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MECHANISMS OF INSECTICIDE RESISTANCE IN FIELD POPULATIONS OF *AEDES AEGYPTI* (L.) FROM QUINTANA ROO, SOUTHERN MEXICO

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ABSTRACT. Potential insecticide-resistance mechanisms were studied with the use of biochemical assays in *Aedes aegypti* (L.) collected from 5 municipalities representing the north part of Quintana Roo: Benito Juarez, Cozumel, Isla Mujeres, Lazaro Cardenas, and Solidaridad. The activities of α and β esterases, mixed-function oxidases (MFO), glutathione-S-transferase (GST), acethylcholinesterase (AChE), and insensitive acethylcholinesterase (iAChE) were assayed in microplates. Three replicates were performed for each enzyme and 60 males and 60 females were analyzed in each population. The New Orleans (NO) susceptible strain of *Ae. aegypti* was used as a susceptible reference and the threshold criteria for each enzyme were the highest NO absorbance values. In none of the 6 tests were absorbance values correlated in males and females. α esterases were elevated in Benito Juarez, Cozumel females and in Lazaro Cardenas males and females. β esterases were elevated in Benito Juarez, Cozumel females and in Cozumel and Lazaro Cardenas males. Elevated esterases suggest potential insecticide-resistance mechanisms against organophosphate, carbamate, and some pyrethroid insecticides. Slightly elevated levels of MFOs appeared in Lazaro Cardenas females and in Cozumel, Isla Mujeres, and Solidaridad males. Mechanisms involving iAChE or GST were not apparent.

KEY WORDS *Aedes aegypti*, insecticide resistance, insecticide surveillance, esterases, permethrin

INTRODUCTION

Dengue fever (DF) and Dengue hemorrhagic fever (DHF), vector-borne diseases transmitted by the mosquito *Aedes aegypti*, are increasing in prevalence in Quintana Roo state, in southern Mexico (Boletin de Epidemiologia SSA, 2002). In 2002, 65 positive locations (including 12 municipalities) were reported in Quintana Roo. There were 172 cases of DF and 10 of DHF, in 5 municipalities in northern areas of the state and 479 DF cases and 80 DHF cases total.

Insecticides have played an important role in the control of *Ae. aegypti* in Mexico. However, the resistance of vectors to insecticides is one of the most important factors contributing to the ineffectiveness of control programs. Therefore, a critique of the potential for increases in resistance is essential (Georghiou and Mellom 1983). Since 1960 the vector program of the Ministry of Health in Mexico has used a series of insecticides for the control of dengue and malaria (Official Regulations of Mexico, NOM-032-SSA). Dichloro-diphenyl-trichloroethane (DDT) is currently restricted, but had seen prolonged use for indoor house spraying. Malathion was used for ultra-low volume (ULV) space spraying of wide areas for both *Anopheles* and *Aedes* mosquitoes from 1981 to 1999. Ministry of Health vector-control programs have switched to pyrethroids (permethrin) in the last 6 years for adult control. Temephos or 1% Abate® granules have been applied to bodies of water and domestic containers for control of larvae. The insecticides utilized in public health and those directed at agricultural pests have provided prolonged and intense selection pressure for resistance evolution in *Ae. aegypti* and other vectors. Herein we report the first investigations of the principal biochemical mechanisms responsible for resistance in *Ae. aegypti* in Quintana Roo. This information will assist in the management and control of *Ae. aegypti* in Mexico.

MATERIALS AND METHODS

Study sites. Quintana Roo adjoins the states of Yucatan and Campeche, and shares international borders with Guatemala and Belize. The geographic location of Quintana Roo is between latitudes 21°37′N and 17°49′N, and between longitudes 86°44′W and 89°24′52″W. The total area of the state is 50,844 km^2. The history of *Ae. aegypti* larvae and eggs were sampled from 5 municipalities and transported to the Medical Entomology Laboratory of UANL, Mexico. The eggs were placed in plastic containers with water and, after hatching, larvae were provided with dog food, yeast, and brain–heart infusion agar in

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a finely ground mixture. Emerged posteneral adults were provided with water and cotton soaked in 10% honey. Females were provided with rat blood for egg production. Once the colonies were established, 60 F_1 unfed 1-day-old females and 60 males were placed individually in numbered vials, and stored at −70°C.

