Molecular Biology and Evolution of Resistance to Toxicants

Martin Taylor and René Feyereisen

Department of Entomology and Center for Insect Science, University of Arizona

To the prevailing biochemical/physiological classification of mechanisms of organismal resistance to toxicants, an additional molecular dimension is proposed. Predictions are developed regarding the relative prevalence of different classes of mutations and are found to compare favorably with reports from the literature. In particular, point mutations in target loci were the dominant form of resistance for both lab and field selection. Amplifications of target loci were less common than structural mutations, and more common for lab-selected than for field-selected strains. Amplification was the most common mechanism of up-regulation of metabolizing enzymes. In comparison, only one mutation involving *cis*-regulation and several involving *trans*-acting regulation were found. Mutations involving gene disruption and down-regulation were uncommon, but were found in appropriate cases, i.e., when toxicants stimulated rather than inhibited target function and when metabolizing enzymes converted toxicants into more toxic metabolites. Additional phenomena of likely but uncertain importance are genetic "succession," recombinational limitation, and negative cross-resistance. More work on these phenomena and on quantification of fitness costs of resistance is recommended.

Introduction

The last 50 years have witnessed a massive transition in medicine and agriculture toward almost total dependence on toxic chemicals designed to control unwanted organisms. This arsenal of toxicants has been directed at numerous taxa in every kingdom: bacteria, protozoa, weeds, fungi, insects, and rodents. The use of such chemicals in everyday life is now widespread. Who has not heard of antibiotics, pesticides, or chemotherapeutic agents, and which large chemical company is not involved in one way or another in the production of such compounds? Evolution of resistance of target organisms to toxicants has been observed in every specialized field,

Key words: toxicant resistance, molecular biology, mutation, evolution, population genetics.

Address for correspondence and reprints: Martin Taylor, Department of Entomology and Center for Insect Science, University of Arizona, Tucson, Arizona 85721. E-mail: taylor@biosci.arizona.edu.

Mol. Biol. Evol. 13(6):719–734. 1996

and there is an extensive literature on antibiotic, drug, and pesticide resistance. Even viruses and cancer cells have become resistant to drugs used to control them. This literature addresses mostly detection, mechanisms, and operational problems of resistance. Only a small proportion of studies focus on resistance primarily as an evolutionary problem.

Reviews and books on resistance to one or another class of toxicants have rarely crossed disciplinary boundaries. The diagnosis of resistance, the characterization of resistant phenotypes, and the genetic and sometimes molecular identification of resistance is usually juxtaposed with some theoretical modeling of resistance evolution, with primary relevance to a goal of management. Rarely is progress in one field seen to influence the other. This disciplinary fragmentation of research on different taxa has led to parallel developments and idiosyncratic vocabularies. In this review we will refer to the diverse array of xenobiotics such as antibiotics, drugs, herbicides, insecticides, and acaricides collectively as *toxicants*. The population biologies of different organisms are another source of diversity. The spread of genes in populations of sexually reproducing organisms, such as DDT-resistant mosquitoes, obeys different rules than that in clonal organisms, such as AZT-resistant or protease-inhibitor resistant HIV virus. Our aim in this review is to attempt to integrate knowledge of resistance mutations in a variety of organisms and to a variety of toxicants so that common themes may emerge and contribute to a better understanding of resistance.

First, we propose a molecular classification of resistance that is meant to improve upon rather than replace the classical biochemical or physiological classifications of resistance phenotypes. Second, we discuss how the initial population frequency of resistance genes (i.e., prior to application of toxicant) is expected to relate to the molecular basis of resistance and review how the available literature matches these predictions. We conclude by offering some suggestions for future research.

Molecular Mechanisms of Resistance

There are many possible adaptations that permit an organism to survive lethal doses of a toxicant and can be classified as either mechanisms of decreased response to the toxicant or mechanisms of decreased exposure. These two major categories are also sometimes referred to as pharmacodynamically derived resistance and pharmacokinetically derived resistance. Under pharmacodynamically derived resistance are the various mechanisms of target insensitivity to toxicant, and under pharmacokinetically derived resistance are included behavioral avoidance, reduced uptake, increased detoxification, elimination, or sequestration. A third and less commonly discussed mechanism is circumvention, a mechanism by which the organism can bypass inhibited processes with alternate metabolic pathways. This classification of resistance mechanisms is also very useful from an operational point of view and it has been discussed in numerous reviews (e.g., for insecticides, Brattsten et al. 1986; for herbicides, Powles and Holtum 1994; for antibiotics, Davies 1994).

We propose to add a molecular dimension that classifies the types of mutations leading to resistance to the existing biochemical/physiological classification of resistance mechanisms. Mutations, whether in receptors, transporters, or enzymes, may be classified into those that alter binding/catalysis by structural changes, up-regulation (including gene amplification), or down-regulation (including gene disruption or silencing). Regulation can be altered either by *cis*- or *trans*-acting control of expression, by duplication or amplification, or by posttranslational modification of, for example, the level of glycosylation, phosphorylation, or cellular localization. This molecular biological dimension in the classification of resistance mechanisms is best expressed as a two-dimensional table which we believe is an innovation in our understanding of the biology of resistance.

Little is known about posttranslational modification as a resistance mechanism. If such modifications have any heritable basis they are expected to appear as *trans*-acting regulatory factors. Similarly, the genetic control of avoidance behavior is not as well understood as biochemical mechanisms, although information is accumulating (Sparks et al. 1989; Riesgoescovar et al. 1992). Hence, for simplicity, these mechanisms have been omitted from our scheme.

Prior to selection, the size and composition of the pool of potential resistance mutants, comprising the set of loci and the set of alleles at each locus that *could* confer resistance, depends crucially on the mode of action, on chemical similarities of the toxicant to other compounds that the organism commonly contacts, and, of course, on the intensity of selection. In this review we develop a set of necessarily broad and speculative predictions about the initial size and composition of this pool of resistance mutants, arising from the integration of biochemical and molecular classifications (table 1). Although we will discuss this basic *genetic* component, it is clear that this approach also lends itself to a reappraisal of the *operational* factors involved (Georghiou and Taylor 1977; Mani and Wood 1984), and perhaps to the addition of an *ecology/life history* dimension to the evolution of resistance (Cook 1981; Roush and McKenzie 1987). Such further analysis is, however, beyond the scope of this review.

Patterns of Resistance Mutations: General Considerations

The first step in predicting the rate of evolution of toxicant resistance is to estimate the number and initial population frequencies of resistance-conferring mutations. Unlike most phenotypic traits that have been studied, resistance is accessible to thorough genetic analysis. This enhances the prospect of developing a predictive theory of initial gene frequency. Several generalizations are possible based on existing knowledge.

The mode of action of toxicants involves binding to a unique major target molecule, although some pes-

Table 1
Major Mechanisms of Resistance to Toxicants, and Predictons of Relative Commonness of Different Mutations

Class of Molecular Mutation	Biochemical Mechanism	
	Target Modification	Metabolism or Sequestration
Structural	C—Single mutations that reduce toxicant affinity can give large resistance effect. Fitness costs highly variable, however. U—If multiple targets exist.	R—Increased catalytic capacity for toxicant less effective than up-regulation. Fitness costs high for enzymes with primary function.
Up-regulation	C—Compensates for loss of target molecules to poisoning. This is less effective if toxicant binds irreversibly.	C—Competent loci likely to be present already and thus up-regulation sufficient. Less common for plants than animals, which deal with more ingested toxins.
Amplification	C—Mutation rate high, and lowest fitness costs. R—For heteromeric targets.	C—Mutation rate high, and lowest fitness costs.
cis-acting	U—Many mutants, but high costs of deregulating expression.	U—Many mutants, but high costs of deregulating expression.
trans-acting	U—Many mutants, but high costs of pleiotropic effects.	U—Many mutants, but high costs of pleiotropic effects.
Down-regulation	U—Only in cases that toxicant enhances rather than inhibits target activity.	U—Only in few cases that enzyme converts toxicant to more toxic form in vivo.
Disruption	R—Many mutants, very high cost of lost function.	R—Many mutants, high cost of lost function.
cis-acting	U—Many mutants, but high costs of reduced function.	U—Many mutants, but moderate costs of reduced function.
trans-acting	U—Many mutants, but high costs of pleiotropic effects.	U—Many mutants, but moderate costs of pleiotropic effects.

Note.—C = common, U = uncommon, R = rare.

ticides are thought to have more than one major target (e.g., carbamate and chloracetamide herbicide/fungicide chemistries; Powles and Holtum 1994). In such cases, evolution of resistance would be more rapidly achieved by mutations resulting in decreased exposure to the toxicant than by simultaneous mutations at two target sites.

Mutational rate is clearly of first importance. When considering net mutation rate to resistance, it must be recalled that there is a pool of mutant alleles at one or more loci, any of which *could* confer resistance for a given dose of toxicant. It is generally assumed that the weaker the dose, the larger the pool of mutants that can confer some resistance and, thus, the more polygenic is the basis of the resistance phenotype. Experiments have lent some support to this prediction for insects (McKenzie and Batterham 1994).

Experimental ("lab") selection is often assumed to be weaker than field selection for the same organism and toxicant. (This view is not, however, well supported for insects. See Follett, Gould, and Kennedy 1993; Groeters and Tabashnik 1995.) Consequently, the numerous mutations that might have little fitness cost, but also only a minor resistance benefit, should be more common in lab-selected than in field-selected populations. As a corollary, it is clear that even mutations with substantial fitness costs, but that provide a high degree of resistance, will be found in field-selected populations, at least in the early stages of resistance, but will be found less commonly in lab-selected populations.

Population frequency prior to toxicant selection is determined by the balance between mutation rate to resistance and natural selection against heterozygotes relative to the average wild genotype, under equilibrium conditions and assuming that frequency of resistant homozygotes and back-mutation rate are both negligible.

Dominance and fitness of resistant alleles under natural selection is, therefore, critical. Dominant deleterious alleles will be much less common than recessive alleles. To the extent that dominance can be inferred from molecular biology, we may be able to make more precise predictions. Alleles with both minor fitness costs and large resistance effects are likely to be very few, perhaps nonexistent, or we would commonly find that resistance evolution was virtually immediate for a novel toxicant. This is essentially Fisher's explanation for the predominance of micromutational change in Darwinian evolution (Fisher 1958; Orr and Coyne 1992).

