Quantitative Trait Loci That Control Dengue-2 Virus Dissemination in the Mosquito Aedes aegypti

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ABSTRACT

The mosquito Aedes aegypti is the most important vector of yellow fever and dengue fever flaviviruses. Ae. aegypti eradication campaigns have not been sustainable and there are no effective vaccines for dengue viruses. Alternative control strategies may depend upon identification of mosquito genes that condition flavivirus susceptibility and may ultimately provide clues for interrupting transmission. Quantitative trait loci affecting the ability of Ae. aegypti to develop a dengue-2 infection in the midgut have been mapped previously. Herein we report on QTL that determine whether mosquitoes with a dengue-2-infected gut can then disseminate the virus to other tissues. A strain selected for high rates of dengue-2 dissemination was crossed to a strain selected for low dissemination rates. QTL were mapped in the F2 and again in an F5 advanced intercross line. QTL were detected at 31 cM on chromosome I, at 32 cM on chromosome II, and between 44 and 52 cM on chromosome III. Alleles at these QTL were additive or dominant in determining rates of dengue-2 dissemination and accounted for ~45% of the phenotypic variance. The locations of dengue-2 midgut infection and dissemination QTL correspond to those found in earlier studies.

Aedes aegypti is the most important vector of yellow fever and dengue fever (serotypes 1–4) flaviviruses in humans (Gubler 2002). It is a container-breeding mosquito with a wide distribution throughout tropical and subtropical regions of the world. Large-scale mosquito eradication programs began in the 1940s and effectively reduced Ae. aegypti numbers. Unfortunately eradication was never achieved, and with the dissolution of these programs in the late 1960s Ae. aegypti reestablished itself throughout tropical and subtropical areas of the Americas. Failure to curb the disease vector would be of little consequence if suitable vaccines were available and used effectively. However, yellow fever remains an important public health problem in much of Africa and South America despite the fact that a safe and effective vaccine is widely available (Barrett and Monath 2003). No effective vaccines are available for dengue virus infection, and this places >2 billion people at risk for infection with dengue fever; each year an estimated 100 million human infections occur (Gubler 2002). Indeed, dengue fever is one of the most rapidly expanding diseases in the tropics, and now that all four serotypes of the virus are circulating in the Americas, there is an added increase in risk for the occurrence of dengue hemorrhagic fever.

Vector competence refers to the intrinsic permissiveness of an arthropod vector to infection, replication, and transmission of a pathogen (Hardy 1988; Woodring et al. 1996). Ae. aegypti exhibits global variation in vector competence for flaviviruses. In sub-Saharan Africa, a black “sylvan” subspecies, Ae. a. formosus, predominates. This subspecies has low vector competence for flaviviruses due primarily to a midgut infection barrier. In tropical and subtropical regions outside of Africa, a lighter-colored “domestic” subspecies, Ae. a. aegypti, is more common and is relatively susceptible to flavivirus infection (Gubler et al. 1979; Tabachnick et al. 1985).

Once ingested by its mosquito host, a virus must overcome several obstacles if it is to be transmitted to a subsequent host. First, the virus must establish a productive infection in the mosquito midgut by overcoming a midgut infection barrier (MIB). Bosio et al. (2000) mapped the quantitative trait loci (QTL) that affect the MIB for dengue-2 virus using an F1 intercross. QTL for the MIB were detected on chromosomes II and III and accounted for ~30% of the phenotypic variance ($\sigma^2_p$) in dengue-2 infection. These respectively accounted for 44 and 56% of the overall genetic variance ($\sigma^2_g$). Gomez-Machorro et al. (2004) mapped QTL for MIB in the F2 generation of an advanced intercross line established from a strain of Ae. aegypti previously selected for dengue-2 susceptibility and an Ae. a. formosus strain selected for refractoriness to midgut infection. A new sex-linked
QTL and a second QTL on chromosome II with genotypes subject to balancing selection were detected. Alleles at these QTL contributed additively in determining susceptibility and accounted for \( \sim 24\% \) of \( \sigma^2 \).