Biochemical assay. Each mosquito was homogenized in 100 µl 0.01 M potassium phosphate buffer, pH 7.2, and then diluted to 2 ml with the same buffer. Aliquots of 100 µl were transferred to individual microtiter plate wells. Sixty female and 60 male adults per location were analyzed in triplicate per plate. Six different resistance enzymes were evaluated for each mosquito (Brogdon and McAllister 1988a, 1988b, 1997): α and β esterases, mixed-function oxidases (MFO), glutathione-S-transferase (GST), acethylcholinesterase (AChE), and insensitive acethylcholinesterase (iAChE). A minimum of 2 positive and negative controls, depending upon the test, were used per plate. Absorbance was measured with a Benchmark spectrophotometer (Biorad Laboratories, Philadelphia, USA). Results were expressed as a frequency distribution of the absorbance values. The New Orleans strain (NO) was used as the susceptible reference standard. The maximum absorbance value for NO for females and males was used as the susceptibility threshold.

Protein concentration was determined to correct for size variation among the specimens (Brogdon 1984). Statistical analysis was performed with ANOVA ($\alpha = 0.05$) to test for differences in enzymes among populations and between sexes.

RESULTS AND DISCUSSION

Maximum absorbance values for the NO strain appear on Table 1. Table 2 lists, by location and sex, the percentage of mosquitoes that exceeded the NO thresholds. Benito Juarez females exceeded the thresholds for α and β esterases; however, in males, none of the enzyme activities was greater than the susceptibility threshold. All Cozumel females had increased levels of α and β esterases, but only a few individuals had increased iAChE and MFO. Most Cozumel males had an increase in β esterase and just 35% had elevated MFO activity, whereas increases in iAChE and α esterase were negligible. Neither Isla Mujeres females nor males exceeded the threshold. Lazaro Cardenas females did not exhibit increased esterase activity, but MFO activity was elevated. In contrast, Lazaro Cardenas males had increased α and β esterase activities and a few had increased MFO activity. Solidaridad females did not exhibit higher activity in any enzyme system, but one quarter of males had increased MFO activity.

Table 1. Susceptibility threshold based on maximum absorbance of different detoxification mechanisms in New Orleans strain.

Biochemical test	Females	Males
α-esterases	0.65	0.60
β-esterases	0.95	1.02
MFO	0.16	0.12
GST	0.23	0.16
AChE	0.28	0.27
iAChE	0.02	0.02

Correlation coefficients were calculated between population means for males and females for each enzyme system. In none of the 6 systems was absorbance value correlated in males and females (Fig. 1). Benito Juarez and Cozumel females had significantly greater α esterase activities than NO (Fig. 2). Only Lazaro Cardenas males had significantly greater α esterase activities (Fig. 3). Benito Juarez and Cozumel females also had the highest β esterase activities, and Lazaro Cardenas and Cozumel males had significantly higher β esterase activities (Figs. 2 and 3). The rest of the populations also had significantly greater mean activity levels, but differences were only slight (Figs. 2 and 3, Table 3).

Broad-spectrum organophosphate resistance is conferred by the elevated esterases of *Culex* sp. Work is in progress on esterase-resistance mechanisms in a range of *Anopheles* and *Aedes* species. All of these esterases act by rapidly binding and then slowly metabolizing the insecticide (Kadous et al. 1983). Esterases have diverse functions in insects, including proteolysis, nervous system

Table 2. Percentage of female and male *Ae. aegypti* that exceeded the susceptibility threshold established in New Orleans.

	B. Juarez		Cozumel		I. Mujeres		L. Cardenas		Solidaridad	
Biochemical test	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
α esterases	100.00	0	100.00	5.00	0	0	1.67	100.00	0	1.67
β esterases	98.33	0	100.00	65.00	1.67	0	0	65.00	3.33	0
MFO	10.00	3.33	18.34	66.67	11.66	16.67	98.33	20.00	0	58.34
GST	0	0	0	6.67	0	0	0	0	0	0
AChE	0	0	0	0	0	0	0	0	0	0
iAChE	5.00	0	1.67	1.67	0	0	5.00	1.67	0	1.67

