

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

SP Foster¹, S Young², MS Williamson², I Duce³, I Denholm¹ and GJ Devine¹

¹Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden AL5 2JQ, UK; ²Biological Chemistry Division, Rothamsted Research, Harpenden AL5 2JQ, UK; ³School 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.

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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

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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

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

Clone	Esterase resistance phenotype ^a	<i>kdr</i> genotype (<i>kdr</i>) ^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
1302M [‡]	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) [‡]	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.

^aS: susceptible, R₁: moderately resistant, R₂: highly resistant, R₃: extremely resistant forms.

^bGenotype determined by direct sequencing of sodium channel fragments amplified from single aphids.

^cRevertant clone carrying unexpressed, highly amplified E4 genes, that is phenotypically indistinguishable from esterase-susceptible clones with respect to esterase production.

experiments. All aphids were maintained at 21 ± 1°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, R₁, R₂, and R₃) 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).

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 (G₁) adults were removed after they had produced approximately 30 G₂ offspring per box (normally after about 10 days). The G₂ aphids were grown to adulthood and transferred, using a fine paintbrush, to inverted 2 cm diameter Chinese cabbage discs (four G₂ adult apterae per disc) held on 1.1% agar inside plastic tubs. The plastic tubs were left at 21 ± 1°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 ± 1°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 $10 \pm 1^\circ\text{C}$ (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 $6 \pm 1^\circ\text{C}$, 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 $28 \pm 1^\circ\text{C}$. Adults were kept in aluminium cages ($0.3\text{ m} \times 0.15\text{ m} \times 0.15\text{ m}$) 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 M918T) 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 $1\ \mu\text{l}$ 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

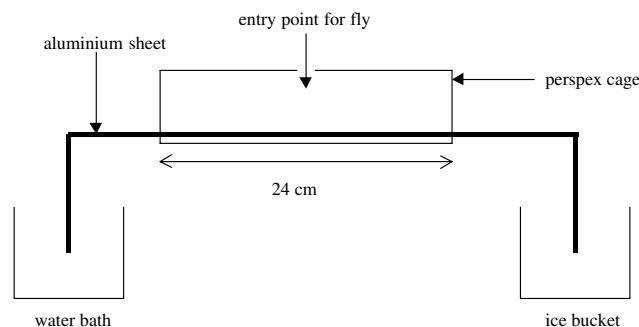


Figure 1 Apparatus for measuring temperature preference in houseflies.

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

All experiments were carried out in a well-ventilated, temperature-controlled room ($26 \pm 1^\circ\text{C}$) 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

Table 2 Origins of housefly strains

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

^aAll strains were homozygous for the alleles listed.

^bDerived by repeated backcrossing of *kdr* or *super-kdr* flies to the Cooper strain and reselecting for resistance (Farnham *et al*, 1987).

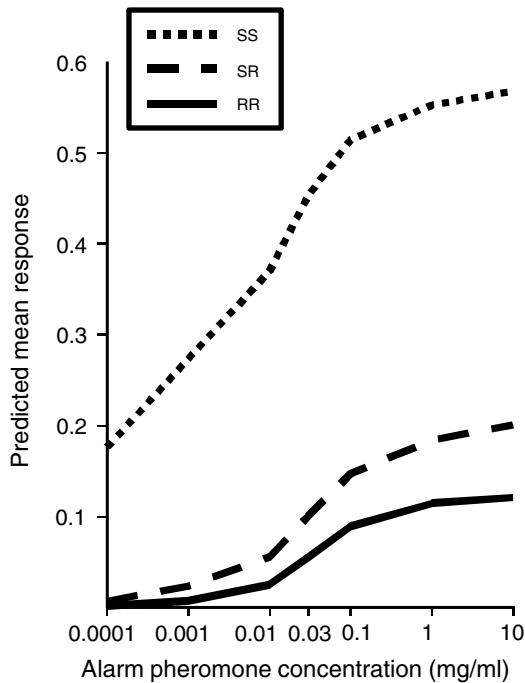


Figure 2 Predicted mean response to alarm pheromone of *Myzus persicae* clones with *kdr*-SS, -SR, and -RR genotypes (clones within each genotype pooled).

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

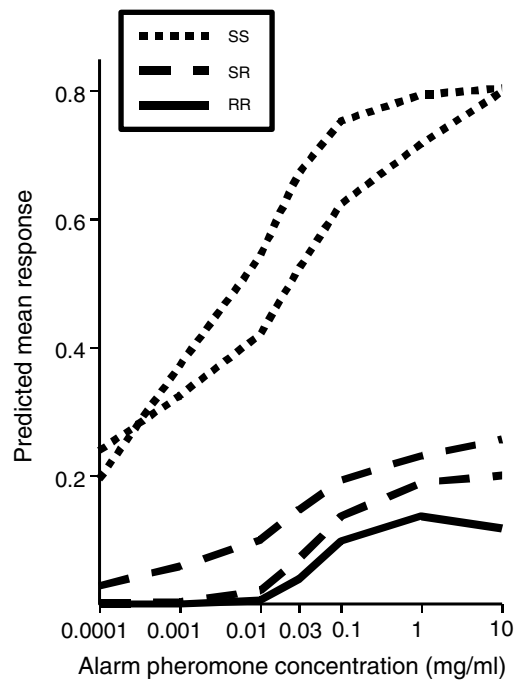


Figure 3 Predicted mean response to alarm pheromone of esterase- R_2 *Myzus persicae* clones with *kdr*-SS, -SR, and -RR genotypes (individual clones shown).

