

Comparative Linkage Maps for the Mosquitoes (*Culex pipiens* and *Aedes aegypti*) Based on Common RFLP Loci

A. Mori, D. W. Severson, and B. M. Christensen

We report construction of a comparative linkage map for the mosquito (*Culex pipiens*) based on restriction fragment length polymorphisms (RFLPs) using cDNA clones from *Aedes aegypti* as probes to Southern blots of *Cx. pipiens* genomic DNA. Seventy-one cDNA clones were screened for hybridization and genetic diversity among three *Cx. pipiens* strains. Fifty-two of 71 cDNA clones, isolated from and previously mapped in *Ae. aegypti* (73.2%), were hybridized under high-stringency conditions with *Cx. pipiens* genomic DNA. Thirty-four clones (47.9%) reflected strain-specific polymorphisms. The map consists of 21 cDNA markers that identify 22 loci covering 165.8 cM. The loci mapped in *Cx. pipiens* cover 7.1 cM on chromosome 1, 80.4 cM on chromosome 2, and 78.3 cM on chromosome 3. Linkage relationships of the RFLP markers for chromosome 1 in *Cx. pipiens* are the same as chromosome 1 in *Ae. aegypti*, indicating that chromosome 1 is highly conserved between the two species. The comparative RFLP linkage maps for chromosomes 2 and 3 in *Cx. pipiens* and *Ae. aegypti* reflect apparent whole-arm translocations. RFLP markers for chromosome 2 in *Ae. aegypti* identified homologous loci on one arm of chromosomes 2 and 3 in *Cx. pipiens*, and RFLP markers for chromosome 3 in *Ae. aegypti* identified homologous loci on the opposite arms of chromosomes 2 and 3 in *Cx. pipiens*.

Culex pipiens is one of the most medically important mosquito species because it has a broad global distribution and is one of the primary vectors for periodic Bancroftian filariasis. Numerous investigators have examined the basic biology of this mosquito, including several genetic aspects. Narang and Seawright (1982) listed as many as 103 genetic mutants of *Cx. pipiens* and were able to determine linkage associations and linear orders for several loci by integrating genetic data extracted from the literature. Munstermann and Craig (1979) compared the linkage associations of the same seven isozyme loci in both *Aedes aegypti* and *Cx. pipiens*. Three isozyme loci located on linkage group II in *Ae. aegypti* were located on linkage group III in *Cx. pipiens*, while two isozyme loci located on linkage group III in *Ae. aegypti* were located on linkage group II in *Cx. pipiens*. Matthews and Munstermann (1994) examined chromosome evolution in mosquitoes and identified extensive conservation in linkage associations among groups of isozyme loci across species. They suggested that chromosome divergence between species largely reflects whole arm rearrangements. They ob-

served that isozyme loci on chromosome 1 seemed to be conserved between *Ae. aegypti* and *Cx. pipiens*. Also, one arm of chromosome 2 and one arm of chromosome 3 in *Ae. aegypti* seemed to be conserved with one arm of chromosome 3 and one of chromosome 2, respectively, in *Cx. pipiens*. A limited amount of available data suggested that some rearrangement existed between opposite arms of chromosome 2 and 3 in the two species.

Severson et al. (1993) constructed a restriction fragment length polymorphism (RFLP) linkage map for *Ae. aegypti* using cloned cDNA sequences isolated from *Ae. aegypti* as probes for identifying polymorphism. They also confirmed that some of these cDNA clones would hybridize with genomic DNA of other mosquito species (Severson et al. 1994b). They then successfully constructed a comparative linkage map for *Ae. albopictus* using common RFLP markers developed for *Ae. aegypti* (Severson et al. 1995). The linkage group associations and linear orders for the 18 common markers examined were conserved completely between the two species. Because *Ae. albopictus* and *Ae. aegypti* are members of the same subgenus

From the Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, Wisconsin. A. Mori and D. Severson are currently at the Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556-5645. We thank L. Christensen, V. A. Kassner, and J. Chiles for technical assistance, and M. Ferdig for helpful discussion and advice. This research was supported by NIH grant no. AI 28781 (to B.M.C.) and Burroughs Wellcome Fund (to B.M.C.). Address correspondence to Akio Mori at the address above.

