QTL Mapping of Genome Regions Controlling Temephos Resistance in Larvae of the Mosquito *Aedes aegypti*

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**Abstract**

**Introduction:** The mosquito *Aedes aegypti* is the principal vector of dengue and yellow fever flavviruses. Temephos is an organophosphate insecticide used globally to suppress *Ae. aegypti* larval populations but resistance has evolved in many locations.

**Methodology/Principal Findings:** Quantitative Trait Loci (QTL) controlling temephos survival in *Ae. aegypti* larvae were mapped in a pair of F3 advanced intercross lines arising from temephos resistant parents from Solidaridad, México and temephos susceptible parents from Iquitos, Peru. Two sets of 200 F3 larvae were exposed to a discriminating dose of temephos and then dead larvae were collected and preserved for DNA isolation every two hours up to 16 hours. Larvae surviving longer than 16 hours were considered resistant. For QTL mapping, single nucleotide polymorphisms (SNPs) were identified at 23 single copy genes and 26 microsatellite loci of known physical positions in the *Ae. aegypti* genome. In both reciprocal crosses, Multiple Interval Mapping identified eleven QTL associated with time until death. In the Solidaridad×Iquitos (SLD×Iq) cross twelve were associated with survival but in the reciprocal Iq×SLD cross, only six QTL were survival associated. Polymorphisms at acetylcholine esterase (AchE) loci 1 and 2 were not associated with either resistance phenotype suggesting that target site insensitivity is not an organophosphate resistance mechanism in this region of México.

**Conclusions/Significance:** Temephos resistance is under the control of many metabolic genes of small effect and dispersed throughout the *Ae. aegypti* genome.

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**Introduction**

*Aedes aegypti* is the principal vector of Dengue Fever (DENV) and Yellow Fever (YFV) flavviruses throughout tropical and subtropical regions of the world and 2.5 billion people are at risk for DENV infection [1]. Currently DENV vaccines have low efficacy [2,3] so that vector control remains the only option to reduce or prevent DENV transmission. Adult control depends largely on the use of pyrethroid insecticides. However, resistance to pyrethroids has been rising globally [4,5,6,7,8,9]. More sustained control can potentially be achieved through the placement of insecticides in water containers that are known to harbor developing *Ae. aegypti* larvae in and around human habitations. For larval control, the three most widely used compounds are *Bacillus thuringiensis* israelensis (*Bti*), methoprene, and temephos. Globally, temephos is the most widely used of these three due to its very low vertebrate toxicity, relatively low cost, the fact that methoprene is a growth regulator with greatest effectiveness against older (third and fourth instar) larvae [10] and, because *Bti* must be ingested to be effective, it does not affect late larval or pupal stages when active feeding has ceased. Temephos is one of a few organophosphates registered to control *Ae. aegypti* larvae, and is the only organophosphate with any appreciable larvicidal use.

Temephos was first registered in the United States for mosquito control in 1965. It was quickly adopted as a larvicide because it was effective in polluted water, had a long residual activity, was available in several use-specific formulations, had a different mode of action than alternatives, and could be used on any larval instar. Temephos is toxic to many mosquito vector species that grow in a diversity of stagnant, saline, brackish and temporary water bodies. It remains an important management tool for mosquito abatement programs. The most widely used commercial preparation of temephos is Abate (EPA Registration No. 8329-60, Clarke Mosquito Control Products, Inc., Roselle, IL).

Temephos was used for 30 years before initial reports of resistance appeared in 1995. Initial studies reported less than a 5-fold resistance ratio (RR) in *Ae. aegypti* collections from Falcon...
Author Summary

The mosquito *Aedes aegypti* is the principal vector of dengue and yellow fever flaviviruses. Due to a lack of effective drugs or vaccines, if an epidemic of dengue fever occurs in the near future, the first line of defense will involve the use of insecticides to suppress adult populations of *Ae. aegypti*. Unfortunately, the species has become resistant to most of the insecticides that can be safely applied. The authors have worked extensively on the mechanisms of resistance to the various insecticides commonly used for suppression of *Ae. aegypti* populations. Temephos is an organophosphate insecticide used globally to suppress *Ae. aegypti* larval populations but resistance has evolved in many locations. In this study we show that temephos resistance is under the control of many metabolic genes of small effect and dispersed throughout the *Ae. aegypti* genome. This information will be of general interest to field workers involved in the suppression of field populations of *Ae. aegypti*.

and Aragua states of Venezuela [11]. In 1995, larvae from 34 strains of *Ae. aegypti* from 17 Caribbean countries were bioassayed and there were fairly high levels of temephos resistance in Tortola, British Virgin Islands (RR = 10–12) and Antigua (RR = 6–9) [12]. In 1999 a Tortola collection of *Ae. aegypti* was tested and a RR = 47 was identified [13]. After 13 generations of temephos laboratory selection, the RR increased to 181 fold [13]. Since 2000, temephos resistance has been reported from Cuba and Venezuela [14,15], Thailand [16], the Brazilian states of Sao Paulo [17], Espirito Santo, Rio de Janeiro [18], Sergipe, Alagoas, [19], Ceara [20], and Paraiba [21]. Most recently reports have appeared from El Salvador [22], Martinique Island in the French West Indies [23], Argentina [24,25], India [26], Colombia [27], and Trinidad [28,29]. Although resistance to temephos has been demonstrated in many areas of the world, it is the only remaining organophosphate larvicide with any appreciable use. As such, it is an important tool in resistance management programs that depend on alternative larvicides. Alteration in the registration status or availability of temephos would have a large negative impact on our ability to control DENV transmission globally.

The purpose of the present study was to develop a better understanding of the genetics underlying temephos resistance in *Ae. aegypti* using QTL mapping in recently collected strains. A strain previously established from Solidaridad, Mexico was selected to have 290 fold higher temephos resistance than another strain that had been established from Iquitos, Peru. Parents from these two strains were reciprocally crossed to generate F₁ siblings which were then intercrossed to generate an F₂. The F₂ generations were not large enough to assay for temephos resistance and so an F₃ was generated through additional sib mating. F₃ larvae were exposed to a discriminating dose of temephos and then checked every two hours up to 16 hours. Dead mosquitoes were preserved for DNA isolation at each time point and those surviving longer than 16 hours were considered resistant.

Methods

**Aedes aegypti strains**

Two strains of *Aedes aegypti* were used. A F₃ strain collected from Iquitos, Perú was kindly provided by Dr. Amy Morrison (University of California, Davis). A second strain raised during two generations in the lab was collected by the authors from the neighborhood of Solidaridad, in the city of Chetumal, in the state of Quintana Roo, México. Eggs were hatched in deoxygenated water from egg papers and then fed brewer’s yeast. Adults were provided 10% (w/v) sucrose solution and were blood fed on citrated sheep blood in an artificial membrane feeder every three days. Incubators were set to a 14:10 photoperiod, 30°C water temperature for larvae and 20°C for adult with a relative humidity of 85%.

