Heredity (2003) 91, 98–106

www.nature.com/hdy

Analogous pleiotropic effects of insecticide resistance genotypes in peach–potato aphids and houseflies

SP Foster[1], S Young[2], MS Williamson[2], I Duce[3], I Denholm[1] and GJ Devine[1]
[1]*Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden AL5 2JQ, UK;* [2]*Biological Chemistry Division, Rothamsted Research, Harpenden AL5 2JQ, UK;* [3]*School of Life and Environmental Sciences, University of Nottingham, University Park Nottingham NG7 2RD, UK*

We show that single-point mutations conferring target-site resistance (*kdr*) to pyrethroids and DDT in aphids and houseflies, and gene amplification conferring metabolic resistance (carboxylesterase) to organophosphates and carbamates in aphids, can have deleterious pleiotropic effects on fitness. Behavioural studies on peach–potato aphids showed that a reduced response to alarm pheromone was associated with both gene amplification and the *kdr* target-site mutation. In this species, gene amplification was also associated with a decreased propensity to move from senescing leaves to fresh leaves at low temperature. Housefly genotypes possessing the identical *kdr* mutation were also shown to exhibit behavioural differences in comparison with susceptible insects. In this species, resistant individuals showed no positional preference along a temperature gradient while susceptible genotypes exhibited a strong preference for warmer temperatures.
Heredity (2003) **91,** 98–106. doi:10.1038/sj.hdy.6800285

Keywords: pleiotropy; insecticide resistance; point mutation; gene amplification; behaviour

Introduction

In the peach–potato aphid, *Myzus persicae* (Hemiptera: Aphididae), gene amplification leads to the overproduction of carboxylesterases that enhance the detoxification of insecticidal esters such as organophosphates (OPs) and carbamates (Devonshire and Moores, 1982; Field *et al*, 1988). In the same species, a point mutation of a voltage-gated sodium channel gene confers cross-resistance to pyrethroids and DDT (knockdown resistance or kdr) (Martinez-Torres *et al*, 1999). This latter mutation is common to many pyrethroid-resistant insect species, including *Musca domestica* (Diptera: Muscidae) (Vais *et al*, 2001).

It has long been proposed that the majority of insecticide-resistant organisms should show some differential survival in comparison with 'wildtype' organisms (eg Crow, 1957). However, despite the extensive literature on the subject, there are relatively few empirical studies that confirm that investment in such adaptations exacts a cost (Minkoff and Wilson, 1992; Carriere *et al*, 1994, 1995; Yamamoto *et al*, 1995; Chevillon *et al*, 1997; Alyokhin and Ferro, 1999; Boivin *et al*, 2001). Some authors have failed to reveal any detrimental effects of insecticide resistance (Follett *et al*, 1993; Tang *et al*, 1997, 1999; Baker *et al*, 1998) and some have shown such organisms to be even more successful than their susceptible counterparts in the absence of insecticides (Omer *et al*, 1992; Bloch and Wool, 1994; White and Bell, 1995; Mason, 1998; Haubruge and Arnaud, 2001). In other studies, some measures of fitness have been negatively affected, others positively (Brewer and Trumble, 1991) and different strains of insect, exhibiting resistance to the same compound, can show opposite associations (Chevillon *et al*, 1997; Hollingsworth *et al*, 1997; Oppert *et al*, 2000).

It is possible that the lack of any clear pattern in the literature results from the fact that evolutionary pathways can compensate for fitness disadvantages through the development of modifier genes (McKenzie and O'Farrell, 1993; Charpentier and Fournier, 2001) or through the replacement of mutant alleles by less costly ones (Guillemaud *et al*, 1998), but it may also reflect the fact that fitness costs are generally investigated by comparing biological parameters such as development times (Boots and Begon, 1993; Baker *et al*, 1998; Boivin *et al*, 2001), fecundity (Hollingsworth *et al*, 1997; Brewer *et al*, 1999; Boivin *et al*, 2001), and intrinsic rates of growth (Yamamoto *et al*, 1995) usually under optimal laboratory conditions. By concentrating purely on such obviously detrimental parameters and by failing to consider suboptimal conditions (stressful environments in which fitness differentials are at a premium), subtle pleiotropic effects on biology or behaviour are often overlooked (McKenzie, 1996). Examples of behavioural effects that have been shown to be correlated with resistance are therefore rare but include mating disruption and oviposition preference in dieldrin-resistant Anopheline mosquitoes (Rowland, 1991a,b), mating success in *Bacillus thuringiensis* (Bt)-resistant diamondback moths (Groeters *et al*, 1993), response to alarm pheromone in

Correspondence: GJ Devine, Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden AL5 2JQ, UK;
E-mail: greg.devine@bbsrc.ac.uk
Received 27 September 2002; accepted 12 February 2003

aphids (Foster *et al*, 1999), and overwintering success in Bt-resistant pink bollworms (Carriere *et al*, 2001), insecticide-resistant aphids (Foster *et al*, 1996, 1997) and blowflies (McKenzie, 1993). There is little understanding of the genotypic basis of these resistance-associated traits, but some may be examples of true pleiotropies, whereby one gene influences more than one aspect of an organism's phenotype. In pest-management terms, such effects clearly have the potential to exert a large influence on the selection and dynamics of genes conferring insecticide resistance.

The work reported here expands upon previous research on clones of *M. persicae* that showed behavioural effects to be correlated with elevated titres of carboxylesterases and with the presence or absence of *kdr* (Foster *et al*, 1996, 1997, 1999). It also reports on behavioural characteristics associated with *kdr* in the housefly (*M. domestica*). We present strong evidence that resistant genotypes may suffer pleiotropic fitness costs in the absence of insecticides, and that in the case of the *kdr* mutation, these are often likely to be identified in behavioural assays because of the effect of the resistant mutation on nerve function.

Methods and materials

M. persicae clones and diagnosis of resistance

In total, 22 *M. persicae* clones, collected from England and mainland Europe, showing different combinations of *kdr* and esterase resistance (Table 1) were used in the experiments. All aphids were maintained at $21 \pm 1^{\circ}C$ on single excised leaves of Chinese cabbage (*Brassica rapa* L. var. *pekinensis*) in small perspex boxes under a 16:8 L:D regime. In *M. persicae*, kdr is caused by a single-point mutation (L1014P) in the *para*-type sodium channel gene. The genotypes of individual clones were determined by DNA sequencing of PCR-amplified *para* gene fragments as described previously (Martinez-Torres *et al*, 1999). Genotypes were scored as either susceptible homozygote (*kdr*-SS), heterozygote (*kdr*-SR), or resistant homozygote (*kdr*-RR) with the latter two being associated with resistance to pyrethroids and DDT (Martinez-Torres *et al*, 1999). Esterase levels, representing increasing levels of resistance (S, R_1, R_2, and R_3) to OPs and carbamates (Devonshire *et al*, 1982, 1986) were based on the relative activities of immunocaptured E4 or FE4 carboxylesterases, the enzyme responsible for esterase-based insecticide resistance (Devonshire *et al*, 1986). None of these clones carried a third insecticide resistance mechanism known in *M. persicae*, termed modified acetylcholinesterase (MACE) (Moores *et al*, 1994).

