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Cytochrome P450 monooxygenase-mediated permethrin resistance confers limited and larval specific cross-resistance in the southern house mosquito, *Culex pipiens quinquefasciatus*

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Abstract

The cytochrome P450-dependent monooxygenases (P450s) are an important enzymatic system that metabolizes xenobiotics (e.g., pesticides), as well as endogenous compounds (e.g., hormones). P450-mediated metabolism can result in detoxification of insecticides such as pyrethroids, or can be involved in the bioactivation and detoxification of insecticides such as organophosphates. We isolated (from the JPAL strain) a permethrin resistant strain (ISOP450) of *Culex pipiens quinquefasciatus*, having 1300-fold permethrin resistance using standard backcrossing procedures. ISOP450 is highly related to the susceptible lab strain (SLAB) and the high resistance to permethrin is due solely to P450-mediated detoxification. This is the first time in mosquitoes that P450 monooxygenase involvement in pyrethroid resistance has been isolated and studied without the confounding effects of *kdr*. Resistance in ISOP450 is incompletely dominant ($D = +0.3$), autosomally linked, and monofactorally inherited. It is expressed in the larvae, but not in adults. Cross-resistance to pyrethroids lacking a 3-phenoxybenzyl moiety (tetramethrin, fenfluthrin, bioallethrin, and bifenthrin) ranged from 1.5- to 12-fold. ISOP450 had only limited (6.6- and 11-fold) cross-resistance to 3-phenoxybenzyl pyrethroids with an α-cyano group (cypermethrin and deltamethrin, respectively). Examination of cross-resistance patterns to organophosphate insecticides in ISOP450 showed an 8-fold resistance to fenitrothion, while low, but significant, levels of negative cross-resistance were found for malathion ($RR = 0.84$), temephos ($RR = 0.73$), and methyl-parathion ($RR = 0.55$). The importance and uniqueness of this P450 mechanism in insecticide resistance is discussed.

Keywords: Cytochrome P450-mediated metabolism; Insecticide resistance mechanism; Life stage specific resistance; *kdr*

1. Introduction

Southern house mosquitoes, *Culex pipiens quinquefasciatus,* are able to transmit a multitude of pathogens that infect both humans and other animals. This species is the primary vector of the filarial nematode, *Wuchereria bancrofti* [1]. It is also capable of transmitting West Nile virus (WNV) [2,3], bird malaria pathogens, dog heartworm (*Dirofilaria immitis*), avian pox virus, and St. Louis encephalitis in eastern United States [4]. For mosquito control, pyrethroid insecticides are mainly used as adulticides to treat bed nets and as aerial sprays [5,6], while organophosphates and insect growth regulators are used as larvicides [7]. Some pyrethroid and pyrethroid-like compounds (i.e., etofenprox) are used as larvicides in Japan (personal communication, T. Kozaki), but substantial larval exposure to pyrethroids may occur as a by-product of agrochemical applications [8].

Insecticide resistance is an important problem in controlling medically important pest populations and is extremely detrimental in the on-going struggle to control or eradicate vectors of disease [9]. Pyrethroid resistance in

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the *Culex pipiens* complex has been recorded worldwide. Resistance has been found in larvae from Martinique (10- to 2800-fold) [10], Saudi Arabia (2500-fold) [11], Tunisia (up to >7100-fold) [12], the southeastern USA (Alabama, 100- to 940-fold; Florida, 13- to 50-fold) [13,14], West Africa (9- to 82-fold) [15], Cuba (3- and 20-fold) [16,17], China (up to >20-fold) [6], Tanzania (5- to 10-fold) [18], and New York (6- to 7-fold) [19]. Understanding the genetic basis of resistance, as well as the patterns of cross-resistance, is important to develop effective resistance management strategies [20]. Resistance to pyrethroids in *Culex* mosquitoes occurs due to detoxification by cytochrome P450 monooxygenases [11], as well as target site insensitivity (i.e., *kdr*) [21].

Cytochrome P450 monooxygenases (P450s) are an important biochemical system involved in the metabolism of xenobiotics and endogenous compounds. Insect P450s are important because they are involved in both the detoxification (i.e., limit the toxicity) and bioactivation (i.e., produce more toxic metabolites) of insecticides. There are multiple P450s in eukaryotes which vary dramatically in overall amino acid sequence among species. Based on available insect genome sequences there are 87 P450 genes in *Drosophila melanogaster* [22], 105 in *Anopheles gambiae*, 48 in *Apis mellifera* [23] and 134 in *Tribolium castaneum* [24].

P450s are remarkable because they are able to metabolize diverse substrates and are capable of catalyzing a large array of reactions [25–27]. The extent of possible substrates metabolized by monooxygenases is due in part to the large number of isoforms present and the broad substrate specificity of some isoforms. For example, CYP1A1 can metabolize more than 20 substrates [27] and CYP6D1 can carry out a wide range of reactions on many structurally diverse substrates [28–30]. Not all P450s are able to metabolize a broad range of substrates. For example, CYP7A1 has only one known substrate [27]. Certain P450s have overlapping substrate specificity (e.g., CYP2C subfamily in humans) [27] so that a single compound may be metabolized by multiple P450s. Additionally, some P450s can attack multiple sites on some substrates and produce multiple metabolites. Amino acid similarity of two P450s need not indicate they metabolize similar compounds, as a single amino acid mutation can change the P450 substrate specificity [31]. It has also been shown that a small change in substrate structure can dramatically change the turnover rate of insecticides [32].