Under special circumstances (table 1), resistance could derive from disruptive mutations that silence, down-regulate, or perhaps de-repress loci. There is likely to be a very large pool of such deleterious mutations (though each alone may be rare), which may be favored only with high-dose selection. Clark, Wang, and Hulleberg (1995) reported a very high rate for pleiotropic *trans*-acting mutations that modify metabolism for *Drosophila melanogaster.*

High-dose selection was once advocated as a means of defeating resistance evolution. As dominance

of resistance typically declines with increasing doses all heterozygotes can theoretically be killed by a high enough dose. The existence of a critical dose above which a large reservoir of disruptive mutations may confer resistance is reason to expect failure for such a strategy. Such disruptive mutations would be observed to increase and decline rapidly with episodes of high-dose selection. We expect a type of genetic "succession" of such resistance mutations, as distinct from accumulation of mutations into a polygenic phenotype.

Population size, if small, may allow even deleterious resistance alleles to achieve locally high frequencies by neutral drift. This is an unlikely consideration for agricultural pests, however, which as pests typically have large population sizes and migration rates.

Polyploidy has a dampening effect on allelic dominance relations. Thus, we expect that a larger load of deleterious alleles can persist in populations of polyploid organisms. A consequence may be that the pool of suitable mutations can be larger and evolution more rapid following selection by toxicants. Polyploidy is more common among plants than among animals, and thus herbicide resistance may be expected to evolve more rapidly than pesticide resistance in animals, all else equal, with the exception of mutants in the chloroplast genome. This possibility has not been examined specifically in the literature, but rapid (3–4 years) evolution of resistance in weeds is not uncommon (Powles and Holtum 1994). For viruses, mutation rates are commonly much greater than for higher organisms, and resistance evolution is expected and found to be very rapid (e.g., Kellam et al. 1994).

Although empirical data are sparse on fitness and dominance of resistance alleles in the absence of toxicant selection, molecular biology may shed more light. For example, a mutation in a target site that reduces affinity to the toxicant may also reduce turnover efficiency for the natural ligand, and thus entail some fitness cost. Whereas over 10 mutations in the quinone binding site in photosystem II can confer herbicide resistance in various laboratory systems, only one is commonly found in weeds in the field (Comai and Stalker 1986). Single amino acids are often critical for normal function of a given enzyme, such as the active-site triad of esterases. The commonly large proportions of conserved nucleotide sites across taxa suggest that modification of the few key residues is deleterious. Hence, resistance deriving from such modifications is likely to entail fitness costs.

Site-directed mutagenesis promises to provide valuable information about the distribution of fitness effects of mutants. For example, only one of five mutants at different sites in various acetylcholinesterases retained normal function, and two were inactive (Soreq et al. 1992). Similarly, numerous induced mutations in β-lactamase genes on plasmids were found to confer cephalosporin resistance to bacteria, but only a small subset of these were found in clinical isolates (Palzkill and Botstein 1992).

We have mapped a set of predictions of initial frequencies of resistance genes onto our two-dimensional scheme (table 1). In each case, we attempt no more than a crude three-level prediction: "rare," "uncommon," and "common." Predictions will be explained further below.

Patterns of Resistance Mutations: General Methods and Problems

Collection Criteria

Studies appropriate to testing the predictions of table 1 would ideally have all the following components: (1) resistant individuals sampled from diverse populations both early and late in resistance evolution, (2) single genes contributing to resistance isolated in lines backcrossed to a single isogenic reference strain, (3) toxicological classification of mutations, (4) linkage analyses and tests of allelism, (5) field–cage competition experiments to estimate fitness costs of each mutant relative to "wild types," (6) identification of molecular mutations, and (7) complementation analyses to prove function. Such studies do not exist. Instead, many independent studies have been gathered from the literature to examine the hypotheses advanced.

Papers were included only if they identified recently evolved mutations at loci with a known functional relationship to the toxicant used in selection. Loci were excluded if, clearly, they had evolved over a long period and most likely were fixed for many different mutations as a result of a long history of selection. For example, monarch butterflies, which have low Na^+,K^+-ATPase sensitivity to host-plant cardiac glycosides, have a substitution in the ouabain-binding site that could account for insensitivity relative to other, sensitive species (Holzinger, Frick, and Wink 1992). This is unlikely to have been a recent event, and is usually referred to as "tolerance" rather than "resistance." There was no recorded intraspecific variation in allele frequency for this mutation, and numerous other mutations and modifiers may also have accumulated. Hence, studies such as this were excluded.

Studies of purified target or metabolizing proteins have enabled identification of several mutant molecules. A few such cases have been included when loci with known functional relationships to toxicants were involved. Although the exact molecular mutations were unknown, papers were included if mutations could reasonably be inferred to be point mutations in structural loci.

Libraries of induced mutants were common in the literature. Only papers reporting mutations at loci of known function were included. For example, screening for nitrate-reductase deficiency as chlorate resistance in mutagenized plants uncovered five mutations, all recessive and all at different loci (Schoenmakers et al. 1991). Only one was in the nitrate-reductase enzyme and the rest were at unknown loci and so this study could not be included. In a contrasting case, three cell lines of corn were selected for resistance to imidizolinones and sulfonylureas. Toxicological studies suggested that the mutations were at herbicide-binding sites in the target mol-

ecule, acetolactate synthase (Newhouse et al. 1991). This study was therefore included.

Biases in Discovery of Mutations

The literature is probably biased toward reports of mutations that are more readily discovered. The single greatest bias that affects our survey is the pervasive focus on toxicant target genes. Identification of resistance mutations in target molecules is laborious, but the targets are usually known for well-established toxicant classes. Mutations in factors that regulate targets or metabolizing enzymes are typically less well known, and are often no more than map positions. Such mutations are expected to be underrepresented in the literature.

Amplification mutations are easily detectable by Southern hybridization, although the task of defining distinct amplicons is more difficult, leading to a probable underestimation of the number of distinct mutational events.

Exclusion of Plasmid Mutants in Bacteria

Horizontal plasmid and other gene transfer is so pervasive among bacteria that a species concept special to prokaryotes is necessary (Cohan 1994). Resistance often involves the coordinated action of several specific loci on a plasmid. Acquisition of plasmids by horizontal transfer, sometimes in a contagious manner, can provide resistance, even to many different antibiotics, but the species in which mutations originated are usually unknown. Thus it is difficult to reconstruct the stepwise evolution of such plasmids (O'Brien et al. 1985; Davies 1994).

The prevalence of horizontal transfer and the difficulty of reconstructing mutation events necessitated the virtual exclusion of the large literature on bacterial antibiotic resistance, although these are very useful for any discussion of biochemical mechanisms. Only chromosomal resistance mutations were included. Recent reviews of bacterial drug resistance offer analyses of plasmid-based mechanisms (Davies 1994; Spratt 1994).

Patterns of Resistance Mutations: Predictions and Evidence

Structural Mutations in the Target: Predictions

This was predicted to be the most common molecular mechanism of target-site resistance. The very effect of a toxicant often derives from similarity to natural ligands of target molecules, or to transition states of substrates. Toxicants affect the target either by displacing natural substrate molecules or because the target itself is converted to an inactive or otherwise disrupted form. This distinction is important, as discussed below.

It is conceivable that for any target–toxicant system, there exists as little as a single residue change that could reduce the effect of the toxicant on the target without greatly affecting the target's interaction with natural ligands, whether substrates or allosteric effectors. Single residue changes in the target could completely abolish the toxicant's effect in some systems.

Such mutants would enjoy large fitness benefits in the presence of the toxicant. However, we expect there to be very few amino acid changes, perhaps none, that could selectively reduce target–toxicant affinity and also leave unchanged normal catalysis or allosteric interactions. Consequently, if the target is a highly taxonomically conserved molecule, the same mutation should be found repeatedly favored by selection with the same toxicant in diverse taxa.

Competitive inhibitors are likely to interact with some of the same amino acids in the target as does the normal substrate, and the available pool of mutations would be correspondingly small. In such cases other types of resistance are more likely to appear first. A mutation with a comparatively large fitness cost may be favored if selection is intense enough to outweigh the cost.

Table 2
Numbers of Distinct Mutations Reported for Different Mechanisms of Resistance

Class of Molecular Mutation	Biochemical Mechanism	
	Target Modification	Metabolism or Sequestration
Structural[a]	80	3
Up-regulation		
Amplification[b]	26	19
cis-acting[c]	0	0
trans-acting[c]	1	2
Down-regulation[c]		
Disruption	0	12
cis-acting	0	1
trans-acting	0	0

[a] Table 3.
[b] Table 4.
[c] Table 5.

Structural Mutations in the Target: Evidence

The prediction of commonness is amply borne out in the literature (tables 2 and 3). We expected a research discovery bias favoring studies of target genes, but within target genes discovery of structural mutations typically involves as extensive genetic analysis as does discovery of other types of target-modifying mutations.

There is a paucity of studies that quantify relative fitness costs for such mutations under realistic field conditions (Roush and Daly 1990), and yet this is critical for estimation of natural selection against mutants and, thus, their frequency in the absence of toxicant selection. The well-studied resistance mutations in the quinone-binding subunit of photosystem II of diverse auxotrophs provide the best set of evidence for any one system. Mutations at the four positions other than 264 and 251 did not entail reduced electron transport, but provided only weak atrazine resistance (table 3). The two mutations 264 Ser to Ala or Gly were the commonest: the former for diuron, the latter for atrazine resistance. Both mutations reduced electron transport, suggesting that this residue is the critical residue for function. For toxicants that attack an essential residue, resistance evolution must incur a fitness cost that only later modifier mutations may be able to reduce.