Table 1 *Myzus persicae* clones assessed in leaf deterioration and alarm pheromone response bioassays

Clone	*Esterase resistance phenotype*[a]	*kdr genotype (kdr)*[b]	*Origin*
US1L	S	SS	England 1974
1076A	S	SS	England 1992
4090A	S	SS	Scotland 2000
4106A	S	SS	Scotland 2000
405D	R_1	SS	England 1977
1260R‡	R_2	SS	Greece 1994
2165B	R_2	SS	England 1997
2141A‡	R_2	SS	England 1997
800F‡	R_3	SS	Italy 1978
1190A‡	R_3	SS	Spain 1993
2160D	R_1	SR	England 1997
1302 M‡	R_2	SR	England 1996
100N	R_2	SR	England 1993
108T	R_3	SR	England 1993
2161C‡	R_3	SR	England 1997
2169G‡	R_3	SR	England 1997
T1 V‡	R_2	RR	England 1975
794J‡	R_3	RR	England 1982
2043B‡	R_3	RR	England 1996
2165C‡	R_3	RR	England 1997
794J(rev)[c]‡	R_3 genotype/S phenotype	RR	England 1994
923A[c]	R_3 genotype/S phenotype	RR	England 1990

All clones were used in studies on low-temperature movement. A subset of these, marked ‡, was used in alarm pheromone studies.
[a]S: susceptible, R_1: moderately resistant, R_2: highly resistant, R_3: extremely resistant forms.
[b]Genotype determined by direct sequencing of sodium channel fragments amplified from single aphids.
[c]Revertant clone carrying unexpressed, highly amplified E4 genes, that is phenotypically indistinguishable from esterase-susceptible clones with respect to esterase production.

Response of *M. persicae* clones to synthetic alarm pheromone

The aphid alarm pheromone (*E*)-β-farnesene is released from cornicle secretions exuded by aphids when they are physically disturbed, for example by foraging predators or parasitoids. Aphid response to synthetic alarm pheromone, applied at a wide range of concentrations, was assessed in the absence of insecticides in seven separate experiments. For each aphid clone tested, six first instar nymphs were obtained from laboratory stocks and grown to adulthood (in two small perspex boxes containing three aphids per clone). These first-generation (G_1) adults were removed after they had produced approximately 30 G_2 offspring per box (normally after about 10 days). The G_2 aphids were grown to adulthood and transferred, using a fine paintbrush, to inverted 2 cm diameter Chinese cabbage discs (four G_2 adult apterae per disc) held on 1.1% agar inside plastic tubs. The plastic tubs were left at $21 \pm 1^{\circ}C$ overnight to allow the aphids to settle and produce nymphs. The adults were removed next morning leaving synchronised cohorts of settled offspring on each disc. Each replicate cohort was then assessed for response to alarm pheromone by applying a 1 µl droplet (ranging from 0.0001 to 10 mg (*E*)-β-farnesene ml^{-1} in hexane) to the central part of each leaf disc with a fine-needle syringe. Aphid behaviour was observed for 2 min using a binocular microscope (preliminary experiments showed that this period is sufficient for all responses to occur). Nymphs that withdrew their stylets and walked away were scored as responders. Control treatments with 1 µl droplets of hexane alone did not stimulate aphid movement. Each replicate batch of nymphs was tested once and then discarded.

Movement of *M. persicae* clones from deteriorating leaves at low temperature

Aphid movement from deteriorating excised Chinese cabbage leaves onto oilseed rape plants (*Brassica napus* L.) was assessed in seven separate experiments at low temperature (using one replicate per clone per experiment). Prior to each experiment, *M. persicae* clones were reared at $21 \pm 1^{\circ}C$ under a 16:8 L:D regime in small

perspex boxes. In each replicate, four young apterous adults were allowed to produce nymphs over 2 days on an excised Chinese cabbage leaf. All adults and surplus nymphs were then removed before the boxes, each containing 50 first/second instar nymphs, were transferred to 10±1°C (8:16 L:D regime) for 24 h temperature acclimation. Clones were then allocated (one replicate per plant) to 5-week-old plants (maintained under a 6±1°C, 8:16 L:D regime) arranged in a single row using an incomplete block design. Movement from each deteriorated excised leaf was assessed 5 days after it had been attached by the petiole and apex to the upper face of a healthy oilseed rape leaf using 4 cm clips. Numbers of aphids that moved to any part of the plant or remained on the deteriorated excised leaf were recorded at the end of each experiment.

M. domestica strains and maintenance

The origin of all housefly strains used is detailed in Table 2. They were reared at 28±1°C. Adults were kept in aluminium cages (0.3 m × 0.15 m × 0.15 m) with open ends; one covered with polythene netting, one with a terylene sleeve. Adults were fed on 20% sugar solution and encouraged to oviposit on cotton wool plugs soaked in milk. Eggs and young larvae were used to 'seed' a larval medium (milk, yeast, bran, and water in the ratio 2:2:3:7 by weight). Pupae were collected by flotation, gathered in a sieve, and transferred to the aluminium cages. The *kdr* (mutation L1014P) and *super-kdr* (mutation M918 T) genotypes were scored by DNA sequencing of PCR-amplified *para* sodium channel fragments from adult flies (Williamson *et al*, 1996).

Bioassays on *M. domestica*

All populations were tested for their responses to technical DDT and the pyrethroid deltamethrin. Serial dilutions were made up in acetone, and 1 µl of solution was topically applied to each fly. Control groups were treated with acetone alone and each group consisted of 10–15 female flies. Treated flies were kept in plastic containers with cotton wool soaked in sugar solution. Mortality was scored after 48 h. Each dose was tested against three groups and bioassays were conducted at least twice.

Temperature gradient studies on *M. domestica*

An aluminium sheet, 24 cm long and encased in a perspex cage (see Figure 1) was used to create a temperature gradient. One end of the sheet was kept in an ice bucket in which the ice was continuously replaced

Table 2 Origins of housefly strains

Strain	*Date*	*kdr genotype*[a]	*Origin*
Cooper	c.1950	*kdr*-susceptible[a]	UK
USA1	c.1995	Susceptible	USA
USA2	c.1995	Susceptible	USA
530[b]	1987	*kdr* and super-*kdr*[a]	Denmark
579[b]	1977	*kdr*[a]	Italy
690ab	1984	Unknown	Denmark
213ab	1957	*kdr* and super-*kdr*[b]	Sweden

[a]All strains were homozygous for the alleles listed.
[b]Derived by repeated backcrossing of *kdr* or super-*kdr* flies to the Cooper strain and reselecting for resistance (Farnham *et al*, 1987).

