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Gene mapping and cross-resistance in cyclodiene insecticide-resistant *Drosophila melanogaster* (Mg.).

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Summary

Resistance to the cyclodiene insecticide dieldrin maps to a single gene (*Rdl*) on the left arm of chromosome III in *Drosophila melanogaster* (Meigen). The gene was further mapped by the use of chromosomal deficiencies to a single letter sub-region, 66F, on the polytene chromosome. The cross-resistance spectrum of a backcrossed strain lacking elevated mixed function oxidase activity, a common resistance mechanism, was examined. Levels of resistance similar to those found in other insects were found to dieldrin, aldrin, endrin, lindane, and picrotoxinin. Strong similarity of this single major gene with that found in other cyclodiene resistant insects is suggested by its cross-resistance spectrum and chromosomal location, via homology with other Diptera. The significance of major genes in insecticide resistance is discussed.

1. Introduction

It is generally agreed that single genes play a major role in the evolution of insecticide resistance (Via, 1986; Roush & McKenzie, 1987; Tabashnik & Cushing, 1989). However, many attempts to determine the number of genes controlling resistance rely on analysis of segregation in an F_2 or a backcross, and often yield ambiguous results. The most persuasive evidence for control by a single locus is to map the gene with respect to genetic markers. Unfortunately, this limits the unambiguous determination of the number of genes involved in resistance to a relatively few species where genetic markers are readily available (Roush & Daly, 1990). Further, one may question whether even genetic markers can unambiguously distinguish between two closely linked genes.

The most common kind of pesticide resistance (including resistance to fungicides, herbicides, and rodenticides) is to cyclodiene insecticides, which include dieldrin, aldrin, chlordane, and endrin. At least 276 species of insects and mites are resistant to cyclodienes, which include 62% of all arthropod species resistant to any pesticide (Georghiou, 1986). This resistance appears to be due to a common mechanism and to a single gene in the more than 18 species studied in detail (Oppenoorth, 1985). Thus, the unintentional selection for cyclodiene resistance may be the most frequent kind of evolutionary change ever caused by man.

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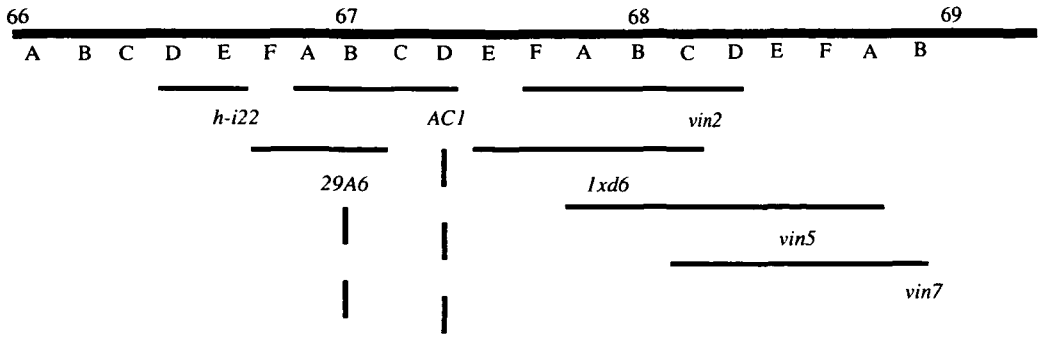
We have recently isolated a dieldrin resistant strain from field populations of *Drosophila melanogaster* (Meigen). No resistance could be attributed to either the *X* chromosome or chromosome II, but was only associated with a single locus on the left arm of chromosome III (ffrench-Constant *et al.* 1990). The left arm of chromosome III in *D. melanogaster* is homologous to chromosome V in the Australian sheep blowfly, *Lucilia cuprina* (Weidemann), and chromosome IV in the housefly, *Musca domestica* (L.), each of which also carries dieldrin resistance (Forster *et al.* 1981).

In this study, we tested whether this resistance was likely to be due to a single gene or multiple, linked genes using deficiency mapping. Next, we examined the cross-resistance spectrum of this gene to other cyclodienes and related compounds, a necessary test of whether the gene shows characteristics similar to cyclodiene resistance in other insects. To do this, we had to separate the resistance gene from genes for elevated mixed function oxidase activity also present in the strain.