Table 3
Resistance Mutations in Structural Loci

Biochemical Mechanism	Selection Regime	Gene	Number of Mutations	Taxa	Toxicants to Which Resistant	Reference(s)[a]
Target modification....	Lab	16srRNA	3	Angiosperm (cell)	Spectinomycin: substrate mimic	1
		Acetolactate synthase (nucleotide synthesis)	2	Angiosperm	Sulfonylurea substrate competitor/imidizolinones noncompetitive inhibitor	2
			3[b]	Angiosperm	Sulfonylurea substrate competitor/imidizolinones noncompetitive inhibitor	3
		Acetylcholine esterase (neurotransmitter catabolism)	1	Insect	Organophosphates: locks enzyme in covalent complex	4
		Aminopeptidase-N (Bt toxin receptor)	1[b]	Insect: 4 spp.	Bt toxins: form midgut lesion when bound to receptor	5
		H+ ATPase (ion transport)	2	Yeast	Vanadate: inhibits ATP hydrolysis	6
		Beta tubulin (microtubule assembly)	1	Yeast	Benomyl: inhibits microtubule assembly	7
			1	Fungus	Rhizoxin: inhibits microtubule assembly	8
			1	Fungus	Benomyl	9
		Cytochrome *b* (respiratory e-flow)	4	Yeast	Antimycin: inhibits cytb electron transfer	10
			1	Cyanobacterium	Antimycin: inhibits cytb electron transfer	11
		Dihydrofolate reductase–thymidylate synthase (nucleotide catabolism)	1	Protozoan, mammal (cell)	Pyrimethamine: competitive inhibitor	12
			1	Mammal (cell)	Pyrimethamine: competitive inhibitor	12
		DNA topoisomerase (DNA synthesis)	1	Mammal	Amsacrine: transition state inhibitor	13
		DNA polymerase (DNA synthesis)	2	Virus	Aphidicolin: dCTP mimic competitive inhibitor	14
			3	Virus	Aphidicolin, acyclovir, foscarnet, PPA: substrate/cofactor analogs, inhibitors	15
		DNA gyrase (DNA synthesis)	2	Bacteria	Nalidixic acid: inhibits subunit A	16
		EPSP synthase (aromatic aa synthesis)	1	Bacteria	Glyphosate: substrate competitor	17
		JH-binding protein (signal transduction)	1[b]	*Insect*	*Methoprene: juvenile hormone analog*	18
		Phytoene desaturase (carotenoid synthesis)	1	Cyanobacteria	Norflurazon: inhibitor	19
		Photosystem II, subunit D1 (photosynthetic electron flow)	5 (3 shared with cyanobacteria)	Alga	Metribuzin, atrazine, diuron: plastoquinone competitors	20
			5	Cyanobacteria	Metribuzin, atrazine, diuron: plastoquinone competitors	21
			aa264 S → G	Angiosperm: 3 spp.	Atrazine	22
			aa264 S → A	Alga, angiosperm, cyanobacteria: 3 spp.	Atrazine, diuron	23
			aa264 S → T	Angiosperm (cell), euglena, cyanobacteria	Atrazine, diuron, phenylureas	24
		Sodium channel "sch" (nerve action)[c]	1	Insect	DDT, pyrethroids: Na+ channel activators	25
	Field	Acetylcholine esterase (neurotransmitter catabolism)	4	Insect	Organophosphates: locks enzyme in covalent complex	4
		DHFR-TS	2 (deletion)	Protozoan	Pyrimethamine: competitive inhibitor	26

Table 3
Continued

Biochemical Mechanism	Selection Regime	Gene	Number of Mutations	Taxa	Toxicants to Which Resistant	Reference(s)[a]
			5 (at 3 sites)	Protozoan	Pyrimethamine: competitive inhibitor	27
		DNA polymerase	1	Virus	Acyclovir, foscarnet: substrate, cofactor analogs/inhibitors	28
		GABA receptor of Cl-channel (nerve modulation)	1	Insect: 5 spp.	Dieldrin: Ligand GABA-competitor	29
		Reverse transcriptase (retroviral DNA synthesis)	5	Virus (collected from one patient)	AZT: nucleotide mimic terminates elongation	30
Metabolism, sequestration	Lab	P-glycoprotein mdr (xenobiotic efflux)	1[d]	Mammal (cell)	Various anticancer drugs	31
	Field	cytochrome P450[c]	1[b]	Insect	Malathion: oxidized form inhibits AChE	32
		Phosphotriester hydrolase[c]	1 (regulatory?)	Insect	Malathion	33

[a] (1) Svab and Maliga 1991. (2) Haughn and Somerville 1986, 1990; Wiersma et al. 1990; Sathasivan, Haughn, and Murai 1991; Hattori et al. 1992. (3) Newhouse et al. 1991. (4) Fournier et al. 1992, 1993; Pralavorio and Fournier 1992. (5) Van Rie et al. 1990; Wolfersberger 1990; Ferré et al. 1991; Gould et al. 1993; Knight, Crickmore, and Ellar 1994. (6) Ghislain, De-Sadeleer, and Goffeau 1992. (7) Thomas, Neff, and Botstein 1985. (8) Takahashi, Kohayashi, and Iwasaki 1989. (9) Orbach, Parro, and Yanofsky 1986. (10) Brunner, Mendoza, and Tuena de Cobos 1987; Coria, Garcia, and Brunner 1989. (11) Bennoun, Delosme, and Kueck 1991. (12) Tanaka et al. 1990. (13) Lee, Wang, and Beran 1992. (14) Taddie and Traktman 1991. (15) Hall et al. 1989; Hall and Woodward 1989. (16) Yamagishi et al. 1986. (17) Comai and Stalker 1986. (18) Shemshedini and Wilson 1990. (19) Linden et al. 1990; Chamovitz, Pecker, and Hirschberg 1991. (20) Erickson et al. 1985; Comai and Stalker 1986; Etienne et al. 1990; Aiach et al. 1992. (21) Johanningmeier, Bodner, and Wildner 1987; Wildner, Heisterkamp, and Trebst 1990. (22) Goloubinoff, Edelman, and Halick 1984; Erickson et al. 1985; Comai and Stalker 1986; Etienne et al. 1990. (23) Erickson et al. 1985; Golden and Haselkorn 1985; Comai and Stalker 1986; Hirschberg et al. 1987; Barros and Dyer 1988; Etienne et al. 1990. (24) Astier et al. 1984; Shigematsu, Sato, and Yamada 1989; Aiach et al. 1992. (25) Amichot et al. 1992. (26) Cowman and Lew 1989. (27) Chen et al. 1987; Cowman et al. 1988; Peterson, Walliker, and Wellems 1988; Snewin et al. 1989; Zolg et al. 1989. (28) Hwang, Ruffner, and Coen 1992. (29) ffrench-Constant et al. 1993; ffrench-Constant 1994. (30) Kellam et al. 1994. (31) Choi et al. 1988; Safa et al. 1990. (32) Konno, Hodgson, and Dauterman 1989; Konno et al. 1990.

[b] Toxicologically defined mutants only.

[c] Toxicant enhances function or is a pro-toxin.

[d] Mutation succeeds amplification, see table 4.

Up-Regulated Target Molecules: Predictions

Up-regulation of targets compensates for the reduction of the pool of functional target molecules by toxicant poisoning. The level of resistance obtainable by overproduction of target is likely to be lower than can be obtained by a structural mutant with reduced inhibition by the toxicant. This is especially true when the toxicant has very low K_i (concentration at 50% inhibition), or when it binds irreversibly to wild-type target.

Such mutants were predicted, therefore, to be more common in lab-selected than in field-selected strains. An exception is that resistance from target overexpression is unlikely for heteromers of products of several loci. In such cases, multiple mutations would be needed to achieve resistance unless loci were linked, in which case a single amplification could suffice (Comai and Stalker 1986), or unless the mutation were in a *trans*-acting factor that regulates target loci coordinately. Double amplification mutants, one for each subunit of a dimeric target, would seem to be quite rare but they have been reported (Hurta and Wright 1990).

Mutations in trans*-acting regulatory factors* were predicted to be uncommon. Such mutations are likely to affect more loci than just the target and as such entail substantial pleiotropic fitness costs. Pleiotropy is fairly common for *trans*-acting modifiers of metabolism in *Drosophila,* and the mutation rate for modifers of any one metabolic locus can be quite high (Clark, Wang, and Hulleberg 1995). We propose that such mutants, with high rates of mutational generation but also with large fitness costs, should be moderately abundant early in resistance evolution, but disappear rapidly with the appearance of less deleterious alternatives such as structural mutations. As emphemeral adaptations we expect a bias against their discovery, relative to mutations with lesser fitness costs.

Mutations in cis*-acting regulatory factors* were also predicted to be uncommon, and more common early than late in evolution of resistance, as for *trans*-acting mutations. In eukaryotes most genes are tissue-specific in expression, and transcription is regulated by a balance of activation of silencer and enhancer elements by diverse regulatory molecules (Clark and Docherty 1993). Disruptive mutations in silencer elements could result in ectopic overexpression. While many mutations could achieve this result, they are likely also to carry a fitness cost from dominant expression in inappropriate tissues. The net mutation/selection balance for such a pool of mutants, while probably at least of the order of that for structural mutations, would make this class of mutations moderately abundant early in resistance evolution. Such mutants would, however, disappear rapidly with the appearance of less deleterious alternatives. No discovery bias was expected between *cis*- or *trans*-acting mutations, as research programs to distinguish among these alternatives involve much the same effort in genetical analysis.

Table 4
Amplified Resistance Genes

Biochemical Mechanism	Selection Regime	Gene	Amplicons	Taxa	Toxicants to Which Resistant	Reference(s)[a]
Target modification	Lab	Adenylate deaminase	2	Mammal (cell)	Coformycin: substrate competitive inhibitor	1
		Aldoketoreductase	1	Nematode	Methotrexate: inhibits DHFR (AKR also a target?)	2
		Dihydrofolate reductase–thymidylate synthase	3	Protozoan	Methotrexate, pyrimethamine: inhibit DHFR and dTTP production	3
			2[b]	Protozoan	Pyrimethamine	4
		EPSP synthase	1	Angiosperm (cell)	Glyphosate: inhibits EPSPS	5
			1	Angiosperm (cell)	Glyphosate	6
			1	Angiosperm (cell)	Glyphosate	7
		Glutamine synthetase	2	Angiosperm	Glufosinate: competitively inhibits GS	7
		GST-AMPD[c]	5	Mammal (cell)	Coformycin	8
		N-acetyl glucosamine transferase	1	Nematode	Tunicamycin: substrate competitive inhibitor	9
		Ribonucleotide reductase (both subunits)	2	Mammal (cell)	Hydroxyurea: enzyme deactivator	10
Metabolism, sequestration . . .	Lab	GST-AMPD[c]	5	Mammal (cell)	Coformycin	8
		P-glycoprotein (mdr)	1	Mammal (cell)	Various anticancer drugs	11
			5+	Insect	Organophosphates: inhibit AChE	12
			1	Protozoan	Mefloquin	13
			1	Nematode	Hydrophobic drugs	14
	Field	Esterase E4	3	Insect	Hydrolyzable insecticides	15
		Esterase B4 and 5	2	Insect	Organophosphates	15
		Metallothionein	1	Insect	Heavy metals	16

[a] (1) Debatisse et al. 1988. (2) Callahan and Beverley 1992. (3) Inselburg, Bzik, and Horii 1987; Hahn, Nevaldine, and Morgan 1990. (4) Cowman and Lew 1989. (5) Shry, Hepburn, and Widholm 1990. (6) Goldsbrough et al. 1990. (7) Comai and Stalker 1986. (8) Robert de Saint Vincent et al. 1990. (9) Detke et al. 1988. (10) Hurta and Wright 1990. (11) Choi et al. 1988; Safa et al. 1990. (12) Mouchès et al. 1986; Raymond et al. 1989; Devonshire and Field 1991; Ferrari and Georghiou 1991. (13) Wilson et al. 1989. (14) Henderson et al. 1992. (15) Field, Devonshire, and Forde 1988; ffrench-Constant, Devonshire, and White 1988; Devonshire and Field 1991. (16) Maroni et al. 1987; Devonshire and Field 1991.

[b] Low intensity selection, see table 3 for high.

[c] Conjugated glutathione transferase (detoxifier) and AMP deaminase (target).

