Evidence for Genetic Hitchhiking Effect Associated With Insecticide Resistance in *Aedes aegypti*

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ABSTRACT

Information on genetic variation within and between populations is critical for understanding the evolutionary history of mosquito populations and disease epidemiology. Previous studies with Drosophila suggest that genetic variation of selectively neutral loci in a large fraction of genome may be constrained by fixation of advantageous mutations associated with hitchhiking effect. This study examined restriction fragment length polymorphisms of four natural *Aedes aegypti* mosquito populations from Trinidad and Tobago, at 16 loci. These populations have been subjected to organophosphate (OP) insecticide treatments for more than two decades, while dichlor-diphenyltrichlor (DDT) was the insecticide of choice prior to this period. We predicted that genes closely linked to the OP target loci would exhibit reduced genetic variation as a result of the hitchhiking effect associated with intensive OP insecticide selection. We also predicted that genetic variability of the genes conferring resistance to DDT and loci near the target site would be similar to other unlinked loci. As predicted, reduced genetic variation was found for loci in the general chromosomal region of a putative OP target site, and these loci generally exhibited larger F_{ST} values than other random loci. In contrast, the gene conferring resistance to DDT and its linked loci show polymorphisms and genetic differentiation similar to other random loci. The reduced genetic variability and apparent gene deletion in some regions of chromosome *1* likely reflect the hitchhiking effect associated with OP insecticide selection.

MOSQUITOES are important vectors for several human pathogens because of their close association with humans. Mosquito habitats often change rapidly as a result of vector control efforts; therefore, successful adaptation to varying human habitats is essential for mosquito reproduction. Adaptation ability of an organism depends on its genetic variability. Information on genetic variation within and between populations is critical for understanding the evolutionary history of mosquito populations and disease epidemiology (Tabachnick and Black 1996). Protein electrophoresis and DNA sequence analyses have revealed remarkable variation in many genes in natural populations of Drosophila and other species, but the genetic variability seems to differ substantially for genes in different genome regions (Aquadro 1992). Distribution patterns of genetic variants in natural populations are the joint effects of various evolutionary forces and demographic factors, including random genetic drift, selection, recombination, mutation, gene flow, mating system and life history (*e.g.*, colonization, range expansions or contractions; Slatkin 1985). Population life history and mating structure influence all loci equally, but selection affects only the target loci (Kreitman and Akashi 1995). Variation of selectively neutral loci may also be constrained by the hitchhiking effect, particularly in genome regions with low recombination rates and under extensive selection (Maynard Smith and Haigh 1974). Recent studies with *D. melanogaster* suggest that the hitchhiking effect may have occurred over a large fraction of the insect genome (Begun and Aquadro 1992). In this study we analyzed restriction site variation of 16 loci in natural populations of the yellow fever mosquitoes, *Aedes aegypti*, and provide evidence that the hitchhiking effect may have reduced genetic variation in the genome regions around a putative insecticide resistance locus.

A. aegypti is an important vector of yellow fever and dengue fever viruses in many tropical countries, including Trinidad and Tobago, West Indies. Control efforts for *A. aegypti* have focused primarily on habitat reduction and chemical treatment, which is based on the destruction of breeding sites and the use of insecticides, including dichlor-diphenyltrichlor (DDT) in the 1950s and several organophosphates (OP) since the 1960s. The wide use of insecticides has been a powerful selection agent, and rapid development of resistance to DDT and OPs is well documented (Gilkes *et al.* 1956; Rawlins and Wan 1995). The genetic mechanisms of insect resistance to various insecticides have been well

