Co-evolution and plant resistance to natural enemies

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Co-evolution between plants and their natural enemies is generally believed to have generated much of the Earth's biological diversity. A process analogous to co-evolution occurs in agricultural systems, in which natural enemies adapt to crop resistance introduced by breeding or genetic engineering. Because of this similarity, the investigation of resistance mechanisms in crops is helping to elucidate the workings of co-evolution in nature, while evolutionary principles, including those derived from investigation of co-evolution in nature, are being applied in the management of resistance in genetically engineered crops.

The process of co-evolution between plants and their natural enemies — including viruses, fungi, bacteria, nematodes, insects and mammals — is believed by many biologists to have generated much of the Earth's biological diversity[1,2]. Co-evolution is a reciprocal evolutionary interaction between a plant and one or more of its natural enemies that occurs in cycles[3–7]. In the first phase of a cycle, natural selection imposed by enemies causes the evolution of a new plant resistance character that reduces enemy attack. Much of the extraordinary chemical and morphological diversity among plant species is believed to reflect this type of defensive adaptation occurring independently in different sets of co-evolving species (Table 1, and see review in this issue by Dixon, pages 843–847). Because most resistance characters reduce the survival or virulence of natural enemies, their evolution generates selection that initiates the second phase of a co-evolutionary cycle: the evolution of counter-resistance by those enemies, that is, the evolution of characters that circumvent the newly evolved plant resistance. Plant natural enemies exhibit a wide variety of physiological, behavioural and morphological characters that seem to have evolved in this way (Table 1).

A process analogous to co-evolution also occurs in agricultural systems. Breeders release a resistant crop variety, and the evolution of counter-resistance typically follows. When breeders respond by introducing another resistant variety, a new cycle is initiated. A typical example of this 'artificial' co-evolution is the attempt to breed wheat resistant to Hessian fly, *Mayetiola destructor*, in Indiana. In 1955, a cultivar carrying a resistance gene was deployed and provided effective resistance. Within six years, however, substantial counter-resistance had evolved in the Hessian fly. A cultivar carrying a second resistance gene was released in 1964, with counter-resistance appearing within eight years. Counter-resistance to a third gene, released in 1971, had evolved within about 10 years[8–10].

Conventional breeding of resistance suffers from a serious limitation: reproductive barriers between species prevent introduction of resistance genes into a crop from any plants except very closely related wild relatives. A potentially effective defence found in, say, teosinte (*Zea mexicana*) can be bred into maize (recently derived from teosinte) but not into rice or soya beans because the latter cannot be crossed with teosinte. The perfection of genetic transformation technology in the 1980s removed this limitation and now allows resistance genes to be transferred into crops from distantly related (even non-plant) species. Such technological advances have stimulated plant molecular biologists to explore the genetic and biochemical control of resistance characters, with the aim of transferring new resistance genes into crops, although they have not alleviated the threat that natural enemies will evolve counter-resistance to whatever resistance genes are deployed.

As I describe below, this threat has stimulated investigations of how evolutionary principles can be applied to slow the evolution of counter-resistance. The molecular analysis of resistance has given renewed impetus to the science of applied evolution, a science that draws upon knowledge gained from the study of natural co-evolution. At the same time, the molecular analysis of resistance has provided new insight into the operation of co-evolution in nature. In particular, it has provided significant evidence for the common operation of the first phase of co-evolutionary cycles, and has facilitated empirical confirmation of one of the main assumptions about how plant defences against enemies evolve.

Natural enemies impose selection for resistance

Evidence indicating that natural enemies generally evolve to overcome the detrimental effects of plant resistance characters (the second phase of co-evolutionary cycles) is abundant: natural enemies exhibit numerous characters that can be interpreted only as having evolved to confer counter-resistance (Table 1). For example, seeds of the tropical legume *Dioclea megacarpa*, which contain the non-protein amino acid L-canavanine, are toxic to most insects because their arginyl-tRNA synthetases also incorporate L-canavanine into proteins. However, the bruchid beetle *Caryedes brasiliensis*, whose larvae feed solely on *D. megacarpa*, has evolved a modified tRNA synthetase that distinguishes between L-canavanine and arginine[11]. Adaptation by natural enemies is also seen at the level of local populations. In parsnip webworms, cytochrome P450 enzymes detoxify furanocoumarins produced by their host plant, wild parsnip (*Pastinaca sativa*). In each population, P450 activity, as well as its specificity towards different furanocoumarins, reflects the concentration and profile of furanocoumarins produced by the host population — each webworm population seems finely adapted to overcoming the particular defences it encounters[12].