Amplification mutations were predicted to be common. At moderate levels, amplification is less likely to carry pleiotropic fitness costs relative to other mechanisms of up-regulation. Also, chromosomal mutation rates such as insertions/deletions, translocations, and duplications can range as high as 10^{-3} per locus per generation, much higher than the base substitution rate (Schimke 1988). Selective overreplication, as distinct from elevated transcription, may be a relatively common method of up-regulation during normal development (Schimke et al. 1986).

Unequal exchange and stable chromosomal reincorporation is more common in plants, whereas overreplication of minute extrachromosomal amplisomes is more common in mammals. These amplisomes are less frequently reintegrated into chromosomes (Schimke 1988; Pauletti, Lai, and Attardi 1990). A mammalian cell transposon has been shown to favor the amplification of dihydrofolate reductase (DHFR) and hence resistance to the DHFR inhibitor methotrexate in cell cultures (McArthur and Stanners 1991). Mutagens which produce double-minute chromosomes and stimulation of the oncogene *c-myc* have both resulted in rapid evolution of methotrexate resistance through enhanced rate of amplification of DHFR (Hahn, Nevaldine, and Morgan 1990; Denis et al. 1991).

We expect a bias favoring discovery of such mutations relative to nonamplification mutations because DNA amplification is easier to detect, whereas discrimination among other regulatory mechanisms requires more laborious genetic analysis.

Up-Regulated Target Molecules: Evidence

The prediction that up-regulation of targets would be moderately common in lab-selected, but uncommon in field-selected organisms is supported by the literature (tables 2, 4, and 5). Amplification was predicted to be the most common means of target overexpression, and this was borne out clearly in the literature (tables 2 and 4). While *cis* mutants were predicted to be uncommon but not rare, no examples were evident from the literature. *Trans*-acting regulatory mutations were found to be uncommon as predicted (table 5).

Down-Regulated Target Molecules: Predictions

Underexpression of target molecules was predicted to be common, but only for toxicants that *aggravate* rather than inhibit the normal function of the target. For example, pyrethroid and DDT insecticides lock nerve

Table 5
Regulatory Resistance Mutations

Biochemical Mechanism	Selection Regime	Molecular Mechanism	Gene Regulated	Mutations	Taxa	Toxicants to Which Resistant	Reference(s)[a]
Target modification...	Lab	*trans*-down-regulated	Sodium channel (para)	1[b]	Insect	Pyrethroids, DDT; sodium channel agonists	1
Metabolism, sequestration ..	Lab	Disruption	Nitrate reductase 2	1 deletion	Angiosperm	Chlorate: substrate competitive inhibitor	2
		trans-up-regulated	Glutathione transferase	1[b]	Insect	DDT; sodium channel agonist	3
	Field	*trans*-up-regulated	Cytochrome P450 (CYP6A1)	1[b]	Insect	Diazinon: AChE inhibitor	4
	Clinical	Disruption	Thymidylate kinase	1 bp deletion	Virus	Acyclovir: purine mimic terminates DNA polymerization	5
		Disruption	Peroxidase-catalase (katG)	10+ missense mutations	Bacterium	Isoniazid: catalyzed by katG to cytotoxic form	6
		cis-down-regulated	Ferredoxin	2 substitutions	Bacterium	Metrodinazole: reduced by ferredoxin to cytotoxic form	7

[a] (1) Hall and Kasbekar 1989; Kernan et al. 1991. (2) Wilkinson and Crawford 1991. (3) Grant and Matsumura 1989; Grant and Hammock 1992. (4) Carino et al. 1994. (5) Palu et al. 1992. (6) Heym et al. 1995. (7) Quon, Doliveira, and Johnson 1992.

[b] Precise mutational change unknown.

membrane sodium channels into an open state, causing chronic sodium leakage and unregulated nerve activity (Narahashi 1986). *Bacillus thuringiensis* toxicants bind to insect midgut *N*-aminopeptidase target molecules, forming a destructive transmembrane pore (Knight, Crickmore, and Ellar 1994; Sangadala et al. 1994). In such cases resistance can result from down-regulation of expression, posttranslational inactivation, or even silencing of the target genes.

As the down-regulated gene is expected to have indispensable functions, such mutants are likely to entail fitness costs. The frequency of any one allele is therefore likely to be low, depending on dominance in the absence of selection. However, there may be a large pool of such disruptive alleles each at low frequencies and any of which may achieve some form of down-regulation or gene-silencing leading to resistance. *Trans*-acting down-regulation was predicted to be more common than simple disruption, as pleiotropic fitness costs are less likely with finer reductions in control. Down-regulating mutants are likely to be recessive, and thus relatively common, but also unlikely to provide any resistance to heterozygotes, in the case of enzymes embedded in networks of metabolism (Kacser and Burns 1981; but see Savageau 1992).

Down-Regulated Target Molecules: Evidence

Evidence was scanty and inconclusive. Resistance to pyrethroid insecticides in tobacco budworm (Taylor et al. 1993; Taylor, Shen, and Kreitman 1995), house fly (Williamson et al. 1993; Knipple et al. 1994), and cockroach (Dong and Scott 1994) is genetically linked to the gene that encodes the putative target site, a voltage-gated sodium channel. However, whether the mutation(s) alters structural binding properties or regulation is, as yet, unresolved (Taylor et al. 1993). Lab-selected mutants at a locus that down-regulates sodium channels indirectly have been found to confer weak resistance to pyrethroids, but the occurence of such mutants under field conditions is unknown (Hall and Kasbekar 1989). A structural point mutation in another sodium channel has been reported for DDT resistance in another laboratory-selected *Drosophila* strain (table 3; Amichot et al. 1992).

Bt-toxicant resistance has been traced to reduced midgut receptor-affinity in several lepidoptera (table 3). However, Gould et al. (1993) have also reported a broad-spectrum mechanism that is not related to receptor affinity changes in the tobacco budworm.

Structural Mutations in Metabolizing Enzyme Loci: Predictions

We predicted that mutations which tailor the affinity and turnover efficiency of a detoxification enzyme to the toxicant would be much less common than up-regulated or amplified mutations. There is likely to be a cost of changing affinity to suit a new substrate, since affinity for normal substrates may be lost. On the other hand, the pool of possible resistance mutants may be larger, especially for larger families of genes devoted to a chemical modification that *could* also modify the toxicant (esterases, glutathione transferases, cytochromes P450, etc.).

Only one or a few base substitutions may be capable of substantially enhancing catalytic efficiency for a novel toxicant. We propose that mutants that can better degrade the toxicant should be rarer than up-regulated mutants and should appear as modifiers later rather than

earlier in the history of resistance evolution for a given species and toxicant. Just such a case appears to have occurred for a P-glycoprotein locus in a mammalian tumor cell line selected with various anticancer drugs (Choi et al. 1988; Safa et al. 1990). The first mutation detected was an amplification, on one descendant of which a subsequent point mutation appeared that enhanced efflux activity for certain drugs (tables 3 and 4). This sequence of duplication and divergence is a logical route for evolution of new functions and avoidance of pleiotropic fitness costs. This seems to be the principal route for the diversification of large gene families (Uyenoyama 1986) such as the cytochromes P450 (Gonzalez and Nebert 1990).

Structural Mutations in Metabolizing Enzyme Loci: Evidence

As predicted, structural mutations in enzyme loci that enhance the metabolism of toxicants were less common than were up-regulating mutations (tables 2–5). In one case, a point mutation appeared after the appearance of an amplification mutation, as discussed above. Biochemical mechanisms of resistance to toxicants very commonly involve increased metabolism, with little known of the molecular mechanisms. The extent to which structural mutations or amplifications contribute remains to be seen. We would predict an extension of the pattern observed, with a predominance of amplifications.

Up-Regulated Metabolizing Enzymes: Predictions

We predicted up-regulatory mutations to be most common for metabolizing enzyme loci. The panoply of metabolizing enzymes, classified by the type of modification they catalyze (oxidases, reductases, esterases, transferases, etc.) are highly diversified into large multigene families. Substrate specificities of such metabolizing enzymes can be broad and overlapping. Thus there is likely to be an enzyme with *some* activity against a novel toxicant, depending on chemical similarity to substrates commonly encountered intracellularly or in the environment (diet, soil, host cell, etc.). If such loci are available, resistance may derive simply from increased constitutive expression of the enzyme.

If nontarget enzymes have strong affinity to the toxicant, by chance or by similarity to target, then it is possible that up-regulation could also confer a sequestration form of resistance, rather than metabolism. Sequestration of organophosphorus insecticides by esterases following gene amplification has been observed in mosquitoes (Karunaratne et al. 1993).

Amplifications are expected to be the most common mutation in this class, as mutation rate is high and pleiotropic costs low for moderate levels of amplification. Mutations *cis* to the controlled locus that disrupt repressor binding sites could also achieve up-regulation of detoxification enzymes.

The arguments advanced earlier for up-regulated target molecules for the relative rarity of *trans*- and *cis*-acting mutations are also pertinent here.

Up-Regulated Metabolizing Enzymes: Evidence

The main prediction was strongly supported. Up-regulation is found to be the most common molecular mechanism of metabolic resistance, and amplification the most common form of up-regulation (tables 2–5). There were two clear cases of *trans*-acting up-regulation of a glutathione transferase locus and a P450 locus in resistant insects (table 5), whereas no *cis*-acting mutations were found.

Down-Regulated Metabolizing Enzymes: Predictions

This was predicted to be uncommon and restricted to the specific case of toxicants that are metabolized in vivo to more toxic metabolites. In such a case, resistance may derive from down-regulation or silencing of the gene for the activating enzyme. This could appear quite rapidly as a disruptive mutation in the gene for that enzyme.

Down-Regulated Metabolizing Enzymes: Evidence

The cases available were all consistent with this prediction (table 5). Acyclovir, when kinased, mimics purines and blocks DNA polymerization. A disruptive point mutation in a Herpes virus' thymidylate kinase conferred acyclovir resistance (Palu et al. 1992). Resistance of clinical isolates of *Mycobacterium tuberculosis* derives from numerous missense mutations in the locus for a peroxidase-catalase which converts the drug isoniazid to a more toxic form (Heym et al. 1995). Ferredoxin reduces metrodinazole to its cytotoxic form in *Trichomonas vaginalis*. Resistant strains have 40% lower transcription rate for ferredoxin apparently as a result of point mutations in the upstream untranscribed region which are known to affect binding of a regulatory protein (Quon, Doliveira, and Johnson 1992). This case is the only *cis*-regulatory mutation we found.