**Bioassays and temephos selection**

F₂ or F₃ offspring from the field constituted the F₁₀ generation in the selection experiments. F₁₀ larvae were bioassayed to estimate the concentration of temephos (Chem Service, West Chester, PA) necessary to kill 50% of larvae (LC₅₀). Bioassays were performed in plastic cups containing 100 ml of water with five different concentrations of temephos in 1 mL ethanol as a solvent. Approximately 25 3rd-instar larvae were gently pipetted into each cup. Mortality was recorded every 15 minutes up to two hours. All larvae were then transferred into clean water and mortality was scored at 24 hours. Each bioassay was performed in triplicate to obtain ~75 larvae per concentration. LC₅₀ and confidence limits were calculated using the IRMA quick calculator software (http://sourceforge.net/projects/irmaproj/files/Qcal/beta/QCal_ver_0.1_rev190.msi/download) which performs logistic regression [30].

Selection proceeded in three replicate lines for three generations. In the first round of selection 40–100 third instar larvae from each of the three replicates were exposed to an LC₅₀ of 30 ng temephos/mL for two hours. Larvae were then transferred to clean water and mortality was recorded at 24 hours. Surviving larvae were transferred to 1 cubic foot rearing cages (BugDorm-1, Mega View Science, Co.) and raised to adults who were then blood fed to obtain F₁₀ eggs. We performed an initial bioassay with ~24 larvae in each of the subsequent F₁₀₁–F₁₀₃ generations of selection to calculate the new LC₅₀. From 40–100 larvae from each replicate were then exposed to the new LC₅₀.

**Mapping family crosses**

For the F₁ mapping family, we crossed Solidaridad (SLD) F₁₀ and Iquitos (Iq₁₀) adults. Twenty P₁×Q₁₀ SLD F₁₀×Q₁₀ and twenty reciprocal P₁Q₁₀×Q₁₀ SLD F₁₀ crosses were made. Larvae from each line were hatched and at the pupal stage, a female (larger size) from one strain was transferred to plastic cups in cardboard containers with a male pupa from the other strain. After adults emerged, they were allowed to mate for 3 days and the P₁ male was frozen and held at −80°C. Females were blood fed three times with an artificial membrane feeder over the next ten days and the P₁ female was then frozen and held at −80°C. Egg batches were maintained at room temperature for 7 days and then hatched by submersion in water followed by feeding them on Brewer’s yeast *ad libitum*. For the F₁ intercross families, one female and one male pupa from the same P₁ family were allowed to emerge, mate and blood fed to eventually generate F₂ progeny. F₂ eggs from the largest F₁ families were hatched and siblings were intercrossed in a single cage.

**Resistance phenotyping of mapping families**

Third instar larvae (200 total) were exposed to 250 ng temephos/mL. After 2 hours, larvae that were unresponsive to prodding with a pipette tip were individually transferred to a labeled 1.5 mL microcentrifuge tube and frozen at −80°C. This was repeated every two hours for the next 16 hours. After 16 hours all remaining larvae were recorded as resistant.
DNA extraction
The DNA of the P1 and F1 parents, and the two sets of 200 F3 offspring was individually isolated following the salt extraction method [31] and then suspended in 200 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0). The DNA was divided into 2–100 μl aliquots and stored at −80°C.

PCR of cDNA-Single Strand Conformation Polymorphisms (SSCP) markers
A total of 23 single copy genes [32,33] and 26 microsatellite loci from [34] were amplified and analyzed. Each of these 49 genes has a known physical and linkage map position in the Ae. aegypti genome. A PCR mixture sufficient to perform 100 25-μl reactions was made by mixing 2,114 μl ddH2O, 250 μl 10×Taq buffer (500 mM KCl, 100 mM Tris-HCL pH 9.0), 25 μl of 20 mM dNTPs, and 2,500 pm of each of the primers. This reaction mixture was set under a UV light source (302 nm) for 10 min, dNTPs, and 2,500 pm of each of the primers. This reaction mixture was added into a 1.5% (w/v) agarose gel made with Tris-Borate-EDTA buffer. DNA fragments were size fractionated by electrophoresis for 15–20 min at 112 V. Fragments were visualized by staining with Syber Green and viewing the gel over a UV transilluminator.

Melting curve assay for SNP
Polymorphic SSCP-markers were sequenced in the four P1 and F1 parents to test for SNPs and to determine the inheritance patterns of SNP alleles. Sequences were aligned using CLUSTALW [35]. Allele specific primers were designed at those loci in which genotypes were fully or partially informative in the P1 and F1 parents. Design of primers for melting curve PCR is previously published [36]. Allele specific fragments were detected by melting curve PCR in a CFX-96 Real time PCR detection system (Bio-Rad, Hercules, CA). Table S1 provides previously unpublished oligonucleotide sequences for allele specific determination.

Quantitative trait loci (QTL) analyses
Associations between genotypes at each marker locus and hours until death (HTD) phenotype were initially assessed with ANOVA [37]. Our null hypothesis was that HTD was equal in each genotype. Associations between death (scored 0) or survival (1) (DOA) after 16 hours were initially assessed with Fisher’s exact test (table DOA, “Marker locus name”) in R2.15.2. The null hypothesis was that the proportions of surviving larvae were equal in each genotype class. When the ANOVA or Fisher’s exact test yielded a probability below 0.05, we examined the inheritance of the alleles at that locus. Our a priori hypothesis was that an excess of F1 individuals with an allele inherited from the SLD P1 parent would be resistant while an excess of F3 individuals with an allele inherited from the Iq P1 parent would die.

Multiple Interval mapping (MIM) [38] was then performed using QTL Cartographer 2.5 [39]. Two separate MIM were done. First, mosquitoes were scored as 2, 4, 6, 8, 10, 12, 14, 16 or 24 corresponding to hours until death. Second, F3 mosquitoes were scored as one if they survived to 16 hours or as zero if they died before 16 hours. In either case we created an initial model containing QTL map positions for markers at which ANOVA or Fisher’s exact tests were significant. This model was then refined in MIM by 1) searching for new QTL, 2) estimating QTL effects, 3) obtaining and recording a summary, 4) optimizing QTL position, 5) searching for new QTL interactions, 6) testing for existing QTL main effects, 7) testing for existing QTL interaction effects, and 8) obtaining and recording a final summary. In addition, we used QTL Cartographer 2.5 to perform an initial MIM model selection on all markers using forward and backward selection with a significance level criterion of 0.01. We then compared this model with the model based upon markers identified as significant by ANOVA or Fisher’s exact tests. The models agreed in all four cases: (1) $R_{SLD \times Iq}^2 = \text{HTD}$ (2) $R_{SLD \times Iq}^2 = \text{DOA}$, (3) $R_{Iq}^2 = \text{SLD – HTD}$ and (4) $R_{Iq}^2 = \text{SLD – DOA}$.

