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SSCP Analysis of cDNA Markers Provides a Dense Linkage Map of the *Aedes aegypti* Genome

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ABSTRACT

An intensive linkage map of the yellow fever mosquito, *Aedes aegypti*, was constructed using single-strand conformation polymorphism (SSCP) analysis of cDNA markers to identify single nucleotide polymorphisms (SNPs). A total of 94 *A. aegypti* cDNAs were downloaded from GenBank and primers were designed to amplify fragments <500 bp in size. These primer pairs amplified 94 loci, 57 (61%) of which segregated in a single F_1 intercross family among 83 F_2 progeny. This allowed us to produce a dense linkage map of one marker every 2 cM distributed over a total length of 134 cM. Many *A. aegypti* cDNAs were highly similar to genes in the *Drosophila melanogaster* genome project. Comparative linkage analysis revealed areas of synteny between the two species. SNP polymorphisms are abundant in *A. aegypti* genes and should prove useful in both population genetics and mapping studies.

THE mosquito *Aedes aegypti* has been the subject of extensive genetic research due to its medical importance and the ease with which it can be manipulated in the laboratory. On a worldwide basis, *A. aegypti* is the most common vector of yellow fever and dengue fever flaviviruses (Miller *et al.* 1989; Monath 1991; Gubler and Meltzer 1999). Beginning in the early 1960s, an abundance of visible genetic markers were identified during isolation of isofemale lines from field *A. aegypti* populations (Craig *et al.* 1961). These 87 spontaneous mutants were associated with a wide array of eye color markers, cuticular scale patterns and colors, distortions of the legs and palps, homeotic mutants, loci with recessive lethal alleles, loci affecting sex ratio, and insecticide resistance (Craig *et al.* 1961; Craig and Hickey 1967). Somatic and germ cell cytogenetics are well characterized in *A. aegypti* (Rai 1963, 1966; Mescher and Rai 1966), and chromosomal translocations and inversions have been induced with gamma radiation (Mcgivern and Rai 1972; Rai *et al.* 1973).

Allozymes constituted the next generation of genetic markers (Munstermann and Craig 1979) and provided many additional loci on the *A. aegypti* linkage map. The first intensive map of *A. aegypti* was obtained in the early 1990s through restriction fragment length polymorphism (RFLP) analysis of cDNA clones and there are currently >100 cDNA loci mapped (Severson *et al.* 1993, 1994, 1995a,b). Soon after, Antolin *et al.* (1996) demonstrated that single-strand conformation polymorphism (SSCP) analysis of randomly amplified polymorphic DNA (RAPD) markers could be used to rapidly construct a linkage map from a single F_1 intercross family. However, subsequent analysis indicated that RAPD loci that were polymorphic within one *A. aegypti* family were fixed for dominant or recessive alleles in other families (Bosio *et al.* 2000), precluding their use for comparisons across families or populations. This problem led us to explore several different types of markers.

Microsatellites are abundant in the genome of the mosquito *Anopheles gambiae* (Zheng *et al.* 1991, 1993, 1996; Lanzaro *et al.* 1995; Wang *et al.* 1999). However, isolation and analysis of microsatellites in *A. aegypti* yielded curious results (Fagerberg *et al.* 2000). Various di- and trinucleotide repeats were tested but none were abundant in the *A. aegypti* genome. Furthermore, most of the microsatellite loci that were obtained were either not variable when analyzed in several *A. aegypti* families or alleles at polymorphic loci segregated as band-absent (recessive) or band-present (dominant) markers. Sequence analysis indicated that loci, not alleles, varied in the number of microsatellite repeats and that some amplified loci had no microsatellite repeats at all.

We subsequently explored a variety of techniques for identification of single nucleotide polymorphisms (SNPs) in PCR products. These included RFLP analysis, SSCP analysis (Orita *et al.* 1989), heteroduplex analysis (White *et al.* 1992), denaturing gradient gel electrophoresis (Myers *et al.* 1987), and allele-specific oligonucleotide hybridization (Saiki *et al.* 1986). In our hands SSCP analysis was the most reproducible and sensitive of these techniques and also the most rapid and least expensive (Black and Duteau 1997). SSCP is based on the principle that both size and primary sequence influence the impedance of single-strand DNA molecules in nondenaturing gels. Impedance is a function of primary sequence because several stable shapes or conformations

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are formed when secondary base pairing occurs among nucleotides on a single DNA strand. The length, location, and number of intrastrand base pairs determine secondary and tertiary structure of a conformation. Point mutations that affect intrastrand interactions may therefore change the shapes of molecules and alter their mobility during electrophoresis. The SSCP technique is reported to detect ≥99% of point mutations in DNA molecules 100–300 bp in length and ≥89% of mutations in molecules 300–450 bp in length (Orita *et al.* 1989; Hayashi 1991).

Here we report on the large diversity of *A. aegypti* cDNA genes that are currently available in GenBank and demonstrate that SSCP analysis of these reveals extensive polymorphisms that can be used to develop an intensive linkage map in a single F_1 intercross family. We also compare the locations of these genes to their physical locations in the *Drosophila melanogaster* genome (Adams *et al.* 2000) to examine the degree of synteny between the two species.

MATERIALS AND METHODS

Mosquito breeding and processing: A single F_1 intercross family consisting of 83 F_2 individuals was used to estimate recombination frequencies among cDNA loci. The P_1 individuals of this family originated from two laboratory colonies derived from field collections of eggs. The P_1 female belonged to the subspecies *A. aegypti formosus* collected from Ibo village, Nigeria. Fifth and sixth generation mosquitoes were used. The P_1 male belonged to the subspecies *A. aegypti aegypti* and was collected in San Juan, Puerto Rico. First and second generation mosquitoes were used. F_1 offspring from this cross were collected and intercrossed. The resulting F_2 offspring were reared to adults. All family members were frozen and stored at −70° to await processing.

DNA was extracted from individual mosquitoes (Black and Munstermann 1996) and resuspended in 500 μl TE buffer (50 mm Tris-HCl, 5 mm EDTA, pH 8.0). A 50-μl aliquot of this DNA was overlaid with sterile mineral oil and stored at 4° for daily use in polymerase chain reaction (PCR). The remainder was stored in plastic screw-top vials at −70°.