Cytochrome P450s are one of the major mechanisms of insecticide resistance in insects [33]. JPAL is a strain of *Cx. p. quinquefasciatus* collected from Saudi Arabia and selected with permethrin for 20 generations [34]. JPAL larvae exhibited 2500-fold resistance to permethrin due to P450 detoxification and *kdr* [11]. Metabolism studies showed that NADPH-dependent metabolism of permethrin to 4′-OH permethrin was greater in JPAL compared to a susceptible strain [11]. The P450 responsible for this resistance has not been identified.

Weerasinghe et al. [35] previously examined cross-resistance to pyrethroid insecticides in JPAL. However, since JPAL has two major mechanisms of resistance (P450s and *kdr*) understanding the contribution of each mechanism to the resulting cross-resistance patterns is difficult. In order to more completely identify the cross-resistance patterns conferred by P450-mediated resistance, we studied a strain of *Cx. p. quinquefasciatus* (ISOP450) (produced by repeated backcrossing of JPAL and a susceptible strain), that contains the P450-mediated resistance, but lacks *kdr*. This is the first time in mosquitoes that P450 monooxygenase involvement in pyrethroid resistance has been isolated and studied without the confounding effects of *kdr*. ISOP450 allowed us to answer some previously intractable questions about P450-mediated resistance in mosquitoes, by investigating the genetic basis of the mechanism as well as the cross-resistance spectrum to pyrethroids and organophosphate insecticides commonly used in mosquito control programs.

2. Materials and methods

2.1. Mosquito strains

Three strains of *Cx. p. quinquefasciatus* Say were used. SLAB is a standard susceptible strain [36]. JPAL originated from Saudi Arabia [34] and is highly resistant to permethrin due to *kdr* and cytochrome P450 monooxygenase-mediated (P450) detoxification [11]. Standard backcrossing methods [37] were used to produce ISOP450, a resistant strain with the susceptible background containing the P450 resistance mechanism, but lacking *kdr*. To construct ISOP450, SLAB females were crossed with JPAL males *en masse*, and F_1 males were backcrossed to SLAB females. Fourth instar progeny were treated (see below) with permethrin (0.02 μg/ml, LC_{25} for progeny of SLAB female × JPAL male) to select for resistant heterozygotes. Surviving males were then backcrossed to SLAB females and this procedure was repeated for 11 generations (Table 1). For generations 12, 13 and 14, the reciprocal cross of surviving backcross females × SLAB males was included. Survivors were combined for the next backcross cycle. The resultant strain was then selected with permethrin for eight generations to produce a homozygous resistant strain (Table 2).

All strains were reared by standard methods. Larvae were reared with ample development space in plastic trays containing 2000 ml distilled water for every 400 larvae. Larvae were provided abundant food consisting of a mixture of ground TetraFin® goldfish flakes, rabbit pellets and liver powder (1:2:1) in distilled water. Adults were provided a 20% sugar solution *ad libitum*, and provided a chicken for 30 min two times per week (Cornell University Animal Use Protocol No. 01-56). All life stages were maintained at 27 ± 1 °C, 80% RH, and photoperiod of 14:10 h (L:D) including 2 h of simulated dawn and 2 h simulated dusk.

Table 1
Construction of the ISOP450 strain

Generation	Cross (female × male)	n[a]	(%) Mortality
BC1	SLAB (317)[b] × F_1 (SLAB × JPAL) (201)	1200	66
BC2	SLAB (255) × BC1 (287)	1180	76
BC3	SLAB (175) × BC2 (233)	540	53
BC4	SLAB (117) × BC3 (80)	1200	60
BC5	SLAB (81) × BC4 (36)	240	56
BC6	SLAB × BC5	1500	50
BC7	SLAB (190) × BC6 (150)	590	51
BC8	SLAB (292) × BC7 (60)	880	49
BC9	SLAB × BC8	949	79
BC10	SLAB × BC9	625	57
BC11	SLAB × BC10	4060	62
BC12	SLAB × BC11	2250	59
	BC11 × SLAB	1025	48
BC13	SLAB × BC12	1500	69
	BC12 × SLAB	1780	60
BC14	SLAB × BC13	1400	60
	BC13 × SLAB	1200	62

[a] Number of fourth instar larvae treated with permethrin at 0.02 μg/ml.
[b] Number of individuals included in cross.

Table 2
Selection of the ISOP450 strain

Generation	Concentration[a]	n[b]	(%) Mortality
G1[c]	0.02	2200	79
G2	0.1	3000	89
G3	1.0	2580	85
G4	1.0	10,180	95
G5	1.0	3600	65
G6	1.0	960	66
	5.0	1200	97
G7	1.0	489	84
	3.0	4880	93.5
G8	5.0	15,400	96

[a] Concentration in μg/ml.
[b] Number of fourth instar larvae treated.
[c] BC14 F_1 survivors (see Table 1).

