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A Linkage Map of the Asian Tiger Mosquito (*Aedes albopictus*) Based on cDNA Markers

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Abstract

The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse), is an important vector of a number of arboviruses, and populations exhibit extreme variation in adaptive traits such as egg diapause, cold hardiness, and autogeny (ability to mature a batch of eggs without blood feeding). The genetic basis of some of these traits has been established, but lack of a high-resolution linkage map has prevented in-depth genetic analyses of the genes underlying these complex traits. We report here on the breeding of 4 F_1 intercross mapping families and the use of these to locate 35 cDNA markers to the *A. albopictus* linkage map. The present study increases the number of markers on the *A. albopictus* cDNA linkage map from 38 to 73 and the density of markers from 1 marker/5.7 cM to 1 marker/2.9 cM and adds 9, 16, and 10 markers to the 3 linkage groups, respectively. The overall lengths of the 3 linkage groups are 64.5, 76.5, and 71.6 cM, respectively, for a combined length of 212.6 cM. Despite conservation in the order of most genes among the 4 families and a previous mapping family, we found substantial heterogeneity in the amount of recombination among markers. This was most marked in linkage group I, which varied between 16.7 and 69.3 cM. A map integrating the results from these 4 families with an earlier cDNA linkage map is presented.

Key words: Aedes albopictus, *cDNA markers, linkage map, SSCP analysis*

The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse), is an important vector of dengue fever viruses (DENV1–4) (Rudnick and Chan 1965; Ibanez-Bernal et al. 1997; Chow et al. 1998; Chung et al. 2001; Chung and Pang 2002), LaCrosse virus (LACV) (Cully et al. 1992), and most recently Chikungunya virus (CHIKV) (Jain et al. 2008; Simon et al. 2008; Townson and Nathan 2008; Tsetsarkin et al. 2008). *Aedes albopictus* is also capable of transmitting many other arboviruses of public health importance (Shroyer 1986a, 1986b; Mitchell et al. 1990, 1992, 1997; Mitchell, Mclean, et al. 1993; Mitchell, Savage, et al. 1993; Savage et al. 1993, 1994; Harrison et al. 1995; Mitchell, Morris, et al. 1996; Mitchell, Smith, et al. 1996). *Aedes albopictus* strains vary in their ability to diapause and in the associated critical photoperiod (Hawley et al. 1989; Pumpuni et al. 1992). They also vary in their cold hardiness (Hawley et al. 1989; Hanson and Craig 1994, 1995) and in their ability to undergo autogeny (Mori et al. 2008). The genetic basis of some of these traits has been established (Cully et al. 1992; Pumpuni et al. 1992; Hanson and Craig 1994). However, except for autogeny (Mori et al. 2008), the inheritance and genetic control of these traits are not well understood. This is in part due to the lack of a high-resolution linkage map for genetic analysis of complex traits.

Severson et al. (1995) were the first to publish a linkage map of *A. albopictus*. This map was constructed with 18 restriction fragment length polymorphism (RFLP) markers in cDNA clones from *A. aegypti* (L). The linkage group and linear order appeared to be identical for the 2 species. The size of linkage group I (the linkage group containing the *Sex* locus) was 18.2 cM, whereas linkage groups II and III were 46.1 and 34.1 cM, respectively. The overall length of the linkage map was 98.4 cM compared with 129 cM in *A. aegypti*. However, for neither species did this represent an

Table 1 Families selected for linkage mapping

Family	No. F_2 progeny	No. markers	Total length (cM)	Chromosome 1		Chromosome 2		Chromosome 3	
				No. markers	Length (cM)	No. markers	Length (cM)	No. markers	Length (cM)
Nuevo Leon × Lake Charles									
Family 5.2	28	50	147.1	9	16.7	18	57.2	14	73.2
Family 5.6	28	49	139.5	12	25.7	7	38.0	16	75.8
Lake Charles × Nuevo Leon									
Family 17.3	36	54	176.9	7	69.3	11	40.4	18	67.2
Family 17.4	46	44	156.3	6	37.9	14	50.0	16	68.4
Four families	138	73	169.0	18	39.0	32	69.0	23	61.0
Mori et al. (2008)	221	38	215.8	9	48.3	16	99.4	13	68.1

The number of markers mapped in each family and for each linkage group. The estimated length of the linkage group for each linkage group and family.

intensive linkage map (1 marker/≤5 cM). Mutebi et al. (1997) analyzed random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990, 1993). They performed SSCP (Orita et al. 1989) analyses of these RAPD markers to construct a linkage map of *A. albopictus*. Six RAPD primers amplified 68 polymorphic loci at which alleles segregated in a Mendelian fashion. The sizes of linkage groups I, II, and II were 54.0, 67.4, and 103.5 cM, respectively. The overall size of the linkage map was 225 cM (1 marker/≤3.3 cM). Unfortunately, maps based on RAPD markers have proved to be irreproducible because these markers are so highly variable among different mapping families that it is rare for there to be much overlap in markers between families (Black and Severson 2002).