Annotation of *Aedes aegypti* anonymous cDNAs: The database of expressed sequence tags (dbest) in GenBank currently contains most of the ∼1630 *A. aegypti* genetic markers. These were individually downloaded from GenBank and a BLASTX search was performed against the Drosophila genome project (Adams *et al.* 2000). Those without a significant match ($>e^{15}$) were subjected to a BLASTX search against the nonredundant (NR) database. Remaining unmatched cDNAs were subject to a BLASTN search against the Drosophila genome, NR, and dbest databases. The physical locations of matches in the Drosophila genome were recorded along with the name or, for Drosophila genes of unknown function, accession number.

Primer design: A subset of 94 cDNA sequences of identified function was selected for further analysis. Primers were designed directly from the cDNA sequence using Primer Premier v4.11 (Premier Biosoft International, Palo Alto, CA). Search parameters were set to a primer length of 20 nucleotides, a 100-pm template concentration, a 50-mm monovalent ion concentration, a 1.5-mm free Mg^{2+} concentration, a 250-mm total Na^+ equivalent, and 25° for free energy calculations. Primers were designed to amplify a 200- to 500-bp region of the gene, an amount deemed optimal for SSCP analysis. These primers were optimized for annealing temperatures using a Mastercycler gradient thermal cycler (Eppendorf, Madison, WI) and template DNA mass isolated from ∼500 Puerto Rican larvae. Annealing temperatures (T_a) that yielded single bands with strong amplification were considered optimal.

PCR was completed in thin-walled polycarbonate 96-well plates (Fisher Scientific, Pittsburgh, PA). Each plate contained an entire family, including all four P_1 and F_1 parents, the 83 F_2 offspring, and a negative control (no template DNA added). The remainder of the PCR and SSCP analyses followed Black and Duteau (1997) and Bosio *et al.* (2000). Cloning and sequencing of bands followed Bosio *et al.* (2000).

Linkage mapping: Genotypes at each putative locus were scored and entered in the JoinMap 2.0 (Stam and Van Ooijen 1995) data file format for a cross pollinator cross. These were tested for conformity to Mendelian ratios with a χ^2 goodness-of-fit analysis using the JMSLA procedure in JoinMap. Loci at which Mendelian genotype ratios were observed were separated into individual linkage groups using the JMGRP and JMSPL procedures with a starting LOD threshold of 0.0 that was increased to 8.0 in increments of 0.1. Pairwise distances (Kosambi 1944) were estimated among loci in each of the three linkage groups using JMREC, and the maximum-likelihood map was estimated using JMMAP. The linkage map was plotted using DrawMap1.1 (Van Ooijen 1994).

Other markers: Microsatellite loci amplified by the TAG66 primers (Fagerberg *et al.* 2001) were mapped as were *s*equence-*t*agged *a*mplified *R*APD (STAR) loci (Bosio *et al.* 2000) and *LF* markers (Severson *et al.* 1993) to orient the map derived in our study relative to maps from earlier studies (Severson *et al.* 1993, 1994, 1995a,b; Antolin *et al.* 1996; Bosio *et al.* 2000). Alleles at the TAG66 loci segregate as dominant markers. STAR loci were developed by cloning and sequencing RAPD markers and then designing primers that contained the original RAPD primer at the 5′ end and the next 10 nucleotides in the sequence to the 3′ end. STARs are amplified by targeted PCR and alleles at STAR loci often segregate as codominant markers (Bosio *et al.* 2000).

RESULTS

Ninety-four primer sets were designed from genes of identified function in *A. aegypti* or from a collection of the ∼1530 *A. aegypti* expressed sequence tags (ESTs) in GenBank (indicated with AI in the accession numbers). Primers were designed only from ESTs that had high similarity in a BLASTX search to genes of known function in GenBank. Primers were tested on family DNA and 88 of them amplified products of the anticipated size to yield a total of 94 loci (Table 1). Three primer sets amplified more than a single locus [allatotropin (5 loci), *ADPATPtl* (2 loci), and *Feilai405* (two loci)]. Fifty-seven (61%) of these were polymorphic and alleles segregated as codominant markers at 53 (93%) of the polymorphic loci. Alleles at the 4 (7%) remaining loci segregated as dominant (band-present) and recessive (band-absent) markers.

The inheritance of genotypes was fully informative at 18 loci and was partially informative at the remaining 39 loci. Examples of genotypes segregating among the P_1 and F_1 parents and the first nine F_2 offspring appear for 10 loci in Figure 1. Alleles at *Fxa*, *Hexam2*, *Peroxnc*,