Results
Bioassays and selection
The concentration of temephos sufficient to kill 50% of larvae (LC50) was 50 ng temephos/mL water for the Iquitos strain. The Solidaridad F50 strain initially had an LC50 of 27 ng temephos/mL water. Following three generations of temephos selection, the LC50 increased to 7.9 μg temephos/mL water in the Solidaridad strain. Thus the selected Solidaridad strain had ~160 fold higher temephos resistance than the Iquitos strain. Among the SLD×Iq F3 larvae the LC50 was 6.5 μg temephos/mL water and was 1.9 μg temephos/mL water among the Iq×SLD F3 larvae.

Statistical analyses of phenotype × genotype associations
The generic markers used in constructing maps in both the SLDxIq and Iq×SLD crosses are listed along with their linkage positions in Table S2. Results of the ANOVA to test the null hypothesis that time until death is equal among genotypes are presented in Table 1. Results of Fisher’s Exact Test on proportions of surviving larvae among genotype classes appear in Table 2. Loci with significant results are shown for all three chromosomes in Figure 1.

In the SLDxIq cross there were five QTL on chromosome 1 associated with HTD, four on chromosome 2 and four on chromosome 3. In the same cross there were four QTL on chromosome 1 associated with DOA, four on chromosome 2 and four on chromosome 3. In the Iq×SLD cross there were three QTL on chromosome 1 associated with HTD, four on chromosome 2 and five on chromosome 3. There was one QTL on chromosome 1 associated with DOA, two on chromosome 2 and three on chromosome 3. The two families shared common QTL at loci 192TAAAl and 88GAAal on chromosome 1, at loci 462GA1 and 1132CT1 on chromosome 2 and at locus 86AC1 on chromosome 3. Between the two families there were six, six and nine QTL affecting HTD on chromosomes 1, 2, and 3, respectively or 21 loci in total. In the two families there were four, five and six QTL affecting DOA on chromosomes 1, 2, and 3, respectively or 15 loci in total.

When the ANOVA or Fisher’s exact tests yielded a probability below 0.05, we examined the inheritance of the alleles at that locus. The last columns of Tables 1 and 2 indicate when the allele inherited from the SLD F3 P1 parent was associated with resistance while the allele inherited from the Iq P1 parent was associated with susceptibility. Figure 2 plots HTD among larvae with the three possible genotypes. The first column of plots correspond to chromosomes 1, 2, and 3 in the SLD×Iq cross. SLD
**Table 1.** Analysis of Variance (ANOVA) of the hours until death among the three genotype classes.

| Chromosome position (cM) | Marker Name   | Hours until Death | ANOVA Prob. | Predicted correlation? |
|--------------------------|---------------|-------------------|-------------|------------------------|
|                          |               | Iq/Iq | SLD/Iq | SLD/SLD |
| SLDxIq                   |               |       |       |         |
| Chromosome 1             |               |       |       |         |
| 0.0                      | CathepB       | 13.6  | 13.5  | 13.7   | 0.9894  |
| 0.3                      | 176TG1        | 13.9  | 13.5  | 13.2   | 0.8889  |
| 18.8                     | 12ATG1        | 16.3  | 13.4  | 11.9   | 0.0543  |
| 18.8                     | 12CGT1        | 11.2  | 13.6  | 15.5   | 0.0610  |
| 26.9                     | 71CGT1        | 12.3  | 14.6  | 15.3   | 0.1162  |
| 29.6                     | 192TAAC1      | 10.2  | 13.2  | 19.3   | 0.0008  |
| 40.4                     | 335CCA1       | 15.4  | 13.4  | 17.6   | 0.0523  |
| 40.7                     | AChE-2        | 12.7  | 13.7  | -      | 0.6185  |
| 48.5                     | CCEae1C       | 9.6   | 12.9  | 15.0   | 0.0486  |
| 48.5                     | CCEae2C       | 9.7   | 13.3  | 14.5   | 0.2070  |
| 48.5                     | CCEae5C       | 13.7  | 13.6  | -      | 0.9047  |
| 56.5                     | 88AT1         | -     | 13.0  | 13.7   | 0.6358  |
| 56.5                     | 88AAA1        | 12.9  | 13.3  | 19.5   | 0.0008  |
| 56.8                     | Chitin1       | 13.6  | 13.4  | 13.7   | 0.6974  |
| 69.7                     | 440AAC1       | -     | 12.7  | 15.4   | 0.0108  |
| 70.0                     | AEG122        | 19.5  | 12.4  | -      | 0.0000  |
| Chromosome 2             |               |       |       |         |
| 0.0                      | 45TACA1       | 14.6  | 12.7  | -      | 0.0717  |
| 29.2                     | 462GA1        | 9.3   | 16.7  | 24.0   | 0.0000  |
| 40.8                     | Arc4          | -     | 13.8  | 12.8   | 0.5196  |
| 46.7                     | Carbox        | 9.7   | 13.9  | 19.5   | 0.0000  |
| 48.6                     | 109CT1        | 11.6  | 13.0  | 12.0   | 0.5610  |
| 62.2                     | 25AAG1        | -     | 10.1  | 14.0   | 0.0299  |
| 66.1                     | 121GA1        | 11.7  | 13.6  | 16.0   | 0.3073  |
| 69.8                     | 1132CT1       | 7.9   | 13.5  | 23.5   | 0.0000  |
| 70.0                     | Sin3J         | 12.6  | 13.9  | 14.6   | 0.3507  |
| Chromosome 3             |               |       |       |         |
| 0.0                      | 301ACG1       | 13.5  | 14.1  | -      | 0.6036  |
| 10.2                     | LF396         | 12.7  | 14.1  | 12.3   | 0.2491  |
| 18.3                     | hsp70         | -     | 13.9  | 14.3   | 0.9081  |
| 30.4                     | AChE-1        | 12.7  | 13.7  | -      | 0.6185  |
| 32.5                     | 766AT11       | 12.2  | 15.8  | -      | 0.0011  |
| 34.5                     | 69TGA1        | 16.4  | 12.1  | 14.1   | 0.0021  |
| 44.7                     | LF261         | 14.5  | 13.5  | 12.7   | 0.5103  |
| 48.8                     | para          | 15.2  | 12.1  | 14.6   | 0.0206  |
| 56.9                     | 86AC1         | 9.3   | 14.6  | 8.9    | 0.0003  |
| 64.8                     | 470CT2        | 14.1  | 14.0  | 12.6   | 0.4477  |
| IqxSLD                   |               |       |       |         |
| Chromosome 1             |               |       |       |         |
| 0.0                      | CathepB       | 9.8   | 9.3   | 8.2    | 0.5813  |
| 0.3                      | 176TG1        | 8.1   | 8.6   | 9.7    | 0.4134  |
| 18.8                     | 12ACG1        | 10.1  | 8.1   | -      | 0.0541  |
| 18.8                     | 12ATG1        | -     | 9.3   | 8.1    | 0.3882  |
| 18.8                     | 12CGT1        | 8.4   | 9.9   | 8.4    | 0.3221  |
| 26.9                     | 71CT1         | 8.6   | 9.3   | 10.7   | 0.6232  |
| 26.9                     | 71AT1         | 10.3  | 9.6   | 8.8    | 0.6318  |
alleles conferred slightly greater longevity for the first three marker loci on chromosome 1 but Aeg22 Iq homozygotes had greater longevity than heterozygotes (Fig. 2A). In contrast, SLD alleles confer greater longevity for all marker loci on chromosome 2 (Fig. 2B) and the effects appear to be additive. On chromosome 3, no general trend is evident (Fig. 2C). Iq homozygotes confer slightly greater longevity at marker loci 69TGA1 and para. The opposite trend is seen in markers 766ATT1 and 86AC1.