Following replication in the midgut epithelium, virus must overcome a midgut escape barrier (MEB) and replicate in other tissues. Ultimately, virus must then infect the salivary glands and be shed in the saliva for transmission to the next vertebrate host. The genetics of flavivirus MEBS in \emph{Ae. aegypti} are not well understood. Bosio et al. (1998) performed a standard half-sib breeding experiment and estimated that the heritability for a MEB was 0.39 in \emph{Ae. aegypti formosus} but was much lower in \emph{Ae. aegypti} where MEB appeared to be controlled instead by dominant alleles. Bosio et al. (2000) found weak evidence for a MEB QTL on chromosome III with standard interval mapping but not with composite-interval mapping. Other than these inconclusive studies, few researchers have examined the genetics of barriers to viral dissemination. A common problem has been that MEBS can be studied only in mosquito species or strains without MIBs because MEB phenotypes cannot be defined in mosquitoes that do not develop an infection in the gut. Thus studying MEBS requires breeding strains of mosquitoes with high midgut susceptibility that still retain some of the natural variation in MEB. Bennett et al. (2005) describe a dengue MEB strain \([\text{dengue-}2 (D2\text{MEB})]\) selected from a field population from Houston, Texas in which 75–85\% of mosquitoes had a midgut infection but only 25–35\% had a disseminated infection depending upon the dengue-2 viral genotype examined. The purpose of the present study is to map and characterize QTL that control midgut escape barriers in the \emph{D2MEB} strain.

**MATERIALS AND METHODS**

**Mosquito strains:** Selection of the \emph{Ae. aegypti} dengue-2 susceptible on 3 chromosomes (D2S3) and D2MEB strains is described by Bennett et al. (2005). Briefly, in D2S3 95–100\% of mosquitoes have a disseminated infection and were selected through crosses between Ibo and Puerto Rico mosquitoes, two long-maintained laboratory strains (Bosio et al. 1998). The D2MEB strain was selected beginning with families arising from single-pair matings between F1 parents from the Houston laboratory strain, which originated from eggs field collected in Houston, Texas, during the summer of 1998 (Bennett et al. 2002), and the selected D2S3 strain. In D2MEB, 85\% of mosquitoes had an infected midgut but only 27–37\% had a disseminated infection. The P1 parents of the reciprocal F1 families generated for this study were from D2MEB F0 and D2S7 F0 generations.

All mosquito phenotyping, selection, and mapping was done using dengue-2 JAM1409 virus, a strain originally isolated in 1983 in Jamaica. Oral feeding and dengue-2 infection protocols follow those of Bennett et al. (2002). Following the 14-day extrinsic incubation period, mosquitoes were frozen and heads and heads were severed, squashed onto acid-washed slides, acetone fixed, and assayed for dengue-2 by indirect immunofluorescence assay (Bennett et al. 2002). When viral antigen was not detected in head tissues, the respective abdomens, which had been stored at \(-70^\circ\), were assayed for virus antigen. Phenotypes were scored on a binary system, where 0/0 denotes an uninfectected mosquito, 1/0 denotes a mosquito with an infected midgut but no disseminated infection, and 1/1 denotes a mosquito with a disseminated infection.

**Breeding and experimental design:** Ten crosses between D2S3 and D2MEB parents were made in both directions to generate 20 reciprocal F1 intercross families. After 4–7 days, females were given an infectious blood meal (Bennett et al. 2002) and males were collected and frozen at \(-70^\circ\). Eggs were collected from individual females. After the 14-day extrinsic incubation period, infection phenotypes were determined in F1 females (Bennett et al. 2002).

D2S3 F1 females that had a disseminated infection and D2MEB F1 females that had an infected midgut but no dissemination of the infection were used. Eggs from each of these females were hatched and reared to adults, and individual F1 females from this hatch were paired with individual full-sibling F1 males. After 6–7 days, males were removed and stored at \(-70^\circ\). Females were given several noninfectious blood meals and eggs were collected from individual females. After 3–4 weeks, the F1 females were also stored at \(-70^\circ\). F2 families with the most eggs were hatched and reared to adults. These included six D2S3 \(\times\) D2MEB families and seven D2MEB \(\times\) D2S3 families. Full siblings of these F2 generations were allowed to intermate. After 4–7 days F1 females were provided an infectious blood meal. The F2 males and unfed females were removed and stored at \(-70^\circ\). Fed females were maintained through the extrinsic incubation period and assayed for infections in the midgut and head. The remnants of the abdomen and the thorax were returned immediately to a tube on dry ice and then frozen at \(-70^\circ\) awaiting DNA isolation.