2.2. Chemicals

Deltamethrin (99%) was from Roussel UCLAF and 1-*R*-*trans*-fenfluthrin was from Bayer CropScience (Kansas City, MO). Insecticides obtained from Chem Service (Westchester, PA) included: permethrin (98%), cypermethrin (98%), *S*-bioallethrin (99%), tetramethrin (99.5%), bifenthrin (99.7%), temephos (98%), malathion (99.2%), diazinon (99.5%), and methyl-parathion (98.9%). Fenitrothion was from Sumitomo Chemical (Tokyo, Japan). The synergist piperonyl butoxide (PBO, 90% purity) was from Sigma–Aldrich, Inc. (St. Louis, MO).

2.3. Larval bioassays

For each larval bioassay, 20 fourth instars were placed in 4 oz. waxed paper cups (Sweetheart Cup Co., Owings Mills, MD) with 99 ml of distilled water and 1 ml of insecticide (in acetone) solution (or just acetone for the controls). To evaluate the role of P450 monooxygenases, bioassays were also run as described above, except that 1 ml of PBO solution (0.1 mg/ml) was added. Preliminary experiments indicated that 0.001 mg/ml (final concentration) was the maximum sublethal concentration of PBO. Mortality was assessed after 24 h, and larvae were considered dead if they failed to move or resurface after being probed.

2.4. Adult bioassays

Adult mosquito bioassays were conducted in glass jars (230 ml, internal surface area of 180 cm^2) treated with 1 ml of insecticide solution (or 1 ml of acetone for controls), which was evenly coated on the inner walls. Jars were held (for acetone to completely evaporate) for 30 min under a fume hood and then 10 adult females (2- to 3-day old virgins) were placed in each jar, and the opening was covered with fabric. Adults were provided cotton wicks saturated with 20% sugar water throughout the assay. Adults were considered dead if after 24 h they were ataxic. Adult bioassays with the synergist PBO were also run as described above except that a PBO in acetone solution (1 mg/ml) was used instead of acetone.

2.5. Statistical analysis

Each bioassay consisted of at least three replicates per concentration and at least three concentrations of insecticide were tested, giving greater than 0% and less than 100% kill. All bioassays (larval and adult) were run at 25 °C and replicated a minimum of five times. Bioassay data were pooled and analyzed by standard probit analysis [38], as adapted to personal computer use [39] using Abbott's correction [40] for control mortality.

2.6. Isolation of gDNA

Genomic DNA was extracted from a single adult male mosquito based on the protocol developed by J. Rehm, Berkeley Drosophila Genome Project (http://www.fruitfly.org). An individual mosquito was completely homogenized in 200 μl Buffer A (100 mM Tris–HCl, pH 7.5; 100 mM EDTA; 100 mM NaCl; 0.5% SDS) with a disposable tissue grinder (Kontes, Vineland, NJ). The homogenate was incubated at 65 °C for 30 min, then 400 μl of LiCl/KAc solution (5 M KAc:6 M LiCl) was added and the sample was incubated on ice for 10 min. The mixture was centrifuged for 15 min at room temperature, and 0.5 ml of supernatant was transferred into a new tube. To precipitate the genomic DNA, 300 μl isopropanol was added, mixed and centrifuged at room temperature for 15 min. The supernatant was then aspirated and the DNA pellet was washed with 70% ethanol. All ethanol was aspirated, the pellet was dried for 5 min and the DNA was dissolved in 30 μl ddH_2O. All samples were stored at −20 °C.

2.7. PCR and sequencing

A partial genomic sequence of the voltage sensitive sodium channel (*VSSC*), including the *kdr* mutation site and adjacent intron, was amplified by PCR using the following primers: forward primer CulexkdrF (5′GGAACTT CACCGACTTCATGC3′) and reverse primer CulexkdrR (5′CGCCGACAGACTTGAGGAACC3′). *Taq* polymerase was obtained from New England Biolabs (Beverly, MA). Amplification reactions were performed using the following thermal cycler conditions: 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 5 min. The PCR products were purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA) following the manufacturer's instructions and sequenced at the Cornell University Biotechnology Resource Center.

2.8. Degree of dominance and mode of inheritance bioassays

To determine the degree of dominance and mode of inheritance for the permethrin resistance in JPAL and ISOP450, standard backcross methods were used [41]. Pupae from each strain were individually isolated in plastic tubes. Upon eclosion, the respective sex and strain were released into a cage to create the desired crosses. The adults were released *en masse* with an approximately 3:1 (female:-male) ratio, with at least 200 females used. The following reciprocal crosses were created: SLAB females × ISOP450 males, and ISOP450 females × SLAB males. The backcrosses were constructed using 300 virgin F_1 females (progeny of SLAB females × ISOP450 males and progeny of ISOP450 females × SLAB males) mated to 100 SLAB males.

2.9. Degree of dominance and mode of inheritance analysis

Once probit regression lines were determined for the parental strains (SLAB and ISOP450) and the reciprocal cross progeny, the degree of dominance (D) for the P450 mechanism was calculated using Stone's [42] equation:

$$D = (2X_2 – X_1 – X_3)/(X_1 – X_3)$$

where X_1 is the log LC_{50} of homozygous resistant strain (ISOP450), X_2 is the log LC_{50} of the heterozygous F_1 progeny of each reciprocal cross, and X_3 is the log LC_{50} of the homozygous susceptible strain (SLAB).

