Published in final edited form as:
Insect Mol Biol. 2012 February ; 21(1): 61–77. doi:10.1111/j.1365-2583.2011.01113.x.

Transcription of detoxification genes following permethrin selection in the mosquito *Aedes aegypti*

Karla Saavedra-Rodriguez[*,†], **Adriana Flores Suarez**[†], **Ildefonso Fernandez Salas**[†], **Clare Strode**[§], **Hilary Ranson**[§], **Janet Hemingway**[§], and **William C. Black IV**[*]

[*]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado 80523-1685

[†]Laboratorio de Entomología Médica, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, México

[§]Vector Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK

Abstract

Changes in gene expression before, during and after five generations of permethrin laboratory selection were monitored in six strains of *Aedes aegypti*: five F_2 – F_3 collections from the Yucatán Peninsula of México and one F_2 from Iquitos, Perú. Three biological replicate lines were generated for each strain. The response to selection was measured as changes in the lethal and knockdown permethrin concentrations (LC_{50}, KC_{50}) and in the frequency of the Ile1,016 substitution in the voltage gated sodium channel (*para*) gene. Changes in expression of 290 metabolic detoxification genes were measured using the "*Aedes Detox*" microarray. Selection simultaneously increased the LC_{50}, KC_{50} and Ile1,016 frequency. There was an inverse relationship between Ile1,016 frequency and the numbers of differentially transcribed genes. The Iquitos strain lacked the Ile1,016 allele and 51 genes were differentially transcribed following selection as compared to 10–18 genes in the Mexican strains. Very few of the same genes were differentially transcribed among field strains but ten cytochrome P_{450} genes were upregulated in more than one strain. Laboratory adaptation to permethrin in *Ae. aegypti* is genetically complex and largely conditioned by geographic origin and preexisting target site insensitivity in the *para* gene. The lack of uniformity in the genes that responded to artificial selection as well as differences in the direction of their responses challenges the assumption that one or a few genes control permethrin metabolic resistance. Attempts to identify one or a few metabolic genes that are predictably associated with permethrin adaptation may be futile.

Keywords

Aedes aegypti; detoxification; knock-down resistance; artificial selection; permethrin resistance

Introduction

Dengue is the most important mosquito-borne viral disease affecting humans, with *Aedes aegypti*, the primary mosquito vector, found in nearly 100 tropical countries. Each year, there are an estimated 50–100 million cases of dengue fever (DF) and, depending on the year, 250,000 –500,000 cases of dengue hemorrhagic Fever (DHF) (Gubler 2005). No vaccines or medicines prevent DF, leaving mosquito control as the best strategy to lessen

Correspond with: William C. Black IV, Dept. of Microbiology, Immunology and Pathology, Colorado State University, Ft. Collins, CO 80523-1685, Office: (970)-491-1081, Lab: (970)-491-8530, FAX: (970)-491-1815, wcb4@lamar.colostate.edu.

dengue transmission. Unfortunately, most national programs are not equipped to manage the prevention and control aspects of a dengue program and heavy reliance continues to be placed on chemical control (Lloyd 2003).

Adults are commonly treated by space spraying of insecticides with ultra-low volume (ULV), thermal fogging, or aerial application during dengue epidemics. Pyrethroids have become the preferred group of insecticides for ULV spraying. These neurotoxins disrupt the function of the voltage-gated sodium channels (VGSC encoded by the orthologue of *para* in *Drosophila* (Loughney et al. 1989)) by preventing the re-polarization phase of action potentials through the nerve cell membrane. Pyrethroid resistance has been reported in *Ae. aegypti* in many countries, including México, Brazil, Cuba, Thailand, Vietnam and others (da-Cunha et al. 2005; Flores et al. 2009; Jirakanjanakit et al. 2007; Kawada et al. 2009; Martins et al. 2009; Paeporn et al. 2005; Rodriguez et al. 2005). Resistance is conferred by target site insensitivity and/or increased metabolic detoxification. Target site resistance involves structural changes in the VGSC that cause reduced pyrethroid binding. Depending on pyrethroid dose, insects do not display the characteristic knockdown effect and are referred to as knockdown resistant ('kdr').

Knockdown resistance arises through nonsynonymous mutations in the VGSC gene and eleven replacement mutations have been identified in *Ae. aegypti* (Brengues et al. 2003; Saavedra-Rodriguez et al. 2007; Yanola et al. 2010). Two nonsynonymous mutations in domain II, segment 6 on the VGSC gene have been detected in Latin American *Ae. aegypti* collections. A valine to isoleucine replacement in codon 1,016 (Ile1,016) and a phenylalanine to cysteine replacement in codon 1,534 (Cys1,534) have been definitively associated with permethrin resistance (Harris et al. 2010; Saavedra-Rodriguez et al. 2008; Saavedra-Rodriguez et al. 2007).

Pyrethroids can also be metabolized by two enzyme families, the cytochrome P_{450} mono-oxygenases (*CYP*) and the carboxyl/esterases (*CCE*) (Hemingway et al. 2004). The metabolism of pyrethroids occurs by ester hydrolysis and oxidation at methyl-, methylene-, alkenyl-, or aryl-substituents and ≥ 80 metabolites have been identified from *cis*- and *trans*-permethrin (Casida et al. 1983). Identification and isolation of individual detoxification enzymes in mosquitoes has been difficult because of the vast array of potential enzymes many of which have similar substrates. Recently, new mosquito genomic tools and microarray technology have been developed to assist in identification of elevated transcription of individual detoxification genes. Microarrays containing probes for putative detoxification genes have been developed for the malaria vector *Anopheles gambiae* (David et al. 2005) and also for the dengue vector *Ae. aegypti* (Strode et al. 2008). Candidate detoxification genes have been identified in pyrethroid resistant strains of *An. gambiae* (David et al. 2005; Djouaka et al. 2008; Muller et al. 2007) and *Ae. aegypti* (Marcombe et al. 2009; Strode et al. 2008). These studies support the hypothesis that gene over-expression is not due to transient upregulation caused by the exposure of the adults to permethrin but instead reflect constitutive over-expression of a common subset of detoxification genes in addition to genes that are uniquely expressed in each population (Muller et al. 2008). The challenge in these studies is that susceptible strains with a common genetic background are usually not available to compare, and results are biased by geographical variation or genetic drift in susceptible reference strains.

The objective of the present study was to investigate whether laboratory selection for permethrin resistance in *Ae. aegypti* derived from Latin America is also associated with changes in detoxification gene expression. The identification of genes associated with permethrin resistance could be a starting point for development of insecticide resistance diagnostic markers in mosquito populations from Latin America. Herein we analyze the

response to selection in five mosquito strains from México known to have the Ile1,016 mutation in a range of frequencies and in a strain from Iquitos, Perú without this mutation. We monitored changes in the lethal concentrations (LC_{50}) and knockdown concentrations (KC_{50}) and in the frequency of the Ile1,016 allele. We demonstrate simultaneous increases in LC_{50}, KC_{50} and Ile1,016 and changes in gene expression as a result of selection. However, microarray analyses demonstrated that laboratory adaptation to permethrin in *Ae. aegypti* is genetically complex, largely conditioned by target site insensivity in the *para* gene and dependent upon the geographic origin of the strain.

Results

Bioassays

Six *Ae. aegypti* lines collected from southern México and one collected from Iquitos, Perú (Table 1) were used in all experiments. The F_2 or F_3 offspring from field collections were designated F_{S0} (no prior selection). The KC_{50}, LC_{50} and Ile1,016 allele frequencies were measured in each of the unselected F_{S0} lines (Table 1). Low levels of resistance ($RR_{50} < 5$) were detected in Iquitos, Lázaro Cárdenas, Solidaridad, and Isla Mujeres unselected F_{S0} lines. KC_{50} and LC_{50} values were similar to those obtained in the New Orleans susceptible line and in Iquitos the Ile1,016 replacement was not detected. Moderate ($RR_{50} = 5 – 10$) levels of resistance were detected in the Calderitas and Mérida F_{S0} lines. High ($RR_{50} >10$) levels of resistance were only detected in the Lagunitas F_{S0}. The Ile1,016 allele was detected in all Mexican unselected lines, with frequencies > 0.5 in Lagunitas, Mérida and Lázaro Cárdenas. As in previous studies there was a correlation ($R^2 = 0.60$, $P = 0.025$) between LC_{50} and KC_{50}. Furthermore, LC_{50} and KC_{50} are strongly correlated (R^2 with $LC_{50} = 0.68$, $P = 0.012$; R^2 with $KC_{50} = 0.83$, $P = 0.002$) with the Ile1,016 frequency squared (the expected frequency of homozygotes).

The general response to permethrin selection was an increase in LC_{50}, KC_{50} and Ile1,016 allele frequencies in all mosquito lines (Figures 2 and 3). However each strain exhibited a distinct response pattern. Table 3 shows the realized heritability (h^2) coefficients for KC_{50} and LC_{50} calculated during the selection process. During selection in Iquitos, Solidaridad, and Mérida, there was a large h^2 for LC_{50} but not for KC_{50}. Using the experiment-wise error rate the h^2 for LC_{50} was only significant in the Iquitos and Solidaridad collections. The Ile1,016 frequency was almost fixed in Solidaridad F_{S5} and showed a significant increase in Mérida. In contrast during Lázaro Cárdenas selection, a large h^2 was observed for both KC_{50} and LC_{50} and Ile1,016 went to fixation. The LC_{50} h^2 during selection in Calderitas was not significant while the KC_{50} h^2 was large and significant and Ile1,016 frequency increased after selection. Selection results for the Lagunitas strain are not shown because all three replicates of this strain died simultaneously in the F_{S2} due to low oviposition rates.

Microarray validation

Expression ratios are expressed as M, the $\log_2$ of mean transcription ratios, where $M = \log_2$ (Cy5/Cy3), Cy5 is the adsorption at 649 nm and Cy3 is the adsorption at 532 nm. Expression ratios from eight genes significantly differentially expressed in the microarray comparisons were correlated with the expression ratios obtained by quantitative-PCR using the same amplified RNA samples (Figure 4). We validated the up-regulation of *CYP4J13, CYP325G3, AaeCOE-8, CYP6Nae1* and *CYP9J22v1* and the down regulation of *AaGSTs1-1*. *AAEL004388* and *AAEL004390* were upregulated in Iquitos F_{S5} but were down-regulated in Calderitas F_{S5} and this trend was validated by quantitative-PCR.

Gene expression - F_{S0} versus New Orleans susceptible strain

Comparisons of transcription patterns among the six F_{S0} strains relative to New Orleans identified a total of 41 differentially expressed genes. Seventeen of these 41 genes appeared in two or more unselected mosquito lines (Table 4a). Thirteen genes were up-regulated including three epsilon *GSTs* (*3, 4*, *6*); seven members of the *CYP9J* family (*CYP9J-9, -10, -22, -23, -28, -32*), two *CYP6* genes (*CYP6-Nae1, -Z6*) and one peroxinectin (*Peroxinectin 4390*). *CYP6P12*, *CYP9AE1* and *AaGSTs1-2* were downregulated. Only *GSTd5* showed inconsistency in expression direction. Table 4b lists the 25 genes that were uniquely differentially transcribed in individual strains. No genes were differentially transcribed in Calderitas or Solidaridad and a single epsilon *GSTe7* was upregulated in Lagunitas. Only two *CYP9* and two *CYP6* genes were up-regulated in Mérida. In contrast, eight genes were differentially transcribed in Lázaro Cárdenas and thirteen genes were differentially transcribed in Iquitos. In total, Solidaridad and Calderitas only exhibited differential transcription of 2–3 genes while Iquitos, Lázaro Cárdenas, Mérida, and Lagunitas exhibited differential transcription at from 10–20 loci.