DNA was extracted from all P0, P1, and F1 mosquitoes (Black and DuTeau 1997) and resuspended in 500 \(\mu\text{l}\) TE (50 mm Tris-HCl, 5 mm EDTA, pH 8.0) buffer. A 50-\(\mu\text{l}\) aliquot of DNA was overlaid with sterile mineral oil and stored at 4 \(^{\circ}\) for daily use in the polymerase chain reaction (PCR). The remainder was stored in plastic screw-top vials at \(-70^\circ\). PCR amplification and single-strand conformation polymorphism analysis are as previously described (Black and DuTeau 1997; Bosio et al. 2000). All of the primers and optimal conditions for the PCR in this study are published (Bosio et al. 2000; Fulton et al. 2001; Gomez-Machorro et al. 2004). Ultimately two complete F1 intercross families, D2S3 \(\times\) D2MEB 2.2 and D2MEB \(\times\) D2S3 7.1, were selected for further analysis. These were chosen because they had the largest F1 family sizes, the P1 and F1 parents had greater numbers of polymorphic loci, and in a preliminary screen a low percentage of F1 females had a disseminated infection (D2S3 \(\times\) D2MEB 2.2, 59.2\% disseminated infection; D2MEB \(\times\) D2S3 7.1, 42.5\% disseminated infection). The F1 eggs from these two families were hatched, reared to adults, and allowed to randomly mate to generate F2 eggs. By the F2 generation, \(\sim 2000\) eggs were available in each family.

For QTL mapping in an advanced intercross line, \(\sim 1000\) F1 eggs were hatched and the resulting females were assayed for infection phenotypes as described above. The remainder of the eggs was used to generate the F2 generation.

**QTL mapping:** A. \emph{aegypti} has a low abundance of microsatellites (Fagerberg et al. 2001) so that geneticists commonly use restriction enzyme analyses or single-strand conformation polymorphism analysis of PCR-amplified cDNA genes as markers for mapping. The linkage positions of all marker loci used in this study are published (Severson et al. 2002; Black and Severson 2004). Associations between genotypes at each marker locus and infection phenotypes were initially assessed by a contingency \(\chi^2\) analysis. The null hypothesis was that the numbers of mosquitoes with midgut and disseminated infections were equal in each genotype class. These marginal probabilities were the frequencies of each genotype at a locus in females and the overall proportions of mosquitoes with
midgut and disseminated infections. When a significant χ² was detected, we examined the inheritance of the alleles at that locus. Our a priori hypothesis was that an excess of F₂ individuals with an allele inherited from the D2S3 P₁ parent would have a disseminated infection while an excess of F₂ individuals with an allele inherited from the D2MEB P₁ parent would not disseminate the virus.

Composite-interval mapping (ZENG 1994) and multiple-interval mapping (ZENG et al. 1999) were performed using QTL Cartographer 2.0 (BASTEN et al. 2002). Composite-interval mapping was performed with nₖ set to the number of separate regions indicated in the initial contingency χ²-analyses and wᵢ = 10 cM (the recommended default value). Composite-interval mapping was run with 1000 permutations to estimate the 95% experimentwise threshold. For multiple-interval mapping, an initial model was estimated by forward and backward selection on markers with a probability of a partial R² set to 0.01. QTL positions were then optimized and the QTL model was saved. A secondary search for QTL was performed, their positions were optimized, and the QTL model was saved. Next, a search for epistasis was performed and the QTL model was saved. In the last step, confidence intervals around QTL positions, breeding values, R² partition, additive and dominance effects, and variance and covariance tables were produced using the multiple-interval mapping model summary procedure.

RESULTS

Mapping families: Of 76 F₁ D2S3 × D2MEB 2.2 females 62 had an infected midgut (62/76 = 81.6%), and 72.6% (45/62) of these developed a disseminated
TABLE 1

Multiple-interval mapping estimates of QTL position and associated genetic, environmental, and phenotypic variance and additive and dominance effects associated with MEB and MIB QTL in Aedes aegypti