2. Materials and methods

The dieldrin-resistant strains used in this study were collected from the field in Maryland (*MD-RR*) and New York (*NY-RR*) and made homozygous by 2-4 generations of selection in the laboratory (ffrench-Constant *et al.* 1990). The susceptible standard strain Oregon-*R* (here referred to as Oregon) was obtained



		h-i22	29A6	AC1	lxd6	vin2	vin5	vin7
% Survival 30 μg	R/Df	0 (90)	83 (411)	0 (379)	1 (69)	3 (88)	1 (144)	0 (190)
	R/TM3	2 (48)	1 (424)	0 (323)	0 (73)	0 (90)	0 (147)	0 (177)

Fig. 1. Chromosomal deficiencies encompassing the approximate location of cyclodiene resistance (recombinational map unit 25) in *D. melanogaster*. Numbers (66–69) and letters (A–F) represent cytological regions on polytene chromosomes. For each deficiency, survival data (with number of insects tested given in parentheses) at 30 μg dieldrin are given for *MD-R/Df* and *R/TM3* progeny (see text for explanation). Deficiencies and breakpoint information were provided by the Mid-America Stock Center.

from the Cornell University Section of Genetics and Development. Deficiency stocks were kindly provided by the Mid-America *Drosophila* Stock Center, Bowling Green State University, Ohio.

Bioassays were performed by placing 20–30 adults in 20 ml glass scintillation vials previously coated internally with solutions of insecticide in acetone and allowed to dry for 30 min. Vials were stoppered with cotton plugs moistened in 5% sucrose. Assessment of mortality was typically after 24 h, except for picrotoxinin assays, which were scored after 48 h. All cyclodienes and lindane were re-crystallized before use; picrotoxinin was obtained from Sigma Chemical Company.

Following localization of the resistance gene, *Rdl* (hereafter referred to simply as '*R*') at map unit 25 on the left arm of chromosome III (ffrench-Constant *et al.* 1990), further localization of the gene was performed using a series of overlapping chromosome deficiency stocks with known cytological breakpoints

