MCE activities and malathion resistances in field populations of the Australian sheep blowfly (*Lucilia cuprina*)

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Malathion resistance has been shown to be the result of a single point mutation in the *LcαE7* gene in four independently isolated chromosomes of *Lucilia cuprina*. The resultant amino acid substitution specifies high malathion carboxylesterase (MCE) activity. We have assayed MCE activities and resistance to malathion in three sets of field-derived samples, two sets of isogenic lines and five mass populations, and show that resistance to malathion in these samples is associated with high MCE activity in both sets of isogenic lines and four of the five mass populations. Additional mechanisms contributing to MCE activity or malathion resistance may be present in one of the mass populations. A second point mutation in *LcαE7* is responsible for conferring diazinon resistance by encoding an increased organophosphate (OP) hydrolase activity. We also assayed diazinon resistances from the same three samples and show that diazinon and malathion resistances were in complete disequilibrium, with two exceptions. One exception involves the mass population with additional resistance mechanism(s) and the other involves three isogenic lines that are resistant to both insecticides. The molecular data for these lines suggest that they carry a duplication of the *LcαE7* gene.

Keywords: carboxylesterase, Diptera, genetic variation, malathion, organophosphate, resistance.

Introduction

Resistance to organophosphates (OPs) in *Lucilia cuprina* was first recorded in 1965 as resistance to diazinon (Shanahan & Hart, 1966). The frequency of diazinon resistant flies in the field was reported to be 97% in 1981 (Hughes, 1981) and it has remained high since then (Levot, 1995). In contrast to diazinon resistance, the frequency of malathion resistant flies in the field is generally low, approximately 11% (Hughes *et al.*, 1984; Levot, 1995). However, given that malathion has not been directly used to control *L. cuprina*, this level of resistance is a little surprising.

Resistance to diazinon and malathion is primarily the result of two point mutations in a carboxylesterase gene on the fourth chromosome (*LcαE7*), encoding both OP hydrolase and malathion carboxylesterase (MCE) activities (Campbell *et al.*, 1997; Newcomb *et al.*, 1997; 1998a). The OP hydrolase confers resistance to diazinon

and many other OPs, whereas high MCE activity confers resistance to malathion and a small number of OPs with carboxylester bonds (Raftos, 1986; Campbell *et al.*, 1997; 1998b). Diazinon resistance in *L. cuprina* has traditionally been measured by electrophoretic analysis using α -naphthylacetate as a substrate, and assaying for esterase 3 (E3) activity. The resistance conferring form of E3, the OP hydrolase, lacks any measurable carboxylesterase activity and appears as 'nonstaining' on native polyacrylamide gels (PAGE; McKenzie *et al.*, 1992). The loss of carboxylesterase activity is caused by a single point mutation in the *LcαE7* gene resulting in a Gly¹³⁷–Asp amino acid substitution (Newcomb *et al.*, 1997), which results in the increase of OP hydrolase and confers diazinon resistance (Campbell *et al.*, 1997; 1998a).

Recent evidence shows that malathion resistance is also conferred by a mutation in the *LcαE7* gene. Campbell *et al.* (1998a) found that a Trp²⁵¹–Leu amino acid substitution in E3 is responsible for high MCE activity in four malathion resistant lines of *L. cuprina*. Malathion resistance is caused by increased specificity of E3 for malathion and resistance to inhibition by malaoxon (the active desulphurated form of malathion).

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Biochemical analysis of the MCE activity revealed three different forms of MCE (Smyth *et al.*, 1996). High MCE activity is caused by the Trp²⁵¹–Leu amino acid substitution, which increases MCE activity sixfold, intermediate MCE activity is caused by the wild-type enzyme and low MCE activity is the result of the Gly¹³⁷–Asp amino acid substitution, which greatly reduces the enzyme's carboxylesterase activities (Campbell *et al.*, 1998a). The high phenotype is 1000-fold more resistant to malathion than either of the other two phenotypes, despite the small difference in activities between the high and intermediate types (high has 1.6-fold greater activity than intermediate MCE activity; Smyth *et al.*, 1994).