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(*Stegomyia*), the high level of gene conservation observed between them was anticipated. Subsequently, Ferdig et al. (1998) used *Ae. aegypti* RFLP markers in developing a genetic linkage map for *Armigeres subalbatus*. Twenty-six markers were mapped and only one was not linked in the manner predicted by the *Ae. aegypti* map. Because many cDNA clones isolated from *Ae. aegypti* also readily hybridize at full stringencies with *Cx. pipiens* genomic DNA (Severson et al. 1994b), it was anticipated that we could construct an RFLP genetic linkage map for *Cx. pipiens* using mapped cDNA clones from *Ae. aegypti* in lieu of isolating new cDNA clones from *Cx. pipiens*. This strategy would also allow us to compare chromosome organization between *Cx. pipiens* and *Ae. aegypti*. In this article we report the construction of a genetic linkage map for *Cx. pipiens* using previously mapped cDNA clones isolated from *Ae. aegypti* as probes for RFLP markers.

Materials and Methods

Mosquitoes

Three *Cx. pipiens* strains were used for these studies. The *Cx. pipiens* Iowa strain was established with females collected near Ames, Iowa, in 1995. The *Cx. pipiens* Nile Delta strain was obtained from Dr. H. Farid of Ain Shams University, Abbassia, Cairo, Egypt. It originated with mosquitoes collected in the Nile Delta region of Egypt in 1979 and has been maintained continuously in the laboratory. The *Cx. pipiens* Molestus strain was obtained from Dr. M. Kamei at Earth Biochemical Inc., Tokushima, Japan. For linkage analysis, three F₂ crosses were produced. In cross 1 and cross 2, F₂ progeny were produced by pairwise matings between Molestus strain females and Iowa strain males. In cross 3, F₂ progeny were produced by a pairwise mating between a Nile strain female and an Iowa strain male.

For crossing experiments, pupae from individual colonies were separated by sex before adult emergence. Then one male and five females representing the appropriate strains were introduced to 30 cm × 20 cm × 20 cm cheesecloth cages. These adults were continuously provided a 1 M sucrose solution as a food source. Females from the Molestus strain are autogenous and required no blood prior to first oviposition. Females from the Nile strain are anautogenous and were allowed to blood feed on a mouse to stimulate oviposition. F₁ progeny from single egg rafts were

hatched and maintained in white enamel pans (25 cm × 42 cm × 7 cm) containing distilled water and fed a diet of fine-ground fish food (Tetramin). F₁ pupae were separated by sex, and one male and five female siblings were combined in 30 cm × 20 cm × 20 cm cheesecloth cages. Females in cross 1 and cross 2 were allowed to blood feed on a mouse after 7 days posteclosion; any eggs oviposited prior to blood feeding (autogenous) were discarded. Females in cross 3 were allowed to blood feed on a mouse 7 days after being combined with a male. F₁ females were transferred individually to 20 ml glass containers containing 5 ml water 3 days after blood feeding. F₂ progeny from individual females were reared as described above. All mosquitoes were maintained in an environmental chamber at 26.5 ± 1°C and 80 ± 5% RH under a 16 h light : 8 h dark cycle with a 90 min crepuscular period at the beginning and end of each light cycle.

Molecular Techniques

DNA extractions from individual and bulked mosquitoes, digestion with *EcoRI*, Southern blotting, and hybridizations were performed as described elsewhere (Severson 1997), except that Quiabran Nylon Plus (Qiagen, Inc.) was used as the membrane for Southern blotting. The original sources of the cDNA clones used as probes are described in Severson et al. (1993 and 1994a). Note that the TY7 clone, isolated from genomic DNA as a PCR product, is an open reading frame for an anonymous gene (Ferdig M, unpublished data).

Linkage Analysis

Linkage analysis was carried out using MAPMAKER (Lander and Green 1987; Lander et al. 1987). A minimum threshold LOD score of 3.0 was used to identify linkage between loci. Linkage data from the three independent crosses were integrated using the JoinMap computer program (Stam 1993).