Gene expression - F_{S1} versus F_{S0}

F_{S1} and F_{S0} comparisons identified 53 genes that responded to one generation of selection (Table 5). Forty-seven of these genes were detected in the Iquitos strain and 18 were differentially transcribed in Calderitas. The remaining Mexican F_{S1} lines had 0–5 differentially expressed genes. Twenty of the 53 genes were differentially expressed in two or more lines (Table 5a). Table 5b lists the remaining 33 genes that were uniquely differentially transcribed in Iquitos (28) or Calderitas (5). Transcription patterns in the remaining genes were inconsistent among strains. For example, eight *Red/Ox* genes were upregulated in Calderitas F_{S1} but were down regulated in Iquitos F_{S1}. Down regulation of a group of four *CCE's* occurred in the Mexican F_{S1} lines but the same genes were upregulated in the Iquitos F_{S1} line.

There was a strong negative correlation between numbers of differentially expressed genes in F_{S0} and the frequency of Ile1,016 in F_{S0} (Figure 5a) suggesting that there may be less selection on metabolic genes in mosquitoes that are already protected by the Ile1,016 allele. This result was consistent whether we analyzed genes differentially up-regulated ($r = -0.87$; $P = 0.023$) or down regulated ($r = -0.86$; $P = 0.029$).

Gene expression - F_{S5} versus F_{S1}

Direct comparisons between the F_{S5} and F_{S1} lines identified 34 genes differentially expressed after four additional generations of selection (Table 6). Lagunitas died in F_{S2} (Figure 2 and 3). Again, most (25/34) differentially expressed genes occurred in Iquitos and the second most occurred in Calderitas (13). Solidaridad, Lázaro Cárdenas, and Mérida respectively had ten, seven and two genes differentially expressed. Sixteen genes were differentially expressed after four additional generations of selection in two or more of the F_{S5} strains (Table 6a). Five peroxinectins were upregulated in Iquitos F_{S5} but down-regulated in the Mexican F_{S5} lines.

There was again a negative correlation between the number of differentially transcribed genes in F_{S5} and the frequency of Ile1,016 in F_{S5} (Figure 5b). This correlation was nonsignificant largely because the frequency of Ile1,016 had approached fixation in the four Mexican strains. Figure 5c shows a strong negative correlation between the frequency of the Ile1,016 allele in F_{S0} prior to selection and the number of genes differentially expressed in F_{S5} after 5 generations of selection. This further supports an hypothesis of low amounts of selection acting on metabolic genes in mosquitoes already protected by the Ile1,016 allele.

Gene expression - F_{S5} versus New Orleans (indirect)

We made an indirect (statistical) comparison of F_{S5} with New Orleans using the Limma package (http://bioinf.wehi.edu.au/limma/ available on www.bioconductor.org) following (Muller et al. 2007) in each of the strains. This was done to make a comparison of expression ratios (M) among F_{S5} strains standardized to the uniform genetic background provided by the New Orleans strain. F_{S5} vs. F_{S1} M ratios were adjusted to F_{S1} vs. F_{S0} M ratios to provide an indirect M ratio of F_{S5} vs. F_{S0}. This indirect ratio was then adjusted to F_{S0} vs. New Orleans M ratios to provide an indirect M ratio of F_{S5} vs. New Orleans. This adjusted all 5 F_{S5} M ratios according to initial differences in transcription among the five F_{S0} strains relative to New Orleans.

Twenty four genes were differentially expressed after 5 generations of selection in two or more of the F_{S5} strains (Table 7a). This included two sigma class *GST* (*AaGSTs1-1, -2*) which were independently selected for decreased transcription in Iquitos, Lázaro Cárdenas and Mérida strains. Ten *CYP*s were independently selected for increased rates of transcription. There was little differential transcription of esterases. The transcription rates of five peroxinectins were greatly increased from 3 – 20 fold (linear scale) through permethrin selection in Iquitos. In contrast, in Calderitas and Mérida, the transcription rates of these peroxinectins decreased from 2–6 fold (linear scale) through permethrin selection.

Discussion

Comparative microarray analysis of transcription rates of detoxification genes before, during and after laboratory selection of *Ae. aegypti* strains with permethrin revealed four important trends. First, artificial selection with permethrin simultaneously increased the LC_{50} (Figure 2), KC_{50} (Figure 3) and the frequency of the Ile1,016 allele. Second, there was a consistent inverse relationship between the frequency of the Ile1,016 allele and the numbers of genes that became differentially transcribed, regardless of whether those genes were up- or down-regulated (Figure 5). This relationship was detected for Ile1,016 frequency in F_{S0} compared to F_{S1} (Figure 5a), Ile1,016 frequency in F_{S5} (Figure 5b) or for Ile1,016 frequency in F_{S0} compared to numbers of genes that become differentially transcribed in F_{S5} (Figure 5c). Third, very few of the 290 detoxification genes on the detox chip were differentially transcribed in two or more field strains (Table 4–7a). There were 17 genes (5.8%) differentially transcribed in the F_{S0} versus New Orleans comparisons, 20 genes (6.8%) in the F_{S1} versus F_{S0} comparisons, 13 genes (4.4%) in the F_{S5} versus F_{S1} comparisons and 24 genes (8.2%) in the F_{S5} versus New Orleans.

Instead Tables 4–7b list the many genes that were uniquely differentially transcribed in each of the five strains. Fourth, adaptation to permethrin did not cause a uniform up- or down regulation of detoxification genes. An intuitive *a priori* hypothesis is that upregulation of a detoxification gene should accompany selection of insecticide resistance. For example, this pattern was seen in Table 7 with some of the *CYP* genes. However, none of these *CYP* genes appeared in all five strains and in the case of the peroxinectins, selection caused an increase in Iquitos but a simultaneous decrease in the Calderitas and Mérida strains.

The observation that artificial selection with permethrin simultaneously increased the LC_{50} and KC_{50} along with the frequency of the Ile1,016 allele is not surprising. Table 3 indicates a large and significant h^2 for LC_{50} for all strains except for Calderitas. This suggests that there is a large amount of additive genetic variance for permethrin adaptive genes in the five field strains. In Figure 2, the LC_{50} in Calderitas and Mérida appears to increase up until generation 4 when it suddenly decreases. This might have been associated with lethal or deleterious recessive alleles linked to the *para* locus that approach fixation as the Ile1,016 allele increased in frequency. However similar patterns were not noted in Lázaro Cárdenas

or Solidaridad. The h^2 for KC_{50} was not expected to be as large as LC_{50} because Ile1,016 allele is mostly recessive in its expression (Saavedra-Rodriguez et al. 2008; Saavedra-Rodriguez et al. 2007). This also would explain why h^2 in Lázaro Cárdenas was greater than one. As Ile1,016 approached fixation in Lázaro Cárdenas there was a nonlinear increase in Ile1,016 homozygotes; the response to selection (R) was greater than the selection differential (S) and since $h^2 = R/S$, $h^2 > 1$. h^2 was small in Iquitos where Ile1,016 was effectively absent. Note however that Ile1,016 was present albeit in very low frequency in Iquitos F_{S5} (Figure 3). Eventually selection increased its frequency to 0.03. This predicts fewer than 9×10^{-4} Ile1,016 homozygotes in the F_{S5}, too few for selection to act upon especially when fewer than 500 individuals survived to pass on genes in the next generation. But (Saavedra-Rodriguez et al. 2008; Saavedra-Rodriguez et al. 2007) showed that Ile1,016 heterozygotes have a higher recovery rate following knockdown and this might account for the early initial appearance of Ile1,016 in Iquitos F_{S5}.

The observation of a consistent inverse relationship between Ile1,016 frequency and the numbers of genes that became differentially transcribed through selection is one of the most interesting outcomes of this experiment. The most parsimonious explanation for this observation is that Ile1,016 confers a much larger selective advantage to both homozygous and heterozygous mosquitoes than the metabolic detoxification genes represented on the *Ae. aegypti* detox chip. Mosquitoes lacking or with a low frequency of Ile1,016 (e.g. Iquitos, Calderitas) can only very slowly evolve permethrin resistance by accumulating slight advantages conferred by the many detoxification genes. In contrast, these same genes would confer only slight additional survival advantages to the Mexican mosquitoes with a high frequency of Ile1,016.

Few of the 290 detoxification genes on the detox chip were differentially transcribed in two or more of the field strains. This outcome can be largely attributed to the pre-existing high frequency of Ile1,016 in the Mexican strains. It would be interesting to compare the relatively long list of genes differentially transcribed in Iquitos with other *Ae. aegypti* field strains in which *para* substitutions are in low frequency. The observation that many were uniquely transcribed in individual strains suggests that permethrin adaptation in *Ae. aegypti* is genetically complex. It is clear that this adaptation is largely conditioned by target site insensitivity in the *para* gene but also probably reflects the frequency of various metabolic genes in different populations. This phenomenon occurs among geographically proximate collections; Lázaro Cárdenas, Solidaridad, and Lagunitas are located within the city of Chetumal.

A potential criticism of our experimental design is that it did not test whether differentially transcribed genes were a result of adaptation to the laboratory rather than to permethrin. To do so would have required analyzing microarrays on each of five lines (maintained without selection) in the F_{SO}, F_{S1} and F_{S5} generations and this was not done. However, we have applied the same experimental design to identify genes responsive to temephos (an organophosphate insecticide) in four of the same collection sites (Saavedra-Rodriguez - unpublished). If differential transcription was a result of laboratory adaptation or reflected a xenobiotic metabolism response to insecticide exposure then the same sets of genes should have been differentially expressed in the present study and in the temephos study. This was examined by sorting all of the genes in Table 7 with all of the genes with significant differential expression between the F_{S5} vs. NO in the temephos experiment to identify genes with differential expression in both permethrin and temephos experiments (Table 8). Of the 70 differentially transcribed genes in the current experiment (Table 7) and the 80 differentially transcribed genes in the temephos experiment, there were 15 genes with differential expression in both experiments. Those highlighted in grey in Table 8 were differentially transcribed in the same direction in the same collection in both experiments

and could therefore represent laboratory adaptation. Patterns consistent with lab adaptation were seen in three genes in Mérida and six genes in Iquitos. No comparisons fit these criteria for Calderitas and only one fit in Lázaro Cárdenas. Of the 27 comparisons in Table 8, 10 were consistent with a pattern expected for lab adaptation. But none appeared in all four collections. In general then there was little or no consistent evidence of laboratory adaptation for the majority of the 70 differentially transcribed genes in the current experiment.