The second column in Figure 2 corresponds to chromosomes 1, 2, and 3 in the Iq SLD cross. Again, SLD alleles confer slightly greater longevity on chromosome 1 (Fig. 2D). In contrast, on chromosome 2 SLD alleles at markers 328CTT1, 462GA1, and Arc4 confer only slightly greater longevity (Fig. 2E) while SLD alleles at the 1132CT1 locus appear to act as recessives in conferring much greater longevity. A similar pattern is seen in SLD alleles at 301ACG1 on chromosome 3 (Fig. 2F). However, Iq homozygotes confer slightly greater longevity at marker loci CCEae2c, vitg, 201TTA1 and Apyr1.

Figure 3 plots proportion surviving past 16 hours among larvae with the three possible genotypes. In the SLDxIq cross SLD alleles conferred slightly greater longevity for the first three marker loci on chromosome 1 but Aeg22 Iq homozygotes had greater longevity than heterozygotes (Fig. 2A). In contrast, SLD alleles confer greater longevity for all marker loci on chromosome 2 (Fig. 2B) and the effects appear to be additive. On chromosome 3, no general trend is evident (Fig. 2C). Iq homozygotes confer slightly greater longevity at marker loci 69TGA1 and para. The opposite trend is seen in markers 766ATT1 and 86AC1.

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Figure 3 plots proportion surviving past 16 hours among larvae with the three possible genotypes. In the SLDxIq cross SLD alleles

| Chromosome position (cM) | Marker Name | Hours until Death | ANOVA Prob. | Predicted correlation? |
|--------------------------|-------------|------------------|-------------|------------------------|
|                          |             | Iq/Iq | SLD/Iq | SLD/SLD |                 |
| 29.6                     | 192TAAA1    | 5.5   | 9.6   | 9.7     | 0.0337 +          |
| 48.5                     | CCEae1C     | 8.8   | 9.1   | 9.3     | 0.9562              |
| 48.5                     | CCEae2c     | 7.3   | 9.9   | 10.6    | 0.0230 +           |
| 48.5                     | CCEae5C     | 9.8   | 9.2   | 8.9     | 0.8247              |
| 56.5                     | 88GA1A1     | 10.5  | 7.6   | 16.8    | 0.0000 –           |
| 56.5                     | 88AT1       | 8.2   | 9.9   | 8.5     | 0.3426              |
| 69.6                     | 68ATGG1     | 8.5   | 9.7   | 4.8     | 0.2542              |
| 69.7                     | 440TGT1A1   | 5.8   | 9.1   | 11.1    | 0.1577              |
| Chromosome 2             |             |       |       |         |                     |
| 5.8                      | 328CTT1     | -     | 7.2   | 11.6    | 0.0002 +           |
| 9.7                      | CCEbe20     | 8.9   | 9.1   | -       | 0.8819              |
| 13.6                     | insrecp     | -     | 9.5   | 8.4     | 0.6120              |
| 21.4                     | fxa         | 6.8   | 9.1   | -       | 0.0739              |
| 29.2                     | 462GA1      | -     | 7.5   | 4.9     | 0.0227 –           |
| 36.9                     | Cyp9J32     | 6.7   | 9.4   | 9.8     | 0.1143              |
| 40.8                     | Arc4        | -     | 9.7   | 6.3     | 0.0077 –           |
| 46.7                     | Carbox      | 9.0   | 8.7   | 11.2    | 0.2890              |
| 48.6                     | 109CT1      | 9.6   | 8.9   | -       | 0.5126              |
| 50.6                     | DDC         | -     | 9.4   | 9.2     | 0.0670              |
| 54.4                     | LF357       | 9.0   | 9.5   | -       | 0.6926              |
| 66.1                     | 121GA1      | 9.6   | 8.9   | 7.0     | 0.1776              |
| 69.8                     | 1132CT1     | 4.9   | 9.7   | 24.0    | 0.0000 +           |
| 70.0                     | Sin3J       | -     | 9.1   | 10.4    | 0.4016              |
| Chromosome 3             |             |       |       |         |                     |
| 0.0                      | 301ACG1     | 7.8   | 8.2   | 24.0    | 0.0251 +           |
| 6.1                      | CCEae2D     | 12.5  | 8.6   | -       | 0.0203 –           |
| 18.3                     | hsp70       | -     | 9.5   | 6.7     | 0.3226              |
| 30.4                     | AChE-1      | -     | 8.6   | 9.8     | 0.5156              |
| 34.5                     | 69TGA1      | 9.7   | 9.7   | 8.8     | 0.9058              |
| 42.7                     | vitg        | 16.0  | 8.7   | -       | 0.0000 –           |
| 44.7                     | LF261       | -     | 9.3   | 6.2     | 0.1521              |
| 56.9                     | 86AC1       | 7.6   | 10.2  | 9.3     | 0.1329 –           |
| 64.6                     | 201TTA1     | 8.1   | 10.1  | -       | 0.0361 –           |
| 64.8                     | 470CT1      | 12.4  | 8.8   | 10.9    | 0.0950              |
| 65.0                     | Apyr1       | 11.2  | 8.3   | 8.5     | 0.0216 –           |

The means among the three classes are listed as are the probabilities estimated in the ANOVA. Probabilities from the ANOVA are listed in the sixth column. The last column indicates whether the allele inherited from the SLD F3 P1 parent conferred resistance while the allele inherited from the Iq P1 parent were susceptible.