By contrast, the contention that apparently defensive traits of plants have actually evolved in response to natural

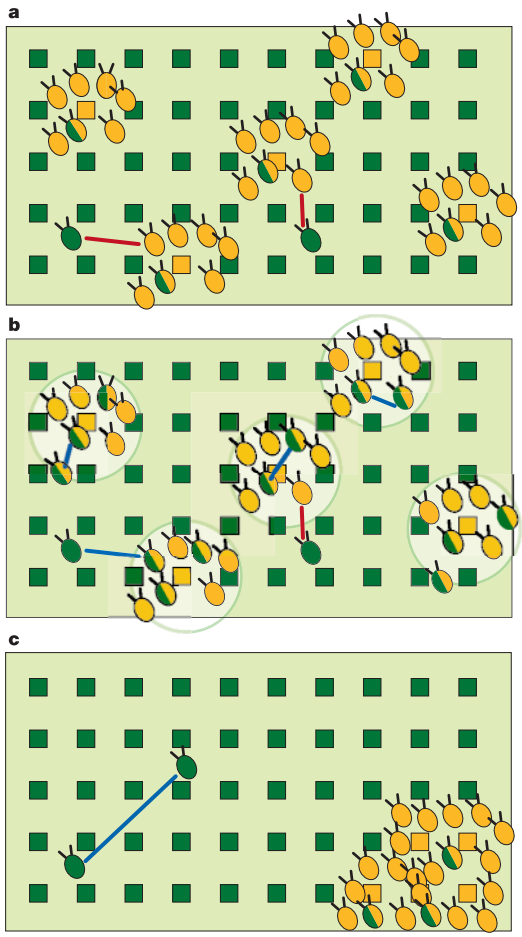


Figure 1 Schematic portrayal of the HDR strategy. Green and yellow squares represent resistant and susceptible (refuge) plants, respectively. Yellow insects, *AA* (susceptible) genotype; yellow and green insects, *Aa* (susceptible) genotype; green insects, *aa* (counter-resistant) genotype. **a**, Refuge plants are interspersed with susceptible plants, and the juvenile insect stage is confined to a single plant. The HDR strategy functions well. The numerous *AA* insects produced on refuge plants screen the few counter-resistant *aa* insects that emerge from resistant plants from each other and ensure that *aa* individuals mate only with *AA* individuals (red lines). This prevents the production of homozygous offspring that would be adapted to feeding on *aa* plants. **b**, Refuge plants are interspersed with susceptible plants, and the juvenile insect stage moves among plants. Circles indicate radius of juvenile insect movement. The HDR strategy functions poorly. Under these conditions, more *Aa* individuals survive because the toxicity of the food plant is diluted. Matings between *Aa* and *aa* or between *aa* and *aa* individuals (blue lines) are thus more frequent and result in the production of *aa* (counter-resistant) offspring. **c**, Refuge plants are clumped, and adult insect dispersal is limited. The HDR strategy functions poorly. Even with movement by juvenile insect stages among plants, most heterozygotes do not feed on susceptible plants and thus die. However, limited dispersal of *AA* insects prevents them from screening matings between the rare *aa* individuals (blue lines), resulting in production of homozygous counter-resistant individuals.

selection imposed by natural enemies is more controversial. For some traits, it is clear that the only function is defence. Thorns and urticating hairs, for example, almost certainly function primarily to protect plants from mammalian herbivores. But in most cases, characters that confer resistance may have additional physiological or ecological functions. For example, although various flavonoids exhibit antifungal and antibacterial properties, most also absorb ultraviolet radiation efficiently and are believed to protect the plant from this environmental hazard[12]. Other functions performed by plant secondary chemicals include conferring frost tolerance, allelopathy, nutrient storage, structural reinforcement, mediation of stigma–pollen interactions, regulation of biochemical processes, and signalling to mutualists[13–17].

As has been repeatedly argued[3,14–16,18–21], the multiplicity of functions attributed to most resistance factors undermines the inference that they have evolved primarily in response to natural enemies. Rather, resistance may simply be a fortuitous side effect of characters that evolved to perform other ecological functions. It has also been argued that in nature, plant enemies are generally too rare to cause the frequent evolution of defensive traits[19,20,22,23]. These arguments call into question the widespread belief that co-evolution between plants and their enemies is common and generates much of the morphological and chemical diversity plants exhibit.

One recent approach to addressing this controversy has been to use manipulative field experiments to compare the pattern of selection on purported defensive characters in the presence and absence of natural enemies[21]. Although the number of cases examined is still small, enemy-imposed selection on resistance has been demonstrated in all investigations conducted, for characters as varied as glucosinolate content and trichome density in *Arabidopsis*[24], alkaloid content in *Datura*[25], resistance to fungal pathogens in *Silene*[21,26], and resistance and tolerance to insects in *Ipomoea*[27,28]. These investigations provide strong evidence for the potential of natural enemies to cause the evolution of plant resistance characters, although they provide little indication of whether resistance characters actually do evolve in response to selection imposed by natural enemies.