A unique outcome of this selection experiment is that many genes exhibited differential expression. Toxicity studies in vertebrates have demonstrated that pyrethroids generate reactive oxygen species (ROS) and cause oxidative stress (Kale et al. 1999; Sayeed et al. 2003). Several studies have demonstrated that pyrethroid intoxication alters the antioxidant system in erythrocytes and causes pyrethroid-induced lipid peroxidation (LPO) (Fetoui et al. 2008; Fetoui et al. 2010). The increased oxidative stress results in an increase in the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Kale et al. 1999). While no similar studies have been made insects, it is interesting that peroxinectins were prominent among the oxidative stress genes listed in Table 7. Peroxinectins are cell adhesive ligands synthesized in invertebrate hemocytes and stored in secretory granules in an inactive form (Schmidt et al. 2010). Peroxinectin is released in response to elicitors, such as bacterial endotoxins (lipopolysaccharide (LPS) or beta-1,3-glucans) and are subsequently activated outside the cells to mediate hemocyte attachment and spreading (Johansson and Soderhall 1989). Peroxinectins are multifunctional molecules involved in encapsulation, opsoninization, and can act as peroxidases (Johansson et al. 1995). In the latter respect, peroxinectins are similar to a family of vertebrate peroxidases that includes myeloperoxidase (Johansson et al. 1995). Peroxinectin is the first protein discovered to have combined adhesive/peroxidase functions. It is possible that peroxinectins have a protective function in insects. If exposure to pyrethroids causes insect tissues to produce ROS, LPOs, and elevate oxidative stress that damage membranes; the peroxidase activity of peroxinectin may protect cells against this damage.

An intuitive *a priori* hypothesis is that upregulation of a detoxification gene should accompany insecticide selection. For example, with its expression mostly restricted to the indirect flight muscle and neurons, Flanagan and Smythe (2011) suggested that *AaGSTs1-1* may serve a protective role in highly aerobic tissues or tissues sensitive to oxidative damage. Thus its down regulation in this study seems counterintuitive. The observation that permethrin laboratory adaptation did not cause a uniform up- or downregulation of detoxification genes suggests the possibility that some detoxification genes may be disadvantageous in mosquitoes with Ile1,016. This might also explain why selection increased transcription of the five peroxinectins in Iquitos strain while decreasing transcription in the Calderitas and Mérida strains. Perhaps, in the absence of pyrethroids or in the presence of Ile1,016, peroxinectin expression is selected against. Peroxinectins were only one of a few mechanisms that the Iquitos strain had for pyrethroid protection. Prior to selection of Ile1,016, expression of peroxinectins was 2–6 fold greater in Calderitas and Mérida but as Ile1,016 increased in frequency increased peroxinectin activity was selected against.

Uniform upregulation was seen with some of the *CYP* genes in Table 7 even though no gene was significant in all five strains. Enzymes of the *CYP6* family are known to be generally involved in detoxification of xenobiotics in insects. In *An. gambiae*, fourteen *CYP6* family genes are clustered on chromosome III. *CYP6Z1* was shown to metabolize DDT, carbaryl and xanthotoxin, while *CYP6Z2* was capable of metabolizing carbaryl (Chiu et al. 2008). Our results show that *CYP6Z6* was upregulated in the Calderitas, Mérida and Lagunitas F_{S0} strains. After selection, this gene was upregulated in Calderitas, Mérida and Iquitos.

CYP6Z6 has a 63% and 59% identity with *An. gambiae CYP6Z2* and *CYP6Z1*, respectively, suggesting that it might have a role in detoxification of permethrin. *CYP6Z6* was also over-transcribed in larval and adult *Ae. aegypti* in a permethrin resistant strain from Martinique (Marcombe et al, 2009). Larvae exposed to sublethal concentrations of the herbicide glyphosate and benzo[a]pyrene significantly induced *CYP6Z6* (Riaz et al. 2009). Paralogues of *CYP6Z6*, *-8* and *-9* also were induced by fluoranthene (Poupardin et al. 2008) and were over transcribed in a permethrin resistant strain from Northern Thailand (Strode et al, 2008). CYP6 gene transcription was also analyzed in different *Ae. aegypti* mosquito tissues, showing that genes in the subfamilies *CYP6Z*, *CYP6M* and *CYP6N* are preferentially transcribed in the alimentary canal, with the exception of *CYP6Z6* which is preferentially transcribed in head and anterior midgut (Poupardin et al. 2010).

A second *CYP6* member that responded to selection was *CYP6Nae1* (AAEL009126, new VectorBase ID = *CYP6N6*). This gene was upregulated in the unselected strains from Mérida and Lagunitas, however, after five generations of permethrin selection, *CYP6Nae1* was significantly up regulated in all Mexican strains. *CYP6Nae1* has not been associated with insecticide detoxification in *Ae. aegypti*, however, paralogues *CYP6N12* (AAEL009124, 87% nucleotide similarity) and *CYP6N11* (AAEL009138, 58% nucleotide similarity) were induced in larvae by the herbicide glyphosate (Riaz et al, 2009). In a microarray analysis, *CYP6N12* was induced in larvae exposed to fluoranthene, however, RT-PCR showed that this gene is also induced by permethrin, temephos and copper (Poupardin et al, 2008).

CYP4J13 was upregulated in larvae from a Isla Mujeres (México) permethrin resistant strain and in adults from a Northern Thailand resistant strain (Strode, et al 2008) and a paralogue of this gene, *CYP4J15* (AAEL013556, 48% nucleotide similarity) was upregulated in larvae in a permethrin resistant strain from Martinique. On the other hand, *CYP325G3* has not been previously identified in *Ae. aegypti* insecticide resistant strains, although, members of this family were upregulated in a permethrin resistant strain of *An. gambiae* (David et al. 2005).

CYP9J family genes are commonly up regulated in insecticide resistant strains. Our results indicate that genes *CYP9J-9*, *-10, -22, -23, -28* and *-32* are upregulated before or after selection in most field strains. Except for *CYP9J32*, most of the *CYP9J* genes occur on chromosome III in two clusters at 4 cM on the *p* arm and at 50 cM in the *q* arm (Strode et al., 2007; Saavedra-Rodriguez et al., 2008). Genes of these clusters were also identified as QTL involved in permethrin resistance in mosquitoes from México (Saavedra-Rodriguez et al. 2008). *CYP9J-9, -22,* and *-23* were also upregulated in a permethrin resistant strain from Martinique (Marcombe et al, 2008). Five *CYP9J* genes were upregulated in a Northern Thailand resistant strain and seven were upregulated in a Mexican permethrin resistant strain (*CYP9J-8, -10, -19, -24, -27, -28,* and *-32*) (Strode et al, 2008). It is possible that *CYP9J* genes in the 4 cM and 50 cM clusters were increased solely by selection for Ile1,016 located at 31 cM on chromosome III. In other words, selecting Ile1,016 could have swept (increased) other alleles on chromosome III that did not necessarily confer resistance. If this were true then we would have expected no increase in *CYP9J* genes in Iquitos in which Ile1,016 does not occur. However Table 7 shows that *CYP9J23 and CYP9J28,* both in the 50cM cluster, increased 2.5 fold in Iquitos. Still the possibility of a selective sweep cannot be excluded for *CYP9J22* and *CYP9J9* (both in the 4cM cluster) which increased 3–5 fold in three of the Mexican strains. Furthermore while there are 12 *CYP9J* genes in the 4 cM cluster and 7 in the 50 cM cluster, only 7 increased in expression during selection.

A common goal of the current study and of the many microarray studies cited above is to identify the principal genes conditioning pyrethroid resistance. However, the lack of uniformity in the genes that responded to artificial and natural selection as well as in the

direction of their responses (e.g. peroxinectins) poses a critical question regarding the assumption that one or a few genes control pyrethroid metabolic resistance. Table 4 compares transcription patterns in six field strains with transcription in the standard susceptible New Orleans strain and basically shows that all collections have very different transcription patterns. Even the proximate collections of Lagunitas, Lázaro Cárdenas, and Solidaridad (Figure 1) exhibited distinct transcription profiles. This result might be a result of genetic drift arising when subpopulations are established by one or a few of the adults that survived (i.e. founder effects) control efforts by permethrin and other tactics. However, Tables 6 and 7 show that application of a uniform artificial selection regime did not cause collections to converge in their transcription profiles. These results collectively suggest that *Ae. aegypti* populations may have a multitude of genes that can respond to pyrethroid selection. If so, attempts to identify one or a few metabolic genes that are predictably associated with pyrethroid adaptation may prove futile.

Experimental procedures

Collection sites and colony rearing conditions

The Mexican lines were collected as larvae from the states of Yucatán (Mérida) and from Quintana Roo (Lázaro Cárdenas, Solidaridad, Calderitas and Lagunitas) in the city of Chetumal (Figure 1). Eggs of the Iquitos strain were from a laboratory strain collected from and maintained in Iquitos by Dr. Amy Morrison. F_1 or F_2 offspring were reared to adults, bloodfed, and eggs were collected and shipped to Colorado State University where additional F_2 or F_3 generations were reared to generate sufficient larvae and adults for bioassay and for initiation of three replicate lines for artificial selection with permethrin. Each replicate line was initiated with 100 males and 100 females placed in a 30 cm^3 cage (BugDorm-1, Mega View Science, Co). Mosquito larvae were reared in plastic containers holding 2 L of water maintained at 30°C and provided with brewer's yeast. Pupae were placed in 30 cm^3 cages and eclosed adults were provided with a 10% (w/v) sucrose solution. Adults were fed on an artificial membrane feeder containing defibrinated sheep blood every three days. Cages were housed in growth chambers held at 14:10 photoperiod, and 28°C with 85% relative humidity.

Bioassays and permethrin selection

F_{S0} adults were bioassayed to estimate the LC_{50} and KC_{50} for permethrin (47.6% *cis* – 50.4 % *trans*; Chem Service, West Chester, PA). The insides of 250 ml Wheaton bottles were coated with 1 ml of acetone containing five different concentrations of permethrin. Twenty-five 3–4 day old adults were gently aspirated into each bottle. The number of mosquitoes unable to fly was scored every 10 minutes for up to one hour. After exposure, all mosquitoes were gently transferred into a 400 ml cardboard carton (Huhtamaki, USA) and mortality was scored after 24 hours. Each bioassay was performed in triplicate to have ~75 mosquitoes per concentration. LC_{50} and KC_{50} were estimated using a logistic regression model in R version 2.11.1 (http://cran.r-project.org/) (Source Code in Supplement 1). Confidence limits were calculated using the IRMA quick calculator software (http://sourceforge.net/projects/irmaproj/files/).

Adult permethrin selection was performed in triplicate for each strain. Replicates were maintained during five generations of permethrin selection. The first selection consisted in exposing 250–700 3–4 day old females and males from each line to the previously estimated LC_{50}. Permethrin exposure time was for one hour and ~100 adults were aspirated into each impregnated bottle. Knocked down and alive mosquitoes were transferred to a 400 ml cardboard carton (Huhtamaki, USA) and mortality was registered at 24 hours after exposure. Survivors for each selection were transferred to insect rearing cages and blood fed. At the

beginning of each of the subsequent four generations of selection, a bioassay was performed to estimate the LC_{50} following the previous generation of selection. From each replicate 300 – 700 adults were then selected using the new LC_{50}. For some mosquito lines in certain generations, a lower permethrin concentration was used for selection, depending upon the damage inflicted by permethrin exposure (i.e. multiple appendage loss or blood feeding appetite loss). Selected line names were designated Fs_1, Fs_2, Fs_3, Fs_4 or Fs_5 indicating the generation of selection.

Realized Heritability

Realized heritability (h^2) is calculated as the ratio of the response to selection (R) to the selection differential (S) and is correlated with the amount of additive genetic variance for a trait. A low h^2 predicts no additive genetic variance for a trait and a poor or very slow response to artificial selection while a high h^2 predicts a large additive genetic variance at one or a few loci that condition a trait and predicts a rapid response to artificial selection. When estimating h^2 for insecticide resistance, R is estimated as the mean difference between the LC_{50} in offspring of surviving parents as compared to the LC_{50} in the parents and S is the proportion of surviving parents.