As an alternative, we developed the use of SSCP analysis of cDNA markers as a means to perform linkage mapping with reproducible markers (Fulton et al. 2001). Variation in cDNAs revealed by SSCP analysis has allowed us to progressively populate the *A. aegypti* map with new markers from different mapping families. Black and Severson (2002) presented one such consolidated map based on RFLP and SSCP analysis of cDNA markers in *A. aegypti*. This map was populated with 55, 69, and 48 cDNA markers, respectively, on linkage groups I, II, and III. In order to begin to compile a similar linkage map based on cDNA markers in *A. albopictus*, we have generated several mapping families. The first of these was constructed by Mori et al. (2008) to develop a quantitative trait locus map of regions of the *A. albopictus* genome that condition autogeny. An anautogenous *A. albopictus* strain (Sri Lanka, SL) was crossed with an autogenous strain Tok-au selected from a strain Tokushima, Japan. Both the SL and Tok strains had been maintained in the laboratory for an unknown number of generations. The final mapping population was initiated from a single P_1 pair but ultimately F_2 progenies were obtained from several F_1 crosses. These included 7 F_1 males each mated with several F_1 females. The resulting F_2 population contained 221 female progenies. The authors developed a linkage map based on RFLP or SSCP analysis of 9 cDNA markers on linkage group I, 16 cDNAs on group II, and 13 cDNAs on III. Many of those markers came from the same cDNA libraries described in the present paper. The sizes of the respective linkage groups were 48.3, 99.4, and 68.1 cM for a combined length of 215.8 cM, very close to the 225 cM RAPD–SSCP map reported by Mutebi et al. (1997).

Herein, we report on the breeding of 4 additional mapping families and the use of these to locate an additional 35 cDNA markers to the *A. albopictus* linkage map. These add 9, 16, and 10 markers to linkage groups I, II, and III, respectively. Joining of the 5 maps yields an overall linkage map with lengths of 64.5, 76.5, and 71.6 cM on linkage groups I, II, and III, respectively, or a combined length of 212.6 cM. Despite conservation in the order of most genes among the 5 families, we found substantial heterogeneity in the amount of recombination among markers in different families. This was most marked in linkage group I, which varied between 16.7 and 69.3 cM. A map integrating the results of all 5 families is presented. This integrated map should prove useful for researchers seeking to map genome regions associated with vector competence for dengue viruses (DENV1–4), CHIKV, and LACV in addition to traits that show extreme variation in adaptive traits such as egg diapause and cold hardiness.

Methods

Mapping Families

Forty reciprocal F_1 intercrosses were constructed between *A. albopictus* parents from a strain collected in Lake Charles, LA, in 2002 and from a second strain collected in Allende in Nuevo Leon, Mexico, in 2003. After mating and oviposition, P_1 and F_1 adults were collected and stored at −80°C for subsequent DNA extractions. The 4 largest F_2 families in each set of reciprocal crosses were eventually selected (Table 1). DNA was extracted from all P_1, F_1, and F_2 mosquitoes using salt extraction (Black and DuTeau 1997) and resuspended in 200 μl Tris–ethylenediaminetetraacetic acid (EDTA) (50 mm Tris–HCl, 5 mm EDTA, pH 8.0) buffer. A 50-μl aliquot of DNA was stored at 4°C for daily use in the polymerase chain reaction (PCR). The remainder was stored in plastic screw-top vials at −80°C.

Five tissue- and development stage–specific cDNA libraries were built using the Lake Charles strain. Mosquitoes

were collected into 1.5-ml microcentrifuge tubes and then killed by flash freezing the tubes in liquid nitrogen. Libraries were made from RNA collected from 1) 50 fourth-instar larvae, 2) 50 female adults within 3 h posteclosion, 3) midguts dissected from fifty 4-day-old adult females and 1 day post–blood feed, 4) ovaries dissected from fifty 4-day-old adult females and 3 days post–blood feed, and 5) heads removed from fifty 3-day-old adult males. Dissections were performed in RNAlater (Qiagen, Valencia, CA), and dissected tissues were then collected into a 1.5-ml microcentrifuge tube containing 200 µl RNAlater. Once all tissues were collected, the tubes were centrifuged at 15 000 × *g* for 15 min, and most of the RNAlater supernatant was removed and replaced with 600 µl Oligotex 1 buffer (Oligotex DIRECT mRNA Micro Kit—Qiagen #72012) to which 18 µl β-mercaptoethanol had been added. RNA was isolated following the manufacturer's instructions. Synthesis and amplification of cDNA used the SMART cDNA library construction kit (PT3000-2, Clontech Laboratories, Inc., Mountain View, CA) and followed the manufacturer's instructions. cDNA products were size fractionated on CHROMA-SPIN—1000 columns (Clontech) following the manufacturer's instructions to remove products <1 kb. The eluent fraction containing the largest fragments was transferred to a Microcon YM-100 filter (Millipore Catalog #42412) to purify and concentrate the fragments in 7 µl distilled deionized H_2O. These were ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) with the TOPO TA Cloning Kit (Invitrogen). Ligated products were then transformed with electroporation into OneShot TOP10 Competent cells. White colonies were picked with 200-µl sterile pipette tips to Luria Broth with kanamycin and grown overnight. Contents of the pipette tip were simultaneously mixed with PCR reagents containing the T7 and M13 reverse primers to determine insert size.