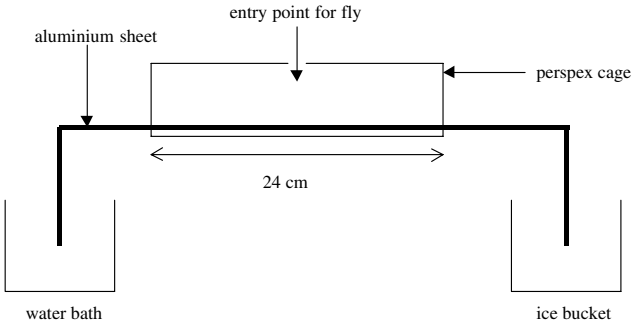

Figure 1 Apparatus for measuring temperature preference in houseflies.

to create a temperature of 19±1.5°C at the 0 cm mark. The opposite end was immersed in a water bath to give a temperature of 29±1°C at the 24 cm mark. The quarter points of the aluminium sheet (6, 12, and 18 cm) were 21.5±1°C, 24±1°C, and 26.5±1°C, respectively.

All experiments were carried out in a well-ventilated, temperature-controlled room (26±1°C) under infrared light to remove any visual cues. Individual female flies were kept in a refrigerator until they were sluggish and easy to manipulate. These were then dropped into the perspex case above the midpoint of the aluminium sheet. Each fly was given 30 s to recover before its position was noted. If a fly had not recovered in that time, it was removed and another released in its place. Between each observation, the aluminium sheet was swabbed with ethanol to remove any semio-chemical cues that could influence the movement of other flies. The orientation of the aluminium plate was also reversed between observations so that any bias resulting from the position of the apparatus was removed. All experiments were conducted between 12:00 and 18:00 h to standardise the part of the diel cycle that individuals were experiencing. Several strains were tested during each experimental period to randomise effects peculiar to that day (eg age of flies, barometric pressure, etc).

Once each fly had recovered, its position was noted 1, 5, 10, 15, and 20 min after introduction. After 20 min, observations were terminated. In total, 12–16 adult females were used for each strain, spread across at least three separate assay dates, and results were pooled for analysis.

Data analyses

Generalised linear models were fitted, using probit transformation, to the proportions of aphids in each replicate responding to alarm pheromone or moving from deteriorating leaves, including effects for clones, experiments, *kdr* genotype, and mean carboxylesterase activity (esterase level).

Housefly bioassays were combined for probit analysis (POLO-PC, 1984). Comparisons of the proportions moving to the warmest quarter of the temperature gradient were analysed using a generalised linear model with a binomial distribution.

Results

Response of *M. persicae* clones to synthetic alarm pheromone

Aphid response increased with higher alarm pheromone concentrations for all three *kdr* genotypes. Fitting a

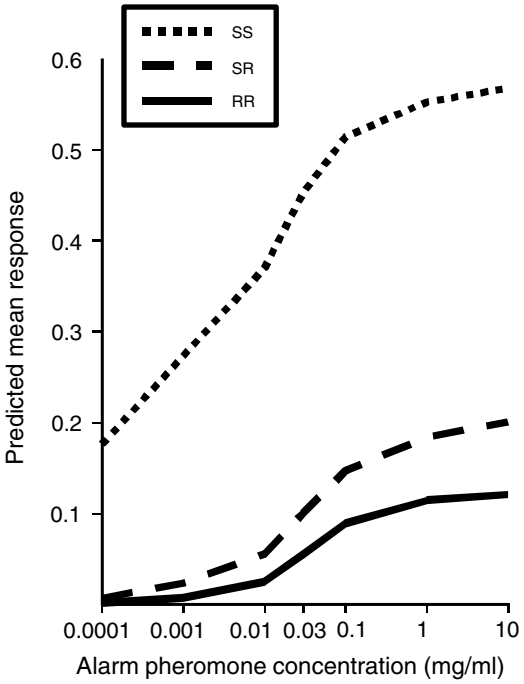

Figure 2 Predicted mean response to alarm pheromone of *Myzus persicae* clones with *kdr*-SS, -SR, and -RR genotypes (clones within each genotype pooled).

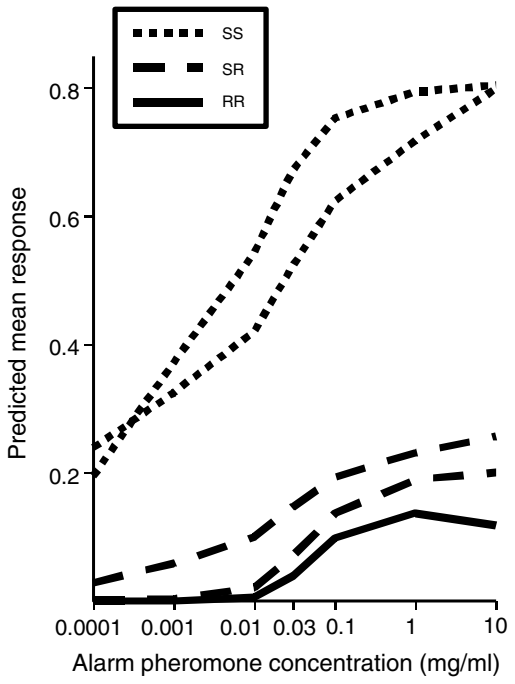

Figure 3 Predicted mean response to alarm pheromone of esterase-R_2 *Myzus persicae* clones with *kdr*-SS, -SR, and -RR genotypes (individual clones shown).

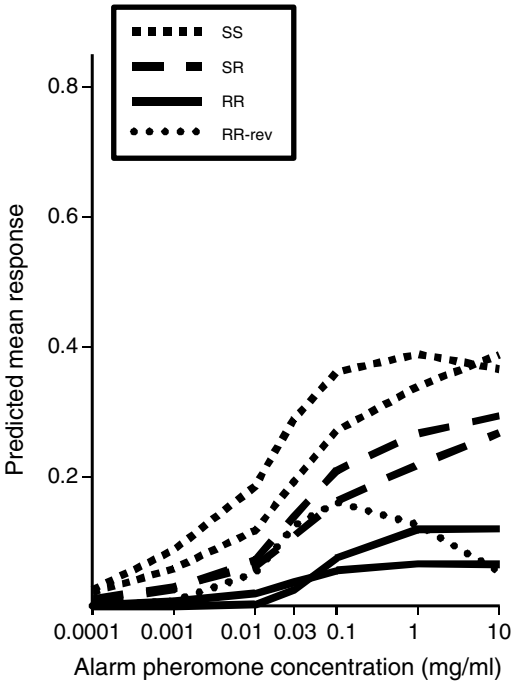

Figure 4 Predicted mean response to alarm pheromone of esterase-R_3 *Myzus persicae* clones with *kdr*-SS, -SR, and -RR genotypes (individual clones shown).

generalised linear model with a probit link and adjusting for esterase level and concentration effects, the tendency to respond was strongly associated with *kdr* genotype (Figure 2); the significance of differences between genotypes being as follows; SS *vs* SR, difference: 1.00, $t_{715}=8.6$, $P<0.001$; SS *vs* RR, difference: 1.75, $t_{715}=14.7$, $P<0.001$; SR *vs* RR, difference: 0.75, $t_{715}=6.6$, $P<0.001$.