Realized heritability was calculated using the method of Tanaka and Noppun (1989). The LC_{50} or KC_{50} were estimated in R version 2.11.1 (Supplement 1) and transformed with the natural logarithm. The proportion surviving (p_t) was the weighted average of the survival rate among the three replicates in generation t. The standard deviation of susceptibility (σ_t) was the reciprocal of the slope from the logistic regression analysis in generation t. The intensity of selection (i_t) was obtained using p_t in Appendix-Table A in (Falconer 1989). S_t was σ_t multiplied by i_t. R_t was the change in $\ln(LC_{50})$ or $\ln(KC_{50})$ from generation t to $t+1$. The cumulative response to selection R was the sum of $R_{t = 1,\ldots 5}$ while the cumulative selection differential S was the sum of $S_{t = 1,\ldots 5}$. Following Tanaka and Noppun (1989), significance of the regression of R on S was tested by ANOVA in R 2.11.1 where the slope ($h^2 = \Delta R/\Delta S$) was treated as a continuous variable with one degree of freedom.

Isoleucine 1,016 allele frequency

Fifty mosquitoes from the unselected F_{S0}, and the selected F_{S1} and F_{S5} lines were genotyped. DNA was isolated from individual mosquitoes by salt extraction (Black and DuTeau 1997) and suspended in 200 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0). The frequency of the Ile1,016 allele was determined by melting curve PCR following (Saavedra-Rodriguez et al. 2007).

Differential expression profiles

The DNA microarray '*Aedes Detox Chip*' v.2 (Strode et al. 2008), was used to follow changes in the expression of detoxification genes. This microarray contains 318 70-mer probes representing 290 detoxification genes including 183 *CYP*, 28 *GST*, 44 *CCE* and 35 additional enzymes potentially involved in response to oxidative stress in *Ae. aegypti*. The three biological replicates from each mosquito line were processed separately. Direct comparisons between three different points of selection were: 1) 'Unselected- F_{S0}' relative to the New Orleans susceptible reference strain; 2) Permethrin selected-F_{S1} relative to F_{S0}, and 3) F_{S5} relative to F_{S1}. RNA isolations, cDNA synthesis and labeling reactions were performed independently for each biological replicate. Total RNA was extracted from batches of thirty 3 day old adults (15 females and 15 males) using the RNeasy ®Midi Kit (Qiagen) according to manufacturer's instructions for total RNA isolation from animal tissues. Total RNA quantity and quality was assessed using a Nanodrop® spectrophotometer. cDNA synthesis, labeling reaction and hybridization to the array were performed as in Strode et al. (2008).

Spot finding, signal quantification and spot superimposition for both dye channels were performed using the Axon Instruments Genepix Personal 4100A laser scanner and Genepix 5.1 software (Axon Instruments, Molecular Devices, Union City, CA, USA). Spots that did not satisfy the criteria described by Strode et al. (2008) were excluded from analysis. Normalization and statistical analyses were performed on R using the limma package http://bioinf.wehi.edu.au/limma/ available on www.bioconductor.org following (Muller et al. 2007). Results are expressed as M, the $\log_2$ of mean transcription ratios where M = $\log_2$(Cy5/Cy3). An arbitrary threshold of M = 1 (i.e. two-fold) was used to identify differentially expressed genes. The probability threshold was set at 3.00 (i.e. $-\log_{10}$ (0.001)).

Quantitative PCR for microarray validation

Transcription profiles of eight differentially expressed genes in the F_{S5} strains were validated by real-time quantitative PCR using the same RNA samples as were used for microarray experiments. Four μg of total amplified RNA (RiboAmpTM RNA amplification kit) were used for cDNA synthesis with Superscript Reverse Transcriptase III (Invitrogen) and oligo-$(dT)_{15–18}$ primer (Invitrogen). Resulting cDNAs were diluted 100 times for real-time quantitative PCR reactions. Primer pairs used for quantitative PCR (Table 2) were optimized and tested by melting curve analysis and agarose gel electrophoresis to insure that they produced a unique amplification product. Real-time quantitative 20 μL PCR were performed in triplicate on a CFX-96 system (BioRad) using iQ SYBR Green Supermix (BioRad), 0.3μM of each primer and 5 μL of diluted cDNAs. For each gene analyzed, a cDNA dilution scale from 1:1,000,000 times was performed to assess efficiency of PCR. Data analysis was performed according to the ΔCt method taking into account PCR efficiency (Pfaffl 2001) and using the gene encoding the ribosomal protein L8 (Vector Base ID *AAEL000987*) for normalization. Results were expressed as average M obtained in the three replicates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Larvae were collected by collaborators at the Universidad Autónoma de Nuevo León and Universidad Autónoma de Yucatán. The Iquitos, Perú collection was provided by Amy Morrison (UC Davis). This work was funded in part by NIH-NIAID U01 AI088647.

References

Black, WC.; DuTeau, NM. RAPD-PCR and SSCP analysis for insect population genetic studies. In: Crampton, JM.; Beard, CB.; Louis, C., editors. Molecular Biology of Insect Disease Vectors: A Methods Manual. London: Chapman & Hall; 1997. p. 362-363.

Brengues C, Hawkes NJ, Chandre F, McCarroll L, Duchon S, Guillet P, Manguin S, Morgan JC, Hemingway J. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. Medical and Veterinary Entomology. 2003; 17:87–94. [PubMed: 12680930]

Casida JE, Gammon DW, Glickman AH, Lawrence LJ. Mechanisms of Selective Action of Pyrethroid Insecticides. Annual Review of Pharmacology and Toxicology. 1983; 23:413–438.

Chiu TL, Wen ZM, Rupasinghe SG, Schuler MA. Comparative molecular modeling of Anopheles gambiae CYP6Z1, a mosquito P450 capable of metabolizing DDT. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:8855–8860. [PubMed: 18577597]

da-Cunha MP, Lima JBP, Brogdon WG, Moya GE, Valle D. Monitoring of resistance to the pyrethroid cypermethrin in Brazilian *Aedes aegypti* (Diptera : Culicidae) populations collected between 2001 and 2003. Memorias Do Instituto Oswaldo Cruz. 2005; 100:441–444. [PubMed: 16113895]

David JP, Strode C, Vontas J, Nikou D, Vaughan A, Pignatelli PM, Louis C, Hemingway J, Ranson H. The *Anopheles gambiae* detoxification chip: A highly specific microarray to study metabolic-based insecticide resistance in malaria vectors. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:4080–4084. [PubMed: 15753317]

Djouaka RF, Bakare AA, Coulibaly ON, Akogbeto MC, Ranson H, Hemingway J, Strode C. Expression of the cytochrome P450s, CYP6P3 and CYP6M2 are significantly elevated in multiple pyrethroid resistant populations of *Anopheles gambiae* s.s. from Southern Benin and Nigeria. Bmc Genomics. 2008; 9 -

Falconer, DS. Introduction to Quantitative Genetics, Third Edition. Harlow, Essex, UK/New York: Longmans Green/John Wiley & Sons; 1989.

Fetoui H, Garoui EM, Makni-Ayadi F, Zeghal N. Oxidative stress induced by lambda-cyhalothrin (LTC) in rat erythrocytes and brain: Attenuation by vitamin C. Environmental Toxicology and Pharmacology. 2008; 26:225–231. [PubMed: 21783916]

Fetoui H, Makni M, Garoui EM, Zeghal N. Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid. Experimental and Toxicologic Pathology. 2010; 62:593–599. [PubMed: 19735994]

Flanagan JU, Smythe ML. Sigma-class glutathione transferases. Drug Metabolism Reviews. 2011; 43:194–214. [PubMed: 21425928]

Flores AE, Solis GR, Salas IF, Ramos FJS, Garcia GP. Resistance to Permethrin in *Aedes aegypti* (L.) in Northern México. Southwestern Entomologist. 2009; 34:167–177.

Gubler D. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. Revista Panamericana De Salud Publica-Pan American Journal of Public Health. 2005; 17:221–224. [PubMed: 15969972]

Harris AF, Rajatileka S, Ranson H. Pyrethroid Resistance in *Aedes aegypti* from Grand Cayman. American Journal of Tropical Medicine and Hygiene. 2010; 83:277–284. [PubMed: 20682868]

Hemingway J, Hawkes NJ, McCarroll L, Ranson H. The molecular basis of insecticide resistance in mosquitoes. Insect Biochemistry and Molecular Biology. 2004; 34:653–665. [PubMed: 15242706]

Jirakanjanakit N, Rongnoparut P, Saengtharatip S, Chareonviriyaphap T, Duchn S, Bellec C, Yoksan S. Insecticide susceptible/resistance status in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera : Culicidae) in Thailand during 2003–2005. Journal of Economic Entomology. 2007; 100:545–550. [PubMed: 17461081]

Johansson MW, Lind MI, Holmblad T, Thornqvist PO, Soderhall K. Peroxinectin, a Novel Cell-Adhesion Protein from Crayfish Blood. Biochemical and Biophysical Research Communications. 1995; 216:1079–1087. [PubMed: 7488183]

Johansson MW, Soderhall K. Cellular-Immunity in Crustaceans and the Propo System. Parasitology Today. 1989; 5:171–176. [PubMed: 15463205]

Kale M, Rathore N, John S, Bhatnagar D. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicology Letters. 1999; 105:197–205. [PubMed: 10355540]

Kawada H, Higa Y, Komagata O, Kasai S, Tomita T, Nguyen TY, Luu LL, Sanchez RAP, Takagi M. Widespread Distribution of a Newly Found Point Mutation in Voltage-Gated Sodium Channel in Pyrethroid-Resistant *Aedes aegypti* Populations in Vietnam. Plos Neglected Tropical Diseases. 2009; 3 -

Lloyd, LS. Best Practices for Dengue Prevention and Control in the Americas Vol. Strategic Report 7. City: U.S. Agency for International Development; 2003. Best Practices for Dengue Prevention and Control in the Americas; p. 106

Loughney K, Kreber R, Ganetzky B. Molecular Analysis of the Para Locus, a Sodium-Channel Gene in Drosophila. Cell. 1989; 58:1143–1154. [PubMed: 2550145]

Marcombe S, Poupardin R, Darriet F, Reynaud S, Bonnet J, Strode C, Brengues C, Yebakima A, Ranson H, Corbel V, David JP. Exploring the molecular basis of insecticide resistance in the dengue vector Aedes aegypti: a case study in Martinique Island (French West Indies). Bmc Genomics. 2009; 10 -

Martins AJ, Lima JBP, Peixoto AA, Valle D. Frequency of Val1016Ile mutation in the voltage-gated sodium channel gene of *Aedes aegypti* Brazilian populations. Tropical Medicine & International Health. 2009; 14:1351–1355. [PubMed: 19735371]

Muller P, Donnelly MJ, Ranson H. Transcription profiling of a recently colonised pyrethroid resistant *Anopheles gambiae* strain from Ghana. Bmc Genomics. 2007; 8 -

Muller P, Warr E, Stevenson BJ, Pignatelli PM, Morgan JC, Steven A, Yawson AE, Mitchell SN, Ranson H, Hemingway J, Paine MJI, Donnelly MJ. Field-Caught Permethrin-Resistant *Anopheles gambiae* Overexpress CYP6P3, a P450 That Metabolises Pyrethroids. Plos Genetics. 2008; 4 -

Paeporn P, Supaphathom K, Sathantriphop SPM, Sangkitporn S. Insecticide susceptibility of *Aedes aegypti* in Tsunami-affected Areas in Thailand. Dengue Bulletin. 2005; 29:210–213.

Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research. 2001; 29 -

Poupardin R, Reynaud S, Strode C, Ranson H, Vontas J, David JP. Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: Impact on larval tolerance to chemical insecticides. Insect Biochemistry and Molecular Biology. 2008; 38:540–551. [PubMed: 18405832]

Poupardin R, Riaz MA, Vontas J, David JP, Reynaud S. Transcription profiling of eleven cytochrome P450s potentially involved in xenobiotic metabolism in the mosquito *Aedes aegypti*. Insect Molecular Biology. 2010; 19:185–193. [PubMed: 20041961]

Riaz MA, Poupardin R, Reynaud S, Strode C, Ranson H, David JP. Impact of glyphosate and benzo[a]pyrene on the tolerance of mosquito larvae to chemical insecticides. Role of detoxification genes in response to xenobiotics. Aquatic Toxicology. 2009; 93:61–69. [PubMed: 19419775]

Rodriguez MM, Bisset JA, De Armas Y, Ramos F. Pyrethroid insecticide-resistant strain of *Aedes aegypti* from Cuba induced by deltamethrin selection. Journal of the American Mosquito Control Association. 2005; 21:437–445. [PubMed: 16506569]

Saavedra-Rodriguez K, Strode C, Suarez AF, Salas IF, Ranson H, Hemingway J, Black WC. Quantitative Trait Loci Mapping of Genome Regions Controlling Permethrin Resistance in the Mosquito *Aedes aegypti*. Genetics. 2008; 180:1137–1152. [PubMed: 18723882]

Saavedra-Rodriguez K, Urdaneta-Marquez L, Rajatileka S, Moulton M, Flores AE, Fernandez-Salas I, Bisset J, Rodriguez M, Mccall PJ, Donnelly MJ, Ranson H, Hemingway J, Black WC. A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. Insect Molecular Biology. 2007; 16:785–798. [PubMed: 18093007]

Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, Haque R, Raisuddin S. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicology and Environmental Safety. 2003; 56:295–301. [PubMed: 12927561]

Schmidt O, Soderhall K, Theopold U, Faye I. Role of Adhesion in Arthropod Immune Recognition. Annual Review of Entomology. 2010; 55:485–504.

Strode C, Wondji CS, David JP, Hawkes NJ, Lumjuan N, Nelson DR, Drane DR, Karunaratne SHPP, Hemingway J, Black WC, Ranson H. Genomic analysis of detoxification genes in the mosquito *Aedes aegypti*. Insect Biochemistry and Molecular Biology. 2008; 38:113–123. [PubMed: 18070670]

Tanaka Y, Noppun V. Heritability Estimates of Phenthoate Resistance in the Diamond-Back Moth. Entomologia Experimentalis Et Applicata. 1989; 52:39–47.

Yanola J, Somboon P, Walton C, Nachaiwieng W, Prapanthadara LA. A novel F1552/C1552 point mutation in the *Aedes aegypti* voltage-gated sodium channel gene associated with permethrin resistance. Pesticide Biochemistry and Physiology. 2010; 96:127–131.

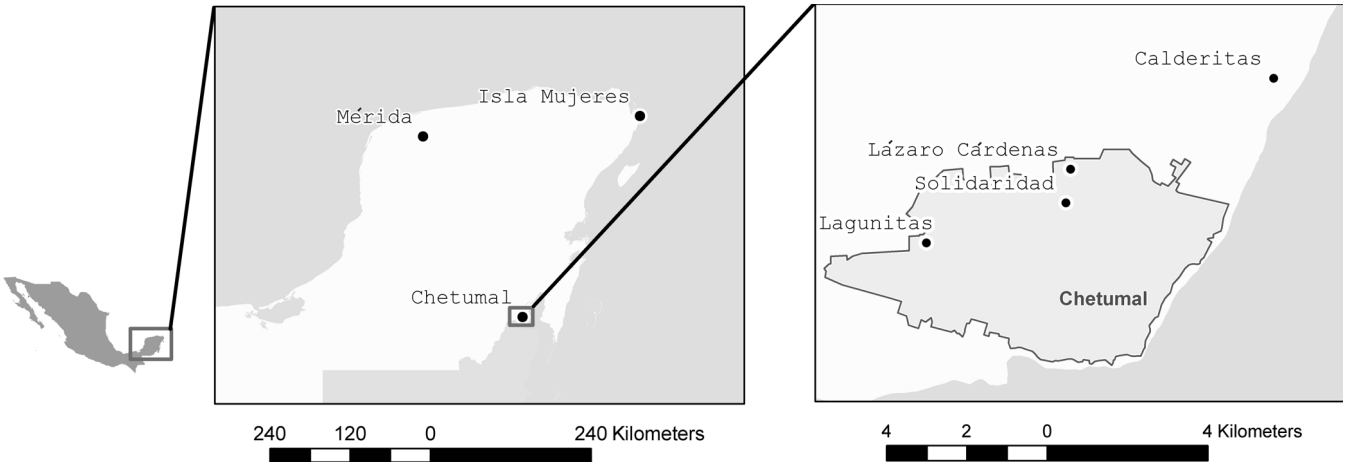

Figure 1.
Map of collections sites in México

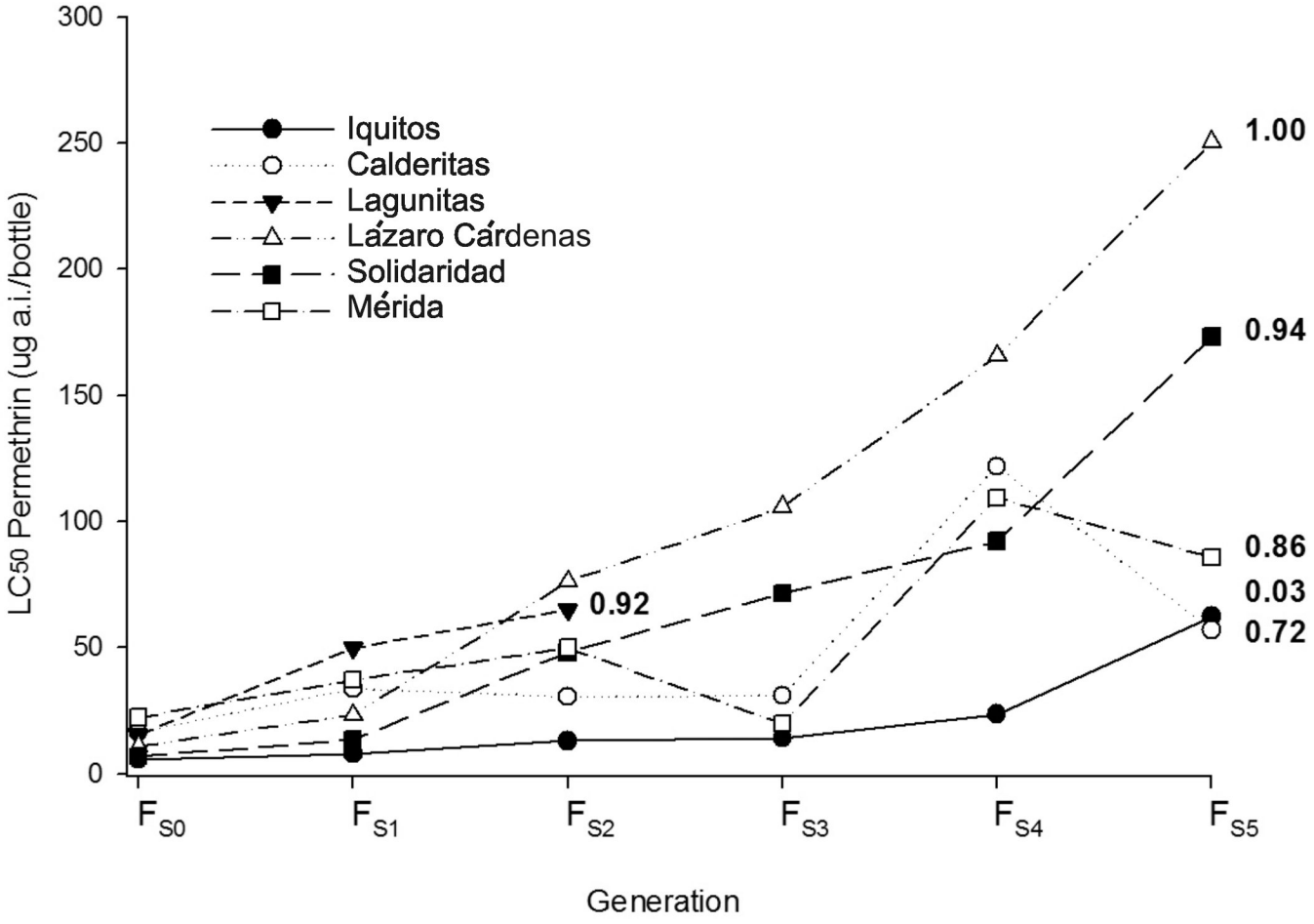

Figure 2.

LC_{50} response of mosquito lines to permethrin selection over five generations. The F_{S5} $Ile_{1,016}$ allele frequencies were calculated from ~50 individuals and appear in the right side of the graph.