Plasmids from clones containing inserts >500 bp in size were purified using the PureLink Quick Plasmid Miniprep Kit (Invitrogen) and then transferred to the Montage PCR 96-well plate (Millipore, LSKMPCR10). Liquid was drawn down using the Millipore Multiscreen Vacuum Manifold (SAVM 384 01, Millipore). Plates were then sent to the Molecular Biology Unit, Laboratory of Malaria, and Vector Research where they were sequenced using a CEQ8000 Genetic Analysis System (Beckman Coulter, Inc.).

Bioinformatics Analysis

A detailed description of the bioinformatics treatment of the data appears in Valenzuela et al. (2002) and Anderson et al. (2008). Briefly, expressed sequence tags were analyzed using a set of customized executable programs written in Visual Basic by José M.C. Ribeiro at the National Institutes of Health. Terminal regions of high N (unidentified nucleotide) content and primer and vector sequences were removed from the raw sequences, and the resultant (cleaned) sequences were assembled into clusters based on 90% similarity over 90 nucleotides, whereas sequences with greater than 5% N's overall were discarded. Clusters were aligned into contiguous consensus sequences using the Consensus Assembly Program version 3 sequence assembly program (Huang and Madan 1999). Using the appropriate basic alignment search tool (BLAST) algorithm (BLASTX, BLASTN, or RPS-BLAST) obtained from the National Center for Biotechnology Information (NCBI) FTP site (Altschul et al. 1998), consensus sequences and singletons were compared with the NR protein database of the NCBI, the GO fasta subset (Lewis et al. 2000), Conserved Domain Database of NCBI (Marchler-Bauer et al. 2002) containing the KOG (Tatusov et al. 2003), Pfam (Bateman et al. 2000), and SMART (Schultz et al. 2000) protein motif databases and with custom-downloaded databases containing the mitochondrial and rRNA nucleotide sequences available at the NCBI. The grouped and assembled sequences and BLAST results were combined in an Excel spreadsheet and manually verified and annotated (available on request).

Linkage Mapping

Primers for PCR were designed using Primer Premier 5.0 (Premier Biosoft International). The optimal amplification temperature was identified using a 45–65°C thermal gradient across 12 wells in an Eppendorf Mastercycler. Next, products were amplified in the 16 parents from the 4 families (2 P_1 grandparents and 2 F_1 parents per family) and then analyzed using SSCP (Black and DuTeau 1997) to test whether genotypes were fully (F_1 genotypes distinct from both P_1 genotypes) or partially (F_1 genotypes distinct from one P_1 genotype) informative. Genes that were partially or fully informative are listed in Table 2, and genotypes were determined at these loci in all F_2 offspring.

The JMSLA program in JoinMap (Stam and van Oojin 1995) was used to test whether F_2 genotype ratios conformed to Mendelian expectations. JMGRP is a program in JoinMap that calculates pairwise recombination distances and associated probabilities (logarithm of the odds [LOD] scores) that loci are linked. LOD scores vary among locus pairs depending on recombination distances and the numbers of informative genotypes among all pairs of loci. JMGRP was run with an LOD ranging from 0.0 to 8.0 with 0.1 intervals. The output file that contained 3 linkage groups was then used as a grouping file. JMSPL in JoinMap is a program that physically splits the original genotype file into separate linkage group files according to the grouping file. JMREC in JoinMap then estimated pairwise Kosambi recombination distances (Kosambi 1943) among all loci in a linkage group and associated LOD scores. JMREC was run with low stringency (LOD = 0.001 and minimum recombination = 49.9 cM). Finally, JMMAP in JoinMap used the output from JMREC to estimate the maximum likelihood solution for the linear order of loci as well as their recombination distances using the approach described by Lander and Green (1987). The stringency in the JMMAP response file was set to LOD = 3.0 with a minimum recombination = 49.9 cM. The maps estimated in each of the 4 families were compared with test for incongruence in gene order, but not in centiMorgans differences. When

Table 2 Genes that were partially or fully informative among the 4 mapping families