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Table 2. Fisher’s Exact Test (FET) of proportions surviving past 16 hours among the three genotypes.

| Chromosome position (cM) | Marker Name | Proportion surviving | Exact Test Prob. | Predicted correlation? |
|--------------------------|-------------|----------------------|------------------|------------------------|
|                          |             | Iq/Iq | SLD/Iq | SLD/SLD |                      |
| Chromosome 1             |             |       |       |         |                      |
| SLD x Iq                 |             |       |       |         |                      |
| 0.0                      | CathepB     | 0.333 | 0.281 | 0.282   | 1.0000                |
| 0.3                      | 176TG1      | 0.333 | 0.275 | 0.263   | 0.6539                |
| 18.8                     | 12ATG1      | 0.300 | 0.321 | 0.176   | 0.2774                |
| 18.8                     | 12CGT1      | 0.281 | 0.252 | 0.438   | 0.1194                |
| 26.9                     | 71CGT1      | 0.254 | 0.340 | 0.364   | 0.4337                |
| 29.6                     | 192TAA1     | 0.091 | 0.268 | 0.632   | 0.0022                |
| 40.4                     | 335CGA1     | 0.464 | 0.277 | 0.478   | 0.0677                |
| 40.7                     | AChE-2      | 0.214 | 0.299 | -       | 0.7611                |
| 48.5                     | CCEae1C     | 0.000 | 0.277 | 0.346   | 0.0562                |
| 48.5                     | CCEae2C     | 0.000 | 0.291 | 0.316   | 0.3045                |
| 48.5                     | CCEae5C     | 0.320 | 0.267 | -       | 0.4388                |
| 56.5                     | 88AT1       | -     | 0.222 | 0.301   | 0.4980                |
| 56.5                     | 88GAA1      | 0.244 | 0.256 | 0.714   | 0.0002                |
| 56.8                     | Chitan1     | 0.304 | 0.255 | 0.429   | 0.4600                |
| 69.7                     | 440AAC1     | -     | 0.234 | 0.381   | 0.0379                |
| 70.0                     | AEG22       | 0.714 | 0.211 | -       | 0.0000                |
| Chromosome 2             |             |       |       |         |                      |
| 0.0                      | 145TAA1     | 0.352 | 0.241 | -       | 0.1165                |
| 29.2                     | 462GA1      | 0.000 | 0.525 | 1.000   | 0.0000                |
| 40.8                     | Arc4        | -     | 0.327 | 0.080   | 0.0097                |
| 46.7                     | Carbox      | 0.101 | 0.267 | 0.650   | 0.0000                |
| 48.6                     | 109CT1      | 0.188 | 0.295 | 0.143   | 0.0586                |
| 62.2                     | 25AAG1      | 0.158 | 0.304 | 0.286   | 0.0782                |
| 66.1                     | 121GA1      | 0.286 | 0.274 | 0.526   | 0.0000                |
| 69.8                     | 1132CT1     | 0.150 | 0.207 | 0.964   | 0.0000                |
| 70.0                     | Sin3J       | 0.194 | 0.329 | 0.360   | 0.0988                |
| Chromosome 3             |             |       |       |         |                      |
| 0.0                      | 301ACG1     | 0.275 | 0.337 | -       | 0.4279                |
| 10.2                     | LF396       | 0.255 | 0.327 | 0.194   | 0.2980                |
| 18.3                     | hsp70       | -     | 0.316 | 0.375   | 0.7110                |
| 30.4                     | AChE-1      | 0.214 | 0.299 | -       | 0.7611                |
| 32.5                     | 766AT1      | 0.227 | 0.400 | -       | 0.0142                |
| 34.5                     | 691GA1      | 0.492 | 0.163 | 0.407   | 0.0000                |
| 44.7                     | LF261       | 0.333 | 0.284 | 0.267   | 0.7858                |
| 48.8                     | para        | 0.351 | 0.212 | 0.407   | 0.0412                |
| 56.9                     | 86AC1       | 0.000 | 0.353 | 0.050   | 0.0002                |
| 64.8                     | 470CT2      | 0.381 | 0.305 | 0.230   | 0.3770                |
| Iq x SLD                 |             |       |       |         |                      |
| Chromosome 1             |             |       |       |         |                      |
| 0.0                      | CathepB     | 0.091 | 0.140 | 0.114   | 0.8592                |
| 0.3                      | 176TG1      | 0.100 | 0.115 | 0.169   | 0.5624                |
| 18.8                     | 12ACG1      | 0.135 | 0.118 | -       | 0.8206                |
| 18.8                     | 12ATG1      | -     | 0.140 | 0.077   | 0.5379                |
| 18.8                     | 12CGT1      | 0.136 | 0.163 | 0.047   | 0.1533                |
| 26.9                     | 71CGT1      | 0.174 | 0.125 | 0.235   | 0.3515                |
| 26.9                     | 71AT1       | 0.000 | 0.117 | 0.138   | 0.7957                |
conferred greater survival at the first three marker loci on chromosome 1 but Aegi2 Iq homozygotes had greater longevity than heterozygotes (Fig. 3A). Note that these are the same markers as in Figure 2A, but with markers 192TAAA1, and 88GAA1. SLD alleles confer a 50% increase in survival. On chromosome 2 (Fig. 3B), with the exception of Arc4, SLD alleles at markers, 462GA1, Carbox and 1132CT1 all greatly increase survival. SLD alleles at 462GA1 appear to act additively in increasing survival from zero in Iq homozygotes to 50% in heterozygotes to 100% in SLD homozygotes. Resistant alleles at markers Carbox and 1132CT1 are recessive with 75–80% greater survival in SLD homozygotes. As with HTD, on chromosome 3 there is no general trend (Fig. 3C). Iq homozygotes confer slightly greater survival at marker loci 69TGA1 and para but the opposite trend is seen in markers 766ATT1 and 86AC1. In the Iq×SLD cross (Fig. 3D) SLD alleles at marker 88GAA1 increase survival by 50% and SLD alleles appear recessive. Similarly, alleles at the 1132CT1 marker increased survival by 90%. Identical patterns were seen in the SLDxIq cross (Fig. 3B). On chromosome 3, Iq homozygotes confer slightly greater survival at marker loci CCEae2D, vitg, and 86AC1.