Furthermore, esterase resistance (adjusted for *kdr* effects and excluding the revertant clone) was inversely associated with pheromone response (Figures 3 and 4) (slope: -1.31, SE 0.13, $P<0.001$). The revertant clone, 794Jrev, was not significantly different from the clone 794J that had retained expression of its highly amplified (esterase-R_3 level) E4 genes ($t_{715}=1.7$, $P=0.09$).

Movement of *M. persicae* clones from deteriorating leaves at low temperature

After adjusting for esterase level, the mean proportions ($\pm$SE) of aphids moving from deteriorating leaves in each genotype, for *kdr*-susceptible homozygotes (*kdr*-SS), *kdr*-heterozygotes (*kdr*-SR), and *kdr*-resistant homozygotes (*kdr*-RR) were, respectively, 0.48 ± 0.03, 0.54 ± 0.04, and 0.52 ± 0.03. There were no significant differences between these three genotypes (*kdr*-SR *vs* *kdr*-SS: $t_{76}=1.25$ $P=0.22$, *kdr*-SS *vs* *kdr*-RR: $t_{76}=0.87$, $P=0.39$, *kdr*-SR *vs* *kdr*-RR: $t_{76}=0.38$, $P=0.71$). Thus, movement from deteriorating leaves was not associated with *kdr* genotype (Figure 5).

There was, however, a significant inverse relation between the tendency to move and esterase level (overall slope: -0.28, SE 0.12, $P=0.027$) (Figure 5). This relation was also apparent within the *kdr*-SS and *kdr*-SR categories (SS slope: -0.62, SE 0.20, $P=0.003$; SR slope: -1.18, SE 0.55, $P=0.036$). However, it was not apparent within the *kdr*-RR category, because all clones in this instance (with the exception of the two esterase-revertant clones) had very high esterase levels (RR slope: 0.08, SE 0.17, $P=0.65$). The two esterase-revertant clones (having the genotype for R_3 amplified esterase, but the susceptible phenotype (Field *et al*, 1989) segregated with other high-esterase clones, in that they exhibited a limited

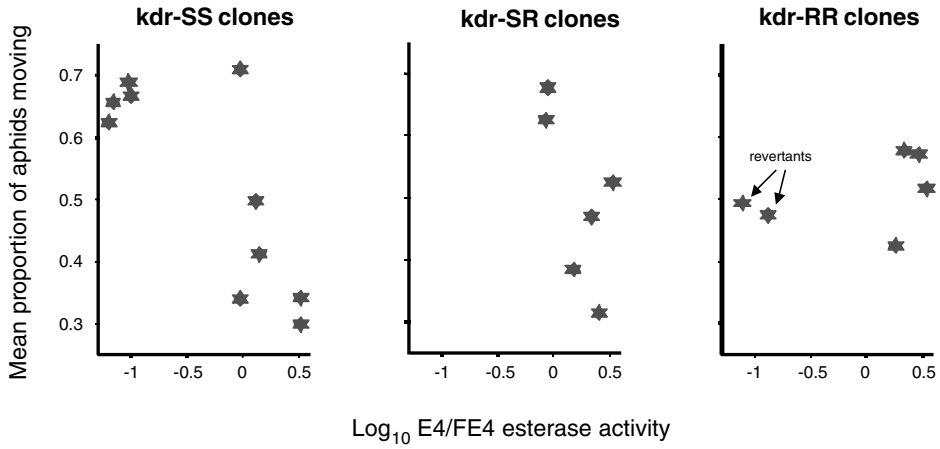

Figure 5 Predicted mean movement from deteriorating leaves of *Myzus persicae* clones with *kdr*-SS, -SR and -RR genotypes and esterase-S to -R_3 levels.

Table 3 Response of housefly strains to μg/μl topical applications of DDT

Strain	*n*	*LD_{50} (95% confidence limits)*[a]	*Slope*	*RF*
Cooper	247	0.07 (0.05–0.11)a	1.5	1
USA1	212	0.03 (0.01–0.05) a	1.1	0.4
USA2	272	0.33 (0.20–0.54) b	1.1	5
690ab	222	0.08 (0.05–0.12) a	1.5	1
579	248	0.51 (0.30–0.76) b	1.7	7
530	241	3.15 (2.03–5.12) c	1.3	43
213ab	278	1.03 (0.64–1.63) b	1.0	14

[a]Values followed by a different letter denote nonoverlap of 95% CLs ($P<0.05$).

Table 4 Response of housefly strains to ng/μl topical applications of deltamethrin

Strain	*n*	*LD_{50} (95% confidence limits)*[a]	*Slope*	*RF*
Cooper	226	0.2 (0.1–0.3) a	1	1
USA1	134	0.5 (0.2–1.0) a	1.1	2.5
USA2	169	0.6 (0.2–1.2) a	0.9	3
690ab	172	2.8 (1.4–5.1) b	1	14
579	189	2.7 (1.4–5.0) b	1	14
530	186	99.4 (55.3–174.4) c	1.1	497
213ab	173	20.9 (6.6–41.9) d	1.2	105

[a]Values followed by a different letter denote nonoverlap of 95% CLs ($P<0.05$).

propensity to move to fresh leaves. This suggests some association between behaviour and the esterase genotype rather than the phenotype.

Bioassays on *M. domestica*

The seven housefly strains could be divided into two groups on the basis of their collective responses to deltamethrin and DDT. Three strains: 213ab, 530, and 579 exhibited resistance to both DDT (resistance factors of 14, 43, and 7, respectively) and deltamethrin (resistance factors of 105, 497, and 14, respectively). Four strains: Cooper, 690ab, USA1, and USA2 were fully susceptible to one or both compounds (Tables 3 and 4). Consistent with these results, the latter four strains were all confirmed as wild type for their domain II sodium channel sequences, whereas all three resistant strains contained the *kdr* (L1014P) mutation, with two strains, 213ab and 530, also carrying the super-*kdr* (M918T) mutation. Previous studies have shown that the *kdr* mutation is associated with moderate (5 to 30-fold) levels of resistance to DDT and pyrethroids, whereas the additional presence of the super-*kdr* mutation increases resistance to over 100-fold for the more potent type II pyrethroids such as deltamethrin (Williamson *et al*, 1996). The USA2 and 690ab strains, showing an inconsistent response to DDT and deltamethrin, probably possessed a metabolic resistance mechanism specific to either DDT or pyrethroids.