Locus name	cDNA library	GenBank #	No. families	Ta	Forward primer/reverse primer	Size (bp)	*E* score	VectorBase # putative orthologue
Chromosome 1								
cecB2	Ten.Mdg	AF394746-B2	3	48	TAGGTACGACGACTCTGTT GCGAGCCTCTGTAGTAAG	328	2.00E-29	AAEL000611—cecropinB2
CecC2	Ten.Mdg	AF394746-C2	1	48	TAGGTACGACGACTCTGTT GCGAGCCTCTGTAGTAAG	328	2.00E-29	AAEL000611—cecropinC2
AALLarv-236	Larval	GR885583	1	51	CATTGCCAACCAAAGAAA AGTCCTGAATTGCAGACA	232	5.00E-60	AAEL013661—thymosin; actin-binding protein
AALLarv-5	Larval	GR885586	4	65	GCGATCCGCGAGATACAC TGCCGATGAGTTCCAGAGTT	177	1.00E-148	AAEL012207—myosin light chain alkali
AALLarv-331	Larval	GR885585	4	57	GTTTGCTTGGCTGTTCTA CGGTGTTCCGATAGTGAT	204	3.00E-43	AAEL006169—cathepsin d
AALLarv-99	Larval	GR885591	3	51	ATTCTTTGTACCGCACTTT TAACAGCATTCGCTTCAT	265	1.00E-100	AAEL003196—carboxylesterase
AALLarv-88	Larval	GR885590	4	53	AGCGAGACAAAGAGTGAT GCATATTATAGGCTACCG	299	5.00E-70	AAEL013678—mannosyl-oligosaccharide alpha-1,2-mannosidase
Chromosome 2								
AALLarv-140	Larval	GR885579	3	55	TCGGAAGTACACTTTGAT GTATTACGCAGTTTGGTG	167	4.00E-36	AAEL003160—conserved hypothetical protein
AALMidg-38	Midgut	GR885568	1	48	GCTGGAAATCGCCCTTAT GGATCTTCATGCCCGTCT	347	2.00E-14	AAEL006007—Kazal; putative secreted peptide
AALLarv-144	Larval	GR885580	2	53	GACAGAACTGAGCCGTTAC TGAGACACCCATCATACCT	280	2.00E-44	AAEL004291—Translin
AALLarv-135	Larval	GR885578	3	60	CGCTTCGGAGACGGAACT CCAACCTGGGTGGTGATT	225	7.00E-93	AAEL012564—lumbrokinase-3(1) precursor
AALHead-67	Head	GR885562	3	57	ACGGAGCCAATCTAACAA CCTGACAGAGCACCATAA	345	1.00E-138	AAEL003586—neuronal cell adhesion molecule
AALLarv-76	Larval	GR885588	4	53	AGCCCATCTAGTCCCTTAA ACTTGTTCGTCATCTTGC	227	6.00E-84	AAEL002253—beta-1,3-*n*-acetylglucosaminyltransferase radical fringe
AALHead-28	Head	GR885561	4	61	CGTTGATCGGGATAGGGA TTGGCGTTGGTGGTCGTT	304	0.01	AAEL004585—putative salivary secreted peptide
X60192	Ten.Mdg	X60192	2	58	TTGATTACCCACAGGATT CATTACGTTTGCCAGAGT	359	1.00E-96	AAEL007370—dihydrofolate reductase
AALTMdg-460	Ten.Mdg	GR885573	4	45	TGTAGTAACAACGAGCA GATAGTTTAGTTCCAGTAG	237	4.00E-38	No transcript—conserved hypothetical protein
AALLarv-86	Larval	GR885589	2	48	AAGCAGTGGTATCAACGCAGAG TTCACCCATCGCCAATCA	320	4.00E-70	AAEL014275—molybdopterin cofactor sulfurase (mosc)
AALLarv-147	Larval	GR885581	3	48	GGAAGCATATCGGTTCTCA AGATGCCCAGGTTCAGTC	204	1.00E-128	AAEL004239—conserved hypothetical protein
AALGrav3-130	G3	GR885559	4	53	AACAGCATCACCCAAACC CCTTCAGGCTATCGGACT	199	8.00E-41	AAEL000218—hypothetical protein

Table 2 Continued

Locus name	cDNA library	GenBank #	No. families	Ta	Forward primer/reverse primer	Size (bp)	*E* score	VectorBase # putative orthologue
AF049228	Ten.Mdg	AF049228	2	58	GGTGTCTCGGGAGGATTG GCGATGAGTTCTTGTGCC	363	0	AAEL007378—synaptic vesicle protein 2
AALGrav3-18	G3	GR885560	4	56	TCGTCGTGTCCCATTCTC TCAGCGTCCTGAAGCAAA	217	1	No similarity
AF283838	Ten.Mdg	AF283838	4	58	GGTTCTGTGGGTGCGTAA AAATGTGCCGATAGTGGG	306	1.00E-152	AAEL009126—CYP6N3v3
AF284782	Ten.Mdg	AF284782	2	58	TCTTCTTGGCTGGGTTCG GTATTCGGCATCGTGGTG	323	1.00E-110	AAEL009124—cytochrome P450
Chromosome 3								
AALMidg-42	Midgut	GR885570	4	48	AATCGTGCCTACAAAGAGTT ATCGCAGTCCCATTATCC	250	4.00E-43	AAEL010202—trypsin
AALMidg-40	Midgut	GR885569	3	61	AGGGAAGACGAACAGACA TTAGCGGGCACTTTACAC	281	1.00E-110	No transcript—unidentified open reading frame
AALLarv-110	Larval	GR885576	4	60	GAAACGCCAACTGTCCCA TCCAAGAGCGCAGACCAG	255	5.00E-27	AAEL002612—hypothetical protein
AALMidg-87	Midgut	GR885571	4	53	TTGAACGCTACGCCATTT TCGATCTCGACCTTGCTC	261	3.00E-61	AAEL011741—glutathione *s*-transferase
AALLarv-104	Larval	GR885575	4	62	GGCGTTTGCATGTTGTAC CTACGGTTCCGCTATCCT	217	9.00E-90	AAEL000758—ubiquitin-activating enzyme E1
AALMidg-33	Midgut	GR885567	4	53	GATCGCCGTTACGTTTGA TCGGTTCCGTATTGTTCG	281		AAEL008441—hypothetical protein
AALTMidg-59	Ten.Mdg	GR885574	1	58	GAAAGCGGAGCGGATAAA TTCGCCGTCAGTTGGTGT	309	8.00E-126	AY433160—conserved hypothetical protein
AALMidg-19	Midgut	GR885564	3	56	GAAAGATCGCCCAGTCCA GAAATCCGGGTTGTAGCC	208	1.00E-137	AAEL006138—vitellogenin-B
AALLarv-130	Larval	GR885577	4	65	CGTCTGCTGTGCCGTAAA CAAAGTCCAGGGTGTCGTAG	199	0	AAEL014292—40S ribosomal protein S24
AALLarv-322	Larval	GR885584	3	65	GAATGTCGTCCTAACTGCG ACAGCGAGCCCTTATTGA	167	1.00E-155	AAEL002595—serine protease
AALMidg-32	Midgut	GR885566	4	53	AAGGCACAGGAGGAGGAA ACCGCCGATTAGTAGAGG	314	2.00E-63	AAEL010801—conserved hypothetical protein
AALMidg-15	Midgut	GR885563	4	48	AATCTCGGATCTCAGCAT GTCGTAACGCCTCAGTAA	301	1.00E-147	AAEL002848—tubulin beta chain
AALLarv-159	Larval	GR885582	2	56	GCCAGACCAAGCTGCCAGTG GTTCGGCTTACGCCCTCA	174	1	No similarity
AALTMdg-398	Ten.Mdg	GR885572	3	48	CTTCACTACCTCTACTTT GTTTGTCCACCCTAC	317	1.00E-27	AAEL009539—acyl-protein thioesterase 1,2 (lysophospholipase i,ii)
AALLarv-69	Larval	GR885587	2	51	ACGATCCGATTGTATTCACA TCGATCAACTTCCTGGTGT	202	1.00E-97	AAEL013221—60S ribosomal protein L10a