We have assayed MCE activities and malathion resistance from three field-derived samples, two sets of lines made isogenic for chromosome 4 and one set consisting of five mass populations, to determine the contribution of the Trp²⁵¹–Leu amino acid substitution in E3 to high MCE activity and malathion resistance in the field. We also explore whether double resistance to both malathion and diazinon can be the result of the two mutations occurring in the *LcxE7* gene.

Materials and methods

Strains

The first sample consists of 34 isogenic lines: four (RM2-6, Q4, LS2 and RM8) from an earlier study of seven strains (Smyth *et al.*, 1994) and 30 new lines generated the same way. Briefly, wild-type males were crossed with three

virgin females heterozygous for a multiply inverted fourth chromosome balancer [In(4)6 + 8 + 12*Sh gl/sv ra tg gl*]. Individual F₁ males heterozygous for the balancer were backcrossed to three virgin balancer strain females and the G₂ progeny [In(4)6 + 8 + 12*Sh gl*/++++] were intercrossed in single-pair matings. Wild-type G₃ progeny from each G₂ cross were then intercrossed to establish the isogenic lines. These 30 new and the four original isogenic lines are termed the unscreened lines because they were not screened with malathion during the extraction procedure. Two low MCE and one intermediate MCE strain from the original survey are not isogenic lines and have been omitted from the statistical analysis of these data (*Sh*, Dua-91 and *sv bu ra*).

The second sample contains 20 isogenic lines generated by the same procedure except that males used as parents in the G₀ and G₁ generations had survived a dose of 0.2% w/v (2 µg/µL) malathion, which discriminates against malathion susceptible individuals (Hughes *et al.*, 1984). Malathion in acetone (1 µL) was topically applied to the upper thorax of each parental male, 3 to 5 days after eclosion. These 20 isogenic lines are termed the screened lines.

The five mass populations were: Finley (collected 1991); Flinders Island (collected 1988); LBB (collected before 1960); Llandillo (collected 1991); Murrumbateman (collected 1977; Foster *et al.*, 1978). All strains were maintained in the laboratory without exposure to insecticides. The distribution of collection sites for the isogenic lines and mass populations is illustrated in Fig. 1.

Fig. 1 Map of Australia showing the collection sites of the strains of *Lucilia cuprina* from which the isogenic lines and mass populations were derived. The unshaded area represents the regions of Australia where sheep are reared (Norris, 1990). The states and capital cities are italicized.

Adult flies were maintained on a diet of sugar and water and given a protein feed prior to oviposition. Flies were assayed within the first week of eclosion.

Biochemical assays

MCE activity was assayed using the radiometric partition method previously described by Whyard *et al.* (1994). Individual flies were homogenized in 600 μ L MCE homogenization buffer (10 mM imidazole HCl, pH 7.0, 1% Triton X-100 (v/v), 1 mM phenylthiourea and 1 mM dithiothreitol) and immediately assayed for MCE activity. Activities were calculated from aliquots of 15 μ L of homogenate for lines expressing high or intermediate MCE activities, and 75 μ L for low lines and individuals from the mass populations. Protein concentrations were determined using the method of Bradford (1976) with ovalbumin as the standard.

The MCE enzymes in adults of six screened isogenic lines were tested for their susceptibility to diagnostic inhibitors that distinguish the low, intermediate and high MCE phenotypes (Smyth *et al.*, 1996): paraoxon (2×10^{-7} M) and triphenylphosphate (TPP; 3×10^{-6} M). Fifteen microlitres of homogenate from each line was preincubated with inhibitor for 30 min at 25°C before assaying for MCE activity. Control assays were preincubated with an equal volume of inhibitor solvent, ethanol.