<table>
<thead>
<tr>
<th>Nearest marker</th>
<th>cM</th>
<th>LR</th>
<th>Effect (%)</th>
<th>(% σ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS × DMB 2.2 F₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEB 2.18 (11.0%)</td>
<td>17.72 (89.0%)</td>
<td>19.90</td>
<td>Peroxnc</td>
<td>47.1</td>
</tr>
<tr>
<td>(Not significant)</td>
<td>(47.0–50.0)</td>
<td>0.8577</td>
<td>D, −3.10</td>
<td>(11.0)</td>
</tr>
<tr>
<td>MIB 0.87 (5.8%)</td>
<td>14.11 (94.2%)</td>
<td>14.16</td>
<td>Chitan1</td>
<td>50.0</td>
</tr>
<tr>
<td>(Not significant)</td>
<td>(49.9–53.6)</td>
<td>0.5238</td>
<td>D, +1.05</td>
<td>(2.7)</td>
</tr>
<tr>
<td>MEB 0.74 (3.0%)</td>
<td>24.18 (97.0%)</td>
<td>24.92</td>
<td>Chym2</td>
<td>42.7</td>
</tr>
<tr>
<td>(Not significant)</td>
<td>(42.6–54.4)</td>
<td>0.9921</td>
<td>D, +1.11</td>
<td>(1.3)</td>
</tr>
<tr>
<td>MIB 0.23 (0.9%)</td>
<td>24.56 (99.1%)</td>
<td>24.79</td>
<td>Chym2</td>
<td>38.7</td>
</tr>
<tr>
<td>(Not significant)</td>
<td>(38.6–42.6)</td>
<td>0.5238</td>
<td>D, +0.43</td>
<td>(0.0)</td>
</tr>
<tr>
<td>DMB × DMS 7.1 F₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEB 8.59 (34.4%)</td>
<td>16.39 (65.6%)</td>
<td>24.98</td>
<td>Amy2</td>
<td>45.2</td>
</tr>
<tr>
<td>(Not significant)</td>
<td>(45.1–47.0)</td>
<td>0.0000</td>
<td>D, +4.36</td>
<td>(25.5)</td>
</tr>
<tr>
<td>MIB 1.30 (9.0%)</td>
<td>13.14 (91.0%)</td>
<td>14.44</td>
<td>Defa</td>
<td>51.9</td>
</tr>
<tr>
<td>(Not significant)</td>
<td>(51.8–52.0)</td>
<td>0.2016</td>
<td>D, −4.16</td>
<td>(13.0)</td>
</tr>
<tr>
<td>MEB 10.96 (45.1%)</td>
<td>13.32 (54.9%)</td>
<td>24.28</td>
<td>Riboptl-1</td>
<td>34.4</td>
</tr>
<tr>
<td></td>
<td>(31.0–44.5)</td>
<td>0.6164</td>
<td>D, +1.24</td>
<td>(5.0)</td>
</tr>
<tr>
<td></td>
<td>(31.8–37.7)</td>
<td>10.9450</td>
<td>A, +1.77</td>
<td>(8.6)</td>
</tr>
<tr>
<td>MIB 2.84 (18.7%)</td>
<td>12.29 (81.3%)</td>
<td>15.13</td>
<td>TrypAbun</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>(44.0–52.0)</td>
<td>10.1491</td>
<td>D, −5.58</td>
<td>(7.4)</td>
</tr>
<tr>
<td></td>
<td>(31.8–37.7)</td>
<td>0.5628</td>
<td>D, −0.46</td>
<td>(0.2)</td>
</tr>
<tr>
<td></td>
<td>(25.0–25.7)</td>
<td>2.9061</td>
<td>A, +0.88</td>
<td>(2.7)</td>
</tr>
<tr>
<td></td>
<td>(44.0–60.0)</td>
<td>1.4398</td>
<td>D, −0.92</td>
<td>(2.1)</td>
</tr>
<tr>
<td></td>
<td>4.5978</td>
<td>AD, +2.94</td>
<td>(6.7)</td>
<td></td>
</tr>
</tbody>
</table>

Confidence intervals for QTL position are in parentheses below the position estimate. A, additive; D, dominance.

infection. Of 436 F₂ females 56.0% (244/436) had an infected gut and 52.0% (127/244) of these further disseminated the infection. Of 40 F₂ D2MEB × D2S3 7.1, 33 females had infected guts (33/40 = 82.5%) and 51.5% (17/33) of these disseminated the infection. A large percentage of the 296 F₂ females had infected midguts (241/296 = 81.4%), but only 41.1% (99/241) of these disseminated the infection.

Genotype-phenotype associations: The results of χ²-goodness-of-fit tests indicated that marker genotypes were not distributed independently of infection phenotypes (Figure 1). Values to the left and right of the chromosomes are from analysis of the F₂ and F₃ offspring, respectively. Loci that are statistically associated with disseminated infection appear in boxes containing the associated type I probability and those associated with midgut infection appear in dotted-line boxes.