The name of the locus is listed alongside the cDNA library from which it was obtained and its GenBank accession number. The number of families in which the markers were fully or partially informative is listed with the annealing temperature during the PCR (Ta). Optimized PCR primer sequences are listed with the size of the amplicon in base pairs (bp). Each sequence was subjected to a BLAST search on VectorBase. The *E* score from that search is listed alongside the VectorBase accession number and the putative orthologues.

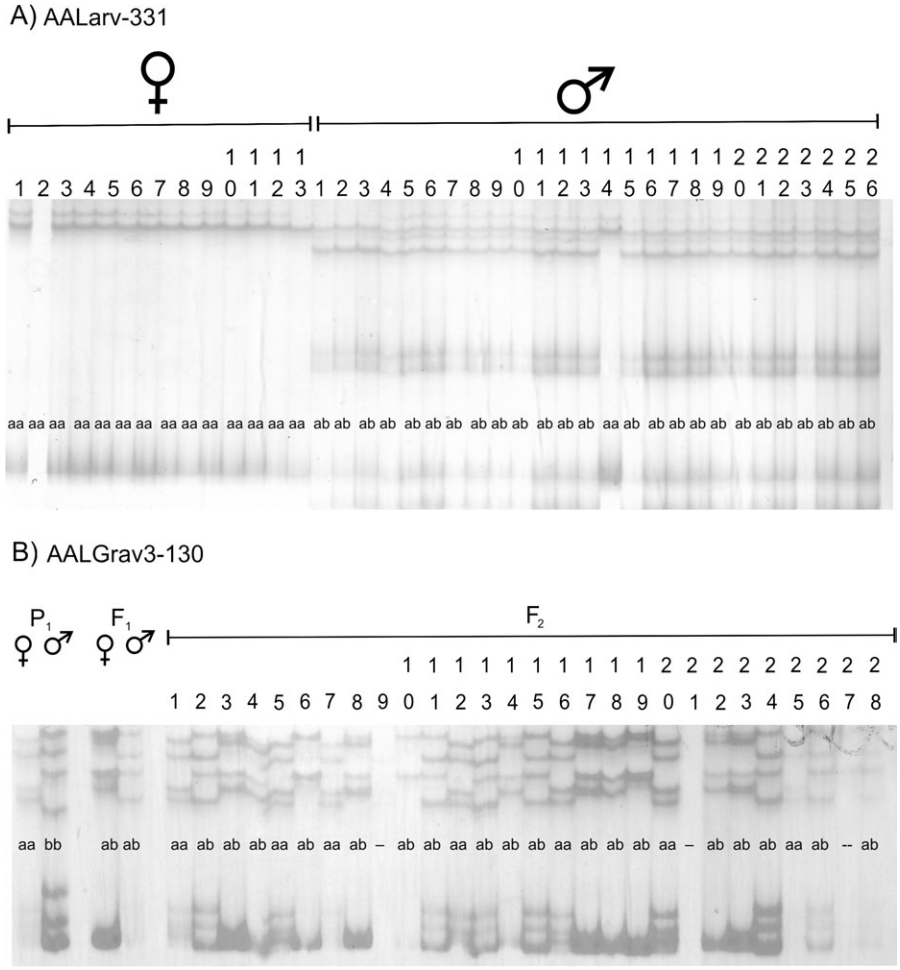

Figure 1. Segregation of SSCP genotypes is shown for locus AALarv-331 in family 17.4 (Figure 1A) and for locus AALGrav3-130 in family 5.2 (Figure 1B).

gene-order estimates were congruent, the JMREC files were combined and JMMAP was run a second time to obtain a single, composite linkage map for all 4 families.