Toxicology

Dose–mortality relationships were determined for a representative subset of the 54 isogenic lines. One microlitre of the appropriate concentration of OP dissolved in acetone was topically applied to the upper thorax of 3–5 day old adults. Twenty-five females were tested and mortality was recorded after 24 h. Malathion was applied to the unscreened lines at seven concentrations ranging from 0 to 0.072% (w/v) and to the screened lines at six concentrations ranging from 0 to 40% (w/v). Diazinon was applied to both the unscreened and screened lines at six concentrations ranging from 0 to 0.04% (w/v). Control adult flies were tested with acetone only. The concentration of each insecticide at which 50% of the flies died was estimated by probit analysis (Finney, 1971) as implemented in SAS, version 6.03 (SAS, 1988).

Discriminating dose assays for malathion were carried out on adults from four additional screened lines (25 flies of each sex per line) and for both malathion and diazinon in the five mass populations (50 flies of each sex per population). The doses were chosen to discriminate susceptible homozygotes from resistant heterozygotes and homozygotes: 0.2% (w/v) for malathion (Hughes

et al., 1984) and 0.01% (w/v) for diazinon (McKenzie *et al.*, 1980), applied topically as described above. Control flies were treated with acetone and mortality was recorded after 24 h.

Diazinon larval toxicology was determined using a dose that again discriminates susceptible homozygotes from resistant heterozygotes or homozygotes (McKenzie *et al.*, 1992). Two hundred first instar larvae were placed on meat meal media containing 0.0003% diazinon and the number of emerging flies counted to determine larval resistance. Control assays were performed without diazinon, and three replicates of each assay were performed.

Genetics

Crosses between several isogenic lines were set up to determine the dominance relationships among the high, intermediate and low MCE specific activities. Virgin females from one MCE specific activity line were crossed with males from a different MCE specific activity line (the lines used and crosses established are described in the Results). For each cross, 20 F_1 individuals of each sex were assayed for MCE specific activity using 15 μ L of homogenate per individual.

Results

Unscreened isogenic lines

MCE specific activities for 28 of the 30 new unscreened lines ranged from 4.0 ± 0.5 to 14.2 ± 0.6 pmole malathion/min/mg (Fig. 2a) and were comparable to that of the two low MCE lines (6.6 ± 0.2 and 9.6 ± 0.8) previously described in Smyth *et al.* (1994). There was significant variation in activity among all low MCE activity isogenic lines ($F_{29,200} = 7.45$, $P \leq 0.0001$). No obvious correlate explained the variation (e.g. geographical origin, time of collection). The remaining two unscreened lines fell into the intermediate MCE group (Fig. 2a). The values for the two new intermediate MCE lines (112 ± 5 and 138 ± 5 pmole malathion/min/mg) were similar to the previously described intermediate line (139 ± 3). There was no significant variation of activities among these isogenic lines ($F_{2,28} = 0.91$, $P > 0.05$). Combining the data for all the new lines and the four isogenic lines from the initial survey (RM2-6, Q4, LS2 and RM8 on Fig. 2a) yielded frequencies of $88 \pm 2\%$ for low MCE, $9 \pm 2\%$ for intermediate MCE and $3 \pm 1\%$ for high MCE activity lines.

LD₅₀ values for malathion in a subsample of five unscreened isogenic lines (three low MCE activity and two intermediate MCE activity lines) were similar to those previously reported (Smyth *et al.*, 1994). Adult

Fig. 2 Distribution of MCE specific activity (pmole malathion min/mg $\pm$ SE). Low MCE activity lines are indicated by clear bars, intermediate MCE activity lines by grey bars and the high MCE activity line by the black bar. The asterisks indicate the four isogenic lines of *Lucilia cuprina* originally described in Smyth *et al.* (1994). (a) Unscreened isogenic lines. (b) Screened isogenic lines.

LD₅₀ values differed 1000-fold between the previously described high MCE activity, malathion resistant line and the low and intermediate MCE activity, malathion susceptible lines (Table 1). The LD₅₀ values for adults of diazinon resistant lines were approximately eightfold higher than those of diazinon susceptible lines. The levels of resistance to the two insecticides concur with those reported earlier with other strains.