Temperature gradient studies on *M. domestica*

The majority of individuals (82–94%) of all four strains lacking *kdr* had, within 20 min, settled between temperatures of 26.5 and 29°C in the warmest quarter of the gradient (Figure 6a). Conversely, those containing sodium channel mutations (Figure 6b) exhibited no positional preference (20–25% in the warmest quarter–the fraction that would be expected if individuals had distributed themselves randomly). A generalised linear model showed that slopes of the lines fitted to the two groups of flies (*kdr* and non-*kdr*) were significantly different from each other ($F_{1,80}=17.93$, $P<0.001$) as a result of an increasing number of non-*kdr* flies moving to the warmer part of the temperature gradient. The intercepts were similar ($F_{1,80}<3.84$, $P>0.05$) because, at the beginning of each observation, flies were introduced at the centre of the temperature gradient.

Discussion

Intuitively, some types of resistance mechanism are more likely to confer fitness disadvantages than others. Attempts to review the relations between fitness and resistance mechanism (Roush and McKenzie, 1987) concluded that mechanisms based on the overproduction of detoxifying enzymes (eg carboxylesterases) often lead to serious disadvantages whereas *kdr*-type mechanisms of pyrethroid resistance may confer little or no effect on

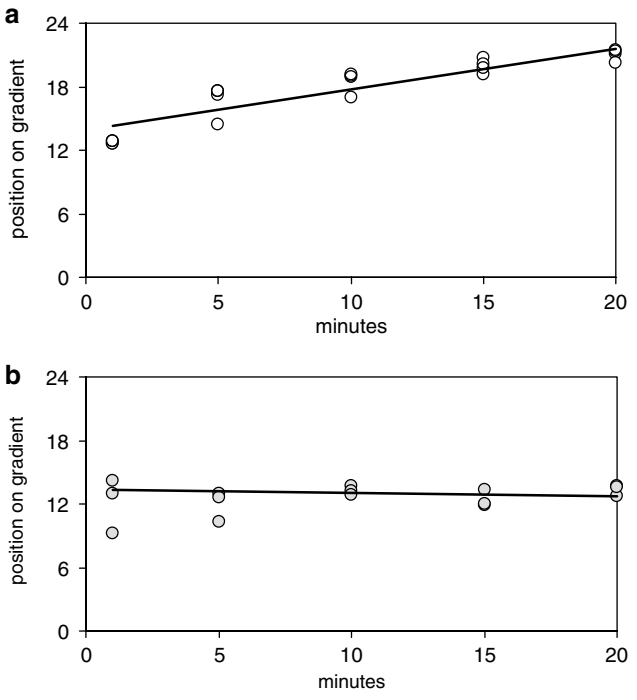

Figure 6 Responses of (**a**) the four strains without *kdr*/*super-kdr* mutations and (**b**) the three strains with *kdr*/*super-kdr* mutations on exposure to a 19–29°C temperature gradient (0–24 cm). Each point represents the mean response of a single housefly strain, the collective movement of which is charted over a 20 min period.

overall fitness. The results of our study concur with this conclusion in that genotypes conferring high carboxylesterase titres were shown, in aphids, to be associated with an inability to move from senescing leaves at low temperature. However, work on the behavioural correlates of resistance genotypes in both aphids and houseflies suggests that *kdr* mutations are also associated with detrimental behaviour.

Our study confirms earlier reports of biological and behavioural effects associated with high levels of carboxylesterase resistance in *M. persicae* (Foster *et al*, 1996, 1997), but the use of clones differing in both esterase titre and *kdr* genotype enabled a clearer resolution for the role of each mechanism in influencing responsiveness to environmental cues (either leaf deterioration or the presence of alarm pheromone). Increased esterase production was significantly associated with reduced movement from deteriorating leaves. A possible explanation of this and other side effects of esterase resistance in *M. persicae* is the large investment of resources in carboxylesterase production which in the most resistant (R_3) clones account for ca. 3% of the total body protein (Devonshire and Moores, 1982). It has been shown that such high carboxylesterase titres reflect a proportionate increase in gene copy number, rising to approximately 80 copies of either E4 or FE4 in R_3 *M. persicae* (Field *et al*, 1999).

Interestingly, esterase-revertant clones, carrying large numbers of unexpressed esterase genes, did not affiliate with true esterase-susceptible forms but instead showed similar responses, to alarm pheromone and leaf deterioration, to clones expressing high levels of esterase. This suggests that reduced response in this case was not directly related to phenotypic esterase overproduction, but rather to the presence of amplified carboxylesterase genes. It is worth noting that the *kdr* genotype was not associated with reduced movement from deteriorated leaves. This may reflect the complexity of the behavioural response in this instance. Mutations such as *kdr*, with direct effects upon nerve function, may be more likely to disrupt the perception of simple stimuli such as temperature or pheromones. Dispersal from deteriorating leaves, however, is probably a more intricate response dependent upon complex stimuli, and so may be less obviously associated with nerve function.

In a prior study (Foster *et al*, 1999), showing a correlated response to alarm pheromone with the presence or absence of *kdr*, only a single dose of alarm pheromone was used and those clones utilised did not display the same range of genotypes, nor were they as well characterised as the current subset. In the current study, the proportion of aphids disturbed by the presence of alarm pheromone increased with stimulus concentration for all three *kdr* genotypes, but there were clear differences between them. Heterozygotes (*kdr*-SR) were less responsive than homozygous susceptible forms (*kdr*-SS), and *kdr*-RR forms were least responsive of all. Once adjusted, statistically, for the presence of kdr, response to alarm pheromone was also inversely associated with carboxylesterase titres; with R_3 aphids showing a lower response than R_2 forms. An esterase-revertant clone (794J-rev) showed a response similar to the R_3 clone from which it was derived. Although both possess the esterase-R_3 genotype, only the nonrevertant clone (794J) expressed this enzyme, implying that it is the genotype to which the pheromone response is allied, and not the phenotype. This reduced response to alarm pheromone is likely to be a highly maladaptive behaviour, as the dispersal of aphids in response to this stimulus is considered to be an important behavioural adaptation for avoiding attack by natural enemies (Pickett *et al*, 1992)

Of the *M. domestica* strains used in this study, 213ab, 530, and 579 were confirmed as carrying *kdr* and/or super-*kdr* mutations and, as expected, this correlated with phenotypic resistance to both DDT and deltamethrin. The other four strains were susceptible to at least one of these compounds, and did not carry the *kdr* mutation. Our LD_{50} values for the strains Cooper 530 and 579 compare favourably with figures published previously. Farnham *et al* (1987) showed that *kdr* alone conferred 17- and 34-fold resistance to DDT and deltamethrin and 63- and 221-fold resistance, respectively, when *super-kdr* was also present. Our data showed 7- and 13-fold resistance conferred by *kdr* alone to DDT and deltamethrin, and 45- and 497-fold resistance, respectively with the addition of the *super-kdr* mutation. These mechanisms are therefore clearly stable in unselected laboratory populations.