Results

The number of markers mapped onto each linkage group and the estimated length of the linkage group are listed in Table 1 for each family. Genotypes were in Mendelian proportions for all 50 loci in the Nuevo Leon × Lake Charles family 5.2 (NL5.2) and for all 49 loci in family 5.6 (NL5.6). Mendelian proportions were also detected among all 54 loci in the Lake Charles × Nuevo Leon family 17.3 (LN17.3) and at all but the *Sex* locus of the 44 loci in family 17.4 (LN17.4). This family had 13 F_2 females and 33 males ($\chi^2 = 8.70$, $P_{[1 \text{ degrees of freedom}]} \leq 0.001$). Segregation of SSCP genotypes is shown for locus AALarv-331 in family 17.4 (Figure 1A) and for locus AALGrav3-130 in family 5.2 (Figure 1B). Inspection of genotypes shows clear co-segregation of AALarv-331 genotypes with sex. The P_1 have distinct genotypes at the AALGrav3-130 locus and the F_1 genotypes are different from the P_1 and the genotypes of the F_2 offspring all match the P_1 mother (labeled "aa") or exhibit one of the 2 F_1 genotypes (labeled "ab").

The composite linkage map obtained from all 4 families is shown alongside the linkage map of Mori et al. (2008) in Figure 2. Markers mapped in Mori et al. (2008) and in the present study are indicated with an asterisk. The composite *A. albopictus* linkage map contains 73 cDNA markers, with 18 markers on linkage group I, 32 markers on group II, and 23 markers on linkage group III. The length of the composite map from all 4 families was 169 cM. Linkage group I was 39 cM, group II was 69 cM, and III was 61 cM. This is substantially shorter than the 215.8 cM map of Mori et al. (2008), 9 cM shorter on linkage group I, 30 cM on II, and 7 cM on III.

The 3 markers shared by both studies (AF145803, AF394745, and AALLarv-54) on linkage group I are collinear in both maps but are 17 cM apart in the composite

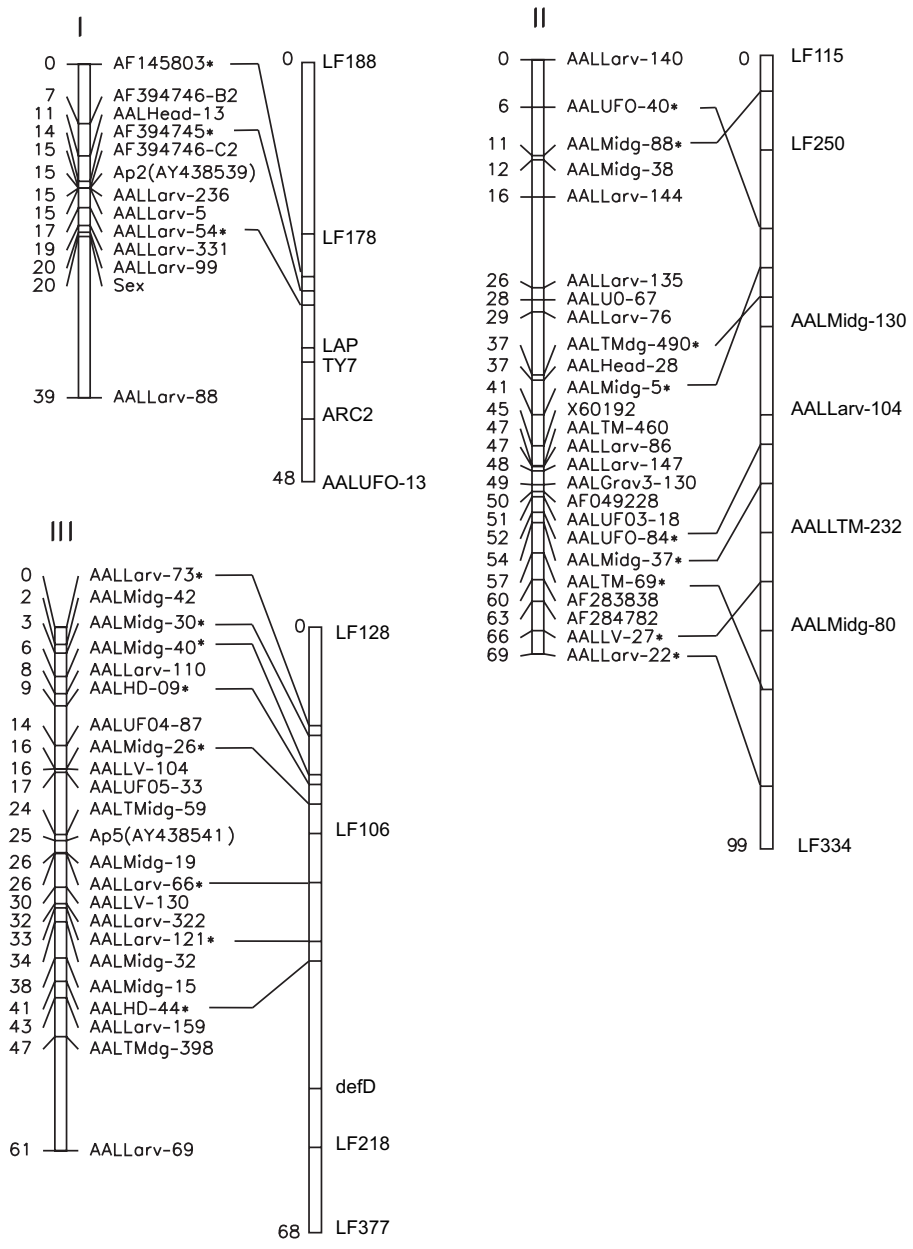

Figure 2. A composite linkage map obtained from all 4 F_1 intercross families. The maps are aligned on 0.0 cM. The linkage map of Mori et al. (2008) appears to the right of each linkage map. Markers mapped in Mori et al. (2008) and in the present study are indicated with an asterisk.