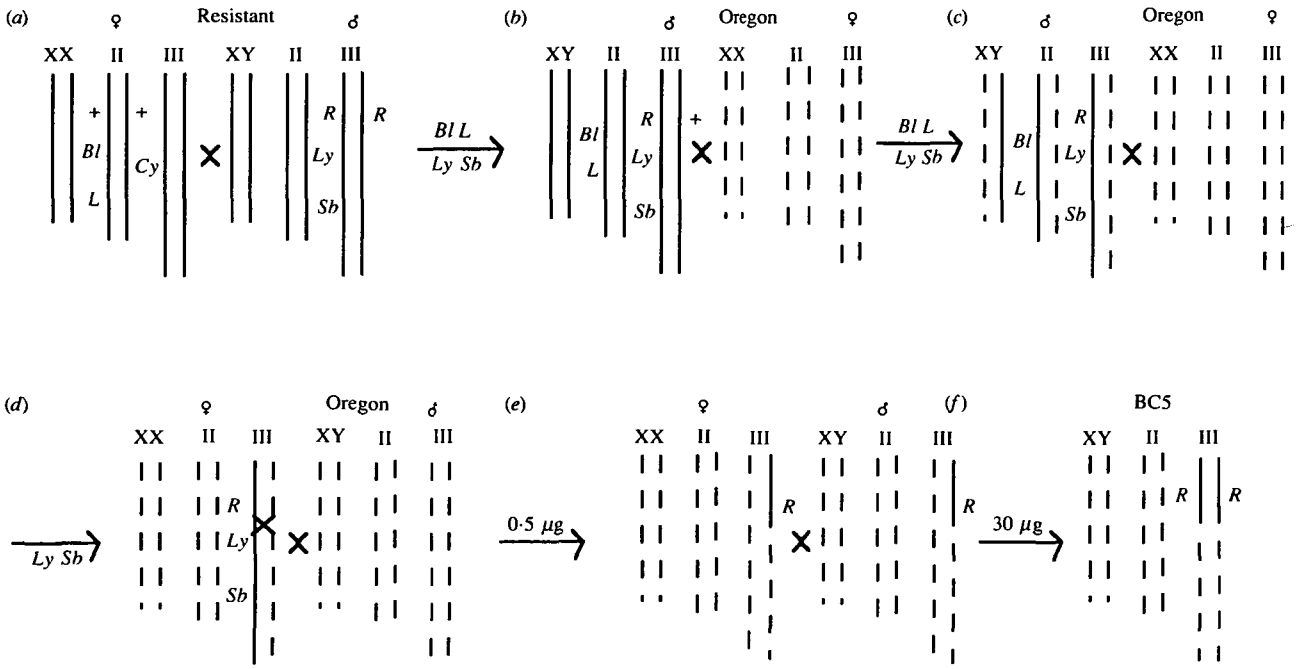


Fig. 2. Backcrossing scheme for MD-RR designed to replace all MD-RR chromosomes (solid lines) with Oregon chromosomes (broken lines) through the use of selection for dominant markers. Annotated arrows indicate the genotypes or dose of dieldrin selected following each cross. Although the ends of chromosomes III in BC5 (*f*) from *R* to the tip were not actively replaced, some recombination probably occurred in this region in (*e*) and (*f*), causing further replacement with Oregon chromosomes. The initial *RLyS/R* resistant males in (*a*) were selected by 30 μg dieldrin from a cross of heterozygous *LySb/R* females to *R/R* males.

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corresponding to the approximate recombinational map unit location 25 (Fig. 1). These deficiency stocks contained third chromosomes with deletions of known cytological location, heterozygous with a TM3 balancer chromosome. The balancer chromosome is lethal when homozygous and has an inversion to prevent recombination, thereby maintaining homozygous lethal deficiencies in a true-breeding heterozygous strain. The TM3 chromosome also carries a marker, usually stubble bristles (*Sb*). The working hypothesis was that deficiency (*Df*) chromosomes missing the susceptible gene (and its sensitive product) would display full levels of resistance (equivalent to *R/R*) when combined with the resistance gene (*R/Df*). In order to test this, each deficiency stock (*Df/TM3*) was crossed to the *MD-RR* strain. The resulting *Df/R* (morphologically wild-type) and *R/TM3* (*TM3* usually marked with *Sb*) progeny were separated and assayed at 30 μg dieldrin.

In preliminary bioassays of the cross-resistance spectra of the *MD-RR* strain, we observed significant resistance to propoxur. This was consistent with previous observations that a major gene conferring resistance to DDT, malathion, propoxur, and other compounds [similar to that studied by Waters & Nix (1988)] occurs at high frequency in the field (our unpublished data). In order to measure the effects of the cyclodiene resistance gene independently of any mixed function oxidase or other potentially resistance conferring genes, all chromosomes from the field-collected *MD-RR* strain were replaced by backcrossing to the standard susceptible strain, Oregon (here denoted as +/+). Dominant markers were used to ensure sequential replacement of chromosomes from the resistant strain with those from Oregon and resistance was then made homozygous again (Fig. 2). The backcrossed resistant strain (MD-BC5) was compared to Oregon in all subsequent bioassay studies. Bioassay data were analysed with the POLO computer program (Robertson *et al.* 1980).

3. Results

Crosses with only one deficiency strain (29A6) produced hemizygote (*R/Df*) flies displaying levels of resistance equivalent to *R/R* homozygotes (approximately 15% mortality at 30 μg). The *R/TM3* flies from the same cross and all progeny from the other deficiency crosses showed only 0–3% survival at this dose (Fig. 1). Therefore, we conclude that the gene in *MD-RR* lies within the deficiency 29A6 but not within the overlapping deficiency AC1 and is thus localized to the polytene subsection, 66F. Similar results were obtained with *NY-RR*, with 78% ($n = 276$) survival of *R/Df* flies for 29A6 and 0% ($n = 89$) survival for *Df/TM3*, suggesting that resistance in *NY/RR* is allelic with that in *MD-RR*, although this awaits confirmation by direct complementation. Although we had previously mapped *Rdl* to map unit 25,

Table 1. *Cross-resistance of dieldrin-resistant (MD-BC5) and susceptible (Oregon)* Drosophila melanogaster *to selected insecticides*