**D253 × D2MEB 2.2:** Among the 62 D253 × D2MEB 2.2 F₂ females (Figure 1), there were significant associations between disseminated infection and genotypes at Riboptl-1 at 31 cM and at markers from 35 to 47 cM on chromosome I and at sin3 at 70 cM on chromosome II. There were no associations between midgut infection and genotypes among F₂ females. Among the 244 F₂ females, there were significant associations between disseminated infection and genotypes at LF090, TSFM, and Ferri- large percentage of the 296 F₂ females had infected midguts (241/296 = 81.4%), but only 41.1% (99/241) of these disseminated the infection.

Composite-interval mapping for disseminated infection was performed in both F₂ and F₃ females (results not shown). In neither generation did the original LOD value exceed the 95% experimentwise threshold and thus no QTL were inferred. Similarly, composite-interval mapping didn’t detect midgut infection QTL in F₂ or F₃ females. Multiple interval mapping estimated disseminated infection QTL in F₂ or F₃ females using for-
The numbers of alleles inherited from the D2S3 P1 parent were plotted against disseminated infection (Figure 2) to test the \textit{a priori} assumption that alleles associated with a higher rate of disseminated infection are inherited from the D2S3 parent. Among F2 females, genotypes at Riboptl1, Ferritin, Amy2, Peroxnc, and sin3 follow the predicted pattern. However, at LF314 and Ace the opposite pattern was seen. Among F2 females, genotypes at only TFSM, LF090, and mtATPSyn follow the predicted pattern. Apparently at some loci alleles inherited from the D2MEB parent yielded more disseminated infections than alleles inherited through the D2S3 parent.

The numbers of D2S3 alleles were also plotted against midgut infections to determine if D2S3 alleles yielded more midgut infections (Figure 3). The observed patterns were consistent with this prediction. However, with the exception of Ace, the effects of genotypes were slight. QTL alleles inherited from the D2S3 parent conferred only slightly more infected midguts than alleles inherited through the D2MEB parent.

\textbf{D2MEB × D2S3 7.1:} Among the 33 D2MEB × D2S3 7.1 F2 females, there were significant associations between disseminated infections and genotypes from 20 to 59 cM on chromosome I and at Apolipo2 on chromosome III (Figure 1). There was also an association between midgut infections and genotypes at DefA on chromosome III. Among the 241 F5 females, there were significant associations between disseminated infections and genotypes from 20 to 50 cM on chromosome I, at AEGI18 and TrypAbun on chromosome II, and at Elastase, RNAHelic, and AspSyn on chromosome III. There were significant associations between midgut infections and genotypes at TrypAbun on chromosome II and at Apolipo2 and RNAHelic on chromosome III.

The results of composite-interval mapping for disseminated infections in F2 and F5 D2MEB × D2S3 females are shown in Figure 4. In the F2, the original LOD values exceeded the 95\% experimentwise thresholds at 47 cM on chromosome I. With multiple-interval mapping, $\sigma^2_g$ accounted for 34.4\% of the $\sigma^2_p$ for disseminated infections in the F2 (Table 1) but none of the effects were significant. In the F5, the original LOD values exceeded the 95\% experimentwise thresholds at 30–45 cM on chromosome I, at 25–35 cM on chromosome II, and at 40–55 cM on chromosome III. With multiple-interval mapping, $\sigma^2_g$ accounted for 45.1\% of the $\sigma^2_p$ for disseminated infections in the F5 (Table 1).

For midgut infections, the original LOD values reached the 95\% experimentwise thresholds at 25 cM on chromosome II in the F2 and at 44 cM on chromosome III in the F5 (Figure 5). With multiple-interval mapping in the F2, $\sigma^2_g$ accounted for 9\% of $\sigma^2_p$ for mid-
gut infections (Table 1) but none of the effects was significant. However, with multiple-interval mapping in the F₅, σₛ² accounted for 18.7% of σₑ² (Table 1).

Numbers of alleles inherited from the D2S3 P₁ parent were plotted against rates of disseminated infection. The observed patterns in the F₂ offspring were entirely consistent with a larger number of disseminated infections being associated with alleles from the D2S3 strain for markers on all chromosomes (Figure 6).

With the exception of Amy2, susceptibility alleles on the chromosome I QTL were additive in conditioning disseminated infections in the F₂ (Figure 6). In multiple-interval mapping, more variance in σₛ² associated with Ribopt11 was attributable to additive effects (16.1%; Table 1). Susceptibility alleles were also additive in conditioning disseminated infections on the chromosome II QTL (Figure 6). In multiple-interval mapping, all of the variance in σₛ² associated with TrypAbun was attributable to additive effects (8.6%; Table 1). In contrast, susceptibility alleles are recessive in the chromosome III disseminated infection QTL (Figure 6). In multiple-interval mapping, equal amounts of variance in σₛ² associated with RNAHelic were associated with additive and dominance effects (8.0 and 7.4%, respectively; Table 1).