map and were only 2.5 cM apart in the map of Mori et al. (2008). This is the reverse of expectations based on the overall greater length of the map of Mori et al. (2008). To investigate this further, we calculated Pearson's correlation coefficient (r) to test the hypothesis that the length of the linkage group in centiMorgans was simply a function of the numbers of markers analyzed in the 4 families. The correlation coefficient for linkage group I was −0.51 and nonsignificant. Furthermore, Table 1 indicates that the length of linkage group I varied from 16.7 cM in NL5.2 to 69.3 cM in LN17.3. We plotted the 4 maps alongside one another (Figure 3). All 4 linkage groups are aligned on the *Sex* locus and markers AF145803, AALLV-331, and AALLV-54 or AALLV-5 appear in boxes to assist in comparing maps. Families LN17.4, NL5.2, and NL5.6 are all similar to one another in having AF145803, AALLV-331, and AALLV-54 or AALLV-5 from 0 to 7 cM apart from one another. In contrast, in family LN17.3, AF145803 is 41 cM from AALLV-54 and segregates independently of AALLV-331 (53 cM).

Markers on linkage group II were collinear in both maps (Figure 2) with 3 exceptions: 1) the positions of AALUFO-40

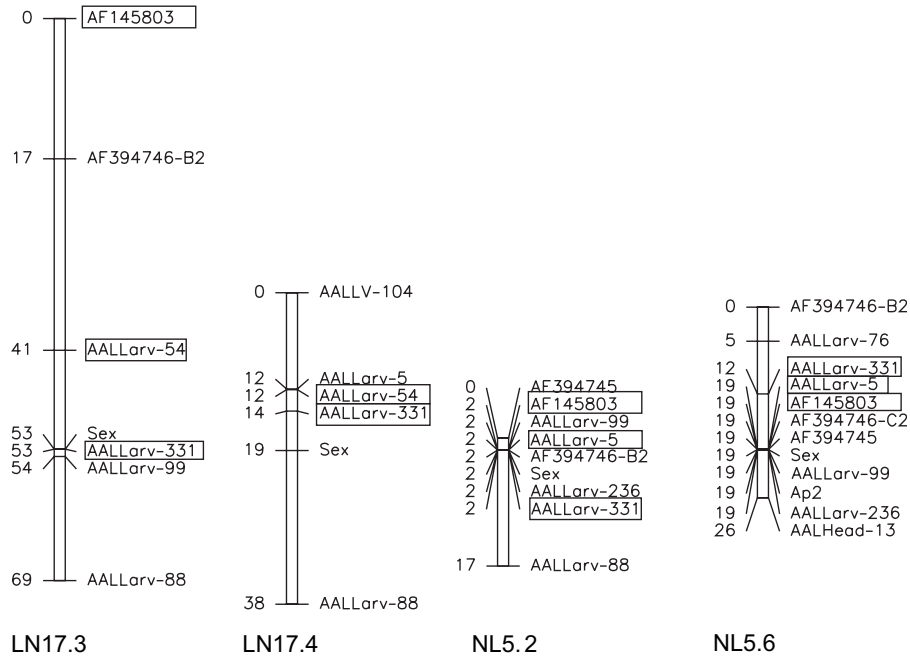

Figure 3. Linkage group I from each of the 4 F_1 intercross families. All maps are aligned on the *Sex* locus, and markers AF145803, AALLV-331, and AALLV-54 or AALLV-5 appear in boxes to assist in comparing maps.

and AALMidg-88 (5 cM apart) are inverted, 2) the positions of AALTMdg-490 and AALMidg-5 (4 cM apart) are inverted, and 3) the positions of AALTM-69 and AALMidg-27 (9 cM apart) are inverted. The correlation coefficient r was again computed to test the hypothesis that the length of the linkage group II was correlated with numbers of markers in a family. Although nonsignificant, $r = 0.62$, suggesting that the estimated map length increased as more markers were added. This trend was confirmed by placing the 4 maps alongside one another (maps not shown) but still did not account for the overall difference of 30 cM in length. The 2 markers LF115 and LF334 added 12.6 cM (4.9 and 7.7 cM, respectively) to the map of Mori et al. (2008) but still failed to account for the additional 17.4 cM in length (30–12.6 cM).

Markers on linkage group III were collinear in both maps (Figure 2). The estimated map length was not correlated with the number of markers $r = -0.16$ (nonsignificant). The 8 markers shared by both studies cover 41 cM in the composite map but are only 26.5 cM (37.5–11.0 cM) apart in the map of Mori et al. (2008). As on linkage group I, this is counter to the longer length of linkage group III in Mori et al. (2008). Figure 4 is a compilation of all markers from the 5 families. This was assembled by combining the orders and distances from Figure 2.