The differential temperature preference shown by the *kdr* and non-*kdr* groups of flies strongly suggests an association between the presence of insecticide-resistant sodium channels and the perception of (or at least the response to) temperature gradients. It is worth noting that the genetic background of the strains 530 and 579 was extremely similar to that of the Cooper strain, having undergone a considerable programme of backcrossing in order to homogenise the genetic background in which the *kdr* and *super-kdr* genes existed (Farnham

et al, 1987). The majority of individuals of all four *kdr*-susceptible strains had, within 20 min, moved to the warmest quarter of the gradient. Conversely, those carrying *kdr* and/or *super-kdr* sodium channel mutations exhibited no positional preference. It is difficult to assess whether this lack of preference is maladaptive, but if it represents a reduced ability to seek out optimal temperatures, then *kdr* strains will suffer decreased longevity, fecundity, and ovarian development rates. All these parameters are affected by temperature in houseflies (Elvin and Krafsur, 1984; Fletcher *et al*, 1990; Lysyk, 1991a, b).

In pest-management terms, the pleiotropic effects described above clearly have the potential to exert a large influence on the selection and dynamics of genes conferring insecticide resistance. Such influences will be most obvious in the absence of selection pressure by insecticides and under stressful environments. It is therefore of note that the incidence of R_2 and R_3 *M. persicae* aphids in the UK decreases over the winter period when insecticidal selection pressure is relaxed, but that the proportion of *kdr*-resistant genotypes in the population has appeared to remain stable in recent years, perhaps reflecting the high amount of pyrethroid use (Foster *et al*, 2002). In northern Europe, *kdr* is the major mechanism of pyrethroid resistance in houseflies (Keiding, 1999). In Danish piggeries, resistance to pyrethroids is increasingly common, and this may reflect the near continuous selection pressure exerted in these environments (Kristensen *et al*, 2001). We are unaware of any attempts to monitor the frequency of *kdr* alleles in areas where pyrethroid use is decreasing.

The results presented above show that although the mechanisms that mediate the reduced responses to both leaf deterioration and alarm pheromone in insecticide-resistant *M. persicae*, and differential movement in response to a temperature gradient in *M. domestica* remain equivocal, there is increasingly sound evidence that they are pleiotropic effects of genes coding for insecticide resistance. In the case of the *kdr* sodium channel mutation specifically, this conclusion is strengthened by evidence that explicitly links qualitative and quantitative changes in the sodium channel structural gene *para* (paralytic) in *Drosophila melanogaster* to behavioural disturbances. For example, the temperature-sensitive paralysis exhibited by individuals carrying the point mutation nap^{ts} (no action potential, temperature sensitive) results from a reduction in the expression of the *para* gene. It is thought that, when temperature rises, an increasing fraction of the available sodium channels is required to maintain propagation of action potentials. Thus, when sodium channel expression is decreased, the number of available channels cannot meet the demands of elevated temperature (Kernan *et al*, 1991). The mutation *tipE* (temperature-induced paralysis locus E) also disrupts *para* expression and confers temperature-induced paralysis as a result of a decrease in sodium channel numbers (Feng *et al*, 1995) The *sbl* (smellblind) mutation, which is not temperature sensitive, is also an allele of the *para* gene (Lilly *et al*, 1994). This associates sodium channel mutations with olfactory and chemotactic defects (Lilly and Carlson, 1990) and with changes in sexual behaviour (Tompkins *et al*, 1982, 1983). In another unrelated point mutation conferring resistance to the cyclodienes, a mutation in the structural gene encoding the γ-aminobutyric acid receptor (*rdl* or resistance to dieldrin) also appears to induce reversible temperature-sensitive paralysis (Ffrench-Constant *et al*, 2000).

Studies on *kdr*/*super-kdr* mutations incorporated into the insect *para* gene and expressed *in vitro* using *Xenopus* oocytes have revealed that these mutations reduce the sensitivity of the channel protein to pyrethroids and DDT and therefore confer resistance. Such mutations almost certainly have the same function in aphids, houseflies, and in all other insects in which they occur (Vais *et al*, 2001). However, in addition to making the channel protein less sensitive to some insecticides, these mutations also alter the normal gating properties of the sodium channel. This causes an elevation in their action potential threshold that, *in vivo*, would cause a general reduction in the excitability of the nervous system (Smith *et al*, 1997; Vais *et al*, 1997; Lee *et al*, 1999; Vais *et al*, 1997), and is likely to cause changes in the way that resistant individuals respond to external stimuli.

Such behavioural associations have wider implications beyond that of the sustainability of pest-management strategies alone. There is currently a debate over whether the pleiotropic effects of adaptive mutations can contribute to assortative mating and, therefore, speciation (Dieckmann and Doebeli, 1999; Emelianov *et al*, 2001). If the collective influence of genes conferring insecticide resistance on behavioural attributes, such as short- or long-range dispersal, can contribute to assortative mating between insects, it is possible that the pleiotropic effects of insecticide resistance might promote wider genetic differentiation than that which occurs at resistance loci alone.

Acknowledgements

We thank James Campbell, Diana Cox, Barbara Hackett, and Neil Mason for their expert technical assistance, and Robin Thompson and Alan Todd for help with statistical analysis. SP Foster was supported by a SAPPIO-Link project (Sustainable Arable Protection through Precision Input Optimisation) secured by Alan Devonshire. This involved the Department of Environment, Food and Rural Affairs, Agrovista UK Ltd, the British Potato Council, QV Foods Ltd and Syngenta. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council.

References

Alyokhin AV, Ferro DN (1999). Relative fitness of Colorado potato beetle (Coleoptera: Chrysomelidae) resistant and susceptible to the *Bacillus thuringiensis* Cry3A toxin. *J Econ Entomol* **92**: 510–515.

Baker JE, Perezmendoza J, Beeman RW, Throne JE (1998). Fitness of a malathion-resistant strain of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). *J Econ Entomol* **91**: 50–55.

Bloch G, Wool D (1994). Methidathion resistance in the sweetpotato whitefly (Aleyrodidae: Homoptera) in Israel: selection, heritability, and correlated changes of esterase activity. *J Econ Entomol* **87**: 1147–1156.

Boivin TC, Chabert D'hieres JC, Bouvier D, Beslay, Sauphanor B (2001). Pleiotropy of insecticide resistance in the codling moth, *Cydia pomonella*. *Entomol Exp Appl* **99**: 381–386.

Boots M, Begon M (1993). Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Funct Ecol* **7**: 528–534.