Insecticide	MD-BC5 (*R/R*)			Oregon (+/+)			RR[c]
	n[a]	LC_{50}[b] (95% CL)	Slope (S.E.)	n	LC_{50} (95% CL)	Slope (S.E.)	
Propoxur	1201	0·16 (0·07–0·24)	1·2 (0·10)	969	0·80 (0·55–1·45)	1·7 (0·13)	0·2
Malathion	717	0·18 (0·16–0·22)	4·6 (0·33)	605	0·29 (0·22–0·52)	3·5 (0·46)	0·6
DDT	1085	3·5 (2·2–5·8)	2·0 (0·15)	1050	3·6 (2·8–47)	2·3 (0·10)	1·0
Dieldrin	1832	235 (146–537)	1·3 (0·77)	1705	0·055 (0·051–0·059)	4·1 (0·16)	4270
Aldrin	2843	194 (151–263)	1·2 (0·06)	2373	1·0 (0·43–1·32)	3·7 (0·26)	194
Endrin	2003	2·9 (2·6–3·3)	3·2 (0·14)	1869	0·088 (0·078–0·098)	4·0 (0·17)	33
Lindane	4602	15·5 (13·6–17·2)	5·5 (0·28)	3166	3·2 (2·1–4·5)	1·5 (0·08)	4·8
Picrotoxinin	430	$6·9 \times 10^{7}$[d]	0·2 (0·19)	339	286[d]	1·1 (0·17)	2×10^{6}

[a] Total number of flies tested (excluding controls).
[b] μg per vial.
[c] RR, resistance ratio (= LC_{50} MD–BC5/LC_{50} Oregon).
[d] Confidence limits could not be calculated due to heterogeneity of response.

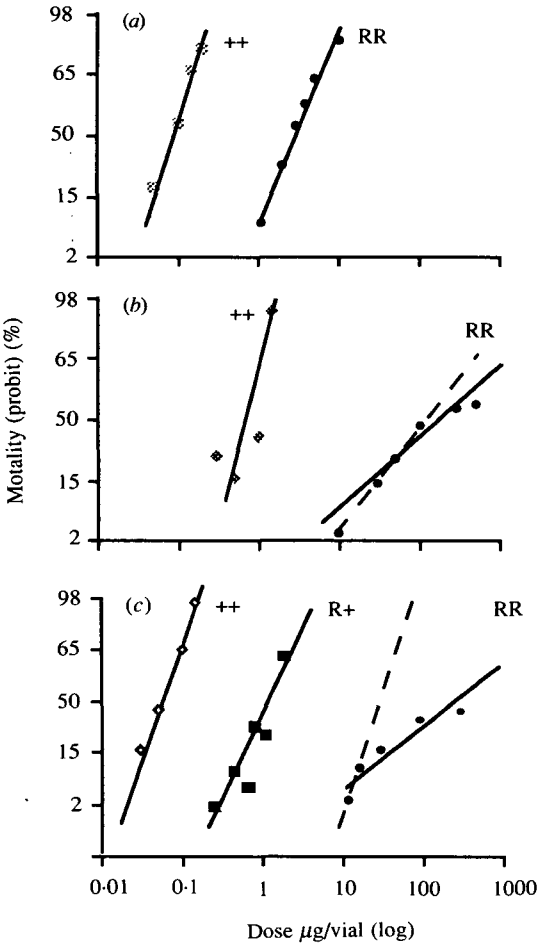

Fig. 3. Dose–mortality regressions (solid lines) for +/+ (Oregon) and MD-BC5 *RR* cyclodiene-resistant *D. melanogaster* for (*a*) endrin and (*b*) aldrin and (*c*) for +/+, MD-BC5 *R/R*, and F1 *R/+* with dieldrin. The broken lines in (*b*) and (*c*) are drawn by eye in order to indicate the expected *R/R* regression lines derived from doses where the response is still linear.

subsection 66F should correspond more closely to map unit 26·5, which is the recombinational map location of *hairy* at cytological location 66D (Lindsley & Grell 1968).

The susceptibility of the MD-BC5 strain to propoxur, malathion, and DDT was equal to our greater than that of Oregon (Table 1), suggesting that the mixed function oxidase genes present in the field-collected strain had been removed by repeated backcrossing. In contrast, resistance to cyclodienes remained high and extended to lindane (γHCH) and picrotoxinin (Table 1). Picrotoxinin is relatively non-toxic even to susceptible *D. melanogaster* via this method of exposure (LC_{50} = 286 μg/vial, Table 1); it proved impossible to kill more than 50% of resistant flies at any dose, accounting for the extremely high LC_{50}. Pre-treatment with piperonyl butoxide (a mixed function oxidase suppressor) did not enhance toxicity, but merely increased heterogeneity of response.