There were also a number of cases for which molecular mechanisms were less certain. Methyl paraoxon is produced in vivo by desulfuration (oxidation) of the less toxic primary insecticide methyl parathion. Resistance from impaired oxidation has been reported, although it remains to be shown whether this is a case of gene disruption, or *cis*- or *trans*-down-regulation (Konno, Hodgson, and Dauterman 1989; table 5). A complementary mutant of unknown molecular basis enhances hydrolysis of the malaoxon metabolite. Angiosperm nitrate reductase is poisoned by chlorate, which is a nitrate analog, and resistance derives from gene silencing through a disruptive mutation (Wilkinson and Crawford 1991).

Circumvention

A biochemical mechanism of resistance that is not commonly reported or discussed in the literature may be termed "circumvention." Pathways blocked by action of a toxicant can be bypassed by alternate pathways if the latter are enhanced or originated by compensating mutations. We predicted limited opportunities for such mechanisms, and thus few, if any, reports. Nevertheless, one case was found which fits this category. Amplification of a short-chain dehydrogenase gene confers

methotrexate resistance to nematodes, probably by providing an alternate folate reductase pathway (White et al. 1988; Papadopoulou, Roy, and Quellette 1992).

Fit of Predictions to Observations

The fit between the predicted distribution of mutations (table 1) and that observed in the literature (table 2) was strikingly good, despite a low total number of cases. The major patterns that emerged from the literature survey may be summarized as follows (table 2):

1. Point mutations in target loci are the dominant form of resistance for both lab and field selection (table 3).
2. Amplifications of target loci were less common than structural mutations (table 4).
3. Amplifications of target loci were more common for lab-selected than for field-selected strains (table 4).
4. Amplification is the most common mechanism of up-regulation of metabolizing enzymes (table 4).
5. Only one mutation involving *cis*-regulation was found, and this form of mutation may be considered rare (table 5).
6. Mutations involving gene disruption and down-regulation were uncommon, but were found in appropriate cases, i.e., when toxicants stimulated rather than inhibited target function and when metabolizing enzyme converted toxicant into more toxic metabolites (table 3).
7. Mutations involving *trans*-acting up-regulation were uncommon as expected (table 3).

Unresolved Issues

Fitness Costs

A central issue is the paucity of information about fitness costs for particular classes of mutants. Our predictions were based on plausible conjectures. There are many studies estimating fitness costs and resistance ratios for specific mutations. However, most of these studies are for target modifications and are conducted under highly artificial laboratory conditions and so suffer from the universal problem of uncertain relevance to field conditions (Roush and Daly 1990). More studies of fitness costs under realistic field conditions are needed to test such conjectures as we and others have made.

Regulatory Mutations

Temperature-sensitive mutants of *D. melanogaster* at *para,* an X-linked voltage-gated sodium channel locus, and *nap,* an X-transcription-regulating locus, are also mildly resistant to pyrethroid insecticides (Kasbekar and Hall 1988; Kernan et al. 1991). Mutations at unknown *trans*-acting regulatory loci enhance expression of a glutathione transferase conferring DDT-resistance in a lab-selected mosquito strain (Grant and Hammock 1992), and a cytochrome P450 linked to resistance in houseflies (Cariño et al. 1994).

While these cases, despite their indefinite status, were included (tables 5), other interesting but less definite cases were not. Scheidel and Stollar (1991) found that several mutants for resistance to ribavirin in Sindbis virus mapped not to the target, RNA guanylyl-transferase, but to a nonstructural protein involved in mRNA capping. Overexpression of *mdr* mRNA in *Entamoeba histolytica* resistant to emetine was not related to DNA amplification (Samuelson et al. 1990). Carbapenem-resistant strains of *Bacteroides fragilis* from hospitals appear to involve a single regulatory mutant that *reactivates* a previously-silent but functional broad-spectrum β lactamase (Podglajen et al. 1992).

The logistic difficulties of locating regulatory mutants means that this class of mutations is highly likely to have been underrepresented in our sample, as discussed above. Only further work focusing on this class of mutations will generate enough cases to resolve uncertainties about fitness costs and relative commonness of regulatory mutations.

Recombinational Limitations

While we have discussed the crude abundance of mutations of a particular class, we have ignored the accumulation of mutations within any one locus. Recombinational accumulation of distinct resistance mutations should result in increased resistance. In *Drosophila* roughly additive increases in resistance to acetylcholinesterase inhibitors appear to have resulted from linkage of several point mutations in the AChE locus (Pralavorio et al. 1993; Mutero et al. 1994). Transfer and recombination among plasmids may be viewed as a means of rapid evolution of bacterial drug resistance (Davies 1994). When individual mutants are relatively common and loci are unlinked, recombination is free and offers no barrier to resistance evolution. For the evolution of resistance *within* a locus, recombinational assembly of a highly resistant haplotype for small loci may be more of a limitation than the mutation rate. Indeed, recombinational coupling of distinct point mutations already present in the population may be less likely than a second mutation, depending on population size, gene frequency, etc. Hence, small loci are expected to evolve more slowly than large loci if several distinct mutations can contribute additively to resistance.

Genetic "Succession"

We have conjectured that in cases where numerous disruptive mutations can confer resistance (whether through derepression, down-regulation, or gene-silencing), high-dose selection may overcome fitness costs associated with disruption and thus favor a large pool of normally deleterious mutations. However, fitness costs also select against such alleles in the absence of toxicant selection, moderated by recessivity and drift. Hence, deleterious alleles, while thay may appear sooner than the few alleles with lower fitness costs, may later be replaced by them. It may be possible to discriminate distinct classes of "pioneer" and "settler" mutations. Deleterious alleles could remain at high frequency only with the appearance of modifier mutations that reduce fitness cost. Without the appearance of modifiers, we expect a succession of resistance genes in populations.

There are a few cases which appear to support this model. An azidovudine (AZT)-resistant mutation in HIV-1 reverse transcriptase (Arg 70 Lys) that always appears early in treatment for AIDS in humans is replaced later in the viral population by other mutations (Thr 215 Tyr/Phe) (Kellam et al. 1994). Colchicine selection on mammalian cell lines first favored a P-glycoprotein (*mdr*) amplification and later a point mutation that enhanced colchicine turnover efficiency through reduced binding affinity (Choi et al. 1988; Safa et al. 1990).

Less clear is the case of pyrethroid insecticide resistance for the moth *Helicoverpa armigera*. The first mechanism to appear in Australian populations was a highly resistant nerve-insensitive form of unknown genetic basis. This seems to have been replaced by a metabolic form of resistance in all populations (Gunning et al. 1991; Daly and Fisk 1992). A similar succession of mechanisms may be happening for *Heliothis virescens* populations in the USA (Ottea et al. 1995). However, whether this results from a successional process cannot be determined without more knowledge of the mutations underlying the traits. To test the model adequately for other organisms, observations must be made over time of the spectrum of resistance-conferring alleles at contributing loci; not a trivial task.

Negative Cross-Resistance

Negative cross-resistance or "collateral sensitivity" refers to the ability of some toxicants to act selectively against mutant organisms that are resistant to another toxicant. In the case of structural mutations affecting the target site, novel compounds may be better adapted to the the mutant target. The extent to which negative cross-resistance is common will determine the extent to which reversal of the evolution of resistance can be brought about. Only a very few cases are evident at this time. *N*-propylcarbamates are active against organophosphate-resistant acetylcholinesterases (Yamamoto, Takahashi, and Kyomura 1983), and *N*-alkylamides are more active against flies with *super-kdr* resistance (Elliott et al. 1986). The Ser264 to Gly mutation in the *psbA* gene confers atrazine resistance in weeds, but also increased sensitivity to the herbicides bentazon and pyridate (Gronwald 1994). Negative cross-resistance has also been observed for fungicides, for instance between benomyl and *N*-phenylcarbamates (Davidse 1987). The molecular mechanism is unknown in this last case.

Future Research

It could be said that development of better theory for resistance evolution, based on more precise estimates of initial frequencies of resistance mutations, is at best a stop-gap measure for agriculture. Indeed, the environmental toll and ultimate futility of the pesticide treadmill has for some time given impetus to biological control and integrated pest management (IPM). Few such alternatives exist, however, for disease control. Ironically, much serious disease is bacterial and outside the scope of this review, as bacterial resistance to drugs is often plasmid-based and thus difficult to analyze from a population–genetic perspective. More analysis of fitness costs associated with different molecular classes of resistance mutations in field conditions is a clear priority, to allow better assessment of prior frequencies in populations. Further questions that we believe need more attention in future research are:

- Are regulatory mutations really as rare in resistance evolution as we expect them to be, and is there a predominance of *trans*- or *cis*-acting mutations?
- What are the fitness costs of specific mutations under field conditions, and are they consistent with the scheme we have proposed?
- Is recombinational limitation important in resistance evolution?
- Is genetic succession important in resistance evolution?
- How common is negative cross-resistance for specific mutations and to what extent can it be exploited?

Acknowledgments

Thanks to Craig Cady, Marten Edwards, and Jake Tu for assistance in data collection. Jonathan Gressel, Bill Plapp, and anonymous reviewers gave very useful commentary, and Eileen Sullivan gave helpful suggestions on layout. This work was supported by USDA competitive grant 9301875 and NIH grant ES06694.

LITERATURE CITED

AIACH, A., E. OHMANN, U. BODNER, and U. JOHANNINGMEIER. 1992. A herbicide resistant *Euglena* mutant carrying a Ser to Thr substitution at position 265 in the D1 protein of photosystem II. Z. Naturforsch. **47C**:245–248.

AMICHOT, M., C. CASTELLA, A. CUANY, J. B. BERGE, and D. PAURON. 1992. Target modification as a molecular mechanism of pyrethroid resistance in *Drosophila melanogaster*. Pestic. Biochem. Physiol. **44**:183–190.

ASTIER, C., I. MEYER, C. VERNOTTE, and A. L. ETIENNE. 1984. Photosystem II electron transfer in highly herbicide resistant mutants of *Synechocystis* 6714. FEBS Lett. **207**:234–238.

BARROS, M. D. C., and T. A. DYER. 1988. Atrazine resistance in the grass *Poa annua* is due to a single base change in the chloroplast gene for the D1 protein of photosystem II. Theor. Appl. Genet. **75**:610–616.

BENNOUN, P., M. DELOSME, and U. KUECK. 1991. Mitochondrial genetics of *Chlamydomonas reinhardtii*: resistance mutation marking the Cytochrome b gene. Genetics **127**: 335–344.

BRATTSTEN, L. B., C. W. HOLYOKE JR., J. R. LEEPER, and K. F. RAFFA. 1986. Insecticide resistance: challenge to pest management and basic research. Science **231**:1255–1260.

BRUNNER, L. A., R. V. MENDOZA, and A. TUENA DE COBOS. 1987. Extrachomosomal genetics in the yeast *Kluyveromyces lactis*: isolation and characterization of antimycin-resistant mutants. Curr. Genet. **11**:475–482.