An alternative source of information regarding this controversy is being provided as a direct result of investigations of the molecular and biochemical basis of resistance to pathogens. Gene-for-gene resistance seems to be mediated by signal cascades that initiate both localized cell death around the site of pathogen infection, and mobilization of systemic induced resistance[29–31]. At the head of the signal cascade is a receptor protein that recognizes some molecular feature of the invading pathogen (the 'elicitor') and activates the cascade.

The high specificity of the receptor to the pathogen elicitor almost unquestionably indicates that the receptor, and its coupling to the signal cascade, represent adaptations to defence against pathogens. Moreover, these receptors have undergone numerous amino-acid substitutions over a relatively short period of time[32–35]. Because the rate of non-synonymous (amino-acid changing) substitution is often higher than the rate of synonymous (non-amino-acid changing) substitutions in the genes coding for these receptors[33,34], much of this evolutionary change seems to be adaptive rather than due to genetic drift[36]. Finally, amino-acid substitutions are concentrated in regions of the receptor that are believed to interact with the elicitor molecule, as is expected if these regions were co-evolving with those elicitors. These patterns indicate that in the progenitors of crop plants as diverse as rice, tomato, flax and sugar beets, receptor genes conferring resistance have in fact evolved in response to selection imposed by bacterial, fungal or viral pathogens.

Chitinase evolution in *Arabidopsis* and related species in the genus *Arabis* exhibits remarkable similarities to receptor evolution[37]. Plant chitinases are believed to defend against fungal infection by attacking chitin, a principal component of fungal cell walls. Class I chitinases in *Arabis* species often exhibit higher rates of non-synonymous than synonymous substitutions, a hallmark of adaptive evolution. Moreover, amino-acid substitutions are concentrated in the molecule's active site, a pattern not usually seen in enzyme evolution. Because the structure of chitin does not evolve, the most reasonable interpretation of this pattern is that plant chitinases are co-evolving with pathogen chitinase inhibitors, small carbohydrate or protein molecules that competitively inhibit the breakdown of chitin by chitinase.

Because receptor proteins and chitinases represent only a small sample of purported plant defences against natural enemies, it may be premature to generalize from these examples that those traits have generally evolved in response to selection imposed by natural

enemies. Nevertheless, it is clear that as plant cell and molecular biologists identify the genes associated with other resistance characters, evolutionary biologists will be presented with many more opportunities to address this issue.

Defensive traits are costly

The idea that adaptation is costly is a deeply entrenched principle in evolutionary biology. In the context of plant defences, this principle states that the incremental fitness benefit associated with genotypes conferring increased defence is accompanied by a decrement in fitness associated with reallocation of resources away from other fitness-enhancing functions[38].

For example, an increase in the production of alkaloid compounds as defences against herbivorous insects removes nitrogen from the pool available to a plant for growth, and is thus expected to reduce plant size and seed production. Because such costs are an integral component of the standard evolutionary model for the evolution of resistance[39,40], the validity of this model depends on whether such costs normally exist.

Recent experiments on natural populations of plants as diverse as *Arabidopsis*, *Ipomoea* (morning glories), *Pastinaca* and *Trifolium* (clovers) have provided strong evidence for costs[28,41–45]. These experiments typically use quantitative genetic approaches to determine whether, in the absence of enemies, fitness and resistance are inversely correlated. Their interpretation relies on a crucial assumption: because in the absence of enemies, benefits associated with resistance cannot be realized, any fitness differences among genotypes must reflect pleiotropic effects of resistance genes (that is, multiple effects of single genes, which affect more than one phenotypic character). Although this interpretation is reasonable, it has been difficult to rule out an alternative interpretation: fitness differences in the absence of enemies result from linkage disequilibrium between resistance alleles and alleles at linked loci. If this alternative interpretation were correct, apparent costs of defence could be transient historical effects, rather than permanent constraints on the evolution of defences, as linkage disequilibrium is expected to decay over time.

Distinguishing between these alternative interpretations has been difficult because in most natural systems, little is known about genes associated with resistance. However, in a set of experiments made possible by prior molecular characterization of resistance to the herbicide chlorosulphuron in *Arabidopsis thaliana*[46,47], resistance costs have been shown to result from pleiotropy[48,49]. In *A. thaliana*, chlorosulphuron resistance is conferred by a single base-pair substitution in the gene encoding acetolactate synthase (ALS), which catalyses the first step in the biosynthesis of branched-chain amino acids. By transforming the resistant *ALS* allele into a chlorosulphuron-susceptible *Arabidopsis* stock, isogenic lines that differed only in whether the resistance allele was present were created. In the field, in the absence of herbicides, the resistant lines produced 34% fewer seeds, indicating a substantial fitness cost clearly due to pleiotropy. This cost is believed to be due to either increased metabolic drain caused by overexpression of branched-chain amino acids or a build-up of the toxic α-amino butyric acid.