The standard backcrossing method tests the null hypothesis that one locus controls resistance (monofactorial inheritance). To determine if the backcross progeny in this experiment differed significantly from the null hypothesis, the following equations were used [41]. The expected mortality of the backcross offspring (Y_x) at a specific concentration (x) is calculated as:

$$Y_x = 0.50(W_{RS} - W_{SS})$$

Table 3
Comparative insecticide screening of mosquito strains to determine mechanism of resistance in ISOP450

Life stage	Insecticide	SLAB			JPAL			*RR*[c]	ISOP450			*RR*[c]
		n[a]	Slope (SE)	LC_{50}[b] (95% CI)	n[a]	Slope (SE)	LC_{50}[b] (95% CI)		n[a]	Slope (SE)	LC_{50}[b] (95% CI)	
Larvae	Permethrin	320	4.5 (0.6)	0.017 (0.015–0.019)	480	4.6 (0.4)	500 (450–540)	29,000	860	1.8 (0.1)	21 (19–25)	1300
Larvae	Permethrin + PBO	480	8.0 (2.8)	0.011 (0.0090–0.014)	240	3.5 (1.4)	0.77 (0.49–1.2)	70	960	3.0 (0.2)	0.059 (0.053–0.065)	5.4
Adult	Permethrin	670	2.5 (0.2)	0.029 (0.026–0.034)	637	2.1 (0.2)	1.3 (1.1–1.5)	45	360	1.9 (0.2)	0.13 (0.10–0.15)	4.5
Adult	Permethrin + PBO	680	1.6 (0.1)	0.0022 (0.0018–0.0026)	470	4.0 (0.5)	0.17 (0.15–0.18)	77	460	1.1 (0.3)	0.0034 (0.0019–0.0059)	1.6

[a] Total number of animals treated.
[b] Final concentration in ng/ml.
[c] Resistance ratio = LC_{50} resistant strain/LC_{50} susceptible strain.

where W_{RS} is the mortality of the heterozygote (F_1) genotype at concentration x, and W_{SS} is the mortality of the susceptible parental strain at concentration x. Chi-squared statistical analysis was conducted to determine significance between the calculated backcross progeny mortality and the observed mortality by this equation [41]:

$$\chi^2 = (F_1 - pn)^2/pqn$$

where F_1 is the observed backcross progeny mortality at concentration x, p is the expected backcross progeny mortality, $q = 1-p$, and n is the number of backcross progeny assayed with concentration x. The monofactorial null hypothesis is rejected if the p-value <0.05.

3. Results

3.1. ISOP450 strain construction

After 14 generations of backcrosses and permethrin selection followed by an additional eight generations of permethrin selection, we isolated a strain (highly related to SLAB) called ISOP450. Larvae of ISOP450 were 1300-fold resistant to permethrin (Table 3) relative to SLAB. This is lower than the levels found in JPAL (29,000-fold), suggesting a resistance factor present in JPAL may have been lost during isolation of the ISOP450 strain. Resistance to permethrin dropped to 5.4-fold when ISOP450 larvae were treated with PBO. This suggested that the major resistance mechanism in ISOP450 was inhibited by PBO. JPAL larvae treated with PBO had 70-fold resistance to permethrin. When treated with PBO, ISOP450 had significantly lower resistance to permethrin than JPAL. This indicates that a mechanism (one which could not be overcome by PBO (i.e., *kdr*)) in the JPAL strain had been lost in ISOP450. To confirm the bioassay results (that ISOP450 lacked *kdr*) we genotyped individual mosquitoes for the *kdr* mutation in *VSSC* and found that all ($n = 20$) ISOP450 individuals contained the susceptible allele (Fig. 3).

Adult JPAL and ISOP450 were less resistant to permethrin compared to larvae, being only 45- and 4.5-fold resistant to permethrin, respectively (Table 3). Addition of PBO resulted in a 13-fold synergism of permethrin toxicity in SLAB, suggesting there is P450-mediated detoxification of permethrin in this susceptible strain, but that this is much greater in adults, than in larvae. This contrasts with *Aedes aegypti*, where the opposite pattern was observed [43]. For both the JPAL and ISOP450 strains, resistance in adults is comparable to the resistance ratios of the respective larvae treated with permethrin and PBO. These results suggest that the P450 mechanism present in JPAL and ISOP450 larvae is not expressed in the adult stage.

3.2. Inheritance of permethrin resistance in ISOP450

To evaluate the mode of inheritance of the cytochrome P450-mediated permethrin resistance in ISOP450 larvae, we calculated the degree of dominance (D) for the heterozygote progeny of SLAB females × ISOP450 males (F_1a) and ISOP450 females × SLAB males (F_1b). The D value for the F_1 progeny of both crosses was 0.3, which indicates inheritance is incompletely dominant [44]. The F_1 responses did not differ significantly between the reciprocal crosses, indicating that there were no cytoplasmic influences and the resistance was due a chromosomal genetic factor [44]. We also found no differences in susceptibility between the sexes of the F_1s, indicating resistance was not sex-linked [44].