TABLE 1

***Aedes aegypti* cDNA genes analyzed in this study**

Gene name	Accession no.	T_a	Size	Forward/reverse primer sequence	Primer location	*A. aegypti* linkage location	Drosophila genome no.	Drosophila physical location (chromosome no., Mb)
Chromosome 1								
Hemepoly (hemolymph polypeptide)[a]	U11235	60	385	CGACAAGCGCCAGCAGAAGG AGTTGGCCGGGTCCACTTCG	39 423	I, 0	CG6186	1, 29
LF198	T58319	48	284	CTGGCGTAGATTCCGTGCTG TCCGTGTTGACGTAGGTGGC	52 235	I, 1	CG2286	1, 9
Erudi (enhancer of rudimentary)[a]	U66869	46	444	CGGACGAAGCTGAATGAAGA TCCGCTAACTGATCCACGAA	13 456	I, 4	CG1871	1, 10
Sialokin1 (salivary vasodilatory protein-sialokinin1)	AF108099	60	298	CCCGATAAATCCTTTCCTTC AAATGGGTATCCCTTTCCTG	83 380	I, 4	NS	
Immuno (immunophilin)[a]	AI618957	60	360	AAATCTCGCACCCGTAAA GCCCTCGTCAAAGTTCAG	22 381	I, 4	NS	
Cathbp (cathepsin b-like thiol protease)[a]	L41940	60	343	CAAATTCGGAACCTCACCAG TATCCACCCTTGCATCCATC	133 475	I, 7	CG10992	1, 15
LF090	T58320	48	145	AGCAGAATGGCTCCCCGTAA ATGGTTTCCTTGCCGGACAG	31 175	I, 13[b]	CG1527	1, 9
VitgRecp (vitellogenin receptor)	AI650188	60	308	CCTCTGCAAGAAGCCGATGT ACCCAGTCGTGGCTGTTGAT	246 553	I, 13	NS	
Transfer (transferrin precursor)	AF019117	60	309	ATGCGGCCATCCAGGTTCAG CCCGCCGACTTCAGTTTCGT	146 454	I, 14	CG6186	1, 20
Allatod, Allatode (allatotropin), *Rf1-5*[a]	U65314	43	289	GAACGGATGCTAGAAGAAAG TTAGAATGGGACTACCGAGA	193 481	II, 32 I, 44 II, 1 I, 22 III, 14	NS	
Hexam2 (hexamerin 2)	U86080	56	436	TTCCTGGTGAAGCAGAAACA TCATGCCATAGAATCCTTGC	100 535	I, 25	CG6806	
White[a]	U88851	59		TACCTGACSGCACTGCTGATTG TGATGACMGGCGGCCCAAC		I, 30	CG4314	3L, 16
Riboptl1 (Ribosomal protein L1)	AI658439	56	285	AGAATCTTGCGTTGCCGTAC GCTTCAGGGTTCACGTTGAT	137 421	I, 30	CG5502	3R, 48
Feilai-405 (SINE)[a], *Rf6*[a]	AF107667	46	206	GATGTTCGACGCTCAGTTGT GTTGGTGATGATACCGTGCT	13 218	I, 30 II, 19	CG5409	2R, 39
Aamy2 (α-amylase 2)	U01208	60	330	ATGACGTTGGAGTGCGAATC ACCAGGTTGCCGTAGATGAA	40 369	I, 32	CG18640	2R, 39
BMIOP (blood meal-induced ovarian protein)[a]	U84248	60	337	TTGAAGTCGTCGTTGCTGTT CTGCCTTTGTCATGTTTGCT	196 532	I, 32	CG5709	2R, 46
Chitan 1 (chitinase 1)[a]	AF026491	60	327	AAACTGACCTACGCCCAAAG GTCTACGCCGATGAACGAAT	687 1013	I, 33	CG9357	2R, 45

(*continued*)

TABLE 1

(Continued)

Gene name	Accession no.	T_a	Size	Forward/reverse primer sequence	Primer location	*A. aegypti* linkage location	Drosophila genome no.	Drosophila physical location (chromosome no., Mb)
CG18355	AI650010	59	258	GATGCTAATGGGAAACAAAT TGATACCGTTTAAGGCAAAG	250 507	I, 35	CG18355	2L, 8
Peroxnc (peroxinectin)	AI657546	58	305	GCATTTCAGCAGGGTAGA AAGATCGGCAAGAACTCA	130 434	I, 35	CG7660	3R, 38
PPallost (preproallatostatin)	U66841	60	326	AAGAAGAAGATTACGACGAT CAATTTCCCACTATCACTTA	16 341	I, 36	NS	
AbdA (abdominal a)	X67132	48	367	AGCGGTAGATCCTGTTGTCT AACCTCCAACTCACCTGTTC	868 1234	I, 59	CG10325	3R, 37
Chromosome 2								
Vmem 15a1 (vitelline membrane protein 15a-1)	S54555	54	383	TCTTGGCAATCTTCGCTCTG ATCGCGTTCCGTGTCTCATA	74 456	II, 0	NS	
Vmem15a (vitelline membrane protein 15a)	U91682	54	332	TGAGCGACGGATAGAACTAA TACGCCAGCTAGGAAATGTA	313 644	II, 5	NS	
TrypB (trypsin–Barillas Mury)	M77814	60	339	ACGGCTACCCTCGGTCAGTT CTACCTCGCGGTCGGTAAAG	93 431	II, 6	CG11529	3L, 12
Fxa (FXA-directed anticoagulant precursor)	AF050133	46	200	TTAGCACCAATCCAGCCTCA TGGCACAACTGTTGGGAAGA	214 413	II, 7	NS	
LF233	T58327	48	218	AAAGCGCCCACCTTTGCC ATCGCCCGTCAGCTTCAGTC	38 255	II, 9	NS	
ADPATPtla, ADPATPtlb (ADP/ATP translocase)	AI657540 AI650176 AI650113	57	284	CTGGCGCTACTTCATGGGTA ATCGAGGTGTTCTTCGGGTC	14 297	II, 9 II, 27	CG16944	1, 12
Aamy1 (α-amylase 1)	AF000569	60	437	GGACTTTGTACGCGAATCTG TAAGCTGGAGCATGTGGATC	350 786	II, 10	CG17876	2R, 39
Mle (male-less)	AI650222	59	303	GTTTGATGACCCACCAGAA ACAGACCAACGCAGAGCA	40 342	II, 14	CG11680	2R, 25
D7 (D7 salivary gland protein)[a]	M33156	60	342	CCTAGATTTGGCCCAGTTGT ACTCGGGCTCTGATTTGGTA	753 1094	II, 16	NS	
LF138	T58332	60	192	AATCTGTTCGACGTGGTATG CCGGCAATTCGGTGATGTT	1 192	II, 19[b]	CG7269	2L, 6
TrypEarl (trypsin-early)	X64362	60	459	CCAACGGTGGCATCATAGTGAAAG GATCCATTGGCGAACAGTGGAGAC	295 753	II, 19	CG3229	2L, 3
MtATPsyn (mitochondrial ATP synthase subunit-α)	AI650137	58	251	CCACAGCCGTCGAAGAAACC CGTCACCGATGCTCAACACG	83 333	II, 19	CG3612	2R, 47
InsRecp (insulin receptor)[a]	U72939	46	313	AGCAGCGGCAGGATCGGTAG CGCGGAACAGCAGCAGGTAC	24 336	II, 19	CG3837	3R, 35

(*continued*)

TABLE 1

(Continued)