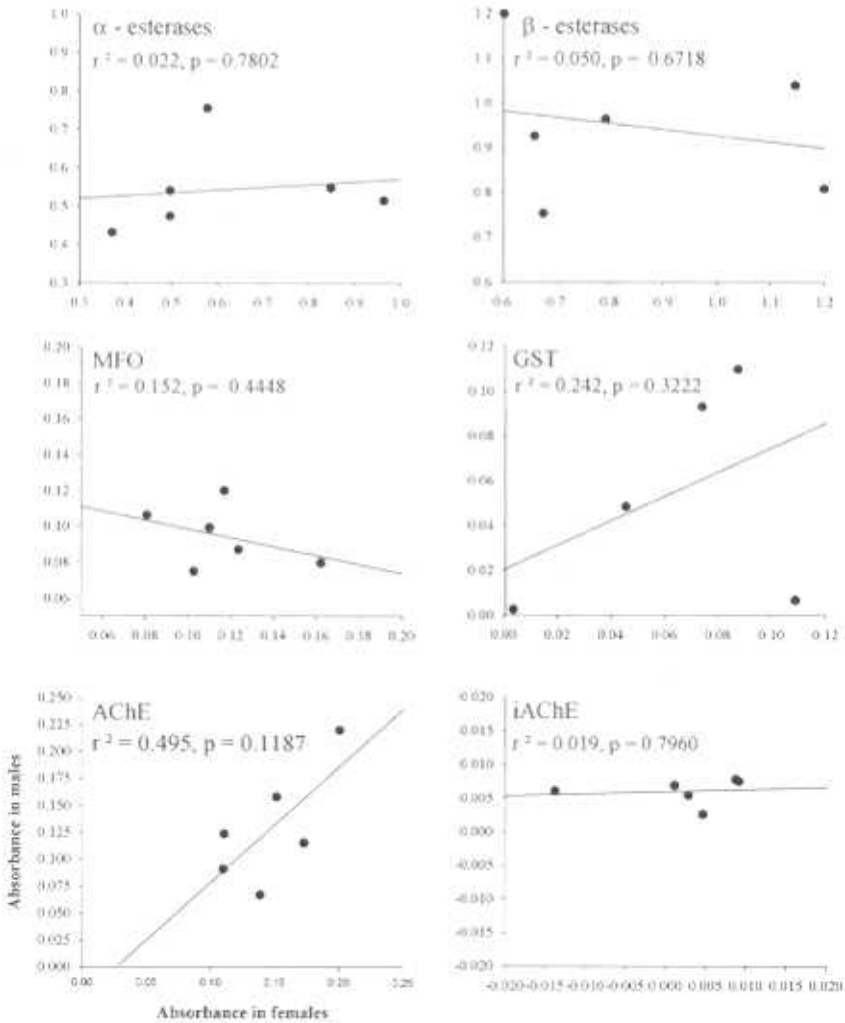

Fig. 1. Correlations between males and females according to absorbance values.

function, hormone metabolism, and xenobiotic metabolism/sequestration (Aldridge 1993). Studies of insecticide resistance have indicated the specific relevance of esterases with regard to xenobiotic metabolism in several insect species, including *Ae. aegypti*. The resistance of vectors to insecticides has indicated that it is one of the most important factors contributing to the ineffectiveness of mosquito control programs. Nonspecific esterases have been reported to be involved in pyrethroid metabolism in several insects (Soderlund et al. 1983, Ruigt 1985) and could play a role in the metabolism of permethrin in *Ae. aegypti*. Even in some cases, there is evidence that esterases are involved in conferring cross resistance to fenitrothion and deltamethrin in the larvae and adults of *Anopheles albimanus* in Guatemala (Brogdon and Barber 1990). Other reports indicate that populations of *Cx p. quinquefasciatus* that were highly resistant to lambda-cyhalothrin and deltamethrin were also highly cross resistant to malathion (Bisset et al. 1997, Bisset et al. 1998). All the populations studied showed the consistent presence of α-esterases, with elevated levels in permethrin-selected populations.

Only Lazaro Cardenas females had a mean MFO absorbance value greater than that of the NO strain (Fig. 2). Cozumel, Solidaridad, and Isla Mujeres males displayed greater MFO activity than the NO strain (Fig. 3). Mixed-function oxidases (MFOs) and nonspecific esterases (NSEs) are commonly involved in the detoxification of permethrin (Miller 1988, Zerba 1988, Ishaaya 1993). Biochemical assays have been developed to measure levels of these enzymes in mosquitoes, and elevated levels of such enzymes are known to enhance insecticide tolerance (Brogdon and McAllister 1988a). Vulule et al. (1999) found evidence that elevated