Although there is a large disparity between the dieldrin resistance ratios of the *R/R* strain (4270) and the F1 *R/+* heterozygotes (26; LC_{50} = 1·43, 95% CL = 1·25 — 1·71, n = 1372), this is probably exaggerated by limits to pesticide pick-up and penetration into resistant insects. The extremely high resistance to dieldrin in the *R/R* homozygotes is associated with a plateau in mortality observed above a dose of 30 μg of dieldrin (Fig. 3*c*). This may be a product of an inability of insects to pick-up more insecticide via contact exposure despite the increase in dose. At concentrations greater than 30 μg, the pesticide residues are readily visible in the vials. Thus the calculated LD_{50} (235 μg) is somewhat higher than the value of 50 μg that might be expected from a regression based on doses below 30 μg (see broken line in Fig. 3*c*). This plateau effect can also be seen in the *R/R* dose–response for aldrin (Fig. 3*b*), but not in that for endrin where lethal doses are lower (Fig. 3*a*). These observations suggest that dieldrin resistance in *D. melanogaster* is intermediate or semi-dominant in inheritance, where each of the three genotypes can be distinguished from one another by bioassay (e.g. by doses 0·1 and 10 in Fig. 3*c*).

4. Discussion

Resistance to cyclodienes in the *D. melanogaster* strains *MD-RR* and *NY-RR* maps cytologically to a single polytene chromosome subsection, containing approximately 100 kb of DNA (our unpublished data). Thus, in all probability resistance is due to a single gene rather than a complex of closely linked genes. Maintenance of high levels of resistance to cyclodienes in the absence of mixed function oxidase genes, and cross-resistance to lindane and picrotoxinin supports the contention that this gene is strongly analogous to that found in other cyclodiene-resistant insects. Cyclodiene-resistant insects typically show cross resistance to the closely related lindane (γHCH) and to picrotoxinin (Kadous *et al.* 1983; Tanaka, 1987), as shown here. Resistance to picrotoxinin has been shown in cyclodiene-resistant cockroaches (Kadous *et al.* 1983) and mosquitofish *Gambusia affinis* (Baird and Girard) (Yarbrough *et al.* 1986). At least as important, we have found levels of cross-resistance to cyclodienes that parallel those in other insects. Resistance to lindane is typically 3- to 20-fold and endrin 30- to 100-fold, whereas resistance to aldrin and dieldrin is much higher, roughly 300- to 1000-fold (Oppenoorth 1985), similar to the values reported here (Table 1). Cyclodiene resistance in insects and mosquitofish also tends to show semi-dominant inheritance (Yarbrough *et al.* 1986), in contrast to many other resistance mechanisms that tend to show nearly completely recessive or nearly completely dominant inheritance (i.e. the heterozygotes cannot be readily distinguished by bioassay from either the susceptible or resistant parent) (Oppenoorth, 1985, and references therein).

Three characteristics of this resistance, (1) chromosomal homology with the location of resistance in

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M. domestica and *L. cuprina* (Foster *et al.* 1981), (2) semi-dominant resistance, and (3) cross-resistance spectrum, strongly imply that the resistance gene in our strains is similar to that conferring cyclodiene resistance in other insects. Although segregation and linkage data suggest that a single major gene is responsible for resistance in several species and strains of mosquitoes, flies, ticks, cockroaches (Oppenoorth, 1985), and mosquitofish (Yarbrough *et al.* 1986), we provide the first direct cytological evidence of a single gene (rather than multiply linked loci) through deficiency mapping. Thus, cyclodiene resistance, the most common kind of resistance, may be caused by a single major gene in all of these species.

The mechanisms of resistance to other classes of insecticides are often much more diverse than to cyclodienes. Even within one species, resistance of different populations can be due to entirely unrelated mechanisms (Oppenoorth, 1985). For example, different mechanisms of resistance to parathion were found in European and American populations of a spider mite, *Tetranychus urticae*, perhaps because different insecticides were used during selection (Herne & Brown, 1969). In the context of cyclodiene resistance, the adaptation of so many species by an apparently similar mechanism (Oppenoorth, 1985) suggests that a similar mutation must be common in most insect species.