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characterized. For example, a point mutation in the *para* sodium channel gene confers one form of resistance to DDT (Williamson *et al.* 1996), and esterase (*EST*) gene amplification is associated with resistance to OPs in Culex mosquitoes (Mouchès *et al.* 1990). A genetic linkage map, based largely on random cDNA sequences, has been constructed for *A. aegypti* (Severson *et al.* 1993), and several insecticide resistance genes have been mapped (Severson *et al.* 1997). In this study, we used cDNA markers distributed across the mosquito genome to examine DNA polymorphism and population genetic differentiation, and to examine mosquito genome structural changes associated with strong selection imposed by insecticides. Several studies have investigated the genetic variation of various genera of mosquito populations with isozyme, RAPD-DNA, microsatellite and mitochondrial DNA markers (Powell *et al.* 1980; Tabachnick and Wallis 1985; Conn *et al.* 1993; Chevillon *et al.* 1995; Apostol *et al.* 1996). Restriction fragment length polymorphism (RFLP) markers are particularly suitable for population genetic studies, because they are presumably neutral, highly polymorphic, segregate as codominant markers, and can be used for studies of other mosquito species (Severson *et al.* 1994a). We chose Trinidad and Tobago populations because the population history is known and surveillance programs have been well established there. Population historical information is important for the interpretation of genetic data. Because the mosquito populations have been under selection of OP insecticides, genetic variation of loci closely linked to an esterase locus conferring resistance would be reduced if the hitchhiking effect has occurred. The hitchhiking effect would be prominent in genome regions with strong linkage disequilibrium and intense selection (*e.g.*, insecticides). In contrast, gene diversity at the *para* locus and other neighboring loci is expected to be similar to unlinked loci in the genome, because selection pressure has been removed at the *para* locus since DDT was abandoned more than two decades ago.

MATERIALS AND METHODS

Natural history of *A. aegypti* in Trinidad and Tobago: It is generally believed that domestic *A. aegypti* originated from an African sylvan ancestor, and was introduced to the New World from West Africa via transoceanic trade during the fifteenth to seventeenth centuries (Tabachnick 1991). Caribbean populations probably represent the initial introduction of the mosquito species into the New World in the course of New World colonization. The first outbreak of yellow fever in Trinidad was recorded in 1796, and in 1820 in Tobago.

In the 1950s, intensive vector control programs aimed toward mosquito eradication were adopted, primarily by the widespread usage of DDT. In the early 1960s, Trinidad was considered free of *A. aegypti*, but was reinfested in 1962. Overall, mosquito populations in Tobago have been exposed to fewer insecticides than Trinidad populations. *A. aegypti* is now widely distributed in Trinidad and Tobago, despite continued intensive vector control efforts through the use of OP insecticides. Insecticide applications not only impose strong selection on the target loci, but also lead to recurrent reductions of population sizes.

Collection of samples: In conjunction with the *A. aegypti* surveillance program, in April, 1995, we collected three geographically-distinct samples from Trinidad and one sample from Tobago (Figure 1). These four villages share similar climates, including temperature and the annual amount of rainfall. For each village, 100 ovitraps were distributed, and about half of the village's residential area was covered (approximately two traps every five houses). Each ovitrap consisted of a black plastic container roughly half-filled with water into which a rectangular masonite strip was placed in an upright manner. Female *A. aegypti* mosquitoes will readily oviposit on the masonite strip, near the water interface. After 2–3 days, the masonite strips were removed, transported to the laboratory, where attached eggs were allowed to hatch, and reared into adults. All adults were identified as *A. aegypti* by microscopic examination, and were frozen for subsequent DNA analysis. Previous studies with *A. aegypti* in Puerto Rico suggest that the mean number of families represented per ovitrap was 4.7 (*e.g.*, several female mosquitoes frequently oviposit in the same container; Apostol *et al.* 1994). Therefore, it is unlikely that siblings within a subpopulation would be sampled.

RFLP and probe selection: We genotyped a total of 870 mosquitoes for four populations (n = 150 for Curepe, 262 for Couva, 258 for San Fernando, and 200 for Tobago). DNA extraction from individual mosquitoes, digestion with *Eco*RI, Southern blotting and hybridization were as previously described (Severson *et al.* 1993). Fifteen mapped RFLP markers were selected to provide broad coverage of the *A. aegypti* genome with an average resolution of 10.6 cM (Figure 2). All clones used were random cDNA clones with the exception of the *para* and *Mal* I clones. *Mal* I is a gene specifically expressed in the salivary glands, and its putative function is related to