Gene name	Accession no.	T_a	Size	Forward/reverse primer sequence	Primer location	*A. aegypti* linkage location	Drosophila genome no.	Drosophila physical location (chromosome no., Mb)
CarboxA (carboxypeptidase A)[a]	AF165923	54	378	TTGAATTGTAATGGGTTGAG	68	II, 20	CG17633	2L, 10
				TTATGATAGGAATCGCTTTG	445			
Glusyn (glutamine synthetase)[a]	AI649983	54	441	ATTCAGAGTTGTGGGATTAT	306	II, 22[b]	CG1743	1, 13
				CATTAAAGCACGTTTGTTAG	746			
Sin3 (transcription factor, sin3)[a]	AI561370	58	454	GTATCTGTTCCTGCGGTTGC	46	II, 39	CG8815	2R, 35
				CCTGAAGTGCTGCTTCTGCT	499			
Chromosome 3								
Apolipo2 (apolipophorin 2)[a]	AF038654	54	329	GCTGGAATCGGTCAAACTCG	24	III, 0	CG11064	4, 1
				CCGGCCTTAACTTGCTGGTA	352			
Apyr1 (apyrase1)[a]	L12389	54	470	GGAATGTGACGGCGGATTT	351	III, 0[b]	CG4837	2R, 40
				TGGATCATGCGGCTGTTTG	820			
AspSyn (asparagine synthetase)[a]	U84118	60	297	GGTCGAACAATGTGCGGTAT	88	III, 2	NS	
				TGATTTCCTGTGCCATCAGC	384			
Dynein (cytoplasmic dynein heavy chain)	AI618900	56	276	ATGGGATGCTTTGGTTACTC	110	III, 6	CG7507	3L, 5
				TACTTTCTCAGCGTTGTTCG	385			
UGALS (UGALS vitellogenin)[a]	U02548	60	328	AGGGCTACAATCCTGGCTAT	80	III, 7	CG3886	2R, 35
				GTATTCTGGCTGCTTGACGT	407			
Hsp70 (heat-shock protein 70)	AI658418	56	342	CCCGTCCTACGTGGCGTTCA	257	III, 8	CG4264	3R, 36
				GGTGGCCTGACGTTGCGAGT	598			
LF227	T58323	51	189	AAAGTTCGTCCGGTGTCCAA	43	III, 10	NS	
				TCTTCTTCAGGAGAACCGTG	231			
RNAhelic (ATP-dependent RNA helicase 46)[a]	AI650162	58	399	TTTGACTTCATGGACCCTCC	109	III, 11	CG11107	2R, 30
				AACTGTGACGCACATTGTCG	507			
Gpd-1 (glycerol-3-phosphate dehydrogenase)	AI648308	59	239	TGTTCAAATGGAGGAAATGC	132	III, 12	CG8256	2R, 38
				CACCACCGTGGATCAGCT	370			
VitgConv (vitellogenin convertase)[a]	L46373	60	287	TGCACAGAAGACCACCAATG	30	III, 12	CG10772	3R, 46
				TCGACTGTTCCGCTGAGTTA	316			
Malt (maltase)	M30442	48	234	GGACTGGTGGGAACATGGAA	72	III, 13	CG8696	2R, 31
				CTTATCGGACAACCGCTGGA	305			
Vitg (vitellogenin)[a]	L41842	60	296	AGATGGCGTCTTCGGTAAAG	41	III, 15	AE003820	
				AGTGAGCACGGAACCTTTGT	336			
DefA1 (defensinA1)[a]	AF156088	54	193	CATTTGTTTCCTGGCTCTGT	88	III, 18	CG1385	2R, 32
				GAGCAGCACAAGCACTATC	280			
TrypLate (trypsin-late)	X64363	60	325	TGGCTTTGAAGTGCCCGTTGAG	44	III, 20	CG9564	2L, 9
				CAAGTTCCTTCGTTGACCGGAGTG	368			
Apyr2 (apyrase2)[a]	L41391	54	317	TGATTGCATCGTCGTTGATT	103	III, 37	CG1961	1, 12
				CAACTTGCGCTGTTTGTTTT	419			

(*continued*)

TABLE 1

(Continued)

Gene name	Accession no.	T_a	Size	Forward/reverse primer sequence	Primer location	*A. aegypti* linkage location	Drosophila genome no.	Drosophila physical location (chromosome no., Mb)
Monomorphic Loci								
Aahr31 (steroid hormone receptor homolog aahr31)	U87543	60	384	TGGGAGGGAGAAACCAATAC	292	M	CG11823	2R, 32
				AACTCCAGCTTGCCAACAAC	675			
Aahr33 (steroid hormone receptor homolog aahr33)	AF106703	60	332	AGATCCTCCGATTATTCCTA	543	M	CG11823	
				TCATACCTCAAATCCCTTCT	874			
ATPaseB (V-ATPase B)	AF092934	56	195	GGTGTCACGCGGCAAGTTTA	110	M	CG11154	4, 1
				AGTCGTGGCTGCGAGATGAA	304			
ATPaseC (V-ATPase C)	AF008924	51	408	TTCCAGCGGGACCGAACAGT	94	M	CG3161	2R, 26
				CACCGGCATCACCGACGATA	501			
Atub (α-tubulin)	AI649995	58	264	CGGTGTCCAGATCGGTAATG	137	M	CG1913	3R, 28
				ACCACGGGCGTAGTTGTTAG	400			
Btub (β-tubulin)	AI657538	58	266	AACATGGAATCGACGCCACC	294	M	CG9277	2R, 41
				TTGCGGACAACGTCCAACAC	559			
Carbox (carboxypeptidase)	M79452	54	403	CAAGAAGCTAATGCGAGGAT	111	M	CG4572	3R, 40
				TATCGGTGAAACTGAATCCC	513			
Chitan2 (chitinase2)	AI612670	60	344	TGCGTCTATGGTGAGTTCAA	21	M	NS	
				TATCGCAGCTCTTATGAGGA	364			
Chymotrp (chymotrypsin)	AI618956	60	319	CCAGTTTGGCACTCGCTTCC	55	M	CG7142	
				GACGGCAATGTCATCGGGAC	373			
Cinnabar (kynureninehydroxylase-white)	AF040957	48	202	TGTGCGCTAAGAACTACCAT	79	M	NS	
				TTTAGCTGACTACGCCCATT	280			
Ddc (dopa decarboxylase)	AI638914	60	386	CGTACCGAAATGCAAGCC	113	M	CG3686	2R, 26
				GAACTCCTCTGGCAGTCCAA	498			
Ecdyrecp (ecdysteroid receptor)	U02021	60	283	GACTCGCGTGGATTGAACGG	393	M	CG1765	2R, 25
				TCTGCACCAGCCGAAGAAGC	675			
Ef1α (elongation factor 1α)	AI658459	56	172	AGCCCAGGAAATGGGTAAGG	206	M	CG1873	3R, 52
				CCTGCGATGTTCCGGTAATC	377			
Ef2 (elongation factor 2)	AI658391	58	200	TCCGATCCTATGGTGCAGTG	158	M	CG2238	2R, 21
				CAGACGGTTGTGCTTGTTGG	357			
Ferritin	L37082	56	367	AGGTGGAAGCAATACGACTG	24	M	CG2216	3R, 50
				GCGGCATACTTCAGGTAGAT	390			
GST2 (glutathiones-transferase-2)	S43311	56	112	GCTTTATCACTTTCCGATGT	3	M	NS	
				AATTCACAAACGGTTCTTGC	114			
G3PDH (glyceraldehyde-3-phosphate dehydrogenase)	AI650112	60	218	TTCCTGTACCACCAACTGCT	332	M	CG8893	1, 17
				CTGGAATGACCTTACCGACA	540			
Hexam1 (hexamerin 1)	U86079	60	294	TCCAGCATGTCCACCAGCAC	65	M	CG2559	1, 14
				AGCATGGCGACCGTTACAGC	358			
Hsp71 (heat-shock protein 71)	AI658418	56	240	GCCATGCAGCGTCTGAAGGA	190	M	CG4264	
				CATACCACCGGCCAGGAGAA	429			