Larvae from the RM8, LS2 and RM2-6 lines were also tested for resistance to diazinon (data not shown). The RM2-6 larvae were resistant, the RM8 and LS2 larvae were not, mirroring the adult resistance phenotypes of the three lines.

The dominance relationships between the low, intermediate and high MCE activities were determined to allow assignment of these activities within the five nonisogenic mass populations. Dominance relationships for MCE activity among six of the unscreened isogenic lines were the same as those evident among the strains used previously to map MCE activity (Fig. 3; Smyth *et al.*, 1994). The crosses of the three low MCE lines with the high MCE line yielded midparental values in the F₁ ($t_{155} = 0.64$, $P \geq 0.05$). However, crosses between two of the low and two of the intermediate MCE lines gave F₁ activities significantly less than midparental ($t_{155} = 3.14$, $P \leq 0.05$), as did crosses between two intermediate MCE lines and the high MCE line

Table 1 Female malathion and diazinon LD₅₀ values (% w/v) for the isogenic lines of *Lucilia cuprina*

Line	Malathion			Diazinon		
	LD ₅₀	LD ₁₀ , LD ₉₀	Resistance status	LD ₅₀	LD ₁₀ , LD ₉₀	Resistance status
(a) Unscreened isogenic lines						
RM2-6†	0.04	0.02, 0.09	S‡	0.04	0.02, 0.06	R
Inverrell 22	0.03	0.02, 0.05	S	0.03	0.02, 0.03	R
Llandillo 103	0.05	0.04, 0.07	S	0.02	0.01, 0.03	R
Sunbury 5.2†	0.02	0.01, 0.03	S	0.02	0.01, 0.04	R
LS2†	0.05	0.03, 0.07	S	0.004	0.002, 0.007	S
LBB 101	0.05	0.04, 0.07	S	0.005	0.003, 0.006	S
Llandillo 104	0.04	0.03, 0.06	S	0.005	0.004, 0.006	S
RM8†	35	24, 50	R	0.008	0.006, 0.01	S
(b) Screened isogenic lines						
Beverly 4.2	7	5, 10	R	NT§	NT	S¶
Beverly 6.2	5	3, 9	R	NT	NT	S¶
Hampton Hill 6.1	15	12, 19	R	0.002	0.001, 0.003	S
Hampton Hill 6.2	9	7, 11	R	0.003	0.002, 0.004	S
Tooronga 2.2	23	15, 36	R	0.06	0.02, 0.08	R
Tooronga 2.3	25	16, 41	R	NT	NT	S¶
Woodside 5.2	17	13, 23	R	0.005	0.003, 0.007	S
55SE 14.1	12	5, 27	R	NT	NT	R¶
60NE 1.1	7	4, 13	R	0.013	0.007, 0.02	R

†Originally described in Smyth *et al.* (1994).

‡Susceptible (S) vs. Resistant (R).

§Not Tested.

¶Resistance status determined by discriminating dose.

($t_{155} = 10.19$, $P \leq 0.001$). F_1 values for the latter cross overlap that of intermediate MCE homozygotes (121 ± 6 vs. 129 ± 7 pmole malathion/min/mg, respectively). There were no differences among any of the reciprocal crosses.

Application of Duncan's multiple range test (Duncan, 1975) to the total data on MCE specific activity for all the unscreened lines and the crosses among them revealed only four groups of phenotypes. Those that were not significantly different at $P < 0.05$ were intermediate homozygotes, high/intermediate heterozygotes and high/low MCE heterozygotes. The high MCE homozygotes, intermediate/low MCE heterozygotes and low MCE homozygotes were all different from one another and from the above group.

Screened isogenic lines

The screened isogenic lines were created to recover as many different malathion resistance alleles as possible within the field populations from which these flies were collected. There was significant variation among the MCE activities of the 20 screened lines (164.8 ± 7.2 to 335.2 ± 6.9 pmole malathion/min/mg; $F_{19,197} = 25.5$, $P \leq 0.001$) but even the lowest activity levels were above those for the three intermediate activity lines (Fig. 2b).