To determine if resistance was monofactorial or polyfactorial, standard backcrosses were conducted. The resulting

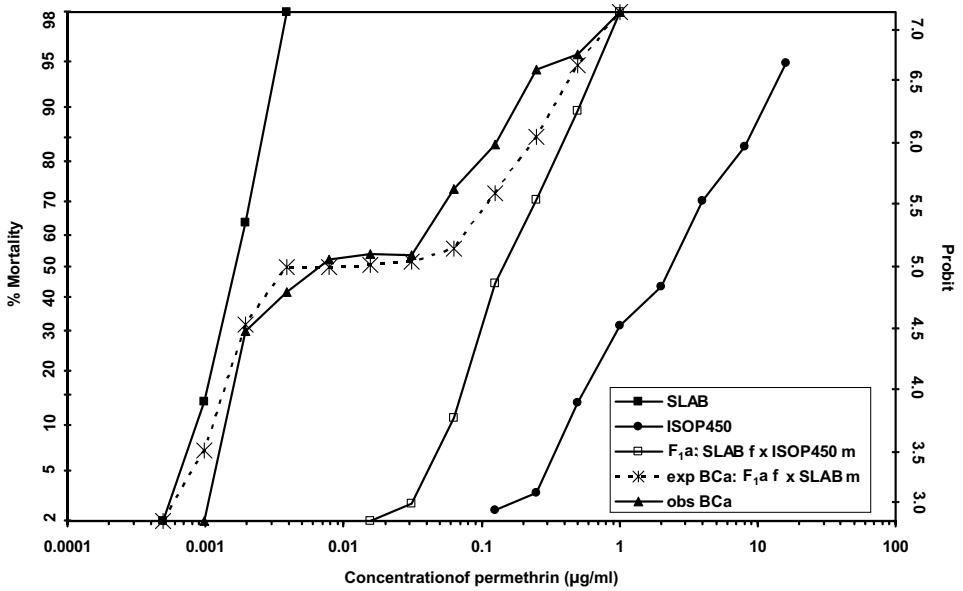

Fig. 1. Probit graph for mode of inheritance determination of the cytochrome P450 resistance mechanism in ISOP450, reciprocal cross A: SLAB f (female) × ISOP450 m (male). Obs, observed; exp., expected.

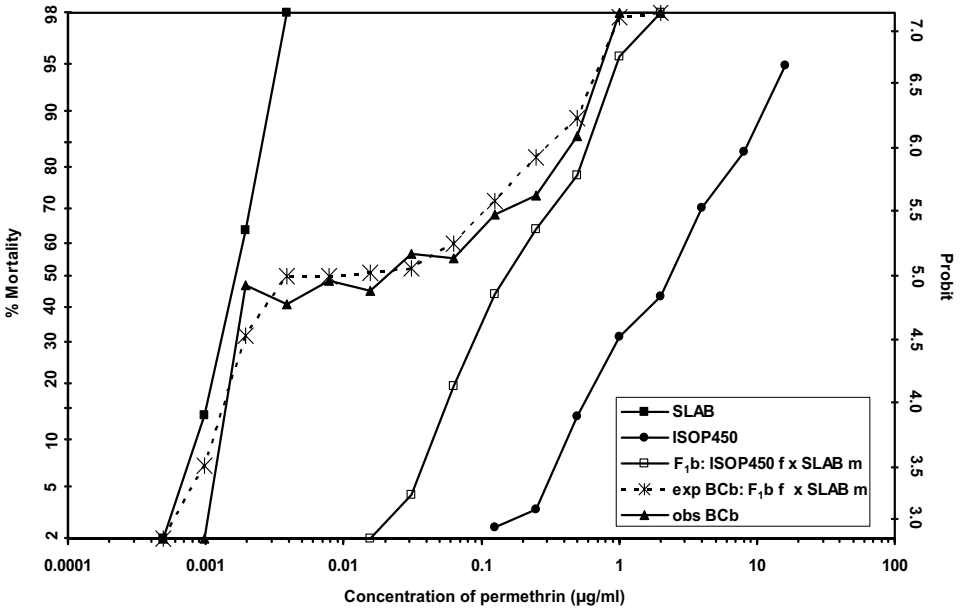

Fig. 2. Probit graph for mode of inheritance determination of the cytochrome P450 resistance mechanism in ISOP450, reciprocal cross B: ISOP450 f (female) × SLAB m (male). Obs, observed; exp., expected.

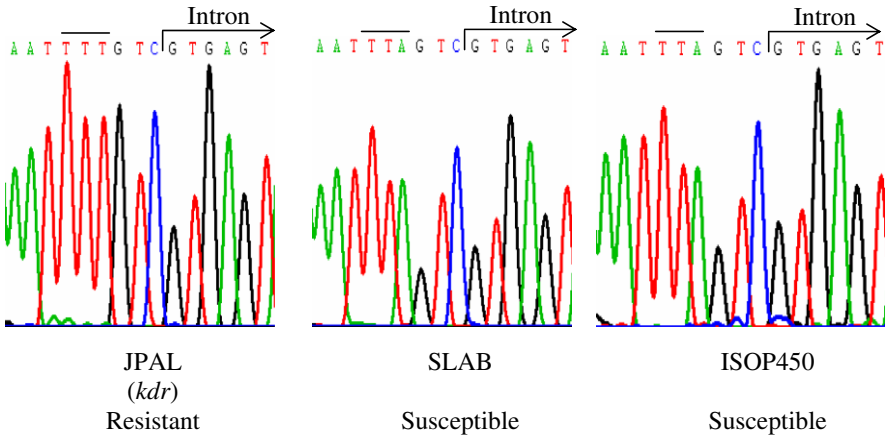

Fig. 3. Genotyping of *VSSC* for the *kdr* mutation in mosquitoes. TTA (Leu) = susceptible allele, TTT (Phe) = *kdr*. Accession Nos. BI918677, BI918687 and BI919768 for the SLAB, ISOP450 and JPAL strains, respectively.