(*continued*)

TABLE 1

(Continued)

Gene name	Accession no.	T_a	Size	Forward/reverse primer sequence	Primer location	*A. aegypti* linkage location	Drosophila genome no.	Drosophila physical location (chromosome no., Mb)
Hsp83 (heat-shock protein 83)	AI658441	56	163	ACATGGAAATCAACCCTGAC	10	M	CG1242	3L, 3
				CTTGACCATACGGTAAATGC	172			
LAP (lysosomal aspartic protease)	M95187	56	437	TCGTTTGCTTGGCCGTTCTA	141	M	CG1548	2R, 30
				TGGCTTCAGCGAAGGTTTGT	577			
Mucin	AF125984	60	408	GACAGCACCCACAGGCAAAT	114	M	NS	
				GCTCCTTTCAACGGGACCTT	521			
NucTrn4a (nuclear transcription factor 4a)	AI618954	48	418	CTGTGGCTACATACCTTCGC	344	M	NS	
				TACTATCCACGGACTGCTCC	761			
Odh-1 [octanol dehydrogenase (E.C.1.1.1.73.)]	AI649985	59	249	CCGAAGGCTGGTGAGGTA	190	M	CG6598	3R, 31
				CGGGCAAAGGTTGGTTTT	438			
OER (ovarian ecdysteroidogenic hormone)	AI619029	48	367	AGCCATCCAGGATCAATCTC	17	M	NS	
				CACCGAAGACCAACTGTGAA	383			
Perox (peroxidase)	AF098717	57	433	CTACGGGTGTCGGGAAGCAA	5	M	CG4009	3R, 37
				TAGGCGCTCAGGAATGGGAC	437			
Polyubq (polyubiquitin)	AI648334	58	359	AGCTCCTCATTGTGCAGTTT	155	M	CG11624	3L, 4
				CGTTATCACTTTCCGTTGGT	513			
PyrCarb (pyruvate carboxylase)	L36530	56	451	CCCCGTGTCAGAATTTGGTC	117	M		
				CCATCAGTTCCGGGTACGAT	567			
Rdl (dieldrin resistance)	U28803	48	441	GGGGTGACCAATAAAGCAAG	97	M	CG10537	3L, 9
				TGCGTCGTCTAAATGGATGG	537			
Retro (retroposon reverse transcriptase pseudogene)	U09359	58	149	ATGGTCAAATGGCAGCGTAC	127	M	NS	
				CAAACTGGACATTCGGGAGA	275			
Riboptl3 (ribosomal protein L3)	AI658429	56	347	ATTGCGTTGCCGTCACCAAG	307	M		
				ACCGATGCAGCAGCCTTTGA	653			
SGA30k (salivary gland allergen–30 kd)	AF001927	58	334	ATTCTGTCTGGTAGGCATTG	41	M	NS	
				TGTCTTCAGCGTTAGCTTCT	374			
SOD (superoxide dismutase)	Cloned	48	151	TGACAACACCAACGGATGCA	37	M		
				CGGACAAGGAAATCTGACTG	187			
Ty7 (ras-like GTPase)	R19560	54	218	GCTTGCTGGATTAGAAACTC	170	M	CG5915	3R, 44
				TAACCACTTATTGAAGGCAC	387			
VCP (vitellogenic cathepsin-b-like protease)	AF127592	60	360	GCCCGCTACCAGGACAATCA	122	M	CG10992	1, 15
				CTGAAACGGCCCAGCAGGAT	481			
Vmem15a2 (vitelline membrane protein 15a-2)	S54556	60	277	GTCCTCTTCACCGCCGTCAT	10	M	NS	
				ATCGGGTTGGGTGGTCTGGT	286			

Listed with each gene are the GenBank accession number, optimal annealing temperature (T_a) and the approximate size of the amplified product. Also listed are the sequences and locations of the oligonucleotide primers relative to the GenBank sequence, the location of the genes in the *A. aegypti* linkage map, the accession number of the homologue in the Drosophila Genome Project, and its physical location in the Drosophila genome.

[a] During SSCP analysis of PCR products, we usually heat product/buffer mixtures at 95° for 5 min and plunge them in ice for an additional 5 min to allow for intrastrand complex formation before loading onto the gel. However, for the loci indicated, better resolution was obtained without heating and cooling.

[b] These loci were not used in mapping because their genotypes failed to fit expected Mendelian ratios.