Results and Discussion

We previously demonstrated that some cDNA clones from *Ae. aegypti* readily hybridized with *Cx. pipiens* genomic DNA and that some of them showed RFLP among *Cx. pipiens* strains (Severson et al. 1994b). In this study, 71 cDNA clones isolated from and previously mapped in *Ae. aegypti* were screened for hybridization and genetic diversity among three *Cx. pipiens* strains. Fifty-two clones (73.2%) hy-

Table 1. Segregation of markers in the F₂ from three crosses used to construct an RFLP linkage map

Marker	Number of individuals P ₁ :H:P ₂	χ ²
Crossing 1		
Chromosome 1		
LF188a	36:41:5 36:31:3 female 10:2 male	23.44**
LF284	42:56:14 42:39 female 17:14 male	14.00**
TY7	43:56:4 43:34 female 22:4 male	30.32**
Chromosome 2		
LF335	18:58:50	17.05**
LF203	15:71:31	9.72**
LF108	5:89:31	33.29**
Chromosome 3		
LF115	26:48:19	1.15
LF264	51:67:25	10.02**
LF106	26:37:29	3.72
LF128	40:50:23	5.82
Crossing 2		
Chromosome 2		
LF335	52:97:41	1.41
DDC	44:71:30	2.77
LF203	53:90:44	1.31
LF108	45:100:45	0.53
LF386	34:115:41	8.94*
Chromosome 3		
LF264	37:99:53	3.14
LF99c	34:76:35	0.35
LF272	37:99:54	3.38
LF106	32:101:54	6.38*
LF99a	32:105:52	6.57*
Crossing 3		
Chromosome 2		
LF334	33:109:53	5.83
LF335	35:110:45	5.79
LF203	17:135:54	33.17**
LF129	18:140:33	43.83**
LF108	31:146:65	19.88**
LF168	26:155:61	29.23**
LF377	37:143:60	13.22**
LF323	33:136:50	15.47**
Chromosome 3		
LF250	44:101:46	0.68
LF124	69:122:50	3.03
LF264	76:114:51	5.89
LF106	67:139:37	12.45**

* $P < .05$; ** $P < .01$.

Phenotypic designations: P₁ = female parental type, H = heterozygote type, P₂ = male parental type.

bridized under high-stringency conditions with *Cx. pipiens* genomic DNA, and 34 of them (47.9%) showed strain-specific polymorphisms. From these 34 clones, 21 clones were chosen for recombinational mapping with segregating F₂ progeny. The 21 clones segregated at 22 independent loci. All loci were scored with respect to the parental RFLPs as homozygous for the maternal or paternal type or as heterozygous. Segregation ratios for each locus are given in Table 1.