Table 4
LC_{50} values of pyrethroid insecticides to susceptible and resistant strains of *Cx. p. quinquefasciatus* fourth instar larvae

Insecticide	SLAB			ISOP450			*RR*[c]
	n[a]	Slope (SE)	LC_{50}[b] (95% CI)	*n*[a]	Slope (SE)	LC_{50}[b] (95% CI)	
Permethrin	320	4.5 (0.6)	1.7 (1.5–1.9)	860	1.8 (0.1)	2100 (1900–2500)	1300
Tetramethrin	960	9.4 (0.6)	47 (46–48)	640	4.3 (1.3)	560 (450–700)	12
Fenfluthrin	960	5.7 (0.7)	0.68 (0.61–0.75)	1180	2.2 (0.2)	5.2 (4.8–5.8)	7.7
Bioallethrin	900	2.5 (0.2)	67 (61–79)	1000	7.5 (1.8)	330 (280–370)	4.9
Bifenthrin	480	3.7 (0.4)	3.5 (3.1–4.0)	600	4.1 (0.9)	5.2 (3.9–6.8)	1.5
Deltamethrin	720	3.6 (0.2)	0.26 (0.32–0.28)	600	4.0 (0.3)	2.9 (2.7–3.2)	11
Cypermethrin	500	7.3 (0.8)	0.79 (0.74–0.85)	480	5.1 (0.4)	5.2 (4.8–5.7)	6.6

[a] Total number of fourth instar larvae treated.
[b] Final concentration in ng/ml.
[c] Resistance ratio = LC_{50} resistant strain/LC_{50} susceptible strain.

lines of both BCa (F_1a females × SLAB males) and BCb (F_1b females × SLAB males) displayed very distinct plateaus at 50% mortality (Figs. 1 and 2). Chi-squared analysis of the backcross progeny indicated that the null hypothesis of monofactorial inheritance could not be rejected (BCa: $\chi^2 = 13.76$, df = 11, p-value >0.1; BCb: $\chi^2 = 11.51$, df = 12, p-value >0.1).

3.3. Cross-resistance to pyrethroids in ISOP450

The ISOP450 strain allowed us the unique opportunity to determine the level of resistance conferred solely by P450-mediated permethrin resistance to pyrethroid insecticides with various structures. LC_{50} values of the seven pyrethroid insecticides to SLAB and ISOP450 strains are listed in Table 4. The resistance ratios were dramatically lowered (compared to permethrin) when either an α-cyano group was present (deltamethrin $RR = 11$, cypermethrin $RR = 6.6$) or when the pyrethroid lacked a 3-phenoxybenzyl moiety (tetramethrin $RR = 12$, fenfluthrin $RR = 7.7$, bioallethrin $RR = 4.9$ and bifenthrin $RR = 1.5$) (Fig. 4).

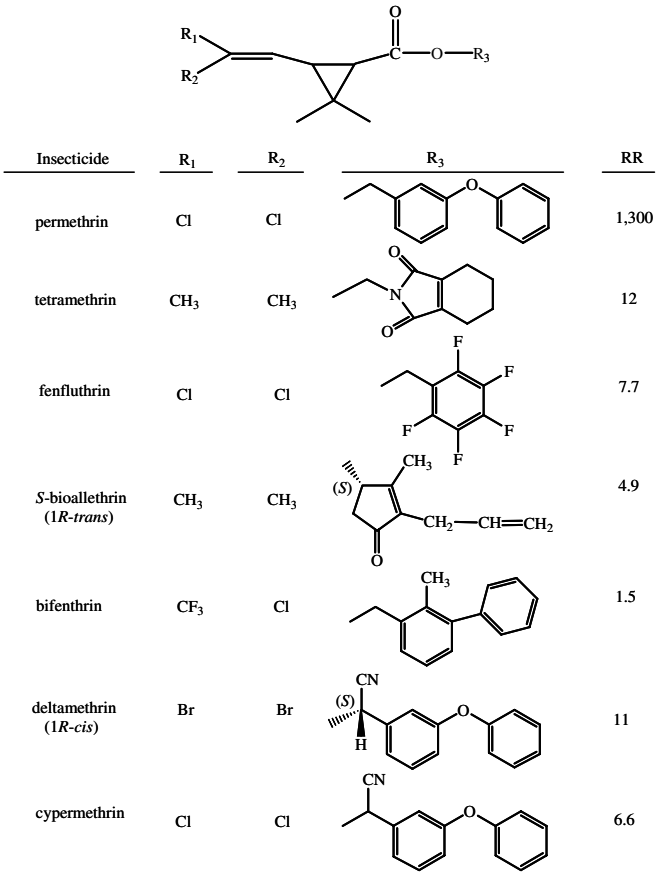

Fig. 4. Levels of resistance to seven pyrethroid insecticides in larvae of the ISOP450 strain.