Table 2. Cont.

| Chromosome position (cM) | Marker Name | Proportion surviving | Exact Test Prob. | Predicted correlation? |
|--------------------------|-------------|----------------------|------------------|------------------------|
|                          |             | Iq/Iq                | SLD/Iq           | SLD/SLD                |
| 29.6                     | 192TAAA1    | 0.000                | 0.123            | 0.171                  | 0.1246                | +            |
| 48.5                     | CCEae1C     | 0.125                | 0.138            | 0.121                  | 0.9253                |             |
| 48.5                     | CCEae2c     | 0.067                | 0.172            | 0.130                  | 0.1865                | +            |
| 48.5                     | CCEae5C     | 0.097                | 0.146            | 0.113                  | 0.7965                |             |
| 56.5                     | 88GAA1      | 0.231                | 0.012            | 0.600                  | 0.0000                |             |
| 56.5                     | 88AT1       | 0.143                | 0.144            | 0.096                  | 0.7535                |             |
| 69.6                     | 68ATGG1     | 0.050                | 0.176            | 0.000                  | 0.3281                |             |
| 69.7                     | 440TGT1A1   | 0.000                | 0.115            | 0.278                  | 0.1000                |             |

Chromosome 2

|                          |             | Iq/Iq                | SLD/Iq           | SLD/SLD                |
| 5.8                      | 328CTT1     | -                    | 0.065            | 0.253                  | 0.0034                | +            |
| 9.7                      | CCEbe20     | 0.091                | 0.134            | -                      | 0.4755                |             |
| 13.6                     | insrecep    | -                    | 0.133            | 0.100                  | 1.0000                |             |
| 21.4                     | fha         | 0.032                | 0.138            | -                      | 0.1254                |             |
| 29.2                     | 462GA1      | 0.090                | 0.000            | -                      | 0.3349                |             |
| 36.9                     | Cyp9J32     | 0.038                | 0.146            | 0.134                  | 0.4101                |             |
| 40.8                     | Arc4        | -                    | 0.145            | 0.031                  | 0.0863                |             |
| 46.7                     | Carbox      | 0.125                | 0.093            | 0.217                  | 0.3054                |             |
| 48.6                     | 109CT1      | 0.161                | 0.112            | -                      | 0.3614                |             |
| 50.6                     | DDC         | -                    | 0.118            | 0.144                  | 0.6672                |             |
| 54.4                     | LF357       | 0.160                | 0.134            | -                      | 0.7547                |             |
| 66.1                     | 121GA1      | 0.164                | 0.090            | 0.033                  | 0.1415                |             |
| 69.8                     | 1132CT1     | 0.000                | 0.120            | 1.000                  | 0.0000                |             |
| 70.0                     | Sin3J       | -                    | 0.131            | 0.100                  | 1.0000                |             |

Chromosome 3

|                          |             | Iq/Iq                | SLD/Iq           | SLD/SLD                |
| 0.0                      | 301ACG1     | 0.100                | 0.074            | 1.000                  | 0.0788                | +            |
| 6.1                      | CCEae2D     | 0.313                | 0.098            | -                      | 0.0253                |             |
| 18.3                     | hsp70       | -                    | 0.142            | 0.000                  | 1.0000                |             |
| 30.4                     | ACHE-1      | -                    | 0.098            | 0.182                  | 0.3177                |             |
| 34.5                     | 69TG1A      | 0.167                | 0.157            | 0.154                  | 1.0000                |             |
| 42.7                     | vitg        | 0.438                | 0.108            | -                      | 0.0019                |             |
| 44.7                     | LF261       | -                    | 0.132            | 0.000                  | 0.6161                |             |
| 56.9                     | 86AC1       | 0.000                | 0.200            | 0.138                  | 0.0394                |             |
| 64.6                     | 201TTA1     | 0.143                | 0.110            | -                      | 0.5247                |             |
| 64.8                     | 470CT2      | 0.250                | 0.119            | 0.000                  | 0.2132                |             |
| 65.0                     | Apyr1       | 0.193                | 0.098            | 0.103                  | 0.2245                |             |

The means in each of the three genotypes are listed. Probabilities from the Exact Test are listed in the sixth column. The last column indicates whether the allele was inherited from the SLD F_{3} P_{1} parent conferred resistance while the allele inherited from the Iq P_{1} parent was associated with susceptibility.

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QTL analysis

The results of Multiple Interval Mapping with the HTD and DOA phenotypes are shown for both crosses in Table 3. Eleven QTL were identified in the SLD×Iq cross and these accounted for 68% of the phenotypic variance in HTD. There were nine QTL that accounted for 63% of the phenotypic variance in DOA. These nine were also all associated with HTD. The QTL that accounted for most (48%) of the genetic variation in HTD were at 47 cM and 70 cM on chromosome 2. The QTL that accounted for the most variation in DOA was at 62 cM on chromosome 2. QTL at 30 cM and 70 cM on chromosome 1 affected both phenotypes.

Genetic factors accounted for less of the variation in HTD and DOA phenotypes in the Iq×SLD cross. Eleven QTL were identified that accounted for 58% of the phenotypic variance in HTD. There were only two QTL that accounted for 31% of the variance in DOA and these were also associated with HTD. The QTL that accounted for most of the variation in HTD were at 57 cM on chromosome 1, 64 cM on chromosome 2 and 43 cM on chromosome 3. The only QTL that accounted for negligible variation in DOA was at 62 cM on chromosome 2. QTL at 57 cM on chromosome 1 affected both phenotypes. QTL at 30 and 57 cM on chromosome 1, and at 23.5 and 70 cM on chromosome 2 were common to both families.

Discussion

QTL mapping indicates that resistance to temephos is conditioned by many regions of the Ae. aegypti genome and therefore appears to behave as a classic quantitative genetic trait that is controlled by many loci each of minor effect. This pattern is supported by a recent parallel study in which we tracked changes in transcription of metabolic detoxification genes using the Ae. aegypti ‘Detox Chip’ microarray [40] during five generations of temephos selection [41]. We selected for temephos resistance in three replicates in each of six collections, five from México, and one from Peru. We used the esterase inhibitor DEF (S.S.S-tributylphosphorotrithioate) to show that esterases were the major metabolic source of resistance. However, the microarray data indicated that expression of many esterase genes increased with selection and that no single esterase was consistently upregulated among the six selected lines.