CALLAHAN, H. L., and S. M. BEVERLEY. 1992. A member of the aldoketo-reductase family confers methotrexate resistance in *Leishmania*. J. Biol. Chem. **267**:24165–24168.

CARIÑO, F. A., J. F. KOENER, F. W. PLAPP JR., and R. FEYEREISEN. 1994. Constitutive overexpression of the cytochrome P450 gene CYP6A1 in a house fly strain with metabolic resistance to insecticides. Insect Biochem. Mol. Biol. **24**: 411–418.

CHAMOVITZ, D., I. PECKER, and J. HIRSCHBERG. 1991. The molecular basis of resistance to the herbicide norflurazon. Plant Mol. Biol. **16**:967–974.

CHEN, G., C. MUELLER, M. WENDLINGER, and J. W. ZOLG. 1987. Kinetic and molecular properties of the dihydrofolate reductase from the pyrimethamine-sensitive and pyrimethamine-resistant clones of the human malaria parasite *Plasmodium falciparum.* Mol. Pharmacol. **31**:430–437.

CHOI, K., C. CHEN, M. KRIEGLER, and I. B. RONINSON. 1988. An altered pattern of cross-resistance in multidrug resistant human cells results from spontaneous mutations in the mdr1 (P-glycoprotein) gene. Cell **53**:519–529.

CLARK, A. G., L. WANG, and T. HULLEBERG. 1995. Spontaneous mutation rate of modifiers of metabolism in *Drosophila.* Genetics **139**:767–779.

CLARK, A. R., and K. DOCHERTY. 1993. Negative regulation of transcription in eukaryotes. Biochem. J. **296**:521–541.

COHAN, F. 1994. Genetic exchange and evolutionary divergence in prokaryotes. Trends Ecol. Evol. **9**:175–180.

COMAI, L., and D. STALKER. 1986. Mechanism of action of herbicides and their molecular manipulation. Oxf. Surv. Plant Mol. Cell Biol. **3**:166–195.

COOK, L. M. 1981. The ecological factor in assessment of resistance in pest populations. Pestic. Sci. **12**:582–586.

CORIA, R., M. GARCIA, and A. BRUNNER. 1989. Mitochondrial cytochrome b genes with a six-nucleotide deletion or single-nucleotide substitutions confer resistance to antimycin A in the yeast *Kluyveromyces lactis.* Mol. Microbiol. **3**:1599–1604.

COWMAN, A. F., and A. M. LEW. 1989. Antifolate drug selection results in duplication and rearrangement of chromosome seven in *Plasmodium chabaudi.* Mol. Cell. Biol. **9**: 5182–5188.

COWMAN, A. F., M. J. MORRY, B. A. BIGGS, G. A. M. CROSS, and S. J. FOOTE. 1988. Identification of amino acids linked to pyrimethamine resistance in the dihydrofolate reductase–thymidylate synthase gene of *Plasmodium falciparum.* Proc. Natl. Acad. Sci. USA **85**:9109–9113.

DALY, J. C., and J. H. FISK. 1992. Inheritance of metabolic resistance to the synthetic pyrethroids in Australian *Helicoverpa armigera* (Lepidoptera: Noctuidae). Bull. Ent. Res. **82**:5–12.

DAVIDSE, L. 1987. Mechanisms of resistance in fungi to benzimidazoles, inhibitors of sterol biosynthesis and phenylamides. Pp. 216–227 *in* M. G. FORD, D. W. HOLLOMAN, B. P. S. KHAMBAY, and R. M. SAWICKI, eds. Combating resistance to xenobiotics: biological and chemical approaches. Horwood, New York.

DAVIES, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science **264**:375–382.

DEBATISSE, M., I. SAITO, G. BUTTIN, and G. R. STARK. 1988. Preferential amplification of rearranged sequences near amplified adenylate deaminase genes. Mol. Cell. Biol. **8**:17–24.

DENIS, N., A. KITZIS, J. KRUH, F. DAUTRY, and D. CORCOS. 1991. Stimulation of methotrexate resistance and dihydrofolate reductase gene amplification by *c-myc.* Oncogene **6**: 1453–1458.

DE SAINT VINCENT, B. R., O. HYRIEN, M. DEBATISSE, and G. BUTTIN. 1990. Coamplification of μ class glutathione s-transferase genes and an adenylate deaminase gene in coformycin-resistant Chinese hamster fibroblasts. Eur. J. Biochem. **193**:19–24.

DETKE, S., G. CHAUDBURI, J. A. KINK, and K. P. CHANG. 1988. DNA amplification in tunicamycin-resistant *Leishmania mexicana.* J. Biol. Chem. **263**:3418–3424.

DEVONSHIRE, A. L., and L. M. FIELD. 1991. Gene amplification and insecticide resistance. Ann. Rev. Ent. **36**:1–23.

DONG, K., and J. G. SCOTT. 1994. Linkage of *kdr*-type resistance and the para-homologous sodium channel gene in German cockroaches (*Blattella germanica*). Insect Biochem. Mol. Biol. **24**:647–654.

ELLIOTT, M., A. W. FARNHAM, N. F. JONES, D. M. JOHNSON, D. A. PULMAN, and R. M. SAWICKI. 1986. Insecticidal amides with selective potency against a resistant (*super-kdr*) strain of houseflies (*Musca domestica* L.). Agric. Biol. Chem. **50**:1347–1349.

ERICKSON, J. M., M. RAHIRE, J. D. ROCHAIX, and L. METS. 1985. Herbicide resistance and cross resistance: changes at three distinct sites in the herbicide-binding protein. Science **228**:204–207.

ETIENNE, A. L., J. M. DUCRUET, G. AJLANI, and C. VERNOTTE. 1990. Comparative studies on electron transfer in photosystem II of herbicide resistant mutants from different organisms. Biochim. Biophys. Acta **1015**:435–440.

FERRARI, J. A., and G. P. GEORGHIOU. 1991. Quantitative genetic variation of esterase activity associated with a gene amplification in *Culex quinquefasciatus.* Heredity **66**:265–272.

FERRÉ, J., M. D. REAL, J. VAN RIE, S. JANSENS, and M. PEFEROEN. 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. Proc. Natl. Acad. Sci. USA **88**:5119–5123.

FFRENCH-CONSTANT, R. H. 1994. The molecular and population genetics of cyclodiene insecticide resistance. Insect Biochem. Mol. Biol. **24**:335–345.

FFRENCH-CONSTANT, R. H., A. L. DEVONSHIRE, and R. P. WHITE. 1988. Spontaneous loss and reselection of resistance in extremely resistant *Myzus persicae* (Sulz.). Pestic. Biochem. Physiol. **30**:1–10.

FFRENCH-CONSTANT, R. H., J. C. STEICHEN, T. A. ROCHELEAU, K. ARONSTEIN, and R. T. ROUSH. 1993. A single amino acid substitution in a g-aminobutyric acid subtype-A receptor locus is associated with cyclodiene insecticide resistance in *Drosophila* populations. Proc. Natl. Acad. Sci. USA **90**: 1957–1961.

FIELD, L. M., A. L. DEVONSHIRE, and B. G. FORDE. 1988. Molecular evidence that insecticide resistance in peach-potato aphids (*Myzus persicae* Sulz.) results from amplification of an esterase gene. Biochem. J. **251**:309–312.

FISHER, R. A. 1958. The genetical theory of natural selection. Dover, New York.

FOLLETT, P. A., F. GOULD, and G. G. KENNEDY. 1993. Comparative fitness of three strains of colorado potato beetle (Coleoptera: Chrysomelidae) in the field: spatial and temporal variation in insecticide selection. J. Econ. Entomol. **86**:1324–1333.

FOURNIER, D., J. M. BRIDE, F. HOFFMANN, and F. KARCH. 1992. Acetylcholinesterase: two types of modifications confer resistance to insecticide. J. Biol. Chem. **267**:14270–14274.

FOURNIER, D., A. MUTERO, M. PRALAVORIO, and J. M. BRIDE. 1993. *Drosophila* acetylcholinesterase: mechanisms of resistance to organophosphates. Chem. Biol. Interact. **87**:233–238.

GEORGHIOU, G. P., and C. E. TAYLOR. 1977. Operational influences in the evolution of insecticide resistance. J. Econ. Entomol. **70**:653–658.

GHISLAIN, M., M. DE-SADELEER, and A. GOFFEAU. 1992. Altered plasma membrane proton-ATPase from the Dio-9-resistant *pmal*-2 mutant of *Schizosaccharomyces pombe.* Eur. J. Biochem. **209**:275–279.

GOLDEN, S. S., and R. HASELKORN. 1985. Mutation to herbicide resistance maps within the *psbA* gene of *Anacystis nidulans* R2. Science **229**:1104–1207.

GOLDSBROUGH, P. B., E. M. HATCH, B. HUANG, W. G. KOSINSKY, W. E. DYER, K. M. HERRMANN, and S. C. WELLER. 1990. Gene amplification in glyphosate tolerant tobacco cells. Plant Sci. **72**:53–62.

GOLOUBINOFF, P., M. EDELMAN, and R. B. HALICK. 1984. Chloroplast coded atrazine resistance in *Solanum nigrum*: psbA loci from susceptible and resistant biotypes are isogenic except for a single codon change. Nucleic Acids Res. **12**:9489–9496.

GONZALEZ, F. J., and D. W. NEBERT. 1990. Evolution of the P450 gene superfamily: animal–plant warfare, molecular drive, and human genetic differences in drug oxidation. Trends Genet. **6**:182–186.

GOULD, F., A. MARTINEZ-RAMIREZ, A. ANDERSON, J. FERRE, F. J. SILVA, and W. J. MOAR. 1993. Broad-spectrum resistance to *Bacillus thuringiensis* toxicants in *Heliothis virescens.* Proc. Natl. Acad. Sci. USA **89**:7986–7990.

GRANT, D. F., and B. D. HAMMOCK. 1992. Genetic and molecular evidence for a trans-acting regulatory locus controlling glutathione s-transferase-2 expression in *Aedes aegypti.* Mol. Gen. Genet. **234**:169–176.

GRANT, D. F., and F. MATSUMURA. 1989. Glutathione S-transferase 1 and 2 in susceptible and insecticide resistant *Aedes aegypti.* Pest. Biochem. Physiol. **33**:132–143.

GROETERS, F. R., and B. E. TABASHNIK. 1995. Insecticide resistance. Trends Ecol. Evol. **10**:164.

GRONWALD, J. W. 1994. Resistance to photosystem II inhibiting herbicides. Pp. 27–60 *in* S. B. POWLES and J. A. M. HOLTUM, eds. Herbicide resistance in plants: biology and biochemistry. Lewis, Boca Raton, Fla.