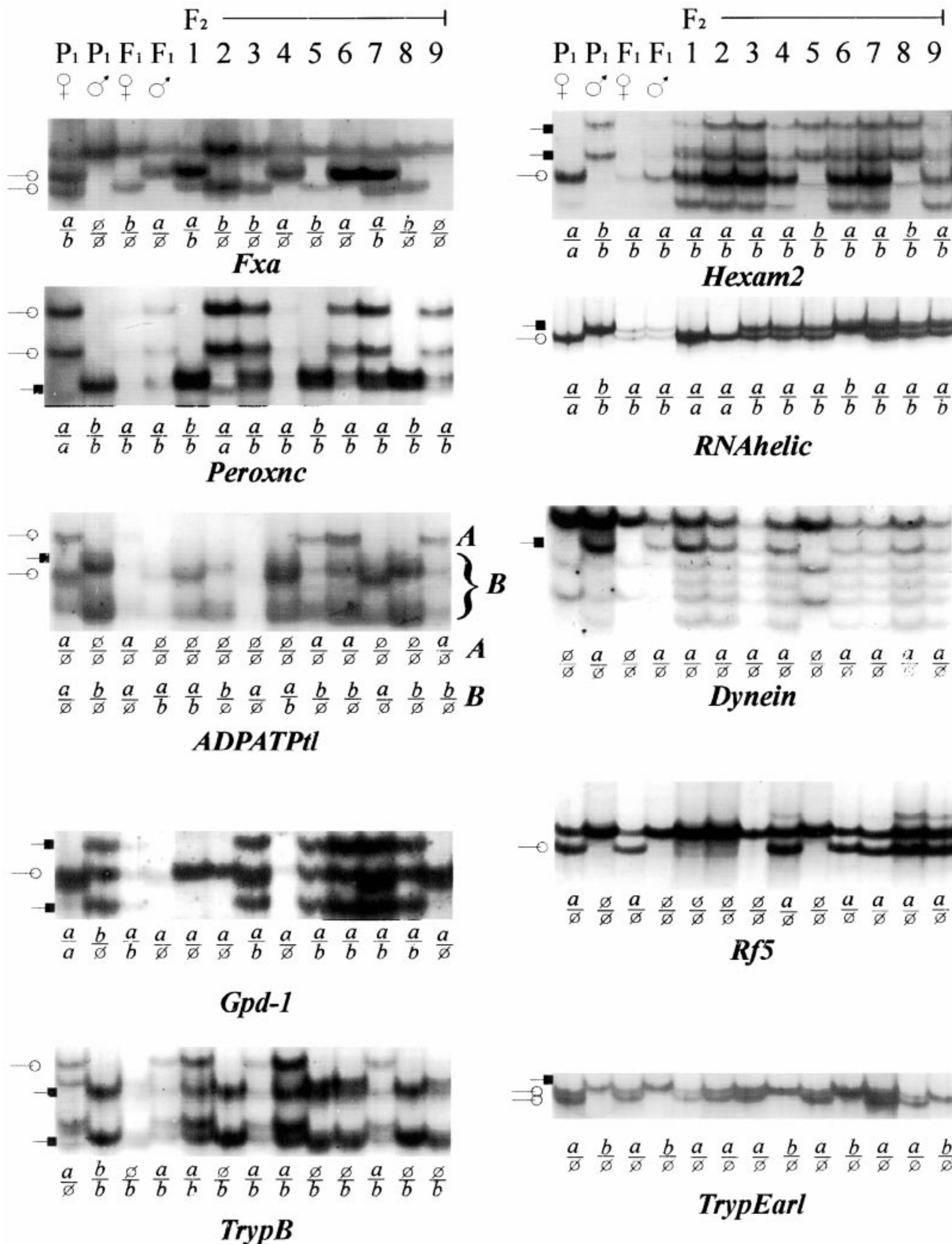

Figure 1.—SSCP genotypes at 10 cDNA loci analyzed in the F_1 intercross family. Displayed are genotypes of the two P_1 parents, the two F_1 parents, and the first nine F_2 offspring. Alleles inherited through the P_1 female are indicated to the left of each gel by an open circle while alleles inherited through the P_1 male are indicated by a solid square. The genotypes of all individuals are indicated below each gel. Ø, a null allele.

and *RNAhelic* segregated as codominant markers whose genotypes were fully informative in this F_1 intercross family. At *Fxa* all four P_1 and F_1 parents had unique genotypes that were recovered in the F_2 offspring and the P_1 male appeared to be homozygous for a null allele. At *Hexam2*, *Peroxnc*, and *RNAhelic*, the P_1 parents had unique genotypes and F_1 parents were heterozygous. All three genotypes were recovered in the F_2 offspring. Alleles at *ADPATPtla* segregated as a dominant marker arising from the P_1 mother and a recessive marker in the P_1 father. Genotypes were only partially informative for mapping because the P_1 mother and her F_1 daughter shared the same genotype. Alleles at the *ADPATPtlb*, *Dynein*, *Gpd-1*, *Rf5*, *TrypB*, and *TrypEarl* loci segregated as codominant markers but a P_1 parent and at least one of its F_1 offspring shared the same genotype and were thus only partially informative for mapping.

Genotype frequencies at all loci fit expected Mendelian ratios except *LF138*, *LF90*, *Glusyn*, and *Apyr1* and these were excluded from mapping. The remaining 53 cDNA-SSCP markers were mapped among the 83 F_2 individuals. In addition, 9 TAG66 microsatellite markers (Fagerberg *et al.* 2000), 6 STAR markers (Bosio *et al.* 2000), and the *Sex* locus were used (Figure 2). The total map consists of 134 cM (58 + 39 + 37), with a marker density of 1.9 markers/cM. Three linkage groups were