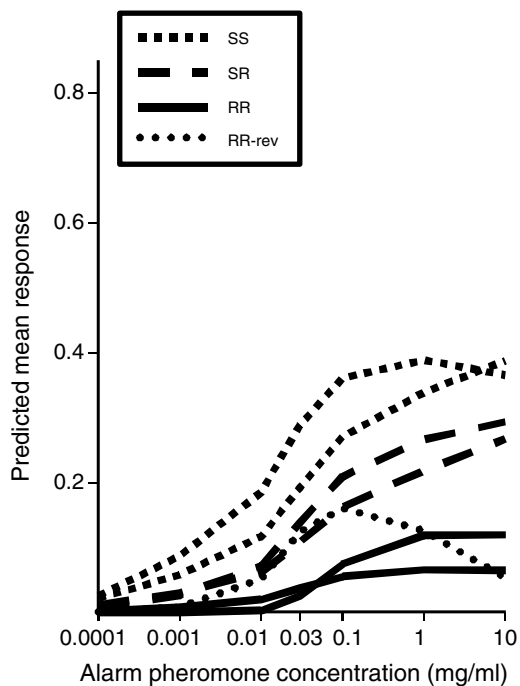


Figure 4 Predicted mean response to alarm pheromone of esterase- R_3 *Myzus persicae* clones with *kdr*-SS, -SR, and -RR genotypes (individual clones shown).

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

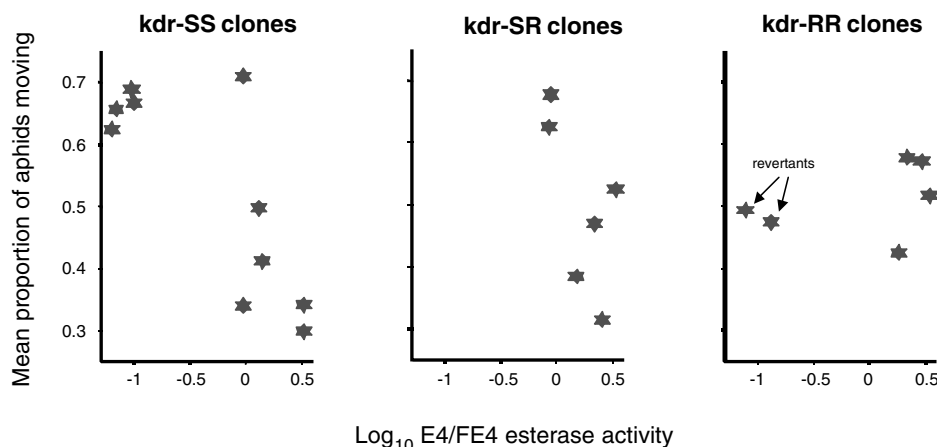


Figure 5 Predicted mean movement from deteriorating leaves of *Myzus persicae* clones with *kdr*-SS, -SR and -RR genotypes and esterase-S to -R₃ levels.

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

^aValues 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.

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

^aValues followed by a different letter denote nonoverlap of 95% CLs ($P < 0.05$).

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

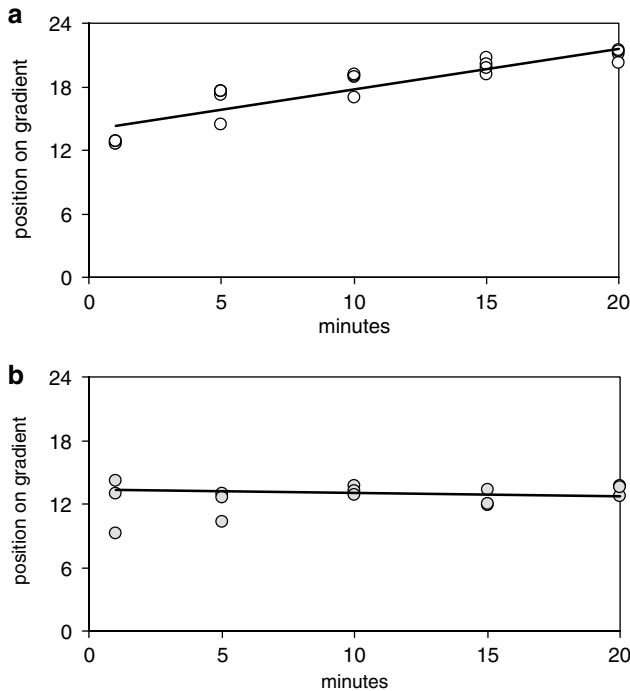


Figure 6 Responses of (a) the four strains without *kdr/super-kdr* mutations and (b) the three strains with *kdr/super-kdr* mutations on exposure to a 19–29°C temperature gradient (0–24 cm). Each point represents the mean response of a single housefly strain, the collective movement of which is charted 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 LD₅₀ 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 Krafusur, 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 R₂ and R₃ *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.

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