GUNNING, R. V., C. S. EASTON, M. E. BALFE, and I. G. FERRIS. 1991. Pyrethroid resistance mechanisms in Australian *Helicoverpa armigera.* Pestic. Sci. **33**:473–490.

HAHN, P., B. NEVALDINE, and W. F. MORGAN. 1990. X-ray induction of methotrexate resistance due to DHFR gene amplification. Somat. Cell Mol. Genet. **16**:413–423.

HALL, J. D., Y. WANG, J. PIERPONT, M. S. BERLIN, S. E. RUNDLETT, and S. WOODWARD. 1989. Aphidicolin resistance in *Herpes simplex* virus type I reveals features of the DNA polymerase dNTP binding site. Nucleic Acids Res. **17**: 9231–9244.

HALL, J. D., and S. WOODWARD. 1989. Aphidicolin resistance in *Herpes simplex* virus type I appears to alter substrate specificity in the DNA polymerase. J. Virol. **63**:2874–2876.

HALL, L. M., and D. P. KASBEKAR. 1989. *Drosophila* sodium channel mutations affect pyrethroid sensitivity. Pp. 99–114 *in* T. NARAHASHI and J. E. CHAMBERS, eds. Insecticide action: from molecule to organism. Plenum Press, New York.

HATTORI, J., R. RUTLEDGE, H. LABBE, D. BROWN, G. SUNOHARA, and B. MIKI. 1992. Multiple resistance to sulfonureas and imidazolinones conferred by an acetohydroxyacid synthase gene with separate mutations for selective resistance. Mol. Gen. Genet. **232**:167–173.

HAUGHN, G. W., and C. SOMERVILLE. 1986. Sulfonylurea-resistant mutants of *Arabidopsis thaliana.* Mol. Gen. Genet. **204**:430–434.

———. 1990. A mutation causing imidizolinone resistance maps to the csr1 locus of *Arabidopsis thaliana.* Plant Physiol. **92**:1081–1085.

HENDERSON, D. M., C. D. SIFRI, M. RODGERS, D. F. WIRTH, N. HENDRICKSON, and B. ULLMAN. 1992. Multidrug resistance in *Leishmania donovani* is conferred by amplification of a gene homologous to the mammalian mdr1 gene. Mol. Cell. Biol. **12**:2855–2865.

HEYM, B., P. M. ALZARI, N. HONORE, and S. T. COLE. 1995. Missense mutations in the catalase-peroxidase gene, katG, are associated with isoniazid resistance in *Mycobacterium tuberculosis.* Mol. Microbiol. **15**:235–245.

HIRSCHBERG, J., N. OHAD, I. PECKER, and A. RAHAT. 1987. Isolation and characterization of herbicide resistant mutants in the cyanobacterium *Synechococcus* R2. Z. Naturforsch. **42C**:758–761.

HOLZINGER, F., C. FRICK, and M. WINK. 1992. Molecular basis for the insensitivity of the Monarch (*Danaus plexippus*) to cardiac glycosides. FEBS Lett. **314**:477–480.

HURTA, R. A. R., and J. A. WRIGHT. 1990. Amplification of the genes for both components of ribonucleotide reductase in hydroxyurea resistant mammalian cells. Biochem. Biophys. Res. Commun. **167**:258–264.

HWANG, C. B. C., K. L. RUFFNER, and D. M. COEN. 1992. A point mutation within a distinct conserved region of the *Herpes simplex* virus-DNA polymerase gene confers drug-resistance. J. Virol. **3**:1774–1776.

INSELBURG, J., D. J. BZIK, and T. HORII. 1987. Pyrimethamine resistant *Plasmodium falciparum*: overproduction of dihydrofolate reductase by a gene duplication. Mol. Biochem. Parasitol. **26**:121–134.

JOHANNINGMEIER, U., U. BODNER, and G. F. WILDNER. 1987. A new mutation in the gene coding for the herbicide binding protein in *Chlamydomonas.* FEBS Lett. **2**:221–224.

KACSER, H., and J. A. BURNS. 1981. The molecular basis of dominance. Genetics **97**:639–666.

KARUNARATNE, S. H. P. P., K. G. I. JAYAWARDENA, J. HEMINGWAY, and A. J. KETTERMAN. 1993. Characterization of a B-type esterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus.* Biochem. J. **294**:575–579.

KASBEKAR, D. P., and L. M. HALL. 1988. A *Drosophila* mutation that reduces sodium channel number confers resistance to pyrethroid insecticides. Pestic. Biochem. Physiol. **32**:135–145.

KELLAM, P., C. A. B. BOUCHER, J. M. G. H TIJNAGEL, and B. A. LARDER. 1994. Zidovudine treatment results in the selection of human immunodeficiency virus type 1 variants whose genotypes confer increasing levels of drug resistance. J. Gen. Virol. **75**:341–351.

KERNAN, M., M. KURODA, R. KREBER, B. BAKER, and B. GANETZKY. 1991. *nap*^{ts} a mutation affecting sodium channel activity in *Drosophila* is an allele of *mle,* a regulator of X chromosome transcription. Cell **66**:949–959.

KNIGHT P. J. K., N. CRICKMORE, and D. J. ELLAR. 1994. The receptor for *Bacillus thuringiensis* CrylA(c) delta-endotoxin in the brush border membrane of the lepidopteran *Manduca sexta* is aminopeptidase-N. Mol. Microbiol. **11**:429–436.

KNIPPLE, D. C., K. E. DOYLE, P. A. MARSELLA-HERRICK, and D. M. SODERLUND. 1994. Tight genetic linkage between the *kdr* insecticide resistance trait and a voltage-sensitive sodium channel gene in the house fly. Proc. Natl. Acad. Sci. USA **91**:2483–2487.

KONNO, T., E. HODGSON, and W. C. DAUTERMAN. 1989. Studies on methyl parathion resistance in *Heliothis virescens.* Pestic. Biochem. Physiol. **33**:189–199.

KONNO, T., Y. KASAI, R. L. ROSE, E. HODGSON, and W. C. DAUTERMAN. 1990. Purification and characterization of a phosphorotriester hydrolase from methyl parathion-resistant *Heliothis virescens.* Pestic. Biochem. Physiol. **36**:1–13.

LEE, M. S., J. C. WANG, and M. BERAN. 1992. Two independent amsacrine-resistant human myeloid-leukemia cell-lines share an identical point mutation in the 170kda form of human topoisomerase-II. J. Mol. Biol. **223**:837–843.

LINDEN, H., G. SANDMANN, D. CHAMOVITZ, J. HIRSCHBERG, and P. BOGER. 1990. Biochemical characterization of *Synechococcus* mutants selected against the bleaching herbicide norflurazon. Pestic. Biochem. Physiol. **36**:46–51.

MANI, G. S., and R. J. WOOD. 1984. Persistence and frequency of application of an insecticide in relation to the rate of evolution of resistance. Pestic. Sci. **15**:325–336.

MARONI, G., J. WISE, J. E. YOUNG, and E. OTTO. 1987. Metallothionein gene duplications and metal tolerance in natural populations of *Drosophila melanogaster*. Genetics **117**: 739–744.

MCARTHUR, J. G., and C. P. STANNERS. 1991. A genetic element that increases the frequency of gene amplification. J. Biol. Chem. **266**:6000–6005.

MCKENZIE, J. A., and P. BATTERHAM. 1994. The genetic, molecular and phenotypic consequences of selection for insecticide resistance. Trends Ecol. Evol. **9**:166–169.

MOUCHÉS, C., N. PASTEUR, J. B. BERGÉ, O. HYRIEN, M. RAYMOND, B. R. DESAINT VINCENT, M. DESILVESTRI, and G. P. GEORGHIOU. 1986. Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito. Science **233**:778–780.

MUTERO, A., M. PRALAVORIO, J. M. BRIDE, and D. FOURNIER. 1994. Resistance-associated point mutations in insecticide-insensitive acetylcholinesterase. Proc. Natl. Acad. Sci. USA **91**:5922–5926.

NARAHASHI, T. 1986. Mechanisms of action of pyrethroids on sodium channel gating. Pp. 37–60 *in* M. G. FORD, G. G. LUNT, R. C. REAY, and P. N. R. USHERWOOD, eds. Neuropharmacology and pesticide action. Horwood, New York.

NEWHOUSE, K., B. SINGH, D. SHANER, and M. STIDHAM. 1991. Mutations in corn (*Zea mays* L.) conferring resistance to imidazolinone herbicides. Theor. Appl. Genet. **83**:65–70.

O'BRIEN, T. F., M. P. PLA, K. H. MAYER, H. KISHI, E. GILLEECE, M. SYVANEN, and J. D. HOPKINS. 1985. Intercontinental spread of a new antibiotic resistance gene on an epidemic plasmid. Science **230**:87–88.

ORBACH, M. J., E. B. PORRO, and C. YANOFSKY. 1986. Cloning and characterization of the gene for β-tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. Mol. Cell. Biol. **6**:2452–2461.

ORR, H. A., and J. A. COYNE. 1992. The genetics of adaptation: a reassessment. Am. Nat. **140**:725–742.

OTTEA, J. A., S. A. IBRAHIM, A. M. YOUNIS, R. J. YOUNG, B. R. LEONARD, and A. R. MCCAFFERY. 1995. Biochemical and physiological mechanisms of pyrethroid resistance in *Heliothis virescens* (F.). Pestic. Biochem. Physiol. **51**:117–128.

PALZKILL, T., and D. BOTSTEIN. 1992. Identification of amino acid substitutions that alter the substrate specificity of TEM-1 β-lactamase. J. Bacteriol. **174**:5237–5243.

PALU, G., G. GERNA, F. BEVILACQUA, and A. MARCELLO. 1992. A point mutation in the thymidine kinase gene is responsible for acyclovir-resistance in *Herpes simplex* virus type 2 sequential isolates. Virus Res. **25**:133–144.

PAPADOPOULOU, B., G. ROY, and M. QUELLETTE. 1992. A novel antifolate resistance gene on the amplified H circle of *Leishmania*. EMBO J. **11**:3601–3608.

PAULETTI, G., E. LAI, and G. ATTARDI. 1990. Early appearance of long-term presistence of the submicroscopic extrachromosomal elements (amplisomes) containing the amplified DHFR genes in human cell lines. Proc. Natl. Acad. Sci. USA **87**:2955–2959.

PETERSON, D. S., D. WALLIKER, and T. E. WELLEMS. 1988. Evidence that point mutations in dihydrofolate reductase–thymidylate synthase confers resistance to pyrimethamine in *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA **85**: 9114–9118.

PODGLAJEN, I., J. BREUIL, F. BORDON, L. GUTMANN, and E. COLLATZ. 1992. A silent carbapenemase gene in strains of *Bacteroides fragilis* can be expressed after a one step mutation. FEBS Lett. **91**:21–30.