Although the *ALS* mutant confers resistance to a synthetic herbicide rather than a natural enemy, there is no reason to believe that alleles for resistance to natural enemies would be less likely to have associated pleiotropic costs. Our growing understanding of the molecular action of genes that control resistance to natural enemies should facilitate similar experiments designed to determine whether the apparent costs detected by quantitative genetics experiments are generally due to pleiotropy. Data obtained from such experiments should eventually reveal whether a basic assumption about constraints on resistance evolution in natural populations is valid.

Table 1 Examples of co-evolution in natural plant–enemy systems

Plant defence	Plant taxon	Natural enemy	Counter-resistance	Refs
Toxic furanocoumarins	Umbelliferae	Black swallowtail butterfly	Cytochrome P450 detoxifying enzymes	95, 96
Toxic amino acids	Various Leguminosae	Bruchid weevil	Modified tRNA synthetase	11
Trichomes	*Solanum*	Ithomiid butterfly	Silk scaffolding	97
Latex	*Asclepias* (milkweeds)	Monarch butterfly and others	Leaf-vein-cutting behaviour	98
Enlarged fruits	Sapindales	*Jadera* bugs	Elongated mouthparts	99, 100
Chitinase	*Arabis* (Cruciferae)	Fungal pathogens	Chitinase inhibitors	37
Hypersensitive response *R* genes	Several taxa	Fungal and bacterial pathogens	Modification of elicitor proteins	33–35
Mutualism with predacious ants	*Acacia*	*Polyhymno* (Gelechiid lepidopteran)	Shelter construction	101, 102

Resistance management by evolutionary engineering

Although evolutionary biology is largely an academic science, the practical benefits of the application of evolutionary principles are beginning to be realized in areas as diverse as disease management[50,51], fisheries management[52–54], conservation[55,56], biomolecular engineering[57,58] and computer design[59]. But perhaps it is in the area of resistance management that this potential has begun to be realized most. Resistance management attempts to prevent natural enemies from evolving counter-resistance to pesticides or resistant crops, a hitherto almost inevitable phenomenon. Although the use of evolutionary principles in this area was aimed initially at preserving the effective lifetime of pesticides and conventionally bred crop resistance, it has taken on added importance for genetically engineered crops because of the greatly increased economic investment required for their development and deployment.

Although completely preventing the evolution of counter-resistance may in most cases be impossible, evolutionary biologists have developed strategies that, at least in theory, will slow the evolution of counter-resistance and thus prolong the usefulness of genetically engineered resistance. With these strategies, biologists hope to redirect the course of evolution.

The high-dose/refuge strategy

Recent research has focused on devising strategies for delaying the evolution of counter-resistance by insect herbivores to toxins, particularly *Bacillus thuringiensis* toxins[60]. Although various approaches have been suggested[61,62], researchers, the United States Environmental Protection Agency, and some large corporations are currently concentrating on the 'high-dose/refuge' (HDR) strategy[60,62–65]. This approach involves engineering a crop to produce high doses of a toxin and planting mixes of resistant and susceptible varieties (Fig. 1a).

The functioning of the HDR strategy relies on several basic evolutionary principles. The first principle is that the rate at which an advantageous allele increases in frequency in a population depends greatly on its degree of dominance (Box 1). A completely recessive allele spreads much more slowly than a dominant or additive allele because initially it is present only in heterozygotes, which are sheltered from selection. The 'high-dose' portion of the strategy is aimed at ensuring that alleles conferring counter-resistance are effectively recessive. Even if the LD_{50} (median lethal dose) of the heterozygote is intermediate between that of the homozygotes (that is, counter-resistance is neither dominant nor recessive), a high enough dose of the toxin will still kill more than 99% of the heterozygotes, rendering counter-resistance effectively recessive.

The second evolutionary principle used by the HDR strategy is that the rate of increase in allele frequency is proportional to the difference in fitness between genotypes (Box 1). The 'refuge' portion of the strategy is designed to slow the spread of a counter-resistance allele by reducing the fitness difference between the homozygote for that allele and the other genotypes. The refuge is provided by the non-resistant plants, which allow the susceptible insects to reproduce

Box 1
Theoretical basis of the HDR strategy

Evolution of resistance conferred by a single mutation can be modelled[74] using the basic population genetic equation for a change in gene frequency at a single locus with two alleles:

$$p' = p(pW_{AA} + qW_{Aa})/(p^2W_{AA} + 2pqW_{Aa} + q^2W_{aa})$$

where p and q are the frequencies of alleles A and a, and W_{ij} is the fitness of genotype ij. Iteration of this equation yields the trajectory of change in gene frequency for allele A[103]. The HDR strategy for resistance management is based on two basic properties of this equation.