3.4. Cross-resistance to organophosphates in ISOP450

Comparisons between SLAB and ISOP450 also allowed us to determine if any cross-resistance was conferred by the P450 mechanism to organophosphate insecticides commonly used in mosquito control (Table 5 and Fig. 5). ISOP450 was 8.1-fold resistant to fenitrothion as compared to SLAB. ISOP450 was then bioassayed with a structurally similar organophosphate, methyl-parathion, which does not contain a methyl substituent at the *meta* position on the benzene ring. The absence of the methyl group at that

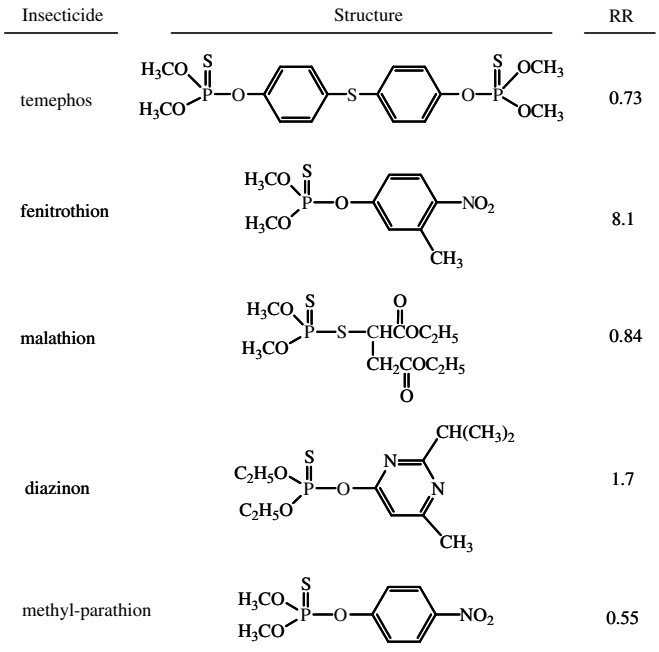

Fig. 5. Levels of resistance to five organophosphate insecticides in larvae of the ISOP450 strain.

Table 5
Toxicities of organophosphate insecticides to susceptible and resistant strains of *Cx. p. quinquefasciatus* fourth instar larvae

Insecticide	SLAB			ISOP450			RR^c
	n^a	Slope (SE)	$LC_{50}{}^b$ (99% CI)	n^a	Slope (SE)	$LC_{50}{}^b$ (99% CI)	
Temephos	1420	11 (0.6)	3.0 (3.0–3.1)	1080	15 (0.8)	2.2 (2.2–2.3)	0.73
Fenitrothion	1100	7.7 (0.4)	4.7 (4.6–4.9)	1060	8.9 (0.52)	38 (36–39)	8.1
Malathion	980	8.5 (1.3)	77 (71–83)	1140	8.9 (1.0)	65 (60–70)	0.84
Diazinon	1040	13 (1.6)	83 (79–88)	850	10 (0.7)	140 (140–150)	1.7
Methyl-parathion	800	7.5 (1.9)	4.0 (3.2–5.0)	800	8.3 (3.0)	2.2 (1.7–2.9)	0.55

[a] Total number of fourth instar larvae treated.
[b] Final concentration in ng/ml.
[c] Resistance ratio = LC_{50} resistant strain/LC_{50} susceptible strain.

site eliminated the resistance (methyl parathion $RR = 0.55$) (Table 5). We found low, but significant, levels of negative cross-resistance to temephos ($RR = 0.7$) and malathion ($RR = 0.8$) as well as significantly low levels of cross-resistance to diazinon ($RR = 1.7$). It has been previously shown that isogenic strains can have LC_{50}s that differ [45]. Thus, the role of the permethrin metabolizing P450 (responsible for resistance) in the low levels of cross-resistance and negative cross-resistance in ISOP450 is unclear.

4. Discussion

Our results show that P450-mediated permethrin resistance in the ISOP450 strain of *Cx. p. quinquefasciatus* is monofactorial. Similarly, monofactorial inheritance was found in *Helicoverpa armigera* resistant to fenvalerate due to metabolic factors [46]. Our results differ from the polygenic nature of P450-mediated pyrethroid resistance in houseflies [47–50].

Our study has demonstrated that the P450 responsible for resistance in the ISOP450 strain has a narrow substrate specificity, with a limited ability to detoxify pyrethroids containing an α-cyano substituent or lacking a 3-phenoxybenzyl group. For example, permethrin resistance in ISOP450 larvae was 1300-fold, while cypermethrin resistance was 6.6-fold (Table 3). This pattern was also observed in JPAL larvae, where resistance to permethrin was 2500-fold and cypermethrin resistance was 47-fold [35]. In JPAL, resistance to pyrethroids with an α-cyano substituent was moderate ($RR = 56$–39) and resistance to those without a 3-phenoxybenzyl group was low ($RR = 6$–9) [35]. The resistance in ISOP450 does not have exactly the same pattern as JPAL. Resistance in ISOP450 is low to pyrethroids with an α-cyano substituent ($RR = 11$ and 6.6) as well as to pyrethroids without a 3-phenoxybenzyl group ($RR = 1.5$–12). It has been shown that *kdr* confers different levels of resistance to various pyrethroids [51,52]. Thus, the higher resistance to α-cyano-pyrethroids found in JPAL, relative to ISOP450, is probably due to the presence of *kdr* in JPAL.