Discussion

The present study increases the number of markers on the *A. albopictus* cDNA linkage map from 38 to 73 and the density of markers from one marker every 5.7 cM (Mori et al. 2008) to one marker every 2.9 cM. Most of the markers in the present study are collinear in order with those in Mori et al. (2008). Mori et al. (2008) used geographically disparate parental strains originally from SL and Japan, whereas our study used more recently established field populations from Lake Charles, LA, and Allende in Nuevo Leon, Mexico. The collinear order of markers shared among the 5 families therefore suggests that gene order is conserved among different geographic collections of the species. The recently published linkage map of *Culex tarsalis* (Venkatesan et al. 2009) also reports substantial variance in recombination rates among different families.

In contrast, there was a large variance in the sizes of linkage groups among collections. The first *A. albopictus* linkage map (Severson et al. 1995) was constructed with parents from New Orleans, LA, and from a Sakamoto, Japan, strain. That study used 18 cDNA markers, and the sizes of the 3 linkage groups were 18.2, 46.1, and 34.1 cM, respectively, and the overall length of the linkage map was 98.4 cM. Linkage group I contained markers LF188, LF178, LAP, and TY7 distributed over 10.7 cM. In contrast, the same 4 markers were distributed over 30.4 cM in Mori et al. (2008). The map of Mutebi et al. (1997) based on SSCP analysis of RAPD markers involved 68 polymorphic loci, and the size of linkage group I was 54 cM, much closer to the 48 cM length in Mori et al. (2008). This same range of variation in the length of linkage group I was found in the present study (Figure 3). Clearly, some process reduces the amount of recombination along linkage group I in different *A. albopictus* populations. Based on recent and past studies in

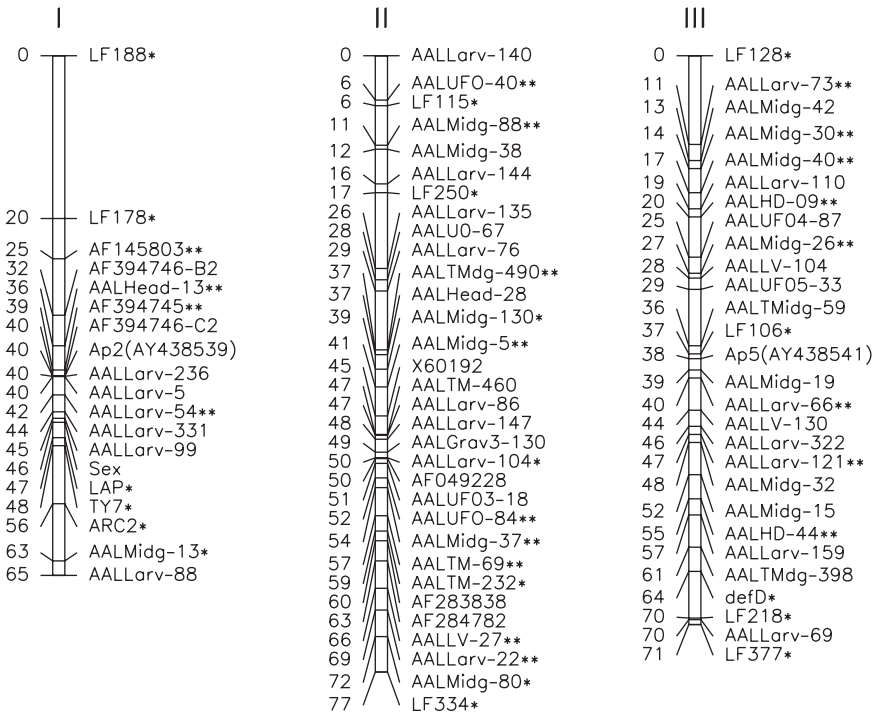

Figure 4. A compilation of all markers from the 4 F_1 intercross families in the present study and the family of Mori et al. (2008). This was assembled by combining the orders and distances from Figure 1. Markers without an asterisk were only mapped in the present study. Markers with a single asterisk were only mapped in Mori et al. (2008). Markers mapped in both studies are marked with double asterisks.

A. aegypti (Macdonald and Sheppard 1965; Bernhardt et al. 2009), it is not unreasonable to suggest the possibility of inversions along linkage group I in *A. albopictus* populations. Also, a great deal of variation in genome size due to variation in the amount of repetitive DNA has been recorded in *A. albopictus* by Feulgen cytophotometry (Kumar and Rai 1990), reverse southern analysis (McLain et al. 1987), and DNA reassociation kinetics (Black and Rai 1988). For example, Kumar and Rai (1990) reported that the haploid nuclear DNA content varied nearly 3-fold from 0.62 pg in Koh Samui from Thailand to 1.66 pg in Houston from the United States. Repetitive regions are known to suppress recombination (Petes 2001), and it is interesting to speculate as to whether variation in this genome fraction could also account for the observed variation in recombination.

Irrespective of these problems, the composite map (Figure 4) should provide workers with a much greater diversity of markers to use in future genetic studies of this medically important vector species. It is hoped that this resource will stimulate more genetic work into this species because it is a model for understanding the basis of adaptation from tropical to temperate regions through diapause and cold hardiness. It is also an important and viable system in which to identify genetic components of vector competence for Flaviviruses (DENV1–4), Alphaviruses (CHIKV), and Bunyaviruses (LACV).

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