The numbers of D2S3 alleles were also plotted against the proportion of mosquitoes with infected midguts.
Figure 5.—Plot of LOD values associated with midgut infection rates along chromosomes I–III in the D2MEB × D2S3 7.1 family. Names of markers are listed to orient QTL positions relative to Figure 1.

(Figure 7). The observed patterns were consistent with a higher number of mosquitoes with infected midguts being associated with alleles from the D2S3 strain. However, the variance in midgut infections among genotypes was slight. Multiple-interval mapping detected and verified a midgut infection QTL at 32 cM on chromosome II and at 25 and 44 cM on chromosome III. In addition, an epistatic interaction was detected between the QTL at 25 and 44 cM on chromosome III. This epistatic interaction is evident in Figure 7. When alleles at the 25 and 44 cM QTL were homozygous for D2MEB alleles, the proportion of mosquitoes with a disseminated infection at the 44-cM QTL exceeded the proportion at the 25-cM QTL. However, when alleles at the 25- and 44-cM QTL are heterozygous or homozygous for D2S3 alleles, the 44-cM QTL rate of dissemination is lower than the 25-cM QTL rate. This interaction appears to occur because D2S3 alleles at the 25-cM QTL are additive but are dominant at the 44-cM QTL. Note also that additive and dominance values (Table 1) are opposite in sign between the 25- and 44-cM QTL.

DISCUSSION

Family-based selection is often necessary to maintain the viability of Ae. aegypti strains due to a high load of deleterious and lethal recessive genes in this and other Aedes species (Gomez-Machorro et al. 2004; Mun-
The genetic diversity in $D2MEB$ generated by family-based selection probably caused the large disparity in the presence and magnitude of QTL between the two reciprocal crosses. Clearly the genome of the $D2MEB \times D2S3$ mother contained distinct QTL with strong effects on disseminated infection or midgut infection while the father’s genome in the $D2S3 \times D2MEB$ cross did not. Furthermore, Figure 2 shows that alleles inherited from the $D2MEB$ parent actually conferred higher disseminated infection rates than alleles inherited through the $D2S3$ parent. These observations are consistent with the presence of a series of hypermorph or hypomorphic alleles at disseminated infection QTL in $D2MEB$.

Advanced intercross lines (Darvasi and Soller 1995) were generated from each reciprocal cross in this study because of the relatively small numbers of F2 females with midgut infections ($62$ $D2S3 \times D2MEB$; $33$ $D2MEB \times D2S3$) that could be used for mapping disseminated infection QTL. This is the most likely reason for the observed increase in the number of QTL from the F2 to the F5 generations of $D2MEB \times D2S3 7.1$. In addition, when analyzing female-limited traits, advanced intercross lines are essential for testing for sex-linked QTL. This is due to insufficient recombination between the sex locus and markers/QTL on chromosome I in F2 progeny to estimate linkage with markers arising in the P1 father. Usually few if any paternal genotypes appear in F2 females. This is probably why the position and magnitude of the chromosome I QTL shifted between the F2 and F5 generations.

The identification of QTL regions associated with dengue-2 infection will become the starting point for future efforts to clone and characterize the actual genes that confer MIBs and MEBs. The limiting factor at this point may be the relatively low rate of recombination observed in the $Ae. aegypti$ genome (1.0–3.4 Mbp/cM) (Brown et al. 2001), as the ability to create a highly resolved map upon which to base map-based positional cloning is directly dependent upon the number of recombination events between a marker and the actual QTL. Advanced intercross lines may become a viable solution to the low recombination rate in $Ae. aegypti$. As the number of generations increases, the amount of recombination observed in a segregating population...
increases, and this in turn increases the mapping resolution that can be obtained. Advanced intercross lines maintained through many generations will provide highly accurate estimates of the order of genetic markers flanking a QTL. D2MEB × D2S3 7.1 is now in the F1 generation and adults generally demonstrate good longevity and fecundity. In contrast, D2S3 × D2MEB 2.2 died out in the F11 generation due mostly to low egg

Barry Miller, Carol Blair, and Carolina Barillas-Mury served on  

LITERATURE CITED


Hardy, J. L., 1988 Susceptibility and resistance of vector mosquitoes,


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