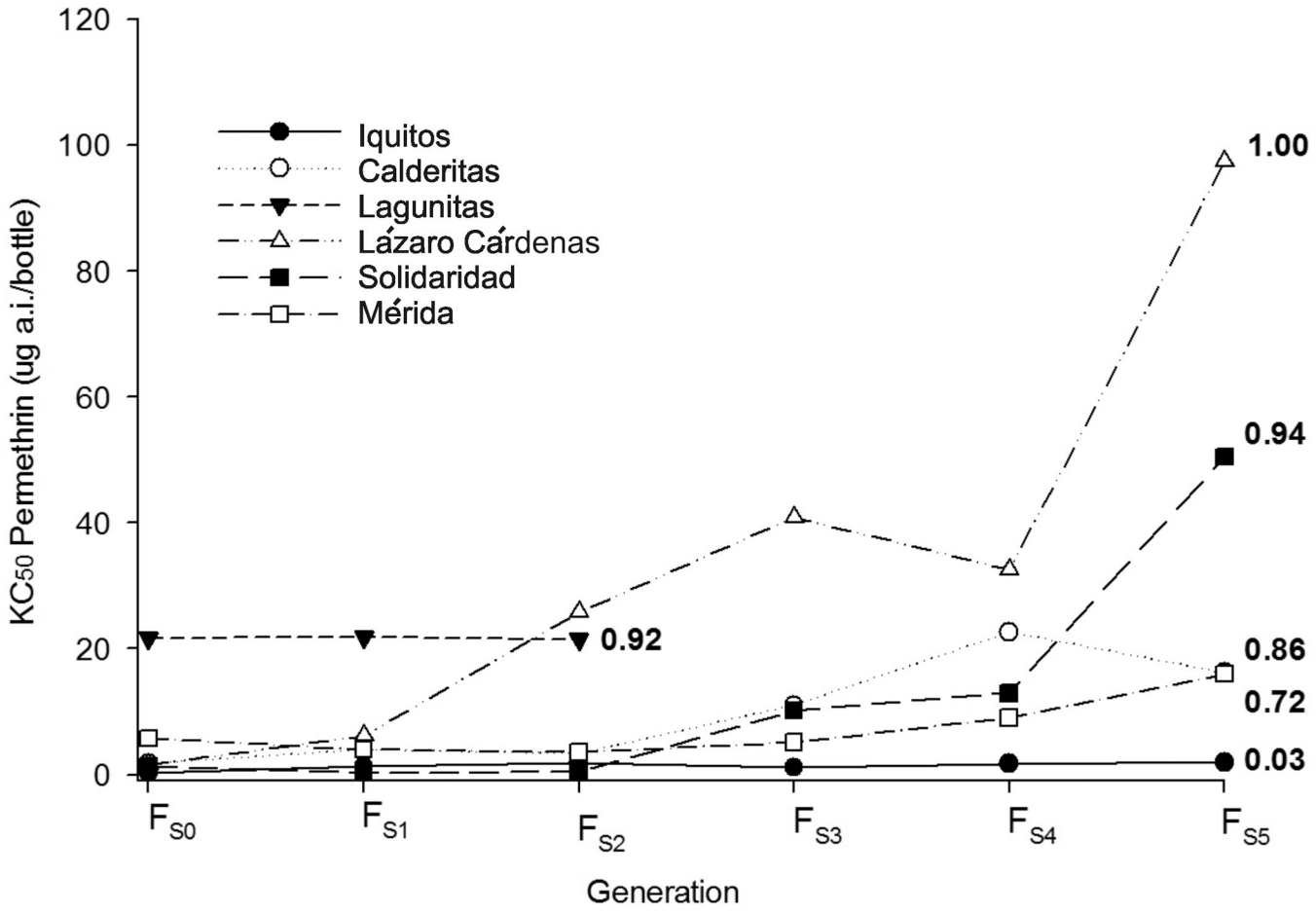

Figure 3.

KC_{50} response of mosquito lines to permethrin selection over five generations. The F_{S5} $Ile_{1,016}$ allele frequencies were calculated from ~50 individuals and appear in the right side of the graph.

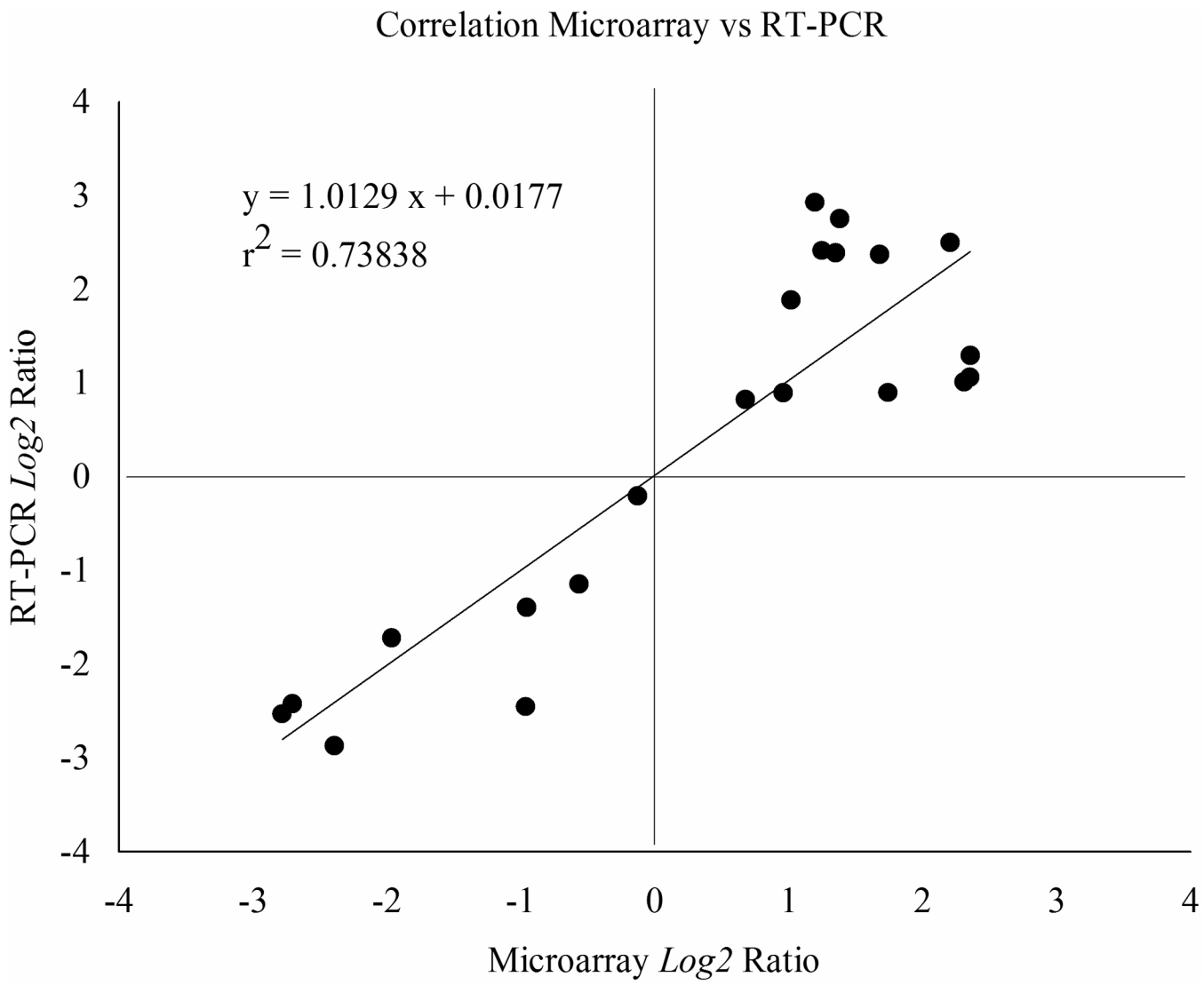

Figure 4.
Correlation between microarray and real time expression ratios. Ratios are display in a $\log_2$ scale.

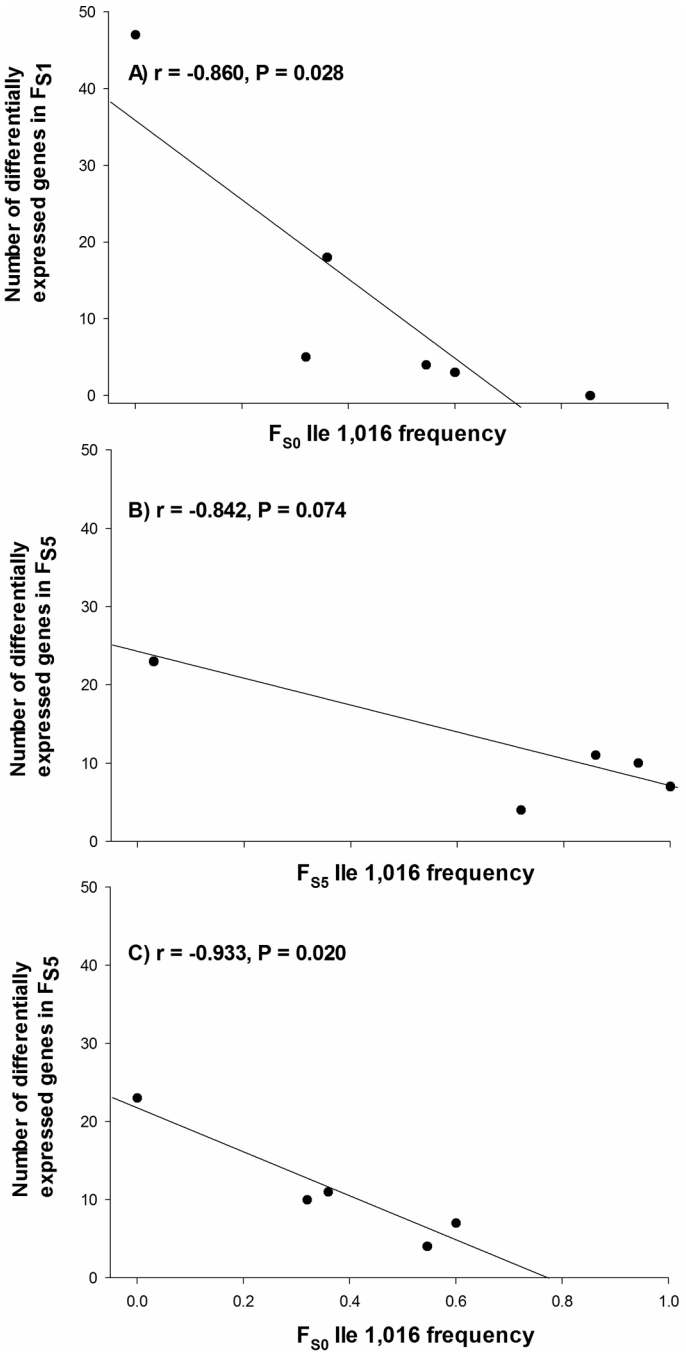

Figure 5.

Correlations between $Ile_{1,016}$ allele frequencies and the numbers of genes differentially transcribed. The Pearson correlation coefficient *r* is displayed alongside the probability that *r* = 0. A) F_{S0} $Ile_{1,016}$ frequencies versus numbers of genes differentially transcribed in F_{S1}. B) F_{S5} $Ile_{1,016}$ frequencies versus numbers of genes differentially transcribed in F_{S5}. C) F_{S0} $Ile_{1,016}$ frequencies versus numbers of genes differentially transcribed in F_{S5}

Table 1

Collection site information and adult permethrin resistance prior to artificial selection. LC_{50} and KC_{50} are µg of permethrin per 250mL Wheaton Bottle. The LC_{50} or KC_{50} resistance ratios are calculated relative to the susceptible New Orleans strain. The initial Ile1,016 allele frequency was calculated from ~50 individuals from each collection site. Pearson's correlation coefficients were calculated between LC_{50} or KC_{50} values and the frequency of Ile1,016 squared.

