed two distinct fitness peaks that corresponded to selection for either high glucosinolate concentration and no trichomes or high trichome densities and low glucosinolate concentrations. It thus appeared that damage by herbivores favored increasing levels of either defense character, but not both. Mauricio and Rausher (1997) interpreted this surface

as representing the benefit of resistance to the plant (herbivory decreases the fitness of plants with high levels of either trait *less* than plants with low resistance).

A second fitness surface for the plants in the sprayed treatment represented the net effect of all selective forces (all other ecological or physiological selective forces) acting on glucosinolates and trichomes *except* those due to herbivores (Fig. 1C, D in Mauricio and Rausher, 1997). In the absence of herbivores, the fitness peak corresponds to absence of both trichomes and glucosinolates. This pattern indicates that, in the absence of herbivores, there is selection acting to decrease both trichome density and total glucosinolate concentration. Therefore, this surface was interpreted by Mauricio and Rausher (1997) as representing the costs of resistance.

The final fitness surface (Fig. 1A, B in Mauricio and Rausher, 1997) showed the combined effects of the costs and benefits associated with glucosinolates and trichomes. This surface was constructed using data from an unmanipulated control treatment. The surface had a single peak corresponding to the absence of trichomes but intermediate levels of glucosinolates. In other words, at the evolutionary equilibrium associated with this fitness surface, glucosinolates were shown to be subject to stabilizing selection. In this case, stabilizing selection resulted from a combination of directional selection favoring a decrease in glucosinolate concentration in the absence of herbivores (the cost of glucosinolate production) and directional selection imposed by herbivores favoring an increase in glucosinolate content (the benefit of glucosinolate production). This result confirmed models of the evolution of resistance suggesting that stabilizing selection on level of resistance may frequently arise from a balance between the costs and benefits of resistance.

By contrast, stabilizing selection on trichome density in the control treatment was absent and indicated that opposing costs and benefits do not necessarily favor an intermediate level of resistance. In this case, the costs of trichome production increases faster with increasing trichome density than

do the benefits, producing an evolutionary equilibrium at which trichomes are absent. Since plants in this experiment obviously had genetic variation for trichome density, the population is evidently not in equilibrium. Temporal and spatial variation in selection on trichomes could explain the lack of congruence between the predicted and observed trichome densities. This difference between trichomes and glucosinolates in the net pattern of selection illustrates how the balance of selection may be tipped in different ways, depending on the relative magnitudes and exact shapes of the cost and benefit functions.

CONCLUSION

In this paper, I have provided evidence for 2 major assumptions of coevolutionary theory. First, in natural populations of *Arabidopsis thaliana*, herbivores are agents of selection on two traits commonly believed to have evolved in response to herbivores: glucosinolates and trichomes. Invoking uniformitarianism, it is likely that herbivores have been, and continue to be, important selective agents on these resistance traits in *Arabidopsis thaliana*. Second, I have provided evidence from two experimental approaches that resistance can incur fitness costs on plants. The mixed results from the mutant study suggests that an understanding of the mechanisms of costs and further dissection of the mechanisms of resistance itself will be fruitful avenues of research. Together, these results illustrate how an understanding of the costs and benefits of resistance traits can lead to a greater understanding of natural plant populations. Finally, although Marlin Perkins, Steve Irwin and David Attenborough provide an entertaining view of evolution, an ecological genetic approach can provide rigorous and powerful tools to the study of coevolutionary interactions between plants and herbivores.

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