Brewer MJ, Mornhinweg DW, Huzurbazar S (1999). Compatibility of insect management strategies: *Diuraphis noxia* abundance on susceptible and resistant barley in the presence of parasitoids. *Biocontrol* **43**: 479–491.

Brewer MJ, Trumble JT (1991). Inheritance and fitness consequences of resistance to fenvalerate in *Spodoptera exigua* (Lepidoptera, Noctuidae). *J Econ Entomol* **84**: 1638–1644.

Carriere Y, Deland JP, Roff DA, Vincent C (1994). Life-history costs associated with the evolution of insecticide resistance. *Proc R Soc Lond Ser B-Biol Sci* **258**: 35–40.

Carriere Y, Ellers-Kirk C, Patin AL, Sims MA, Meyer S, Liu YB *et al* (2001). Overwintering cost associated with resistance to transgenic cotton in the pink bollworm (Lepidoptera: Gelechiidae). *J Econ Entomol* **94**: 935–941.

Carriere Y, Roff DA, Deland JP (1995). The joint evolution of diapause and insecticide resistance–a test of an optimality model. *Ecology* **76**: 1497–1505.

Charpentier A, Fournier D (2001). Levels of total acetylcholinesterase in *Drosophila melanogaster* in relation to insecticide resistance. *Pestic Biochem Physiol* **70**: 100–107.

Chevillon C, Bourguet D, Rousset F, Pasteur N, Raymond M (1997). Pleiotropy of adaptive changes in populations: comparisons among insecticide resistance genes in *Culex pipiens*. *Genet Res* **70**: 195–203.

Crow JF (1957). Genetics of resistance to chemicals. *Annu Rev Entomol* **2**: 227–246.

Devonshire AL, Moores GD (1982). A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach–potato aphids (*Myzus persicae*). *Pestic Biochem Physiol* **18**: 235–246.

Devonshire AL, Moores GD, Ffrench-Constant RH (1986). Detection of insecticide resistance by immunological estimation of carboxylesterase activity in *Myzus persicae* (Sulzer) and cross reaction of the antiserum with *Phorodon humuli* (Schrank) (Hemiptera: Aphididae). *Bull Entomol Res* **76**: 97–107.

Dieckmann U, Doebeli M (1999). On the origin of species by sympatric speciation. *Nature* **400**: 354–357.

Elvin MK, Krafsur ES (1984). Relationship between temperature and rate of ovarian development in the housefly, *Musca domestica* L (Diptera, Muscidae). *Ann Entomol Soc Am* **77**: 50–55.

Emelianov I, Dres M, Baltensweiler W, Mallet J (2001). Host-induced assortative mating in host races of the larch budmoth. *Evolution* **55**: 2002–2010.

Farnham AW, Murray AWA, Sawicki RM, Denholm I, White JC (1987). Characterization of the structure activity relationship of kdr and 2 variants of super-kdr to pyrethroids in the housefly (*Musca domestica* L.). *Pestic Sci* **19**: 209–220.

Feng GP, Deak P, Kasbekar DP, Gil DW, Hall LM (1995). Cytogenetic and molecular localization of tipe–a gene affecting sodium-channels in *Drosophila melanogaster*. *Genetics* **139**: 1679–1688.

Ffrench-Constant RH, Anthony N, Aronstein K, Rocheleau T, Stilwell G (2000). Cyclodiene insecticide resistance: from molecular to population genetics. *Annu Rev Entomol* **45**: 449–466.

Field LM, Blackman RL, Tylersmith C, Devonshire AL (1999). Relationship between amount of esterase and gene copy number in insecticide-resistant *Myzus persicae* (Sulzer). *Biochem J* **339**: 737–742.

Field LM, Devonshire AL, Ffrench-Constant RH, Forde BG (1989). Changes in DNA methylation are associated with loss of insecticide resistance in the peach–potato aphid *Myzus persicae* (Sulz). *FEBS Lett* **243**: 323–327.

Field LM, Devonshire AL, Forde BG (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.

Fletcher MG, Axtell RC, Stinner RE (1990). Longevity and fecundity of *Musca domestica* (Diptera, Muscidae) as a function of temperature. *J Med Entomol* **27**: 922–926.

Follett PA, Gould F, Kennedy GG (1993). Comparative fitness of 3 strains of colorado potato beetle (Coleoptera, Chrysomelidae) in the field – spatial and temporal variation in insecticide selection. *J Econ Entomol* **86**: 1324–1333.

Foster SP, Harrington R, Devonshire AL, Denholm I, Clark SJ, Mugglestone MA (1997). Evidence for a possible fitness trade-off between insecticide resistance and the low temperature movement that is essential for survival of UK populations of *Myzus persicae* (Hemiptera: Aphididae). *Bull Entomol Res* **87**: 573–579.

Foster SP, Harrington R, Devonshire AL, Denholm I, Devine GJ, Kenward MG *et al* (1996). Comparative survival of insecticide-susceptible and resistant peach–potato aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), in low temperature field trials. *Bull Entomol Res* **86**: 17–27.

Foster SP, Harrington R, Dewar AM, Denholm I, Devonshire AL (2002). Temporal and spatial dynamics of insecticide resistance in *Myzus persicae* (Hemiptera: Aphididae). *Pest Manag Sci* **58**: 895–907.

Foster SP, Woodcock CM, Williamson MS, Devonshire AL, Denholm I, Thompson R (1999). Reduced alarm response by peach–potato aphids, *Myzus persicae* (Hemiptera: Aphididae), with knock-down resistance to insecticides (kdr) may impose a fitness cost through increased vulnerability to natural enemies. *Bull Entomol Res* **89**: 133–138.

Groeters FR, Tabashnik BE, Finson N, Johnson MW (1993). Resistance to *Bacillus thuringiensis* affects mating success of the diamondback moth (Lepidoptera, Plutellidae). *J Econ Entomol* **86**: 1035–1039.

Guillemaud T, Lenormand T, Bourguet D, Chevillon C, Pasteur N, Raymond M (1998). Evolution of resistance in *Culex pipiens*: allele replacement and changing environment. *Evolution* **52**: 443–453.

Haubruge E, Arnaud L (2001). Fitness consequences of malathion-specific resistance in red flour beetle (Coleoptera: Tenebrionidae) and selection for resistance in the absence of malathion. *J Econ Entomol* **94**: 552–557.

Hollingsworth RG, Tabashnik BE, Johnson MW, Messing RH, Ullman DE (1997). Relationship between susceptibility to insecticides and fecundity across populations of cotton aphid (Homoptera: Aphididae). *J Econ Entomol* **90**: 55–58.

Keiding J (1999). Review of the global status and recent development of insecticide resistance in field populations of the housefly, *Musca domestica* (Diptera: Muscidae). *Bull Entomol Res: Suppl Ser* **89**: S1–S67.