The MCE activities of six of the screened lines (Beverly 6.2, Hampton Hill 6.1, Hampton Hill 6.2, Tooronga 2.2, Woodside 5.2 and 60NE 1.1; see Fig. 2b) were tested for susceptibility to the diagnostic inhibitors TPP and paraoxon. Previous analysis of three of the original unscreened lines had shown that 3×10^{-6} M TPP and 2×10^{-7} M paraoxon distinguish the different MCE activities and completely inhibit high MCE activity (Smyth *et al.*, 1996). Both TPP and paraoxon also completely inhibited the MCE activities of all six screened lines, suggesting that they were similar to that of RM8.

The LD₅₀ values indicate that all the screened lines were resistant to malathion, as expected (Table 1). The differences in degrees of resistance to malathion correlate closely with the variation in MCE activity among these lines (linear regression, $F_{1,7} = 6.50$, $P \leq 0.01$). The variation in resistance is greater than the variation in activity (fivefold vs. twofold) but is still small compared to the difference between resistance and susceptibility. Three of the nine lines tested were also resistant to diazinon: Tooronga 2.2, 55SE 14.1 and 60NE 1.1. 60NE 1.1 showed substantially less resistance than Tooronga 2.2 (diazinon resistance in 55SE 14.1 was determined by discriminating dose), and than the resistant unscreened lines (1.5- to 5-fold less resistance).

Fig. 3 Dominance relationships between the low, intermediate and high MCE phenotype of *Lucilia cuprina*. The MCE specific activity ($\pm$ SE) is given for each parental isogenic and F₁ line. Low MCE activity lines are indicated by clear bars, intermediate MCE activity lines by grey bars, high MCE activity line by black bars and the F₁ progeny by hatched bars. The female parent is listed first in each cross. (a) Low and intermediate MCE phenotypes. (b) Low and high MCE phenotypes. (c) High and intermediate MCE phenotypes.

Larvae of the Woodside 5.2 line were susceptible to diazinon and those of Tooronga 2.2 were resistant (data not shown), consistent with the respective adult resistance phenotypes of these lines.

Mass populations

MCE activities were determined for 100 individual flies from each of five mass populations (Fig. 4). The distribution of activities was clearly discontinuous in each population. One narrow peak in each population corresponded to the activity of low MCE homozygotes. The frequency of these individuals was high in Finley (86%), the most recently collected population (1991), and comparable to frequencies of low MCE chromosomes in the unscreened lines. However, the frequencies of the putative low MCE homozygotes were substantially lower in the other four populations, ranging from 9% in LBB to 42% in Murrumbateman.

All individuals from each population not classified as low MCE homozygotes had specific activities in the range compatible with the various intermediate and high MCE genotypes found in the isogenic lines. Precise frequencies of intermediate and high MCE homozygotes in the mass populations cannot be calculated because of the evidence from the isogenic lines for complex dominance relationships in heterozygotes. However, most of the 'nonlow' MCE individuals from four of the populations lay in the range of activities for the intermediate MCE homozygotes and the intermediate/high and low/intermediate MCE heterozygotes, whereas the majority of the 'nonlow' MCE individuals from the Finley population had higher values, in the range previously ascribed to high MCE homozygotes. Correcting for variations in protein content did not significantly change the data.

The proportion of flies susceptible to malathion or diazinon in the five mass populations was estimated as the frequency of individuals that did not survive a dose of malathion designed to kill susceptible homozygotes. The frequency of malathion susceptible flies varied from 73% in the Finley population to 96% in Flinders Island (Table 2), and was higher in four populations than the

Fig. 4 The distribution of MCE activities (pmole malathion/min) for 100 individuals from each of the five mass populations of *Lucilia cuprina*. The numbers of individuals with low MCE activity are indicated by clear bars, intermediate MCE activity by grey bars and high MCE activity by black bars.