Significant deviations from the expected

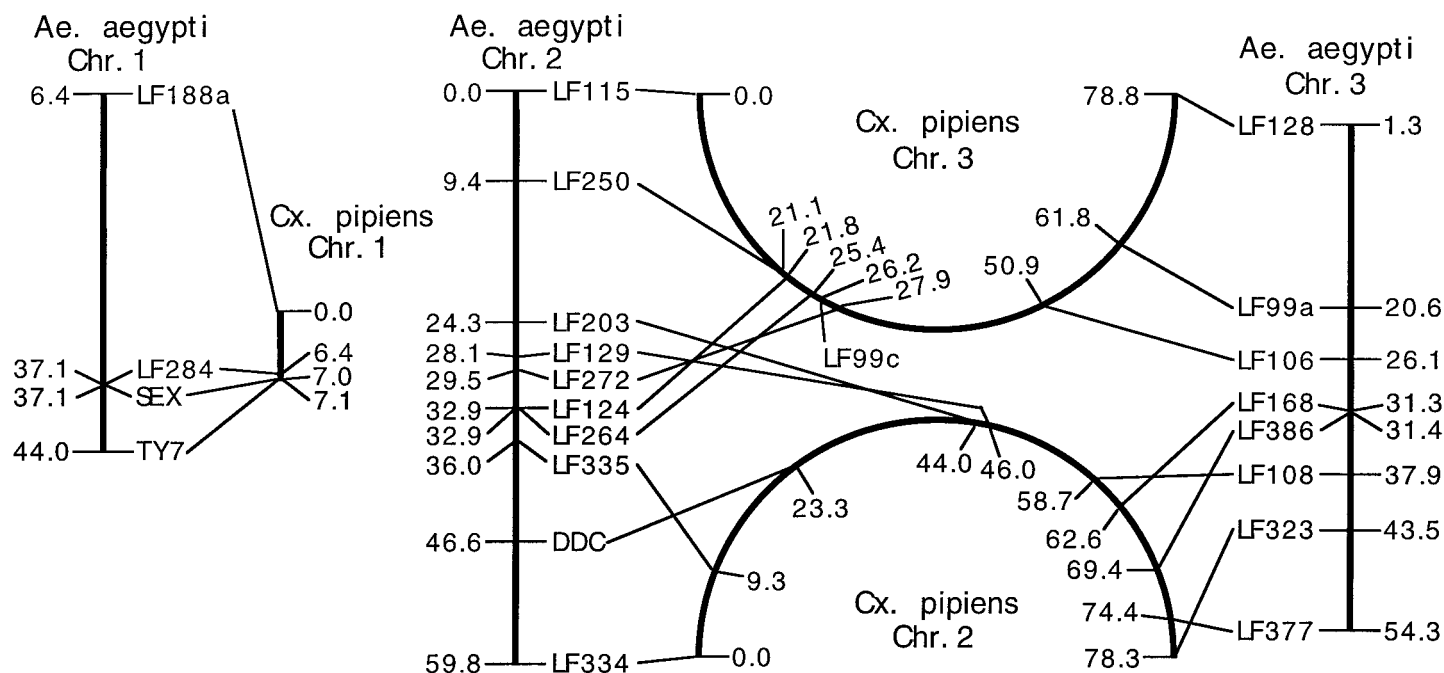


Figure 1. Comparative genetic maps of *Culex pipiens* and *Aedes aegypti* based on common RFLP loci. Map distances are listed in Kosambi centiMorgans.

1:2:1 ratio were observed with 18/33 (54.5%) of the loci examined in at least one of the test crosses. With these loci there was a significant deficit in one of the parental homozygote genotypes. The greatest deficit was observed around the LF129

and LF203 loci on chromosome 2 in cross 3. This deficit likely reflects the effect of a lethal locus located near the LF129 locus. Deleterious alleles at this locus apparently were obtained through the maternal genotype. Similar segregation distortions

were observed previously with *Ae. aegypti* (Severson et al. 1994a), *Ae. albopictus* (Severson et al. 1995), and *Armigeres subalbatus* (Ferdig et al. 1998). This phenomenon has been suggested as a mechanism for maintaining heterozygosity in mosquito strains (Matthews and Munstermann 1994; Munstermann and Craig 1979).

The comparative linkage map for *Cx. pipiens* is presented in Figure 1. This linkage map represents the best fit for all our mapping data using the JoinMap computer program (Stam 1993). Recombination fractions and standard error for the composite data are listed in Table 2. The loci mapped in *Cx. pipiens* cover 7.1 cM on chromosome 1, 80.4 cM on chromosome 2, and 78.3 cM on chromosome 3, for a total map distance of 165.8 cM, compared with 150.8 cM for the same markers in *Ae. aegypti*. Linkage associations and linear orders of RFLP loci located on chromosome 1 seem to be completely conserved between *Ae. aegypti* and *Cx. pipiens*. We also were able to identify the map location of the sex determination locus on this chromosome. Gilchrist and Haldane (1947) reported that maleness in a *Cx. pipiens* autogenous strain (note that this strain is frequently referred to as *Culex molestus* or *Cx. pipiens molestus*) seemed to be determined by a single dominant gene, and Jost and Laven (1971) associated this locus with the morphologically shortest chromosome in *Cx. pipiens*, chromosome 1. The map distance between LF188 and TY7