POWLES, S. B., and J. A. M. HOLTUM. 1994. Herbicide resistance in plants: biology and biochemistry. Lewis, Boca Raton, Fla.

PRALAVORIO, M., and D. FOURNIER. 1992. *Drosophila* acetylcholinesterase: characterization of different mutants resistant to insecticides. Biochem. Genet. **30**:77–83.

QUON, D. V. K., C. E. DOLIVEIRA, and P. J. JOHNSON. 1992. Reduced transcription of the ferredoxin gene in metronidazole-resistant *Trichomonas vaginalis*. Proc. Natl. Acad. Sci. USA **89**:4402–4406.

RAYMOND, M., V. BEYSSAT-ARNAOUTY, N. SIVASUBRAMANIAM, C. MOUCHES, G. P. GEORGHIOU, and N. PASTEUR. 1989. Amplification of various esterase B's responsible for organophosphate resistance in *Culex* mosquitoes. Biochem. Genet. **27**:417–423.

RIESGOESCOVAR, J., C. WOODARD, P. GAINES, and J. CARLSON. 1992. Development and organization of the *Drosophila* olfactory system—an analysis using enhancer traps. J. Neurobiol. **23**:947–964.

ROUSH, R., and J. DALY. 1990. The role of population genetics in resistance research and management. Pp. 423–441 *in* R. ROUSH and B. TABASHNIK, eds. Pesticide resistance in arthropods. Chapman and Hall, London.

ROUSH, R., and J. A. MCKENZIE. 1987. Ecological genetics of insecticide and acaricide resistance. Annu. Rev. Entomol. **32**:361–380.

SAFA, A. R., R. K. STERN, K. CHOI, M. AGRESTI, I. TAMAI, N. D. MEHTA, and I. B. RONINSON. 1990. Molecular basis of preferential resistance to colchicine in multidrug-resistant cells conferred by Gly-185 to Val-185 substitution in P-glycoprotein. Proc. Natl. Acad. Sci. USA **87**:7225–7229.

SAMUELSON, J., P. AYALA, E. OROZCO, and D. WIRTH. 1990. Emetine-resistant mutants of *Entamoeba histolytica* overexpress messenger RNA for multidrug resistance. Mol. Biochem. Parasitol. **38**:281–290.

SANGADALA, S., F. S. WALTERS, L. H. ENGLISH, and M. J. ADANG. 1994. A mixture of *Manduca sexta* aminopeptidase and phosphatase enhances *Bacillus thuringiensis* insecticidal CryIA(c) toxin binding and $^{86}Rb^{+}$-K^{+} efflux in vitro. J. Biol. Chem. **269**:10088–10092.

SATHASIVAN, K., G. W. HAUGHN, and N. MURAI. 1991. Molecular basis of imidazolinone herbicide resistance in *Arabidopsis thaliana* var columbia. Plant Physiol. **97**:1044–1050.

SAVAGEAU, M. A. 1992. Dominance according to metabolic control analysis—major achievement or house of cards? J. Theor. Biol. **154**:131–136.

SCHEIDEL, L. M., and V. STOLLAR. 1991. Mutations that confer resistance to mycophenolic acid and ribavirin on Sindbis virus map to the nonstructural protein nsP1. Virology **181**: 490–499.

SCHIMKE, R. T. 1988. Gene amplification in cultured cells. J. Biol. Chem. **263**:5989–5992.

SCHIMKE, R. T., S. W. SHERWOOD, A. B. HILL, and R. N. JOHNSTON. 1986. Overreplication and recombination of DNA in higher eukaryotes: potential consequences and biological implications. Proc. Natl. Acad. Sci. USA **83**:2157–2161.

SCHOENMAKERS, H. C. H., M. KOORNNEEF, S. J. H. M. ALEFS, W. F. M. GERRITS, D. VAN DER KOP, I. CHÉREL, and M. CABOCHE. 1991. Isolation and characterization of nitrate re-

ductase-deficient mutants in tomato (*Lycopersicon esculentum* Mill.). Mol. Gen. Genet. **227**:458–464.

SHEMSHEDINI, L., and T. G. WILSON. 1990. Resistance to juvenile hormone and an insect growth regulator in *Drosophila* is associated with an altered cytosolic juvenile hormone binding protein. Proc. Natl. Acad. Sci. USA **87**:2072–2076.

SHIGEMATSU, Y., F. SATO, and Y. YAMADA. 1989. A binding model for phenylurea herbicides based on analysis of a Thr264 mutation in the D-1 protein of tobacco. Pestic. Biochem. Physiol. **35**:33–41.

SHYR, Y. Y. J., A. G. HEPBURN, and J. M. WIDHOLM. 1990. Glyphosate selected amplification of the 5-enolpyruvylshikimate-3-phosphate synthase gene in cultured carrot cells. Mol. Gen. Genet. **232**:377–382.

SNEWIN, V. A., S. M. ENGLAND, P. F. G. SIMS, and J. E. HYDE. 1989. Characterization of the dihydrofolate reductase–thymidylate synthase gene from human malaria parasites highly resistant to pyrimethamine. Gene **76**:41–52.

SOREQ, H., A. GNATT, Y. LOEWENSTEIN, and L. F. NEVILLE. 1992. Excavations into the active-site gorge of cholinesterases. Trends Biochem. Sci. **17**:353–358.

SPARKS, T. C., J. A. LOCKWOOD, R. L. BYFORD, J. B. GRAVES, and B. R. LEONARD. 1989. The role of behavior in insecticide resistance. Pestic. Sci. **26**:383–399.

SPRATT, B. 1994. Resistance to antibiotics mediated by target alterations. Science **264**:388.

SVAB, Z., and P. MALIGA. 1991. Mutation proximal to the transfer RNA binding region of the *Nicotiana* plastid 16S ribosomal RNA confers resistance to spectinomycin. Mol. Gen. Genet. **228**:316–319.

TADDIE, J. A., and P. TRAKTMAN. 1991. Genetic characterization of the *Vaccinia* virus DNA polymerase: identification of point mutations conferring altered drug sensitivities and reduced fidelity. J. Virol. **65**:869–879.

TAKAHASHI, M., H. KOBAYASHI, and S. IWASAKI. 1989. Rhizoxin resistant mutants with an altered beta-tubulin gene in *Aspergillus nidulans*. Mol. Gen. Genet. **220**:53–59.

TANAKA, M., H. M. GU, D. J. BZIK, W. B. LI, and J. INSELBURG. 1990. Mutant dihydrofolate reductase–thymidylate synthase genes in pyrimethamine-resistant *Plasmodium falciparum* with polymorphic chromosome duplications. Mol. Biochem. Parasitol. **42**:83–92.

TAYLOR, M. F. J., D. G. HECKEL, T. M. BROWN, M. E. KREITMAN, and B. BLACK. 1993. Linkage of pyrethroid insecticide resistance to a sodium channel locus in the tobacco budworm. Insect Biochem. Mol. Biol. **23**:763–775.

TAYLOR, M. F. J., Y. SHEN, and M. E. KREITMAN. 1995. A population genetic test of selection at the molecular level. Science **270**:1497–1499.

THOMAS, J. F., N. F. NEFF, and D. BOTSTEIN. 1985. Isolation and characterization of mutants in the β-tubulin gene of *Saccaromyces cerevisiae*. Genetics **111**:715–734.

UYENOYAMA, M. 1986. Pleiotropy and the evolution of genetic systems conferring resistance to pesticides. Pp. 207–221 *in* E. H. GLASS et al., eds. Pesticide resistance: strategies and tactics for management. National Academy Press, Washington, D.C.

VAN RIE, J., W. H. MCFAUGHEY, D. E. JOHNSON, B. D. BARNETT, and H. VAN MELLAERT. 1990. Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis*. Science **247**:72–74.

WHITE, T. C., F. FASE-FOWLER, H. VAN LUENEN, J. CALAFAT, and P. BORST. 1988. The H circles of *Leishmannia tarentolae* are a unique amplifiable system of oligomeric DNAs associated with drug resistance. J. Biol. Chem. **263**:16977–16983.

WIERSMA, P. A., J. E. HACHEY, W. L. CROSBY, and M. M. MOLONEY. 1990. Specific truncations of an acetolactate synthase gene from *Brassica napus* efficiently complement ilvB/ilvG mutants of *Salmonella typhimurium*. Mol. Gen. Genet. **224**:155–159.

WILDNER, G. F., U. HEISTERKAMP, and A. TREBST. 1990. Herbicide cross-resistance and mutations of the psbA gene in *Chlamydomonas reinhardii*. Z. Naturforsch. **45C**:1142–1150.

WILKINSON, J. Q., and N. M. CRAWFORD. 1991. Identification of the *Arabidopsis* CHL3 gene as the nitrate reductase structural gene NIA2. Plant Cell **3**:461–472.

WILLIAMSON, M. S., I. DENHOLM, C. A. BELL, and A. L. DEVONSHIRE. 1993. Knockdown resistance (*kdr*) to DDT and pyrethroid insecticides maps to a sodium-channel gene locus in the housefly (*Musca domestica*). Mol. Gen. Genet. **240**:17–22.

WILSON, C. M., A. E. SERRANO, A. WASLEY, M. P. BOGENSCHUTZ, A. H. SHANKAR, and D. F. WIRTH. 1989. Amplification of a gene related to mammalian mdr genes in drug-resistant *Plasmodium falciparum*. Science **244**:1184–1186.

WILSON, T. G., and J. FABIAN. 1986. A *Drosophila melanogaster* mutant resistant to a chemical analog of juvenile hormone. Dev. Biol. **118**:190–201.

WOLFERSBERGER, M. G. 1990. The toxicity of two *Bacillus thuringiensis* δ-endotoxicants to gypsy moth larvae is inversely related to the affinity of binding sites on midgut brush border membranes for the toxicants. Experientia **46**:475–477.

YAMAGISHI, J., H. YOSHIDA, M. YAMAYOSHI, and S. NAKAMURA. 1986. Nalidixic acid-resistant mutations of the gyrB gene of *Escherichia coli*. Mol. Gen. Genet. **204**:367–373.

YAMAMOTO, I., Y. TAKAHASHI, and N. KYOMURA. 1983. Suppression of altered acetylcholinesterase of the green rice leafhopper by N-propyl and N-methyl carbamate combinations. Pp. 579–594 *in* G. GEORGHIOU and P. T. SAITO, eds. Pest resistance to pesticides. Plenum Press, New York.

ZOLG, J. W., J. R. PLITT, G. X. CHEN, and S. PALMER. 1985. Point mutations in the dihydrofolate reductase–thymidylate synthase gene as the molecular basis for pyrimethamine resistance in *Plasmodium falciparum*. Mol. Biochem. Parasitol. **36**:253–262.

JULIAN P. ADAMS, reviewing editor

Accepted February 14, 1996