Table 2 Malathion and diazinon percentage mortalities and percentage low MCE phenotype for each of the five mass populations of *Lucilia cuprina*

Mass population	Malathion percentage mortality†	Percentage low MCE phenotype	Diazinon percentage mortality†
Finley	73 ± 2.0	86	16 ± 1.3
Flinders Island	96 ± 0.4	35	19 ± 1.5
LBB	92 ± 0.8	9	78 ± 1.7
Llandillo	88 ± 1.1	12	45 ± 2.5
Murrumbateman	90 ± 0.9	42	23 ± 1.8

†Results are expressed as percentage mortality ± binomial standard errors.

frequencies of low MCE homozygotes, as expected given that there are low/intermediate MCE heterozygotes and intermediate MCE homozygotes in these populations. However, the frequency of malathion susceptible homozygotes in the Finley population was significantly lower than the frequency of low MCE homozygotes ($\chi^2_1 = 5.22$, $P < 0.01$), suggesting that other malathion resistance mechanisms may be present in the Finley population in addition to high MCE activity.

The frequency of diazinon resistance ranged from 22% in the LBB population to 84% in the Finley population (Table 2).

Discussion

Our analysis of all three field-derived samples of *L. cuprina* revealed only the three levels of MCE activity originally described (Smyth *et al.*, 1994). High MCE activity was almost invariably associated with malathion resistance, whereas low MCE activity was always associated with resistance to diazinon. Consequently, malathion resistance was in almost complete disequilibrium with diazinon resistance. The major exceptions are the Finley mass population, which may contain other mechanisms for malathion resistance, and the three screened isogenic lines that are resistant to both malathion and diazinon. The double resistance of these isogenic lines can be explained by a duplication of the *LcαE7* gene (see below). Taken together, these results indicate that malathion and diazinon resistances in field populations of *L. cuprina* are generally caused by the Gly¹³⁷–Asp and Trp²⁵¹–Leu variants of E3 encoded by alleles of the *LcαE7* gene.

In the 34 unscreened isogenic lines we found all three levels of MCE activity; high, intermediate and low, albeit there was only one high MCE activity line (RM8). Only high MCE activity was found in the 20 screened isogenic lines, and these activities were all similar to the high MCE activity characterized from the RM8 unscreened isogenic line. Anticipating the difficulty of assigning MCE activity phenotypes in the five nonisogenic populations, because of the suggestion of complex dominance relationships from earlier experiments (Smyth *et al.*, 1994), we determined the dominance relationships between the low, intermediate and high MCE activities with the unscreened isogenic lines. The MCE activity phenotypes of F_1 progeny of crosses involving various unscreened lines exhibit the same complex but consistent set of dominance relationships we previously found: no dominance between low and high MCE activities; intermediate dominant to high MCE activities; low semidominant to intermediate MCE activities. These relationships differ from the patterns of dominance among the same MCE phenotypes with respect to malathion resistance. The malathion resistance associated with high activity was semidominant to the malathion susceptibility associated with either the low or intermediate MCE activities.

Although the dominance relationships among MCE activity classes preclude a complete assignment of genotypes in the mass populations, the frequencies of MCE activities in these populations were broadly consistent with those found in the sample of unscreened isogenic lines. The variation in these frequencies among populations may reflect the heterogeneity in their collection times. Low MCE activity was most frequent in the most recently collected population, Finley, and least frequent in LBB, the earliest collected population. The Finley population also appeared to show a marked deviation from Hardy–Weinberg equilibrium as individuals within the range of high homozygotes were more frequent than those in the range of intermediate homozygotes or high and intermediate heterozygotes.

In order to determine if malathion resistance is generally caused by high MCE activity in field populations, we tested the resistance status of all three field-derived samples, and found that resistance to malathion was completely correlated with MCE activities in both sets of the isogenic lines. All low and intermediate MCE activity lines were susceptible to malathion, whereas the high MCE activity lines were resistant. The frequencies of malathion resistance in the five mass populations are also generally consistent with the data on MCE activities in these populations, and malathion resistance in prior surveys of field populations (Hughes *et al.*, 1984). The exception is the Finley population for which the frequency of malathion susceptibility was lower than

expected from the MCE activity data. This finding is consistent with the evidence that intermediate MCE activity is not common in this population. One possible explanation for the skewed distribution of MCE activities in the Finley population is that a different or second genetic basis for high MCE activity may be present, which may also underlie the apparent deviation from Hardy–Weinberg. Another possibility is that additional resistance mechanisms may contribute to malathion resistance in this population. Altered acetylcholinesterase (AChE) and enhanced glutathione S-transferase and mixed function oxidase activities have been shown to confer resistance to malathion in other pest insect species (Takahashi & Yasutomi, 1987; Bull & Pryor, 1990; Scott *et al.*, 1990), and one or more of these mechanisms may be present in the Finley population.