The exact nature of this resistance mechanism remains uncertain as does the resistance gene product. Although cyclodiene insecticides are believed to act primarily at the $GABA_A$ receptor (Eldefrawi & Eldefrawi, 1987), their precise mode of action remains obscure. Resistance could be caused either by an insensitive GABA receptor or by a reduction in the number of receptors (Tanaka, 1987). The precise chromosomal localization outlined here, combined with the molecular method of chromosomal walking (Bender *et al.* 1983), should allow cloning of the gene responsible for cyclodiene resistance in *Drosophila* and an examination of cyclodiene resistance genes in other species via heterologous probing.

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References

Bender, W., Spierer, P. & Hogness, D. S. (1983). Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the bithorax complex in *Drosophila melanogaster*. *Journal of Molecular Biology* **168**, 17–33.

Eldefrawi, A. T. & Eldefrawi, M. E. (1987). Receptors for γ-aminobutyric acid and voltage-dependent chloride channels as targets for drugs and toxicants. *FASEB Journal* **1**, 262–271.

ffrench-Constant, R. H., Roush, R. T., Mortlock, D. & Dively, G. P. (1990). Isolation of dieldrin resistance from field populations of *Drosophila melanogaster* (Diptera: Drosophilidae). *Journal of Economic Entomology* **83**, (in the Press).

Foster, G. G., Whitten, M. J., Konovalov, C., Arnold, J. T. A. & Maffi, G. (1981). Autosomal genetic maps of the Australian sheep blowfly, *Lucilia cuprina dorsalis* R-D. (Diptera: Calliphoridae) and possible correlations with the linkage maps of *Musca domestica* L. and *Drosophila melanogaster* (Mg.). *Genetical Research* **37**, 55–69.

Georghiou, G. P. (1986). The magnitude of the resistance problem. In *Pesticide Resistance: Strategies and Tactics for Management* (ed. National Academy of Sciences), pp. 14–43. Washington, D.C.: National Academy Press.

Herne, D. H. C. & Brown, A. W. A. (1969). Inheritance and biochemistry of OP-resistance in a New York strain of the two-spotted spider mite. *Journal of Economic Entomology* **62**, 205–209.

Kadous, A. A., Ghiasuddin, S. M., Matsumura, F., Scott, J. G. & Tanaka, K. (1983). Difference in the picrotoxinin receptor between the cyclodiene-resistant and susceptible strains of the German cockroach. *Pesticide Biochemistry and Physiology* **19**, 157–166.

Lindsley, D. L. & Grell, E. H. (1968). Genetic variations of *Drosophila melanogaster*. Carnegie Institution of Washington Publication 627.

Oppenoorth, F. J. (1985). Biochemistry and genetics of insecticide resistance. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, vol. 12. (ed. G. A. Kerkut and L. I. Gilbert), pp. 731–773. New York: Pergamon.

Robertson, J. L., Russell, R. M. & Savin, N. E. (1980). POLO: a user's guide to probit or logic analysis. United States Forestry Service General Technical Report PSW 38.

Schofield, P. R., Darlison, M. G., Fugita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Reale, V., Glencorse, T. A., Seeburg, P. H. & Barnard, E. A. (1987). Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. *Nature* **328**, 221–227.

Tabashnik, B. E. & Cushing, N. L. (1989). Quantitative genetic analysis of insecticide resistance: variation in fenvalerate tolerance in a diamondback moth (Lepidoptera: Plutellidae) population. *Journal of Economic Entomology*, **82**, 5–10.

Tanaka, K. (1987). Mode of action of compounds acting at inhibitory synapse. *Journal of Pesticide Science* **12**, 549–560.

Via, S. (1986). Quantitative genetic models and the evolution of pesticide resistance. In *Pesticide Resistance: Strategies and Tactics for Management* (ed. National Academy of Science), pp. 222–235. Washington, D.C.: National Academy Press.

Waters, L. C. & Nix, C. E. (1988). Regulation of insecticide resistance-related cytochrome P-450 expression in *Drosophila melanogaster*. *Pesticide Biochemistry and Physiology* **30**, 214–227.

Yarbrough, J. D., Roush, R. T., Bonner, J. C. & Wise, D. A. (1986). Monogenic inheritance of cyclodiene resistance in mosquito fish, *Gambusia affinis*. *Experientia* **42**, 851–853.