Table 2. Recombination fractions and standard errors for RFLP marker data

Chromosome 1	LF188	LF284	SEX						
LF284	6.13 (2.23)								
SEX	7.14 (3.98)	0.01 (0.09)							
TY7	6.21 (2.26)	1.02 (0.84)	0.01 (0.11)						
Chromosome 2 ^a	LF334	LF335	DDC	LF203	LF129	LF108	LF168	LF386	LF377
LF335	8.84 (1.88)								
DDC	— ^b	14.85 (2.63)							
LF203	37.66 (4.50)	28.89 (2.20)	21.79 (3.08)						
LF129	35.59 (4.74)	27.41 (4.05)	— ^b	2.94 (1.14)					
LF108	43.70 (3.91)	39.99 (2.53)	30.37 (3.77)	15.07 (1.55)	14.05 (2.71)				
LF168	46.95 (4.35)	42.86 (5.30)	— ^b	17.06 (2.64)	15.46 (2.92)	5.35 (1.32)			
LF386	— ^b	42.18 (3.95)	34.75 (4.24)	22.85 (3.08)	— ^b	10.98 (2.22)	— ^b		
LF377	— ^c	— ^c	— ^b	24.47 (3.33)	25.73 (3.93)	15.81 (2.39)	13.00 (2.16)	— ^b	
LF323	— ^c	— ^c	— ^b	27.03 (3.89)	27.41 (4.26)	17.94 (2.72)	15.93 (2.59)	— ^b	4.48 (1.26)
Chromosome 3 ^a	LF115	LF250	LF124	LF264	LF99c	LF272	LF106	LF99a	
LF250	— ^b								
LF124	— ^b	0.79 (0.53)							
LF264	23.44 (4.39)	4.28 (1.25)	3.16 (0.94)						
LF99c	— ^b	— ^b	— ^b	1.04 (0.70)					
LF272	— ^b	— ^b	— ^b	1.59 (0.76)	2.09 (1.00)				
LF106	— ^c	26.65 (3.39)	29.92 (3.14)	24.81 (1.87)	24.40 (3.74)	20.31 (2.84)			
LF99a	— ^b	— ^b	— ^b	27.40 (3.76)	28.95 (3.86)	26.99 (3.77)	14.30 (2.76)		
LF128	— ^c	— ^b	— ^b	37.72 (4.35)	— ^b	— ^b	26.18 (4.23)	— ^b	

Standard errors in parentheses.

^a JoinMap-derived composite data.

^b Not examined in the same population; two-point comparisons not available.

^c Recombination fraction greater than 50%.

in *Cx. pipiens* was only 7.1 cM, compared with 37.6 cM in *Ae. aegypti*. Severson et al. (1995) reported a similar phenomenon in *Ae. albopictus*, where the map distance between LF188 and TY7 was only 10.7 cM. They suggested that the reported variability in haploid nuclear DNA content among *Ae. albopictus* strains may influence recombination frequencies. No evidence currently exists to explain the extremely low recombination frequency observed for chromosome 1 in *Cx. pipiens*.

The comparative RFLP linkage maps for chromosomes 2 and 3 in *Cx. pipiens* and *Ae. aegypti* largely reflect whole-arm translocations, although some putative inversions are also evident (Figure 1). RFLP markers for chromosome 2 in *Ae. aegypti* identified homologous loci on one arm of chromosomes 2 and 3 in *Cx. pipiens*. In addition, loci located near the presumed centromeric region of chromosome 2 in *Ae. aegypti* (LF203 to LF335) are distributed in *Cx. pipiens* in a manner reflecting the occurrence of multiple inversion events in the evolutionary history of the two species. Estimations of the phylogenetic relationships among mosquitoes suggest that the genus *Culex* is basal to the genus *Aedes* (see Besansky and Fahey 1997). The fossil record indicates the presence of the genus *Culex* by the Upper Eocene and both the *Culex* and *Aedes* genera by the Lower Oligocene (Rohdendorf 1974). Of interest, although the genus *Armigeres* is considered basal to both genera (see Besansky and Fahey 1997), the genome structures of *Ar. subalbatus* and *Ae. aegypti* remain highly conserved (Ferdig et al., 1998). RFLP markers for chromosome 3 in *Ae. aegypti* identified homologous loci on the remaining arms of chromosomes 2 and 3 in *Cx. pipiens*. Again, we observed evidence for some inversion events involving loci located near the presumed centromeric region of chromosome 3 in *Ae. aegypti*. These results are consistent with previous comparative interpretations of isozyme linkage associations between the two species (Matthews and Munstermann 1994; Munstermann and Craig 1979).