Diazinon resistance is also conferred by a mutation in the *LcaE7* gene. An amino acid substitution at position 137 results in increased OP hydrolase and decreased carboxylesterase activities (Newcomb *et al.*, 1997; Campbell *et al.*, 1998a). We sought to determine whether resistance to both malathion and diazinon could be conferred by the OP hydrolase and high MCE activities encoded by alleles of the *LcaE7* gene. Diazinon resistance was in complete disequilibrium with malathion resistance in the unscreened isogenic lines tested. The high MCE line was resistant to malathion and all low MCE lines were resistant to diazinon. These lines were also assayed for OP hydrolase activity by scoring for the presence of E3 activity on native PAGE (data not shown), and all the low MCE activity lines lacked any visible E3 staining activity, indicating the presence of the OP hydrolase. Therefore, the diazinon resistance in the low MCE activity lines was consistent with expectations based on the OP hydrolase activity encoded by the Gly¹³⁷–Asp substitution in the *LcaE7* gene.

Only one mass population may contain some flies resistant to both malathion and diazinon. The combined frequencies of malathion and diazinon resistances in the Finley population exceeded 100% (Table 2). We have already noted that this population may contain other malathion resistance mechanisms, such as altered AChE, that have also been shown to contribute resistance to diazinon (Fournier *et al.*, 1992; Waters *et al.*, 1992; Mutero *et al.*, 1994). Thus, with the exception of the Finley population, the frequencies of malathion and diazinon resistances were consistent with the resistances being in disequilibrium in the mass populations. The data for malathion and diazinon resistances also support prior evidence for high frequencies of diazinon resistance and low frequencies of malathion resistance in the field (Hughes *et al.*, 1984; Levot, 1995).

In contrast to the unscreened isogenic lines, malathion and diazinon resistances were not in complete

disequilibrium in the screened isogenic lines. Three of the nine lines tested were resistant to both compounds. Our preliminary analysis of genetic variation around the *Lcx*E7 gene using restriction fragment length polymorphisms has revealed seven different haplotype classes, each one associated exclusively with (i) OP susceptibility (classes A, B and C) (ii) malathion resistance (E and F) or (iii) diazinon resistance (D and G; T.M.B., K.A.S. and J.G.O., unpubl. data). The restriction patterns suggest that two of the three screened isogenic lines resistant to both malathion and diazinon have duplicated *Lcx*E7 genes consistent with their resistance status. Tooronga 2.2 has both the D and E haplotype classes and 60NE 3.1 has classes F and G. The third line has not been characterized. Therefore, for an isogenic fly to be resistant to both malathion and diazinon it appears that it must carry two different *Lcx*E7 genes, one presumptively encoding E3 Gly¹³⁷–Asp and the other presumptively E3 Trp²⁵¹–Leu.

We have found, with a few notable exceptions, that high MCE activity confers almost all malathion resistance in these three field-derived samples, and on this basis we conclude that the Trp²⁵¹–Leu amino acid substitution in the *Lcx*E7 gene is responsible for most malathion resistance in field populations of *L. cuprina*. We also report that double resistance to malathion and diazinon can occur and appears to involve a duplication of the region containing the *Lcx*E7 gene. Double resistance is not common in the field, malathion resistance is about 11% and one-third of those chromosomes may also carry diazinon resistance. Restrictions on the rate of recombination or fitness costs associated with the duplications may account for the low frequency of these doubly resistant chromosomes.

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