Country State	**City**	***Site in City***	**Coordinates**	**Generation**	**LC_{50} ug/a.i. (95% CI)**	**RR LC_{50} P_1/NO**	**KC_{50} ug/a.i. (95% CI)**	**RR KC_{50} P_1/NO**	**Ile1,016 freq. F_{S0}**
Perú									
Iquitos	Iquitos			F_2	5.6 (4.3–7.0)	2.1	0.3 (0.26–0.44)	0.7	0.000
México									
Quintana Roo	Chetumal								
		Calderitas	18.556269° –88.256181°	F_3	16.3 (13.2–20)	6.2	1.8 (1.27–2.63)	3.7	0.360
		Lagunitas	18.519485° –88.333923°	F_3	25.4 (22.6–28.6)	10.2	21.7 (18.01–26.1)	43.4	0.854
		Lázaro Cárdenas	18.535938° –88.301646°	F_3	11.0 (7.9–15.2)	4.2	1.6 (0.49–5)	3.1	0.600
		Solidaridad	18.528407° –88.302693°	F_3	6.9 (5.2–8.9)	2.6	1.4 (0.51–3.7)	2.8	0.320
Yucatán									
	Mérida		20.948942° –89.640526°	F_2	22.4 (19.9–25.1)	8.5	5.7 (3.7–8.8)	11.5	0.546
United States									
	New Orleans				2.6		0.5		0.000

Table 2

Real time PCR primers used for microarray validation

Gene	Vector Base ID	5'-->3' forward sequence	5'-->3' reverse sequence	cDNA size (bp)
Glutathione transferases				
AaGSTe-2	AAEL007951	GCCCGATGATGACGTGAAG	TGGCTTGCTTAACCAGTTCTTTC	246
AaGSTs1-1	AAEL011741	GCCCGATGATGACGTGAAG	TCCAGGATGGCGACAAAGTA	159
Cytochrome P450 monooxygenases				
CYP4J13	AAEL013555	TATGCGTATGTGCCGTTTAGT	ATAGGGCGTATTTCTGTCC	62
CYP325G3	AAEL012772	GCACGTTGGATATGATTTGT	TCTGTCCGATAGTATTTGGTTAGT	178
CYP6Nae1	AAEL009126	GGATTTCCAATACTTCCACGA	TTCCACTTGGCACCCTCC	96
CYP9J22 v1	AAEL006802	CAGAGGCTGTACGAGGAGATAGT	CGAAGGGACTCCGACACTA	116
CYP9J32	AAEL008846	CGATTTCATCCAGACCATTTA	CGTCTTCTTTGTTCGCTCC	176
Carboxyl/esterases				
AaeCOE-8	AAEL008757	CGGAGTCTTTCTTGAAGGGTA	TTCGGAGTGTTTCATTTCGTAG	111
Red/Ox & other				
Peroxinectin 4388	AAEL004388	CAACCTCCACTGGGATGACG	ATGTTCTGAAGTACGGCGATTA	72
Peroxinectin 4390	AAEL004390	TCAGCAGCCAATGGAACAAA	GGTCGCAGGGCAAAGTCA	132
60S ribosomal protein L8	AAEL000987	TGAAGGGAACCGTCAAGCAA	CATCAGACCGATTGGCAGAAC	200

Table 3

Realized heritability (h^2) of LC50 or KC50 resulting from five generations of permethrin selection. An ANOVA was performed to test the hypothesis: $h^2 > 0$.

Selected Line	LC50 h^2	p^*	KC50 h^2	p^*
Iquitos	0.616	0.001	0.181	0.381
Solidaridad	0.864	0.006	0.142	0.008
Mérida	0.740	0.034	0.229	0.066
Lázaro Cárdenas	0.829	0.022	1.397	0.050
Calderitas	0.241	0.573	0.789	0.014

*Bonferroni experimentwise rate = $1-(1-0.05)^{1/5} \simeq 0.01$

Table 4

a. Genes differentially expressed in two or more unselected F_{S0} strains relative to the New Orleans strain. Ratio of the average expression of each comparison is displayed on a linear scale. Probability value is shown as a negative $\log_{10}$ scale.

Activity		Iquitos		Calderitas		Lázaro C		Solidaridad		Mérida		Lagunitas	
Gene	**Vector Base ID**	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***
Glutathione transferases													
AaGSTe3	AAEL007947	2.06	4.29					1.99	9.80			2.93	10.98
AaGSTe4	AAEL007962									2.48	13.09	3.18	10.44
AaGSTe6	AAEL007946					2.23	9.90	2.01	9.53	2.03	10.07	3.25	9.49
AaGSTs1-2	AAEL011741	0.35	4.59			0.27	14.14						
GSTd5	AAEL001071	2.04	4.23							0.46	19.18		
Cytochrome P450 monoxygenases													
CYP6Nae1	AAEL009126									2.20	5.43	2.07	6.18
CYP6P12V2	AAEL014891	0.37	6.82			0.45	11.83					0.48	6.99
CYP6Z6_b	AAEL009123			2.08	7.32					2.25	18.07	2.22	6.27
CYP9AE1	AAEL003748							0.47	4.56			0.37	4.85
CYP9J10 v2	AAEL014614					2.04	3.47			3.01	18.53		
CYP9J22 v1	AAEL006802					2.28	7.86			2.64	13.31	2.27	5.70
CYP9J22 v2	AAEL014619	2.39	7.72							2.66	13.54	2.39	3.62
CYP9J23_b	AAEL014615					2.35	5.54			2.33	15.37		
CYP9J28	AAEL014617	3.14	6.80							2.93	15.98		
CYP9J32	AAEL008846			8.82	12.22					2.95	20.01		
CYP9J9 v2	AAEL014605					2.06	13.54			2.00	17.62		
Red/Ox													
Peroxinectin 4390	AAEL004390	2.03	7.28			3.23	8.61						

b. Genes differentially expressed in one of the unselected F_{S0} strains relative to the susceptible New Orleans strain

Glutathione transferases			
AaGSTd1-1	AAEL001061	4.00	11.45
AaGSTd1-2	AAEL001061	2.38	4.50
AaGSTd1-3	AAEL001061	2.48	8.64

b. Genes differentially expressed in one of the unselected F_{S0} strains relative to the susceptible New Orleans strain

AaGSTe7	AAEL007948							3.25	10.39
AaGSTs1-1	AAEL011741			1.39	10.70				
Cytochrome P450 monoxygenases									
CYP4G36	AAEL004054	0.33	6.00						
CYP6BB2a	AAEL014893			4.66	4.77				
CYP6CB1	AAEL002046					2.81	24.91		
CYP9J19 v1	AAEL006810					2.57	14.59		
CYP9J9 v1	AAEL006793					2.00	15.37		
Carboxyl/esterases									
AaeCOE-10	AAEL001517			1.41	13.24				
AaeCOE-17	AAEL004341	0.21	10.62						
CCae2B	AAEL017553			1.28	11.07				
CCae5C	AAEL003201	0.40	9.75						
CCEunk6o	AAEL000545			1.38	10.21				
Red/Ox									
Peroxinectin 3612	AAEL003612			4.51	8.14				
Peroxinectin 4388	AAEL004388			4.51	9.44				
Dihydrolipoamide dehydrogenase	AAEL006928	0.42	5.84						
Aldehyde oxidase 10367	AAEL010367	2.30	10.57						
Glutaredoxin 13980	AAEL013980	2.71	5.95						
Aldo-keto reductase 15002	AAEL015002			4.08	4.85				
Catalase	AAEL013407	0.32	8.41						
Thioredoxin peroxidase 9051	AAEL009051	2.14	3.70						
Others									
40S ribosomal ptn S17	AAEL004175	2.50	11.88						
60S ribosomal ptn L44 L41	AAEL003942	2.14	7.71						

Table 5

a. Genes differentially expressed in two or more F_{S1} strains relative to the unselected F_{S0}. Ratio of the average expression of each comparison is displayed on a linear scale. Probability value is shown as a negative $\log_{10}$ scale.

Activity		Iquitos		Calderitas		Lázaro C.		Solidaridad		Mérida		Lagunitas	
Gene	**Vector Base ID**	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***
Glutathione transferases													
GSTd5	AAEL001071	0.37	19.11							0.46	19.18		
AaGSTe4	AAEL007962			0.41	10.30					2.48	13.09		
AaGSTs1-2	AAEL011741	20.97	14.85			2.89	14.50	0.17	14.80				
Cytochrome P450 monooxygenases													
CYP6Z6_b	AAEL009123	0.43	14.01							2.25	18.07		
CYP9J9 v2	AAEL014605	2.51	11.65							2.00	17.62		
Carboxyl/esterases													
AaeCOE-2	AAEL002376	2.16	14.29	0.34	9.16								
AaeCOE-9	AAEL012509	2.81	15.15	0.44	8.52			0.44	13.24				
AaeCOE-17	AAEL004341	4.38	7.19			0.46	10.02	0.46	14.26				
AaeCOE-19	AAEL005112	2.25	12.17	0.32	11.28								
Red/Ox													
Aldehyde oxidase 8	AAEL010382	2.14	5.93			0.45	8.30	1.00					
Aldo-keto reductase 11	AAEL015002	0.15	12.86	2.35	8.56								
Aldo-keto reductase 7	AAEL004118	2.04	8.88	0.43	9.28								
Dual oxidase	AAEL007563	0.36	10.96	3.16	8.78								
Peroxinectin 3612	AAEL003612	0.18	16.46	4.44	10.46								
Peroxinectin 4386	AAEL004386	0.30	11.39	3.18	9.00								
Peroxinectin 4388	AAEL004388	0.15	17.99	3.34	7.94								
Peroxinectin 4390	AAEL004390	0.07	21.72	4.96	12.16			2.55	14.98				
Peroxinectin 4401	AAEL004401	0.29	11.49	3.68	7.03								
Thioredoxin peroxidase 5	AAEL009051	0.45	7.10					2.31	10.55				
HypProt	AAEL004400	0.22	16.56	3.48	8.15								

b. Genes differentially expressed in one of the F_{S1} strains relative to the unselected F_{S0}					
Glutathione transferases					
AaGSTd1-1	AAEL001061	0.46	4.09		
AaGSTd6	AAEL010591	0.49	10.41		
AaGSTe7	AAEL007948			0.34	12.21
AaGSTs1-1	AAEL011741	4.63	19.91		
Cytochrome P450 monooxygenases					
CYP305A6	AAEL002071			0.44	5.99
CYP306A1	AAEL004888	0.48	14.33		
CYP325S2	AAEL000325	0.50	8.63		
CYP4G36	AAEL004054	2.19	17.89		
CYP4H28	AAEL003380	0.24	9.47		
CYP6CB2	AAEL002872	0.38	16.05		
CYP6Z8_b	AAEL009131	2.06	17.12		
CYP6Z9	AAEL009129	2.91	13.27		
*HypProt*1055570	AAGE01055570			0.49	8.46
*HypProt*1055570	AAGE01055570			0.50	9.58
Carboxyl/esterases					
AaeCOE-14	AAEL012886	2.08	8.15		
AaeCOE-34				0.48	9.46
CCae2B	AAEL017553	3.14	13.32		
CEunk6o	AAEL000545	2.28	10.02		
Red/Ox					
Peroxinectin 5416	AAEL005416	2.38	12.02		
Heme peroxidase 6014	AAEL006014	2.25	6.4		
Cu-Zn Superoxide Dismutase	AAEL006271	0.48	19.05		
Ubiquinol-cytochrome c reductase complex 14kd	AAEL007868	2.50	21.38		
Ubiquinol-cytochrome c reductase hinge protein	AAEL010801	2.35	15.04		
Heme peroxidase 13171	AAEL013171	2.08	7.43		
Aldehyde oxidase 14493	AAEL014493	0.19	16.5		
Aldoketo reductase 15002	AAEL015002	0.15	12.86		
Catalase	AAEL013407	5.35	12.1		
Peroxiredoxin 7135	AAEL007135	2.57	11.81		

b. Genes differentially expressed in one of the F_{S1} strains relative to the unselected F_{S0}

Others				
60S ribosomal ptn L44 L41	AAEL003942	0.37	15.7	
Acidic ribosomal ptn P1	AAEL005027	0.48	11.11	
ribosomal ptn L34	AAEL009341	0.48	13.23	
40S ribosomal ptn S17	AAEL004175	0.38	12.4	

Table 6

a. Genes differentially expressed in two or more F_{S5} strains relative to the unselected F_{S1}. Ratio of the average expression of each comparison is displayed on a linear scale. Probability value is shown as a negative $\log_{10}$ scale.