Target site resistance in acetylcholine esterase genes is a very common mechanism of resistance to organophosphate and carbamate insecticides [42]. We therefore tested for a significant genotype -phenotype interaction with SNPs in the AChE-2 gene (AAEL012141) at 40.7 cm on chromosome 1 and the AChE-1 gene (EF209048) at 3p1.2 (30.4 cM) on chromosome 3 [43]. Results in Table 1–3 show that no significant associations were
Figure 2. Hours until death among larvae plotted against the three possible genotypes at each of the markers found to be significantly associated with the HTD phenotype (Table 1). Iq/Iq = both alleles inherited from the Iquitos parent, Iq/SLD = heterozygous for alleles inherited from both Iquitos and Solidaridad parents, SLD/SLD = both alleles inherited from the Solidaridad parent. The second column
detected. Similar studies of temephos resistance in field populations of *Ae. aegypti* also failed to detect insensitive acetylcholine esterase [44] despite the fact that these authors were able to generate recombinant clones that produced *Ae. aegypti* insensitive acetylcholine esterases in the laboratory [45]. Another possibility is that temephos in particular fails to select for insensitive

corresponds to chromosomes 1, 2, and 3 in the Iq x SLD cross. Error bars are Bayes 95% highest density intervals (HDI), credible differences exist when the 95% HDI fail to overlap. For the SLD x Iq cross, A) shows the relationship among genotypes at six loci on chromosome 1 and HTD, B) is the relationship among genotypes at four loci on chromosome 2 and HTD, and C) indicates the relationship among genotypes at four loci on chromosome 3 and HTD. For the Iq x SLD cross, D) shows the relationship among genotypes at three loci on chromosome 1 and HTD, E) is the relationship among genotypes at four loci on chromosome 2 and HTD, and F) indicates the relationship among genotypes at five loci on chromosome 3 and HTD.

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Figure 3. Proportion surviving among larvae plotted against the three possible genotypes at each of the markers found to be significantly associated with the DOA phenotype (Table 2). Iq/Iq = both alleles inherited from the Iquitos parent, Iq/SLD = heterozygous for alleles inherited from both Iquitos and Solidaridad parents, SLD/SLD = both alleles inherited from the Solidaridad parent. Error bars are Bayes 95% highest density intervals (HDI), credible differences exist when the 95% HDI fail to overlap. For the SLD x Iq cross, A) shows the relationship among genotypes at four loci on chromosome 1 and proportion surviving, B) is the relationship among genotypes at four loci on chromosome 2 and proportion surviving, and C) indicates the relationship among genotypes at four loci on chromosome 3 and proportion surviving. For the Iq x SLD cross, D) shows the relationship among genotypes at one locus on chromosome 1, two loci on chromosome 2 and 3 loci on chromosome 3 and proportion surviving.

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### Table 3. Multiple-interval mapping estimates of QTL position and associated genetic, environmental, and phenotypic variances.

| SLD x Iq | Hours until Death | Survival |
|----------|------------------|----------|
|          | Genetic $\sigma^2$ | 36.91 | 68.3% |
|          | Residual $\sigma^2$ | 17.14 | 31.7% |
|          | Phenotypic $\sigma^2$ | 54.05 | 0.206 |

#### Chromosome 1

| Marker          | Position | LOD | Effect | Effect(%) | LOD | Effect | Effect(%) |
|-----------------|----------|-----|--------|-----------|-----|--------|-----------|
| Add. 192TAAA1   | 29.7     | 2.1 | 3.02   | 4.2       | 1.4 | 0.16   | 3.4       |
| Dom.            | 0.6      | -2.12 | 0.8    | -0.22     | 4.4 |
| Add. 88GAA1     | 56.6     | 0.1 | 0.52   | 0.6       | 0.10 | 1.5 |
| Dom.            | 0.8      | -2.37 | 2.9    | -0.19     | 4.5 |

#### Chromosome 2

| Marker          | Position | LOD | Effect | Effect(%) | LOD | Effect | Effect(%) |
|-----------------|----------|-----|--------|-----------|-----|--------|-----------|
| Add. 462GA1     | 29.3     | 0.6 | 1.21   | 1.9       | 0.4 | 0.07   | 1.4       |
| Dom.            | 1.9      | 3.25 | 4.8    | 0.17      | 5.0 |
| Add. Carbox     | 46.8     | 8.4 | 3.69   | 17.4      | 0.0 | -0.02  | -0.2      |
| Dom.            | 0.3      | 0.86 | 0.2    | 0.14      | -0.7 |
| Add. 25AAG1     | 62.3     | 0.1 | 5.00   | 3.4       | 5.5 | 0.25   | 17.1      |
| Dom.            | 0.1      | -2.69 | -1.5   | 0.2       | -0.05 | 0.3 |
| Add. 1132CTT1   | 69.9     | 7.0 | 4.21   | 15.5      | 4.1 | 0.20   | 10.1      |

#### Chromosome 3

| Marker          | Position | LOD | Effect | Effect(%) | LOD | Effect | Effect(%) |
|-----------------|----------|-----|--------|-----------|-----|--------|-----------|
| Add. 69TGA1     | 34.6     | 0.1 | -0.34  | 0.4       | 0.1 | -0.02  | 0.3       |
| Dom.            | 0.3      | -0.90 | 1.2    | 1.2       | -0.12 | 3.7 |
| Add. para       | 48.9     | 0.7 | -1.11  | 0.9       | -    | -      |
| Dom.            | 0.5      | -1.28 | 1.7    | -        | -    |
| Add. 86AC1      | 57.0     | 0.3 | -0.95  | 0.5       | 0.1 | -0.02  | 0.1       |
| Dom.            | 0.9      | 1.93 | 2.8    | 1.1       | 0.14 | 3.2 |

#### Iq x SLD

| Marker          | Position | LOD | Effect | Effect(%) | LOD | Effect | Effect(%) |
|-----------------|----------|-----|--------|-----------|-----|--------|-----------|
| Add. 192TAAA1   | 29.7     | 0.4 | -0.84  | 1.0       | -   | -      |
| Dom.            | 0.5      | 1.24 | 0.6    | -        | -   |
| Add. CCEae2c    | 48.6     | 0.6 | -1.38  | 2.2       | -   | -      |
| Dom.            | 0.0      | 0.47 | 0.0    | -        | -   |
| Add. 88GAA1     | 56.6     | 0.9 | -1.27  | 2.8       | 3.55 | -0.15  | 8.7       |
| Dom.            | 1.7      | -3.13 | 6.4    | 8.1       | -0.30 | 20.4 |

#### Residual $\sigma^2$ estimates

| Marker          | Position | LOD | Effect | Effect(%) |
|-----------------|----------|-----|--------|-----------|
| Add. 328CTT1    | 0.1      | 2.1 | -1.95  | 5.6       |
| Dom.            | 0.7      | -1.65 | 3.2    |
| Add. 462GA1     | 23.5     | 0.1 | -0.48  | 0.0       |
| Dom.            | 0.5      | 2.04 | 2.1    |
Acetylcholine esterases. Cuban investigators were able to select *Ae. aegypti* with 13-fold increase in insensitive acetylcholine esterase but using the carbamate insecticide propoxur [46].