1. The rate of increase in the frequency of a new mutant depends on degree of dominance. When a mutant allele first appears, it is rare and occurs only in heterozygotes. The effective magnitude of selection on this allele therefore depends on whether it is expressed in the heterozygote. If the allele is dominant, it is exposed to selection immediately and increases rapidly in frequency, whereas if it is recessive, it is initially shielded from selection and increases only slowly. An example of this effect is portrayed in Fig. B1a, in which the relative fitnesses of the mutant and wild-type alleles are 1 and 0.5 respectively.

2. The rate of increase in the frequency of a new mutant depends on the difference in fitness between genotypes. For the case of a recessive mutant allele, the relevant fitness difference, s, is that between the wild-type and mutant homozygotes. As shown in Fig. B1b, reducing this fitness difference retards the spread of the mutant allele.

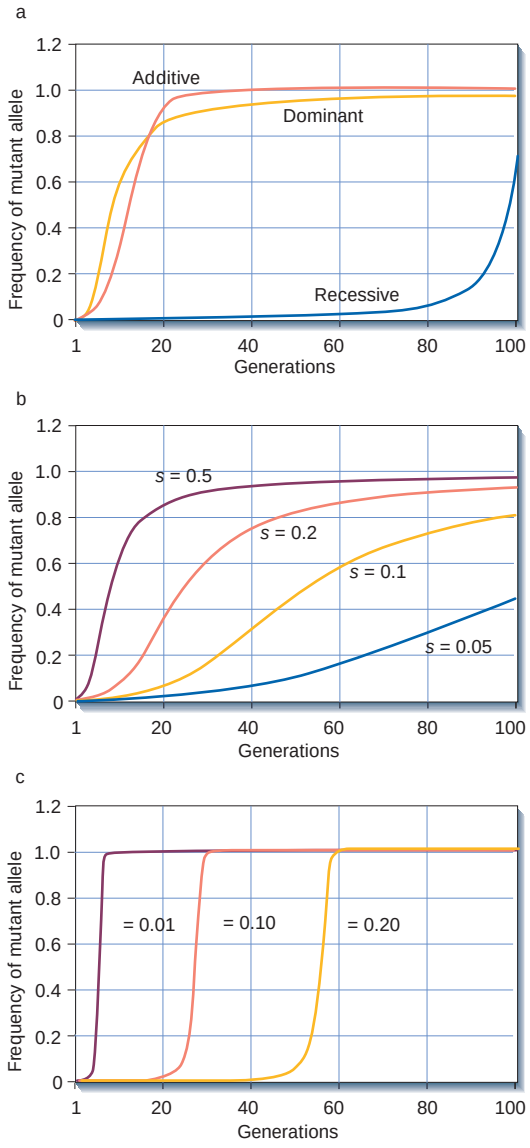
When refuges are used, the fitness of each genotype, W, depends on the proportion of the habitat dedicated to the refuge, symbolized by β (Box 1 Table). With complete recessivity ($h = 0$), the fitness difference between homozygotes is $1 - c - \beta$, indicating that this fitness difference decreases as the size of the refuge increases. For an initial mutant frequency of 0.005, Fig. B1c shows that although counter-resistance evolves to appreciable frequencies within about five pest generations in the absence of a refuge ($\beta = 0.01$), 10% and 20% of refuges delay the development of counter-resistance for more than 25 and 50 generations, respectively.

Box 1 Table Fitness of genotypes *AA*, *Aa* and *aa*

Genotype	Non-refuge	Refuge*	W
AA (susceptible)	0	1	β
Aa (susceptible)	0	$1 - hc$	$\beta(1 - hc)$
aa (resistant)	$1 - c$	$1 - c$	$1 - c$

*c, cost of counter-resistance; h, dominance of heterozygote.

Figure B1 Effects of dominance (**a**), strength of selection (**b**) and refuge size (**c**) on rate of increase of frequency of mutant allele.



substantially. With even a small refuge, the fitness difference is reduced markedly, greatly retarding the rate of increase of the counter-resistance allele (Box 1). In essence, the refuge produces enough susceptible insects to greatly reduce the probability that the rare counter-resistant insects mate with each other and produce counter-resistant offspring (Fig. 1a).