We designated linkage groups as chromosomes 2 and 3 for *Cx. pipiens* based on presumed synteny with *Ae. aegypti*. In *Ae. aegypti*, the gene for larval DDT resistance is near one end of chromosome 2, but the gene for dieldrin resistance is near the opposite end of chromosome 2 (Lockhart et al. 1970). In *Cx. pipiens*, the gene for larval DDT resistance also resides on chromosome 2, but the dieldrin resistance gene is

on chromosome 3 (Tadano and Brown 1967). Because the cloned gene for dieldrin resistance (a GABA receptor) maps near the LF250 locus on chromosome 2 in *Ae. aegypti* (Severson et al. 1997), it seems likely that this gene remains linked with the LF250 locus on chromosome 3 in *Cx. pipiens*.

The LF99 clone identified two independent loci (a and c) on chromosome 3 in *Cx. pipiens*. This clone also mapped to two loci in *Ae. aegypti*, but they are located on chromosome 3 (LF99a) and chromosome 1 (LF99b), respectively (Severson et al. 1993). Based on the observed linkage associations, LF99a seems to be the common locus between the two species. Because the second LF99 locus in *Cx. pipiens* seems to be independent from LF99b in *Ae. aegypti*, we elected to designate it as a novel locus LF99c.

Cx. pipiens is a primary vector of periodic *Wuchereria bancrofti* in urban areas and *Dirofilaria immitis* in select geographic locations. Zielke and Kuhlow (1977) suggested that at least two genetic factors, which seemed to be sex-linked, influence *W. bancrofti* susceptibility in *Cx. pipiens*. This was based on their efforts to select for *W. bancrofti* susceptible or refractory strains. Zielke (1973) successfully selected strains of *Cx. pipiens* and *Ae. aegypti* that were susceptible or refractory to *D. immitis* infection and determined that susceptibility of *Ae. aegypti* to *D. immitis* is determined in part by a sex-linked recessive gene. Although *Cx. pipiens* does not naturally transmit *Brugia pahangi* or *B. malayi*, Obiamiwe (1976) selected a *B. pahangi*-susceptible strain in the laboratory. Further, the gene for *B. pahangi* susceptibility in this strain was linked with the sex determination and red-eye loci on chromosome 1 (Obiamiwe 1983; Obiamiwe and Macdonald 1973). In *Ae. aegypti*, genes for susceptibility to *B. malayi* (*fsb* 1), sex (*M*), and red-eye (*re*) are also located on chromosome 1 (Munstermann and Craig 1979; Severson et al. 1994a). Our results demonstrate that linkage and linear orders of loci on chromosome 1 are highly conserved between *Cx. pipiens* and *Ae. aegypti*. Further, because previous investigations indicate that the major gene determining susceptibility to several filarial parasites seems to reside in a similar genome position in both species, it seems most likely to represent an orthologous gene, highly conserved in mosquito evolution.

Although many mutations have been identified and examined for linkage associations with *Cx. pipiens*, most of the mu-

tant strains were lost because of the difficulty in maintaining them in laboratory culture. Our present results show that cDNA clones originating from *Ae. aegypti* can be used for rapidly constructing an RFLP map for *Cx. pipiens*. These results also provide further evidence that RFLP markers developed from cDNA clones can likely be used to develop linkage maps for any mosquito species and that the comparative maps can be used to infer genome positions of orthologous genes, including those that determine vector competence. Of obvious interest is the isolation and characterization of these genes to determine how specific changes in them influence mosquito vector competence.

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