Kernan MJ, Kuroda MI, Kreber R, Baker BS, Ganetzky B (1991). Napts, a mutation affecting sodium-channel activity in Drosophila, is an allele of Mle, a regulator of X-chromosome transcription. *Cell* **66**: 949–959.

Kristensen M, Spencer AG, Jespersen JB (2001). The status and development of insecticide resistance in Danish populations of the housefly *Musca domestica* L. *Pest Manag Sci* **57**: 82–89.

Lee SH, Smith TJ, Knipple DC, Soderlund DM (1999). Mutations in the house fly *Vssc*1 sodium channel gene associated with super-kdr resistance abolish the pyrethroid sensitivity of *Vssc*1/tipE sodium channels expressed in Xenopus oocytes. *Insect Biochem Mol Biol* **29**: 185–194.

Lilly M, Carlson J (1990). Smellblind – a gene required for Drosophila olfaction. *Genetics* **124**: 293–302.

Lilly M, Kreber R, Ganetzky B, Carlson JR (1994). Evidence that the Drosophila olfactory mutant smellblind defines a novel class of sodium-channel mutation. *Genetics* **136**: 1087–1096.

Lysyk TJ (1991a). Effects of temperature, food, sucrose feeding on longevity of the house-fly (Diptera, Muscidae). *Environ Entomol* **20**: 1176–1180.

Lysyk TJ (1991b). Modelling oviposition of the house-fly (Diptera, Muscidae). *Can Entomol* **123**: 345–352.

Martinez-Torres D, Foster SP, Field LM, Devonshire AL, Williamson MS (1999). A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach–potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Insect Mol Bio* **8**: 339–346.

Mason PL (1998). Selection for and against resistance to insecticides in the absence of insecticide: a case study of malathion resistance in the saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). *Bull Entomol Res* **88**: 177–188.

McKenzie JA (1993). Measuring fitness and intergenic interactions – the evolution of resistance to diazinon in *Lucilia cuprina*. *Genetica* **90**: 227–237.

McKenzie JA (1996). *Ecological and Evolutionary Aspects of Insecticide Resistance*. Academic Press: London.

McKenzie JA, O'farrell K (1993). Modification of developmental instability and fitness – malathion-resistance in the Australian sheep blowfly, *Lucilia cuprina*. *Genetica* **89**: 67–76.

Minkoff C, Wilson TG (1992). The competitive ability and fitness components of the methoprene-tolerant (met) Drosophila mutant resistant to juvenile-hormone analog insecticides. *Genetics* **131**: 91–97.

Moores GD, Devine GJ, Devonshire AL (1994). Insecticide-insensitive acetylcholinesterase can enhance esterase-based resistance in *Myzus persicae* and *Myzus nicotianae*. *Pestic Biochem Physiol* **49**: 114–120.

Omer AD, Leigh TF, Granett J (1992). Insecticide resistance of greenhouse-whitefly (Homoptera, Aleyrodidae) and fitness on plant hosts relative to the San-Joaquin valley (California) cotton agroecosystem. *J Appl Entomol-Z Angew Entomol* **113**: 244–251.

Oppert B, Hammel R, Throne JE, Kramer KJ (2000). Fitness costs of resistance to *Bacillus thuringiensis* in the Indian meal moth, *Plodia interpunctella*. *Entomol Exp Appl* **96**: 281–287.

Pickett JA, Wadhams LJ, Woodcock CM, Hardie J (1992). The chemical ecology of aphids. *Annu Rev Entomol* **37**: 67–90.

Roush RT, Mckenzie JA (1987). Ecological genetics of insecticide and acaricide resistance. *Annu Rev Entomol* **32**: 361–380.

Rowland M (1991a). Activity and mating competitiveness of gamma HCH/dieldrin resistant and susceptible male and virgin female *Anopheles gambiae* and *An. stephensi* mosquitoes, with assessment of an insecticide-rotation strategy. *Med Veterinary Entomol* **5**: 207–222.

Rowland M (1991b). Behaviour and fitness of gamma HCH/dieldrin resistant and susceptible female *Anopheles gambiae* and *An. stephensi* mosquitoes in the absence of insecticide. *Med Veterinary Entomol* **5**: 193–206.

Smith TJ, Lee SH, Ingles PJ, Knipple DC, Soderlund DM (1997). The L1014F point mutation in the house fly *Vssc1* sodium channel confers knockdown resistance to pyrethroids. *Insect Biochem Mol Biol* **27**: 807–812.

Tang JD, Collins HL, Roush RT, Metz TD, Earle ED, Shelton AM (1999). Survival, weight gain, and oviposition of resistant and susceptible *Plutella xylostella* (Lepidoptera: Plutellidae) on broccoli expressing CrylAc toxin of *Bacillus thuringiensis*. *J Econ Entomol* **92**: 47–55.

Tang JD, Gilboa S, Roush RT, Shelton AM (1997). Inheritance, stability, lack-of-fitness costs of field-selected resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae) from Florida. *J Econ Entomol* **90**: 732–741.

Tompkins L, Gross AC, Hall JC, Gailey DA, Siegel RW (1982). The role of female movement in the sexual behavior of *Drosophila melanogaster*. *Behav Genet* **12**: 295–307.

Tompkins L, Siegel RW, Gailey DA, Hall JC (1983). Conditioned courtship in Drosophila and its mediation by association of chemical cues. *Behav Genet* **13**: 565–578.

Vais H, Williamson MS, Devonshire AL, Usherwood PNR (2001). The molecular interactions of pyrethroid insecticides with insect and mammalian sodium channels. *Pest Manag Sci* **57**: 877–888.

Vais H, Williamson MS, Goodson SJ, Devonshire AL, Warmke JW, Usherwood PNR *et al* (2000). Activation of Drosophila sodium channels promotes modification by deltamethrin – reductions in affinity caused by knock-down resistance mutations. *J General Physiol* **115**: 305–318.

Vais H, Williamson MS, Hick CA, Eldursi N, Devonshire AL, Usherwood PNR (1997). Functional analysis of a rat sodium channel carrying a mutation for insect knock-down resistance (kdr) to pyrethroids. *FEBS Lett* **413**: 327–332.

White NDG, Bell RJ (1995). A malathion resistance gene associated with increased life-span of the rusty grain beetle, *Cryptolestes ferrugineus* (Coleoptera, Cucujidae). *J Gerontol Ser A-Biol Sci Med Sci* **50**: B9–B13.

Williamson MS, Martinez-Torres D, Hick CA, Devonshire AL (1996). Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. *Mol General Genet* **252**: 51–60.

Yamamoto A, Yoneda H, Hatano R, Asada M (1995). Influence of hexythiazox resistance on life-history parameters in the citrus red mite, *Panonychus citri* (McGregor). *J Pestic Sci* **20**: 521–527.