A third evolutionary principle — adaptations are generally costly — suggests that in many situations, the HDR strategy may postpone the evolution of counter-resistance indefinitely. Accumulating evidence indicates that counter-resistance to toxins is frequently costly[66–68], as evidenced by the rapid elimination of counter-resistance when pesticide use is discontinued[69–71]. Such costs, if manifested in heterozygotes, render the overall fitness of heterozygotes less than the fitness of the susceptible homozygote, which will tend to prevent the allele conferring counter-resistance from increasing in frequency when rare. Unfortunately, our ignorance about how often counter-resistance is costly in heterozygotes prevents us from being able to predict how likely genetically engineered resistance is to be evolutionarily stable for indefinite periods under the HDR strategy.

Even in the absence of costs, the theoretical considerations described above indicate that this strategy may greatly slow the evolution of counter-resistance. It is this theoretical promise that has convinced the Environmental Protection Agency and some corporations to adopt the HDR strategy as a goal for resistance management. However, the theoretical predictions of this strategy have been examined empirically only rarely, usually under artificial conditions[72,73], and rest on largely untested assumptions about the basic biology of insect pests. One such assumption is that the spatial organization of refuges can be designed to ensure mating panmixia (that is, indiscriminate or random mating) and restriction of juvenile stages to just toxic or just non-toxic plants. Close intermixing of toxic and non-toxic plants may allow mobile individual insects to feed on a mixture, effectively diluting the toxin dose and compromising the effective recessivity of counter-resistance (Fig. 1b). By contrast, although large

Box 2
Evolution of counter-resistance by haploid pests

A simple evolutionary model illustrates that a refuge strategy is not likely to delay the evolution of counter-resistance in haploid pests. This model assumes that there are two pest genotypes, one resistant to a genetically engineered plant toxin (R) and one susceptible to the toxin (S). There are also two crop varieties, one with the toxin and one without. The variety without the toxin is planted in refuges, which constitute a fraction β of the acreage planted. The relative fitness of the susceptible pest genotype is assumed to be 1 on plants without the toxin, and 0 on plants with the toxin. The relative fitness of the resistant pest genotype is taken to be $1-c$ on both crop varieties, where c represents the cost of counter-resistance. Such costs typically range from 0 to 0.2[66,68,104].

Using the general equation for change in allele frequencies in haploid populations[103], the equation that describes the change in frequency, p_S, of the resistant pest genotype from one generation to the next is

$$p'_R = (1-c)p_R / [(1-c)p_R + \beta]$$

This equation can be iterated, starting with an initially low frequency of the resistant genotype ($p_R = 0.0001$), to determine how the rate of spread of the resistant genotype depends on the size of the refuge and on the magnitudes of cost of resistance. Box 2 Table below shows the number of generations needed for the resistant genotype to become common (that is, to reach a frequency of 0.5).

These results indicate that, unless refuges constitute roughly half the acreage planted and costs of counter-resistance are very high, a refuge strategy is not likely to delay the evolution of counter-resistance substantially. Growers typically will not accept refuges that constitute more than 10% of the acreage planted, and the US Environmental Protection Agency mandates a refuge of 4% for transgenic resistant cotton[60]. With refuges of these sizes, substantial counter-resistance is likely to evolve within a few years in haploid pests.

Box 2 Table Number of generations to reach $p_R = 0.5$

β	c	With cost	Without cost
0.1	0.1	4	3
0.1	0.2	4	3
0.1	0.3	4	3
0.3	0.1	9	8
0.3	0.2	10	8
0.3	0.3	11	8
0.5	0.1	16	14
0.5	0.2	20	14
0.5	0.3	28	14

refuge and non-refuge patches may ensure that individuals will remain in the same patch throughout development, mating panmixia may be compromised if the patches are too large, leading to a preponderance of within-patch mating. Such assortative mating produces relatively more counter-resistant homozygotes and fewer heterozygotes, reducing the effectiveness of recessivity in slowing the evolution of counter-resistance (Fig.1c). Although some recent theoretical analyses have attempted to assess the effects of these complications on the effectiveness of the HDR strategy, detailed empirical investigations of insect movement patterns are necessary to determine whether, for any specific crop, an appropriate refuge configuration can be designed that will allow the HDR strategy to be effective[74].

Limitations of the high-dose/refuge strategy

The HDR strategy has been developed largely in the context of delaying the evolution of counter-resistance by sexually reproducing insects. But evolutionary considerations suggest that this strategy may be ineffective in managing counter-resistance in other types of organisms. Panmictic sexual reproduction is crucial for this strategy because it ensures that most copies of the initially rare counter-resistance allele occur in low-fitness heterozygotes. In diploid pests with substantial asexual reproduction (for example, aphids), a rare mutant homozygote can rapidly multiply on resistant hosts, increasing the frequency of counter-resistance.