Activity		Iquitos		Calderitas		Lázaro C.		Solidaridad		Mérida	
Gene	**Vector base ID**	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***
Glutathione transferases											
AaGSTs1-1	AAEL011741	0.19	17.18							0.51	15.61
Cytochrome P450 monoxygenases											
CYP4J13	AAEL013555	2.64	12.18			2.38	8.8	3.20	10.73		
CYP6Nae1	AAEL009126					2.03	6.4	2.08	4.78		
CYP9J22 v1	AAEL006802							2.55	8.37	1.95	17.79
CYP9J22 v2	AAEL014619							2.75	10.43	2.11	19.55
CYP325G3	AAEL012772	2.99	10.59	2.30	5.63			2.58	8.11		
*HypProt*1055570	AAGE01055570	2.10	10.18					2.03	4.18		
Red/Ox											
Peroxinectin 3612	AAEL003612	5.13	14.79	0.17	10.68	0.39	9				
Peroxinectin 4386	AAEL004386	3.34	11.97	0.21	8.05	0.45	5.09				
Peroxinectin 4388	AAEL004388	5.10	17.27	0.15	12.4	0.42	9.17	0.44	12.31		
Peroxinectin 4390	AAEL004390	9.25	18.22	0.26	11.47						
Peroxinectin 4401	AAEL004401	2.66	6.94	0.22	7.19						
*HypProt*4400	AAEL004400	3.76	11.37	0.19	8.39	0.40	4.41				

b. Genes differentially expressed in one of the unselected F_{S5} lines relative to F_{S1}

Glutathione transferases									
AaGSTt4	AAEL004229							0.47	14.1
AaGSTe2	AAEL007951			2.10	6.1				
AaGSTs1-2	AAEL011741	0.30	17.37						
Cytochrome P450 monoxygenases									
CYP6Z6_b	AAEL009123	4.66	18.77						
CYP6CB1	AAEL002046	4.20	11.75						
CYP9J23_b	AAEL014615	2.55	10						

b. Genes differentially expressed in one of the unselected F_{S5} lines relative to F_{S1}

CYP6CB2	AAEL002046	2.13	6.92						
CYP314A1_b	AAEL010946	2.04	6.67						
Carboxyl/esterases									
AaeCOE-8	AAEL008757			2.60	9.4				
AaeCOE-9	AAEL012509					3.16	9.37		
AaeCOE-23	AAEL005122					2.06	1.95		
AaeCOE-25	AAEL002391			0.50	1.93				
AaeCOE-34								2.23	6.19
Ace2	AAEL012141			0.46	6.83				
CCEunk6o	AAEL000545	0.40	9.97						
CCae2B	AAEL017553	0.36	9.97						
Red/Ox									
Aldehyde oxidase 10382	AAEL010382	0.45	15.23						
Aldehyde oxidase 14493	AAEL014493	3.84	15.47						
Aldo-keto reductase 15002	AAEL015002	4.69	8.26						
Glutaredoxin 14064	AAEL014064			0.50	5.52				
Other									
40S ribosomal ptn S17	AAEL004175	2.43	13.47						
Acidic ribosomal ptn P1	AAEL005027	2.08	9.62						

Table 7

a. Genes differentially expressed in two or more F_{S5} strains relative to New Orleans. Ratio of the average expression of each comparison is displayed on a linear scale. Probability value is shown as a negative log10 scale.

Activity	Vector base ID	Iquitos		Calderitas		Lázaro C.		Solidaridad		Mérida	
Genes		Ratio	*p*	Ratio	*p*	Ratio	*p*	Ratio	*p*	Ratio	p
Glutathione S-transferases											
AaGSTs1-1	AAEL011741	0.20	16.08			0.34	27.37			0.39	24.89
AaGSTs1-2	AAEL011741	0.10	10.87			0.31	23.81				
Cytochrome P450 mono-oxygenases											
CYP325G3	AAEL012772	2.85	14.24	2.08	13.11			3.03	15.05		
CYP4J13	AAEL013555	2.14	8.02			2.25	15.15	2.89	18.82		
CYP6CB1	AAEL002046	3.94	14.9							2.51	27.31
CYP6Nae1	AAEL009126			2.79	10.74	3.66	21.23	3.43	18.24	2.07	5.79
CYP6Z6_b	AAEL009123	4.20	18.01	2.41	7.96					2.16	20.19
CYP9J22 v1	AAEL006802					3.07	14.59	2.87	12.16	5.03	27.33
CYP9J22 v2	AAEL014619					3.63	16.73	3.94	27.65	5.54	28.88
CYP9J23_b	AAEL014615	2.20	7.69			2.51	13.36	2.06	12.76	2.85	20.3
CYP9J28	AAEL014617	2.53	4.92							2.58	16.61
CYP9J32	AAEL008846	4.76	10.21			2.75	18.46				
CYP9J9 v2	AAEL014605					3.46	32.5	1.97	12.03	3.12	31.08
Carboxyl/esterases											
AaeCOE-9	AAEL012509					2.01	18.26	2.50	17.01		
CCae2B	AAEL017553	0.29	12.77			0.49	12.2				
Reduction/Oxidation											
Aldo-keto reductase 4118	AAEL004118	0.42	8.47	2.60	7.81						
Dual oxidase	AAEL007563	2.83	14.47	0.30	11.47						
HypProt4400	AAEL004400	4.32	14.82	0.15	21.13					0.42	16.69
Peroxinectin 3612	AAEL003612	6.02	17.12	0.16	19.13					0.47	23.74
Peroxinectin 4386	AAEL004386	3.39	14.54	0.21	13.8					0.46	17.59
Peroxinectin 4388	AAEL004388	8.88	22.93	0.21	15					0.46	21.27
Peroxinectin 4390	AAEL004390	19.56	24.03	0.25	18.85						

a. Genes differentially expressed in two or more F_{S5} strains relative to New Orleans. Ratio of the average expression of each comparison is displayed on a linear scale. Probability value is shown as a negative log10 scale.

Activity	Vector base ID	Iquitos		Calderitas		Lázaro C.		Solidaridad		Mérida	
Genes		**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	**p**
Peroxinectin 4401	AAEL004401	2.45	4.91	0.28	15.34					0.51	8.53
Thioredoxin peroxidase 4112	AAEL004112	2.39	5.57					0.48	20.73		

b. Genes differentially expressed in one of the F_{S5} strains relative to New Orleans.

Glutathione S-transferases											
AaGSTd1-1	AAEL001061	3.63	11.92								
AaGSTd1-3	AAEL001061	2.35	8.01								
AaGSTe2	AAEL007951			2.04	16.52						
AaGSTe3	AAEL007947			2.50	9.16						
AaGSTe4	AAEL007962			2.41	16.97						
AaGSTe6	AAEL007946					2.10	14.71				
AaGSTe7	AAEL007948			3.53	15.75						
AaGSTt4	AAEL004229									0.40	19.63
GSTd5	AAEL001071	3.78	9.2								
Cytochrome P450 mono-oxygenases											
CYP304C1	AAEL014413	0.45	10.36								
CYP305A5	AAEL002043	0.39	10.07								
CYP325S2	AAEL000325	2.06	6.02								
CYP4G36	AAEL004054	0.50	2.73								
CYP4H28	AAEL003380	2.91	9.4								
CYP4K3	AAEL007798	2.23	10.34								
CYP6BB2_a	AAEL014893					1.99	8.38				
CYP6CB2	AAEL002872	3.05	8.8								
CYP6P12V1	AAEL012491	0.45	7.29								
CYP6P12V2	AAEL014891	0.35	6.74			0.50	11.82				
CYP6Z9	AAEL009129	0.28	9.49								
CYP9J19 v1	AAEL006810									2.58	11.04
CYP9J9 v1	AAEL006793									2.16	15.83

b. Genes differentially expressed in one of the F_{S5} strains relative to New Orleans.

CYP9M9_a	AAEL001807						2.04	7.55
Carboxyl/esterases								
AaeCOE-2	AAEL002376			2.87	12.45			
AaeCOE-8	AAEL008757					3.51	25.63	
AaeCOE-17	AAEL004341	0.22	12.63					
AaeCOE-19	AAEL005112			3.53	11.63			
CCEjhe1o	AAEL0155590			0.50	8.1			
CCEunk6o	AAEL000545	0.24	16.91					
CCEae5C	AAEL003201	0.34	12.78					
Reduction/Oxidation								
Aldehyde oxidase 14493	AAEL014493	6.63	15.31					
Aldehyde oxidase 10367	AAEL010367	2.19	9.73					
Aldehyde oxidase 10382	AAEL010382	0.37	10.49					
Aldo-keto reductase 15002	AAEL015002	4.50	10.45					
Aldo-keto reductase 4102	AAEL004102	3.10	8.94					
Catalase	AAEL013407	0.23	13.32					
Dihydrolipoamide dehydrogenase	AAEL006928	0.31	8.88					
Glutaredoxin 12238	AAEL012238	2.08	3.45					
Glutaredoxin 13980	AAEL013980	3.16	7.69					
Peroxinectin 3933	AAEL003933	0.46	6.38					
Peroxinectin 5416	AAEL005416	0.43	10.22					
Thioredoxin peroxidase 9051	AAEL009051	3.63	6.36					
Thioredoxin peroxidase 10777	AAEL010777	2.91	8.36					
Others								
60S ribosomal ptn L44 L41	AAEL003942	2.04	6.67					
Acidic ribosomal ptn P1	AAEL005027	3.12	14.03					
Ribosomal ptn L34	AAEL009341	2.20	11.03					
40S ribosomal ptn S17	AAEL004175	6.02	22.89					
HypProt	AAGE01055570	2.01	8.37					

Table 8

Genes with differential expression in both permethrin and temephos experiments. Those highlighted in grey were differentially transcribed in the same direction in the same collection in both experiments and could therefore represent laboratory adaptation.

Gene	Experiment	Collection			
		Iquitos	Calderitas	Lázaro C.	Mérida
AaGSTs1-1	Permethrin	0.20	-	0.34	0.39
	Temephos	-	-	2.58	0.23
AaGSTs1-1	Permethrin	0.10	-	0.31	-
	Temephos	0.44	-	2.14	-
AaGSTd1-1	Permethrin	3.63	-	-	-
	Temephos	2.39	-	-	-
CYP304C1	Permethrin	0.45	-	-	-
	Temephos	-	0.49	-	-
CYP6BB2_a	Permethrin	-	-	1.99	-
	Temephos	-	-	2.43	-
CYP6Z9	Permethrin	0.28	-	-	-
	Temephos	0.39	-	-	-
CYP9J22 v1	Permethrin	-	-	3.07	5.03
	Temephos	-	-	0.49	4.59
CYP9J22 v2	Permethrin	-	-	3.63	5.54
	Temephos	-	-	0.44	4.08
CYP9J32	Permethrin	4.76	-	2.75	-
	Temephos	2.48	3.73	-	-
CYP9J9 v2	Permethrin	-	-	3.46	3.12
	Temephos	-	-	0.46	-
Aldehyde oxidase 14493	Permethrin	6.63	-	-	-
	Temephos	-	-	0.45	-
Catalase	Permethrin	0.23	-	-	-
	Temephos	0.41	-	-	-
Thioredoxin peroxidase 4112	Permethrin	2.39	-	-	-
	Temephos	-	-	2.46	0.40

Gene	Experiment	Collection			
		Iquitos	Calderitas	Lázaro C.	Mérida
Dihydrolipoamide dehydrogenase	Permethrin	0.31	-	-	-
	Temephos	-	-	-	-
AaeCOE-17	Permethrin	0.22	-	-	-
	Temephos	0.36	-	-	-