Previous studies of esterase isozyme loci identified two genetically mapped loci associated with resistance to the organophosphate insecticide malathion. Elevated activity staining of Esterase-5 located at 57 cM at the base of Chromosome 1 [47] was reported [48]. This may correspond to the 57 cM QTL on chromosome 1 associated with marker 88GAA1 in both families in the current study. Similarly elevated activity staining of Esterase-6 located at 83 cM at the base of Chromosome 2 was reported [49,50]. This may correspond to the QTL at 70 cM on chromosome 2 associated with marker 1132CT1 found in both families in the current study. We have no means to formally check these associations because neither the nucleotide nor amino acid sequences of proteins Esterase-5 and 6 are known.

There are 49 currently identified carboxy/choline esterase genes [40]. With the recent publication of a physical map that contains 45% of the *Ae. Aegypti* genome [51,52] we had hoped to learn the physical locations of many of these esterases. However, other than AChE-1 and AChE-2, there were only six other esterase genes that occurred in mapped supercontigs. These were CCEbe2o (AAEL008757) on 2p3.4 (also mapped in our previous QTL mapping study [36]) and CCEbe2o (AAEL004323) on 2q2.4, and four (CCEhe2F (AAEL005200), CCEhe2F (AAEL005198), CCEhe3F (AAEL005210), and CCEhe4F (AAEL005182)) all located in supercontig 1.145 at 2p4.4. Whether these four are associated with the QTL at 5.8 cM on the top of Chromosome 2 in the *Q1 × SLD* cross (see Tables 1–2) is unknown at this time.

Even though the selected Solidaridad strain had overall ~160 fold higher temephos resistance than the *Iquitos* strain, this pattern wasn’t uniform across the entire genome. This could have affected the locations and relative contributions of QTL. There are many instances in Tables 1 and 2 wherein the mosquitoes homozygous for markers from the “susceptible” *Iquitos* strain were more resistant than heterozygotes or homozygous for markers from the “resistant” *SLD* strain (note especially the bottom of chromosome 3 for both HTD and DOA). This counterintuitive outcome is probably a result of using *Iquitos* mosquitoes taken directly from the field without selecting for a more susceptible phenotype. However, it could also be associated with negative fitness effects associated with resistance alleles in the *SLD* strain that became concentrated during selection.

In our previous QTL mapping study [36] we found resistance to permethrin to be principally (91.8% of genetic effect in MIM) under the control of target site insensitivity in the voltage gated sodium channel gene (orthologue of *paralysis* in *Drosophila* [53]). We have shown that the genetic architecture underlying temephos resistance to be completely different with both families having up to 11 QTL affecting the HTD phenotype in both families and from 2–9 QTL affecting DOA. The practical implications of these findings are that selection for temephos resistance in the field is likely to involve many (principally esterase) loci. It is unlikely that the same genes will be involved in all field populations and that genetic drift may play a large part in determining which combinations of the 49 currently identified carboxy/choline esterase genes [40] become upregulated and assume responsibility for metabolic detoxification of temephos.

**Table 3.** Cont.

| Marker Position | LOD | Effect | Effect(%) | LOD | Effect | Effect(%) |
|-----------------|-----|--------|-----------|-----|--------|-----------|
| Add. Arc4 | 35.1 | 0.0 | 0.02 | 0.0 | - | - |
| Dom. | 0.1 | 1.67 | 1.1 | - | - | - |
| Add. 1132CT1 | 64.1 | 1.9 | -0.04 | 11.2 | - | - |
| Dom. | 0.0 | -0.47 | 0.3 | - | - | - |
| Chromosome 3 Add. 301ACG1 | 0.1 | 0.7 | -3.65 | 1.4 | - | - |
| Dom. | 0.2 | 2.01 | 1.3 | - | - | - |
| Add. CCEae2D | 6.2 | 0.1 | -2.53 | -0.8 | - | - |
| Dom. | 0.1 | -5.34 | 5.2 | - | - | - |
| Add. vtg | 42.8 | 1.7 | 4.79 | 9.2 | 0.01 | 0.03 |
| Dom. | 0.2 | -1.69 | 2.5 | 0.06 | 0.11 | 1.8 |
| Add. 201TTA1 | 64.9 | 0.1 | 0.55 | 0.8 | - | - |
| Dom. | 0.2 | -1.54 | 1.8 | - | - | - |

Additive and dominance effects associated with hours until death and survival QTL in *Aedes aegypti* are also listed.

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In our previous QTL mapping study [36] we found resistance to permethrin to be principally (91.8% of genetic effect in MIM) under the control of target site insensitivity in the voltage gated sodium channel gene (orthologue of *paralysis* in *Drosophila* [53]). We have shown that the genetic architecture underlying temephos resistance to be completely different with both families having up to 11 QTL affecting the HTD phenotype in both families and from 2–9 QTL affecting DOA. The practical implications of these findings are that selection for temephos resistance in the field is likely to involve many (principally esterase) loci. It is unlikely that the same genes will be involved in all field populations and that genetic drift may play a large part in determining which combinations of the 49 currently identified carboxy/choline esterase genes [40] become upregulated and assume responsibility for metabolic detoxification of temephos.

**Supporting Information**

**Table S1** Single nucleotide polymorphic markers, vector base ID (or gene bank accession number), SNP position from cDNA and oligonucleotide sequence. The nucleotide at the 3’ end of primers tagged with [5’-Long tail] and [5’-Short tail] correspond to the SNP of interest. [5’-Long tail] corresponds to the sequence 9-GCGGGCAGGGCGGCGGGGGCGG-3' and [5’-Short tail] to the sequence 9-GCGGGC-3'. These GC rich tails produce amplicons that can be differentiated by melting curve PCR or agarose electrophoresis.
Table S2  Names and locations of markers used in mapping of temephos resistance QTL in *Aedes aegypti*. (DOCX)

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Author Contributions

Conceived and designed the experiments: GeiCRS KSR AFS WCB. Performed the experiments: GeiCRS KSR. Analyzed the data: GeiCRS KSR WCB. Contributed reagents/materials/analysis tools: GeiCRS KSR AFS WCB. Wrote the paper: GeiCRS KSR AFS WCB.
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