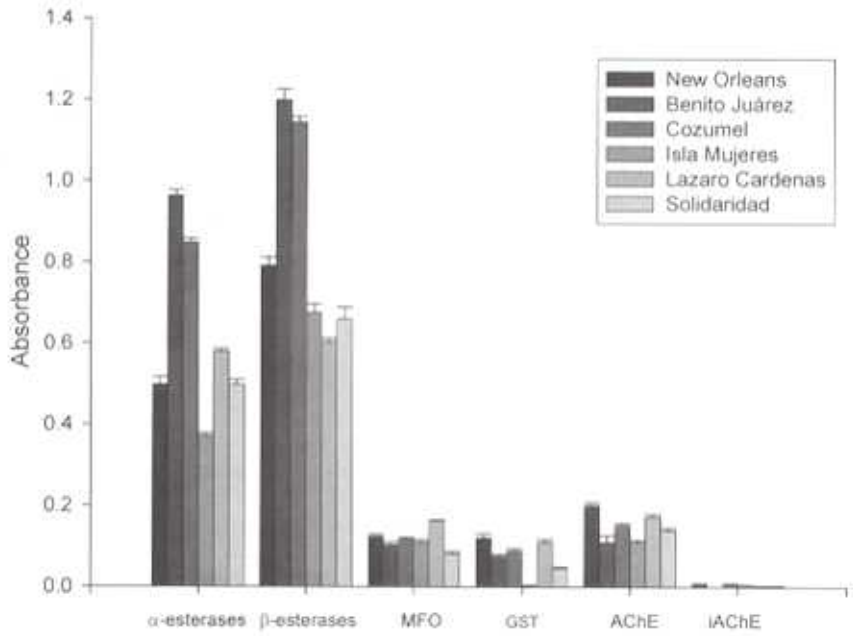

Fig. 2. Mean absorbance values of adult females of *Aedes aegypti* from 5 populations in study and NO strain obtained in biochemical assays.

MFO levels were associated with permethrin tolerance in *An. gambiae*. First, in Kenyan villages (Vulule et al. 1994), after introduction of permethrin-impregnated nets, found that *An. gambiae* males had higher MFO levels than in males from nearby villages where *An. gambiae* remained susceptible to permethrin and impregnated nets were not used. They assumed that high MFO levels in males can be inherited from maternal females selected for permethrin by exposure to permethrin-impregnated nets when foraging for a blood meal. Brogdon et al. (1999) found that only adult females of *An. albimanus* expressed an oxidase mechanism, although they

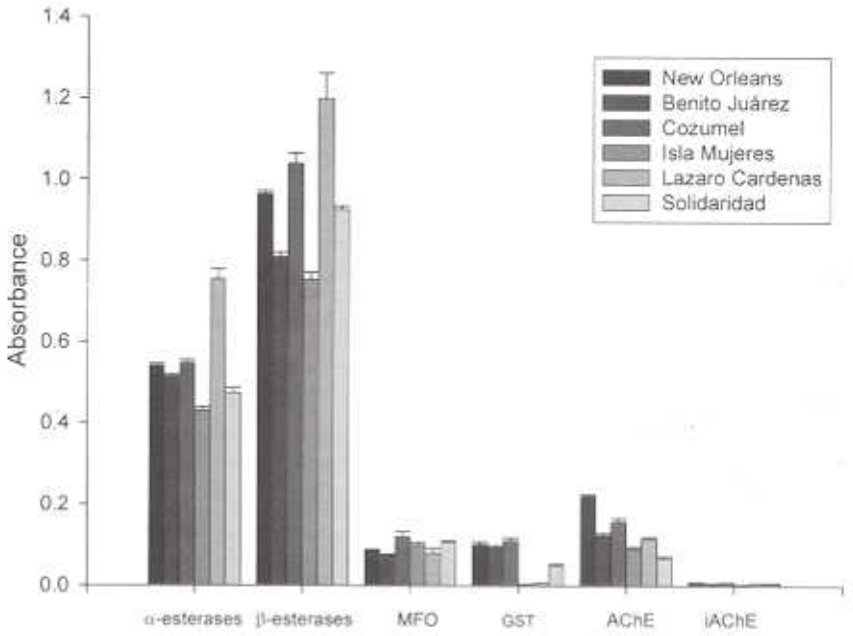

Fig. 3. Mean absorbance values of adult males of *Aedes aegypti* from 5 populations in study and NO strain obtained in biochemical assays.