For haploid pests (for example, viruses, bacteria and some fungi), achieving effective recessivity is by definition impossible. Consequently, slowing the spread of a counter-resistance allele within the HDR paradigm can be achieved only by providing a refuge, which reduces the fitness difference between counter-resistant and non-counter-resistant genotypes. Unfortunately, a simple evolutionary model suggests that even with a refuge constituting 50% of all plants, far greater than is commercially acceptable[60], and a large cost associated with counter-resistance, evolution of counter-resistance will not be substantially delayed (Box 2). It is therefore likely that alternate strategies will be necessary for resistance management in systems with haploid pests.

One augmentation of the HDR approach that has been examined theoretically is pyramiding — engineering plants to produce two unrelated toxins simultaneously. Models with pyramiding indicate that, compared to crops with just one toxin, much smaller refuges are required to achieve the same delay in pest adaptation[75–77]. As with the HDR approach with a single toxin, recessivity is critical in these models, because the added effectiveness of pyramiding is due largely to the rarity of doubly counter-resistant homozygote genotypes in the pest. With high doses of both toxins, such recessivity can theoretically be achieved, but the technical and economic difficulties of pyramiding even two unrelated toxins[78] make this strategy unfeasible for the foreseeable future.

One possible exception to this pessimistic conclusion is suggested by the recent molecular dissection of receptor genes for pathogen resistance (see above, and the review in this issue by Dangl and Jones, pages 826–833). The presence of multiple copies of these genes presumably increases the effectiveness of this type of defence in nature by increasing the likelihood that mutations will produce at least one receptor protein that can recognize a virulent pathogen. This multiplicity could also be used to advantage for resistance management by pyramiding five or six receptors that recognize different pathogen elicitors. Evolutionary stability of resistance would be conferred by the redundancy of the receptors — mutations would be required simultaneously in five or six pathogen elicitor molecules to confer counter-resistance (escape from recognition), an event with infinitesimally low probability. Moreover, by targeting as elicitors pathogen molecules that are involved in vital pathogen life processes, mutations in individual elicitors that render them no longer recognized by the corresponding receptor are likely to be detrimental. This cost would tend to prevent loss of elicitor recognition by genetic drift in the pathogen. In addition, such targeting would minimize the chance that some naturally occurring pathogen strains lack the elicitors, and thus would be virulent on the genetically modified crop.

Several considerations suggest that this type of pyramiding is likely to be more easily achieved than pyramiding unrelated toxins. First, only a single gene — that coding for the receptor — needs be inserted for each resistance factor, as different receptors all initiate the same signal cascade[79]. By contrast, in many cases, inserting new toxins will require inserting the genes coding for all of the enzymes required to make that toxin. (Bt-toxin is unusual in that it is the product of a single gene.) Second, the risk of increased autotoxicity associated with multiple toxins does not arise with receptor proteins, because they are not toxic to the plants that produce them. Finally, each toxin inserted into a plant potentially reduces yield because of costs associated with resistance. By contrast, if engineered receptors can be substituted for the redundant receptors already in a plant genome, it may be possible to completely avoid any costs of resistance.

Of course, there still remain formidable obstacles to implementing this type of strategy. Among these are designing or discovering receptors that target a specific pathogen, and developing techniques for replacing native receptor genes with engineered genes. Nevertheless, the potential payoff of indefinitely lasting resistance to pathogens suggests that this is a strategy to pursue.

Tolerance as an evolutionarily stable defence

In developing approaches for managing resistance, most effort has focused on slowing artificial co-evolution by delaying the evolution of counter-resistance. However, recent investigations of plant defences in nature suggest that an alternate management strategy may be effective in some cases: breaking the co-evolutionary cycle by incorporating tolerance, rather than resistance, into crops.

Whereas resistance reduces the amount of damage or infection a plant experiences, tolerance reduces or eliminates the detrimental effect of a given amount of damage or infection on plant fitness (or on crop yield, in an agricultural context). Agricultural scientists recognized decades ago that crop cultivars could differ in tolerance, and have made some attempts to breed tolerance into crops[80,81]. But only over the past decade have evolutionary biologists discovered that natural plant populations often use tolerance as a defence against natural enemies. Genetic variation for degree of tolerance has been detected in plant families as taxonomically disparate as Piperaceae, Convolvulaceae, Polemoniaceae and Brassicaceae. Within species, populations often diverge in mean level of tolerance, and in some cases it is known that populations with a history of higher herbivore damage have higher tolerance[82]. In most plants, tolerance does not completely prevent damage from decreasing fitness, probably because costs prevent the evolution of maximum levels of tolerance[28]. Nevertheless, some species exhibit overcompensation, a form of tolerance in which enemy damage actually increases plant fitness[83,84].