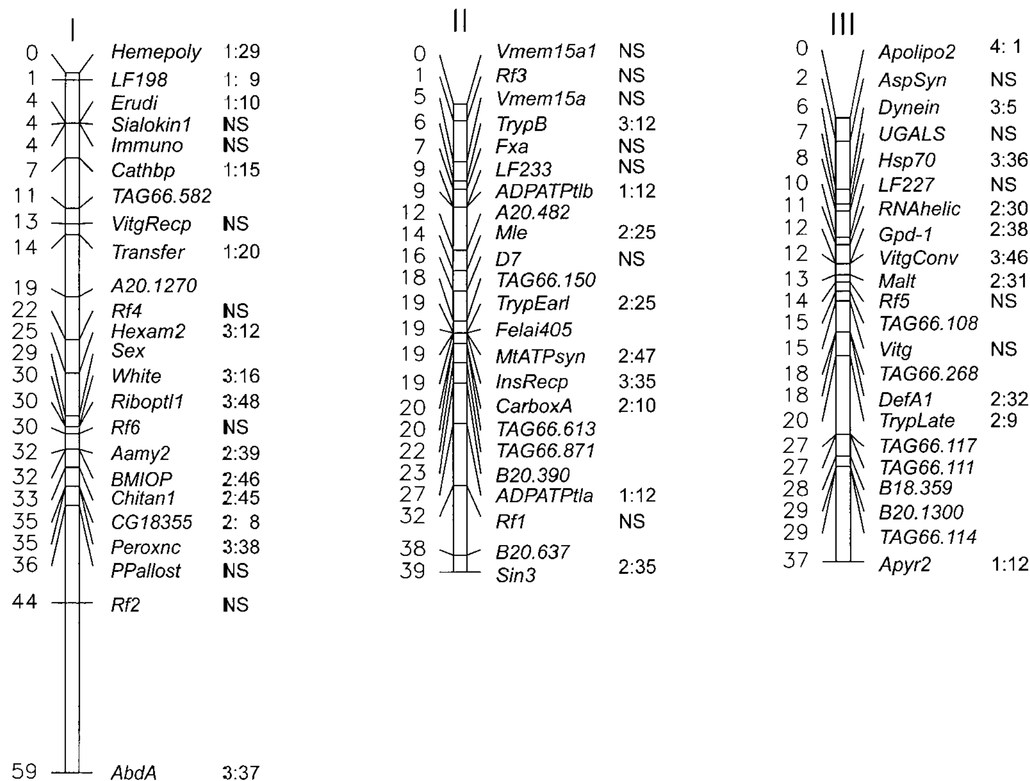

FIGURE 2.—Linkage map of cDNA, STAR, and microsatellite loci in *Aedes aegypti*. The numbers to the right of some loci indicate the chromosome location of the homologous locus in *D. melanogaster* followed by its physical location along the chromosome in megabase pairs. NS indicates that BLAST searches failed to recover similar sequences ($<e^{-15}$) in the Drosophila genome.

detected at a LOD of 2.9. The LOD was increased in 0.1 increments and the three linkage groups remained intact until a LOD of 3.5, when B20.390 and Rf1 formed a separate linkage group. The three linkage groups then remained intact until a LOD of 4.7, when *AbdA* separated from chromosome 1.

Products of various primers were sequenced to determine if they amplified the predicted product. For *A. aegypti* genes of known function these included *Apyr*, *CarboxA*, *D7*, *DefA1*, *Fxa*, all of the *LF* markers (SEVERSON *et al.* 1993), *Malt*, *Sialokin1*, *TrypLate*, and *TrypB*. In every case BLASTN recovered the predicted sequences from the NR database. *AbdA*, *Gpd-1*, and *Hsp70* were designed from ESTs. Sequences amplified from these primers were subjected to a BLASTN search and in every case recovered the original EST and in a BLASTX search recovered the *Abd-A* gene ($3e^{-27}$; CG10325), the glycerol-3-phosphate dehydrogenase gene ($7e^{-22}$; CG8256), and Hsc70-4 ($4e^{-19}$; CG4264) genes from *D. melanogaster*.

We also sequenced any products that appeared as multiple independently segregating alleles. The *ADPATPtl* primers were designed from an EST (AI657540) and amplified two independently segregating bands (Figure 1). A BLASTP search indicated that both were highly similar ($6e^{49}$) to a clone of *A. gambiae* ADP/ATP carrier protein (L11617). Sequence analysis (Figure 3) suggested that *ADPATPtla* is a pseudogene with a premature stop at codon 45 while *ADPATPtlb* may encode a functional mRNA (Figure 3). Interestingly, two *A. aegypti* ESTs AI650113 and AI650176 that were similar in sequence to AI657540 contained insertions between codons 33 and 34 and at codons 41, 57, and 66. These may represent other *ADPATPtl* pseudogenes.

The primers designed to amplify a single allatotropin locus from *A. aegypti* (U65314) instead amplified five loci albeit at a low annealing temperature of 43°. All five amplicons were mapped and sequenced but none were similar to the allatotropin gene in *A. aegypti* or to any other sequences in GenBank and were thus assigned labels *Rf1–Rf5*. The primers that were predicted to amplify actin loci amplified two separate loci. One had no similarity to any sequences in GenBank and was thus labeled *Rf6*. The other amplicon was similar to an *A. aegypti* repetitive element *Feilai 405* (AF107667).

An initial BLASTX search with AI650010 suggested similarity to a region of the Antennapedia complex in *D. melanogaster*. Anticipating that AI650010 would map at an ∼10-cM distance from *AbdA*, as in *D. melanogaster*, we added this marker to our map. It mapped to chromosome 1 at a distance of ∼20 cM from *AbdA*. However, while a BLASTN analysis of the amplified fragment recovered AI650010, a subsequent search of the Drosophila genome database with AI650010 identified it as being more similar ($2e^{20}$) to a gene of unknown function (CG18355 on the right arm of chromosome 2).