Table 3. Means and standard deviations of the mean absorbance in each biochemical test of 6 populations of female and male *Ae. aegypti*.

	α esterases		β esterases		MFO		GST		AChE		iAChE	
Females												
New Orleans	0.4979D[a]*	0.0775	0.7913C*	0.0829	0.1236B*	0.0238	0.1207A*	0.0402	0.2005A*	0.0305	0.0091A*	0.0043
Benito Juarez	0.9632B*	0.0565	1.1990A*	0.1082	0.1027D*	0.0298	0.0739C*	0.0220	0.1106D*	0.0640	0.0138B*	0.0440
Cozumel	0.8479A*	0.0413	1.1444B*	0.0565	0.1170BC*	0.0254	0.0872B*	0.0273	0.1515C*	0.0278	0.0087A*	0.0037
Isla Mujeres	0.3714E*	0.0284	0.6752D*	0.0853	0.1100CD*	0.0220	0.0035E*	0.0043	0.1098D*	0.0265	0.0046A*	0.0043
Lazaro Cardenas	0.5787C*	0.0316	0.6019E*	0.0378	0.1621A*	0.0120	0.1087A*	0.0346	0.1727B*	0.0253	0.0011A*	0.0097
Solidaridad	0.4979D*	0.0490	0.6588D*	0.1208	0.0899E*	0.0238	0.0455D*	0.0174	0.1388C*	0.0271	0.0028A*	0.0033
Males												
New Orleans	0.5408B*	0.0225	0.9645C*	0.0302	0.0872CD*	0.0087	0.1010AB*	0.0276	0.2202A*	0.0193	0.0075A*	0.0069
Benito Juarez	0.5150C*	0.0234	0.8088D*	0.0477	0.0750D*	0.0106	0.0932B*	0.0233	0.1238B*	0.0248	0.0061AB*	0.0031
Cozumel	0.5475B*	0.0338	1.0396B*	0.1008	0.1200A*	0.0533	0.1099A*	0.0319	0.1582B*	0.0353	0.0078A*	0.0030
Isla Mujeres	0.4317E*	0.0342	0.7539E*	0.0703	0.0994BC*	0.0285	0.0027D*	0.0038	0.0909D*	0.0211	0.0026C*	0.0019
Lazaro Cardenas	0.7548A*	0.0947	1.1987A*	0.2476	0.0797D*	0.0522	0.0069D*	0.0021	0.1153C*	0.0189	0.0069AB*	0.0021
Solidaridad	0.4743D*	0.0559	0.9260C*	0.0328	0.1064AB*	0.0207	0.0486C*	0.0196	0.0672E*	0.0217	0.0054B*	0.0070

[a] Different letters in columns mean significant difference.
* $P < 0.01$.

said that further investigation is needed to provide the genetic explanation for the absence of expression in males. Many studies have shown that resistant insects have elevated levels of glutathione S-transferase activity in crude homogenates, which suggests a role for GSTs in resistance (Grant 1991, Grant et al. 1991). Multiple forms of these enzymes have been reported for mosquitoes, house fly, *Drosophila*, sheep blow fly, and grass grub (Clark et al. 1984, 1985; Toung et al. 1990). In *Ae. aegypti* at least 2 GSTs are elevated in DDT-resistant insects (Grant and Hammock 1992, Grant and Matsumura 1989), and in *An. gambiae* a large number of different GSTs are elevated, some of which are class I GSTs (Prapanthadara et al. 1993, 1995). The *Ae. aegypti* and *An. gambiae* GSTs in resistant insects are constitutively overexpressed. The GST-2 of *Ae. aegypti* is overexpressed in all tissues except the ovaries of resistant insects (Grant and Hammock 1992). Only Cozumel males had elevated GST over that of NO.

In examining AChE and iAChE (Figs. 2 and 3) activity, all populations showed lower absorbance than the NO strain.

Both α and β esterase assays showed elevated enzyme levels characteristic of resistance mechanisms. These were detected in females of *Ae. aegypti* and occurred at a much higher frequency in areas of intensive use of insecticide, such as in Benito Juarez and Cozumel (tourist areas). Levels of MFO in females were elevated in Lazaro Cardenas, suggesting that they have been selected as a detoxifying mechanism. These elevated levels were focal, but with a high degree of intersite variability. These data are consistent with the view that these resistance mechanisms have been under selection by the insecticides used in Quintana Roo.

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