In contrast to resistance, tolerance is not believed to adversely affect natural enemies[85–87]. Consequently, the evolution of tolerance does not generate natural selection for counter-adaptation in enemies, and thus breaks the co-evolutionary cycle. These considerations suggest that if crops could be genetically engineered to be tolerant to pests, counter-resistance management might no longer be an issue.

Many obstacles stand in the way of realizing this promise. The most important is that genes involved in conferring tolerance have not been identified at the molecular level for any plant species. A second is that conventional breeding programmes suggest that tolerance may often be a genetically complex trait involving many different plant characters[82]. A final obstacle is that increased tolerance may not prevent unacceptable cosmetic damage to a crop. Despite these obstacles, there is no reason to believe that some crops could not eventually be genetically engineered to be tolerant. As in the case of pyramiding receptor genes, the payoff of a possibly indefinitely stable defence should provide a strong economic incentive for funding the basic research needed to achieve this goal.

Coupling a toxin with non-preference

Approximately 90% of all herbivorous insects have narrow diets, feeding on plants of only one taxonomic family, and many species are confined to a single host species[22,88–90]. Both empirical and theoretical investigations suggest two general reasons for evolving such specialization: (1) variability in fitness on different host plant species favours behavioural genotypes that restrict feeding to the best hosts; and (2) maintaining mechanisms to nullify the disparate defensive adaptations of many different plant species is too costly for generalist herbivores[7].

By contrast, the key to understanding why specialized herbivores remain specialized lies in the observation that specialization involves both behavioural and physiological adaptation. Selection for behavioural genotypes that restrict feeding to a small number of plants increases selection for physiological adaptation to those plants and relaxes selection for physiological adaptation to other (non-host) plants[91,92]. Specialist species thus tend to have low fitness on non-host plants even if they can be induced to feed on them.

Population genetic models indicate that once a herbivore has evolved both behavioural and physiological specialization, the subsequent evolution of a broader diet is likely to be very difficult; if costs are associated with physiological adaptation to the novel host, specialization can be an evolutionarily stable state[91,93]. These theoretical considerations suggest another approach for resistance management: combining the HDR strategy with manipulation of the attractiveness of the toxic crop variety. Under this approach, a crop would be genetically engineered not only to produce a high dose of a toxin, but also to be unrecognized as a potential host by the pest. As in the HDR strategy, a refuge is provided that consists of a non-resistant variety of the crop or an alternative host.

Lack of recognition has long been recognized as a form of resistance, and has been bred into some crops[80], although by itself it is not evolutionarily stable. In many insects, behavioural recognition is based on one or a few 'token stimuli', often plant secondary compounds that stimulate insect feeding or oviposition[94]. In theory, genetically engineering non-preference resistance could in many cases involve simply deactivating one gene coding for an enzyme in the biochemical pathway that produces the token stimulus. Such a manipulation is likely to be simpler than inserting, under a pyramiding strategy, a new toxin that is biochemically even moderately complex to produce, and is likely to be equally effective.

Applied evolutionary research

Although the promise of genetically engineered crops that are resistant to important pests is beginning to be realized (see review in this issue by Stuiver and Custers, pages 865–868), the long-term success of this approach will be determined by economic realities. Because research and development costs are enormous compared to conventional breeding, their widespread use will depend on their having a reasonably long effective lifetime. And because the main threat to longevity is evolutionary change in the targeted pest species, management approaches that actively manipulate the evolutionary process will be required. Although evolutionary biologists have begun to develop approaches that allow such manipulation, most work so far has used simple evolutionary models with possibly unrealistic assumptions about the basic biology and genetics of the targeted pests. Moreover, there is currently little empirical evidence indicating whether the evolution of counter-resistance can actually be slowed or prevented as the theory suggests.

As the examples above illustrate, the continued study of evolution in natural plant–enemy systems is likely to contribute new insights regarding approaches to resistance management. At the same time, refining and improving current strategies such as HDR will require the development of more realistic and sophisticated population genetic models, the conceptual foundation of evolutionary biology. Yet the number of scientists engaged in these activities is small, compared with the number engaged in elucidating the molecular and biochemical causes of resistance. This dilemma should provide sufficient justification to stimulate the private sector, governments and universities to establish and fund a new initiative aimed at fostering increased research effort not only in the area of resistance management, but also in all applied disciplines that involve a significant evolutionary component.

The ultimate irony, of course, is that a call for enhanced funding for applied evolutionary research comes at a time of renewed anti-evolutionary religious zeal. In the United States, citizens in many of the very states whose economic welfare depends on crops susceptible to attack by devastating pests are calling for restricting or banning the teaching of the very science that holds out the most promise for winning the co-evolutionary war between crop plants and their enemies. We can only hope that continued education about the practical implications of evolutionary biology will persuade most citizens to ignore these calls. □

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