The GenBank sequences of all mapped loci were subjected to BLAST searches against the Drosophila genome to compare Aedes linkage locations to Drosophila physical locations (Table 1). The locations of several genes on *A. aegypti* chromosome 1 also mapped to *D.*

```
               1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17
               W   R   Y   F   M   G   N   L   G   S   G   G   A   A   G   A   T
ADPATPla      TGG CGC TAC TTC ATG GGT AAC TTG GGA TCC GGC GGT GCC GCT GGT GCC ACC
ADPATPlb      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
AI657540      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
AI650113      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
AI650176      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
ANOPHELES     ... ... ... ... C.. ... ... C.C ... ... ... ... ... ..C ... ... ..G

              18  19  20  21  22  23  24  25  26  27  28  29  30  31  32  33
              SP   L   C   F   V   Y   P   L   D   F   A   R   T   R   L   G
ADPATPla      CCG CTG TGC TTC GTC TAC CCA CTC GAC TTT GCC CGT ACC CGT CTG GGT -
ADPATPlb      T.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C .
AI657540      T.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C .
AI650113      T.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C C
AI650176      T.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C .
ANOPHELES     T.. ..C ... ... ..G ... ..G ... ... ..C ... ... ... ... ..C ... .

              34  35  36  37  38  39  40   41  42  43  44  45  46  47  48  49  50
               A   D   V   G   R   A   G    A   E   R   E  N*   N   G   L   I   D
ADPATPla      GCC GAT GTT GGC CGT GCC GGA G-CC GAG CGC GAG TAG AAC GGT CTG ATC GAC
ADPATPlb      ... ... ... ... ... ... ... .... ... ... ... A.C ... ... ... ... ...
AI657540      ... ... ... ... ... ... ... .... ... ... ... ..C ... ... ... ... ...
AI650113      ... ... ... ... ... ... ... .C.. ..A ... ... ..C ... ... ... ... ...
AI650176      ... ... ... ... ... ... ... -... ... ... ... ..C ... ... ... ... ...
ANOPHELES     ..G ..C ... ..T .CG .GT .CC ..G. ... ..T ... .TC ... ... ... C.. ...

              51  52  53  54  55  56   57  58  59  60  61  62  63  64  65   66  67
               C   L   K   K   T   V    K   S   D   G   P   I   G   L   Y    R   G
ADPATPla      TGC CTG AAG AAG ACC GTC AA-G TCC GAT GGT CCG ATC GGT CTG TAC C-GT GGA
ADPATPlb      ..T ... ... ... ... ... .... ... ... ... .T. ... ... ... ... .... ...
AI657540      ... ... ... ... ... ... .... ... ... ... .T. ... ... ... ... .... ...
AI650113      ..G ... ... ... ... ... ..A. ... ... ... .T. ... ... ... ... .C.. ...
AI650176      ... ... ... ... ... ... .... ... ... ... .T. ... ... ... ... .C.. ...
ANOPHELES     ..T ... ... ... ..G ... .... ..G ..C ... ATC ... ... ... ... .... ...

              68  69  70  71  72  73  74  75  76  77  78  79  80  81  82  83  84
               F   N   V   S   V   Q   G   I   I   I   Y   R   A   A   Y   F   G
ADPATPla      TTC AAC GTG TCG GTC CAG GGT ATC ATC ATC TAT CGT GCT GCC TAC TTT GGT
ADPATPlb      ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ... ... ...
AI657540      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
AI650113      ... ... ... ... ... ..- --- --- --- --- --- --- --- --- --- --- ---
AI650176      ... ... ... ... ... ..A ... ... ... ... ... ..- --- --- --- --- ---
ANOPHELES     ... ... ... ..C ..T ... ... ... ... ... ... ... ..G ... ... ..C ...

              85  86  87  88  89  90  91  92  93  94  95  96  97  98  99 100
               C   F   D   T   A   K   G   M   L   P   D   P   K   N   T   S
ADPATPla      TGC TTC GAT ACT GCC AAG GGA ATG CTG CCC GAC CCG AAG AAC ACC TCG AT
ADPATPlb      ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ..
AI657540      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
AI650113      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --
AI650176      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --
ANOPHELES     ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ..C ..
```

FIGURE 3.—Sequences of alleles at the *ADPATPtla* and *ADPATPtlb* loci aligned to ESTs AI657540, AI650113, and AI650176 and to the *Anopheles gambiae* ADP/ATP carrier protein gene (L11617). Dots indicate identical sequences to *ADPATPtla*.

melanogaster chromosome 1 (Figure 2). *Hemepoly*, *LF198*, *Erudi*, *Cathbp*, and *Transfer* all mapped to the first 14 cM of chromosome 1 in *A. aegypti* and to the first 29 Mb of Drosophila chromosome 1 albeit not in identical order. In addition, *Aamy2*, *BMIOP*, and *Chitan1* all mapped between 32 and 33 cM on *A. aegypti* chromosome 1 and were located within 39–46 Mb of Drosophila chromosome 2. The remainder of genes appeared to be located on different linkage groups in the two species.

DISCUSSION

The *A. aegypti* genome contains from 750 to 842 Mbp and 40% of this consists of repetitive elements distributed as short repeats (WARREN and CRAMPTON 1991). On the basis of a range of estimated linkage sizes of 134 cM (this report) to 228 cM (MUNSTERMANN and CRAIG 1979), the relationship between physical and recombination distance is between 3.3 and 6.3 Mbp/cM. However, comparison of physical and recombination distances (D. W. SEVERSON, personal communication) suggests that, as with *D. melanogaster* (ADAMS *et al.* 2000), a large proportion of the repetitive elements are clustered in centromeres or along whole arms such that the resolution among coding sequences may be as low as 1 Mb/cM.

This relatively low resolution and lack of well-resolved polytene chromosomes predict that *A. aegypti* genetic studies will continue to rely heavily on linkage mapping and eventually mapped-based positional cloning to

identify genes of interest. Positional cloning depends critically on having a high density of genetic markers. RFLP analysis provides abundant codominant loci (Severson *et al.* 1993, 1994, 1995b; Severson and Zhang 1996) but is limited by the amount of data that can be gleaned from an individual mosquito, since an extraction from one mosquito yields ≤10 μg of genomic DNA (Severson *et al.* 1993). Also, sequence variation outside of restriction sites is undetected. Alternatively, use of PCR-based analyses increases the amount of data that can be acquired from a mosquito such that saturated linkage maps can be constructed with DNA from only a single family.

We demonstrated that detection of SNPs in cDNA loci by SSCP analysis provides an abundance of codominant markers for construction of saturated linkage maps in *A. aegypti*. SSCP analysis detected allelic sequence variation at 61% of the loci examined in a single family. This underestimates the amount of natural variation at these loci: analysis of additional mosquitoes from natural populations identified variation at *Apyr*, *CarboxA*, *D7*, *DefA1*, *Fxa*, *Gpd-1*, *Hsp70*, *Malt*, *Sialokin1*, *TrypLate*, and *TrypB* loci. Furthermore, markers that could be mapped in our 83-member family could also be consistently amplified and mapped in a reciprocal cross (Bosio *et al.* 2000) and in the original RAPD family (Antolin *et al.* 1996).

The linkage map derived in our study is shorter than the earlier maps constructed using RAPD markers [52.3 + 58.2 + 57 = 168 cM in Antolin *et al.* (1996); 61 + 52 + 99 = 212 cM in Bosio *et al.* (2000)]. This may in part be due to fewer markers used in our study (68 as compared to 98 and 83). However, if repetitive DNA is clustered rather than dispersed, then our map may fail to include estimates of recombination among noncoding repetitive sequences. Combined use of cDNA and additional STAR markers may result in a map of more accurate length.

Jennifer Holmes, Amy Fagerberg, and Heather Stevenson assisted in the laboratory. Dr. Norma Gorrochotegui-Escalante provided preliminary sequence results from analysis of some cDNA genes in *A. aegypti* populations. Dr. Chris Bosio constructed the *A. aegypti* family used in this study. Drs. Barry Beaty and Boris Kondratieff served on R.F.'s graduate committee. This research was supported in part by the MacArthur Foundation for the Network on the Biology of Parasite Vectors and by National Institutes of Health grants AI 41436 and AI 45430.

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