LPR is a housefly strain highly resistant to pyrethroids (>6000-fold) [53] due to *kdr* and detoxification mediated by CYP6D1 [49]. LPR showed high levels of resistance to pyrethroids with or without an α-cyano group [49]. This pattern contrasts that observed in ISOP450 and JPAL [35], where the presence of an α-cyano group decreases the resistance. Thus, even though CYP6D1 and the P450 responsible for permethrin metabolism in JPAL (and ISOP450) metabolize the same site on permethrin (and produce the same metabolite, i.e., 4′-OH permethrin) [11,29], they have different apparent substrate specificity to α-cyano pyrethroids, with the former being able to metabolize these pyrethroids and the latter being much less able to metabolize them. Similar to ISOP450 and JPAL, resistance to pyrethroids lacking an unsubstituted phenoxybenzyl group was decreased in LPR (relative to pyrethroids with an unsubstituted phenoxybenzyl group) [49].

Interestingly, ISOP450 was 8.1-fold cross-resistant to fenitrothion, but was not cross-resistant to methyl-parathion. Given that these two insecticides differ only in the presence of a *meta* methyl group (in fenitrothion, but lacking in methyl-parathion) suggests that the cross-resistance is due to metabolism of this methyl group. This suggests that the P450 responsible for permethrin resistance (due to aromatic hydroxylation) is also capable of carrying out the alkyl hydroxylation of fenitrothion. Identification of the P450 responsible for permethrin resistance in ISOP450 will allow us to test this hypothesis.

In both ISOP450 and JPAL the larvae exhibit high levels of permethrin resistance (1300-fold and 29,000-fold, respectively). The adults of these strains have significantly lower levels of permethrin resistance than the larvae (Table 3). This is similar to the recent report of high levels of pyrethroid (deltamethrin) resistance in larval, but not adult, *A. aegypti* [54]. In vector control programs pyrethroids are used as aerial sprays and on bed nets [5,6] where the adult life stage is targeted. Interestingly, the P450 mechanism present in ISOP450 and JPAL larvae is not expressed in the adults. Thus, it appears that either *kdr* is sufficient to survive insecticide exposure in the adult, or that high levels of resistance had not yet evolved in adults of these strains of *Cx. p. quinquefasciatus*. Further studies on pyrethroid resistance levels in adult *Cx. p. quinquefasciatus* would help clarify this. Pyrethroid resistance in mosquito larvae is documented worldwide. Substantial insecticidal pressures in the environment of the larvae exist possibly due to agricultural runoff or misuse. This inadvertent exposure may be selecting for resistance mechanisms essential for larvae to survive constant insecticidal exposure in a body of water, but unnecessary for adults to survive in their terrestrial environment. This may be why the P450-mediated detoxification in JPAL and ISOP450 is exclusively in the larval stage. Since high levels of resistance in ISOP450 seem to be restricted to permethrin, most other pyrethroids, as well as most organophosphates, are still effective control tools against mosquitoes with this resistance mechanism.

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Corrigendum

Corrigendum to "Cytochrome P450 monooxygenase-mediated permethrin resistance confers limited and larval specific cross-resistance in the southern house mosquito, *Culex pipiens quinquefasciatus*" [Pestic. Biochem. Physiol. 89 (2007) 175–184]

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On page 178, Table 3 contains errors in the LC_{50} values. These errors do not alter the conclusions of the paper in any way. The correct Table 3 is given here.

Table 3
Comparative insecticide screening of mosquito strains to determine mechanism of resistance in ISOP450

Life stage	Insecticide	SLAB			JPAL				ISOP450			
		n[a]	Slope (SE)	LC_{50}[b] (95% CI)	n[a]	Slope (SE)	LC_{50}[b] (95% CI)	RR[c]	n[a]	Slope (SE)	LC_{50}[b]	RR[c] (95% CI)
Larvae	Permethrin	320	4.5 (0.6)	1.7 (1.5–1.9)	480	4.6 (0.4)	49,000 (45,000–54,000)	29,000	860	1.8 (0.1)	2100 (1900–2500)	1300
Larvae	Permethrin + PBO	480	8.0 (2.8)	1.1 (0.9–1.4)	240	3.5 (1.4)	77 (49–120)	70	960	3.0 (0.2)	5.9 (5.3–6.5)	5.4
Adult	Permethrin	670	2.5 (0.2)	1.6 (1.4–1.9)	637	2.1 (0.2)	72 (61–83)	45	360	1.9 (0.2)	7.2 (5.6–8.3)	4.5
Adult	Permethrin + PBO	680	1.6 (0.1)	0.12 (0.10–0.14)	470	4.0 (0.5)	9.3 (8.3–10)	77	460	1.1 (0.3)	0.19 (0.11–0.33)	1.6

[a] Total number of animals treated.
[b] Units for larvae are in ng/ml and adults are in ng/cm^2.
[c] Resistance ratio = LC_{50} resistant strain/LC_{50} susceptible strain.

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