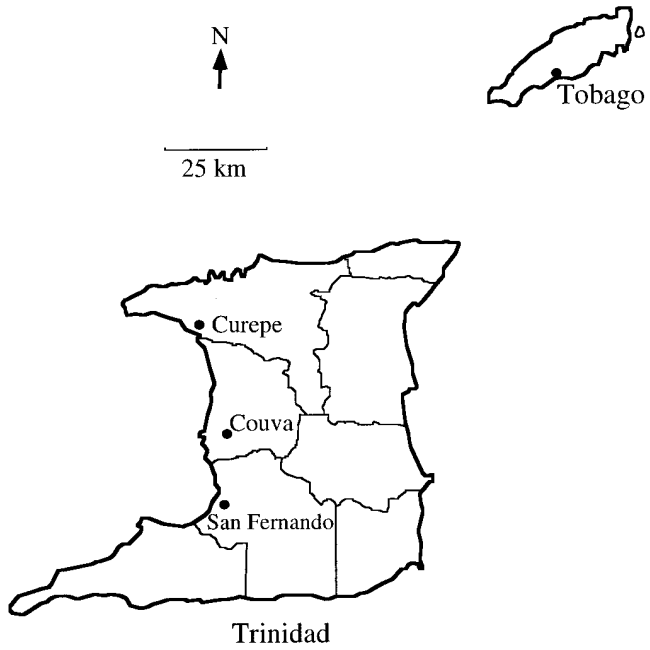

Figure 1.—Map of Trinidad and Tobago. Three samples were collected from Trinidad: Curepe (10°38.62'N, 61°24.23'W), Couva (10°26.12'N, 61°28.19'W), and San Fernando (10°18.11'N, 61°28.21'W). One sample was collected from Tobago (11°11.23'N, 60°44.21'W).

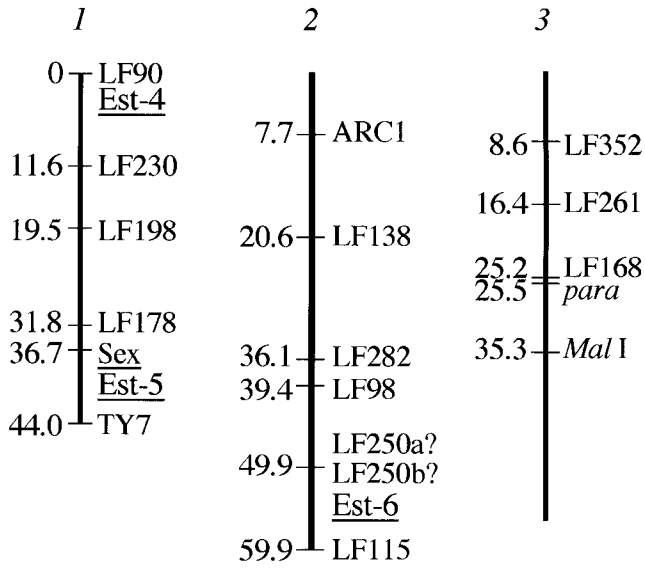

Figure 2.—Relative map positions of the 16 *Aedes aegypti* (2N = 6) RFLP loci used in the study. Chromosome numbers are in italics. Map distances are in Kosambi centimorgans. Underlined loci were not used in the study. Esterase gene amplification is involved with resistance to organophosphate insecticides in mosquitoes (Mouchès *et al.* 1990). Three esterase loci were mapped to the general chromosomal locations as shown in the figure (Munstermann 1990). LF250 represents duplicated loci. Markers in italics are genes with known functions; other markers are random cDNA.

sugar metabolism (James *et al.* 1989). The mosquito genome consists of single or low-copy DNA sequences and repetitive DNA with short-period interspersion (Black and Rai 1988). In this study, we focused on allelic variations of single- or low-copy cDNA sequences.

Data analysis: *DNA polymorphism and Hardy-Weinberg equilibrium (HWE) tests:* Molecular weights of fragments detected by each clone were estimated by comparing them to lambda-*Hin*dIII digest standards included on each gel, using the Eagle Sight image capture and analysis software (Stratagene, La Jolla, CA). DNA polymorphisms may be measured by the proportion of polymorphic loci, number of alleles, and heterozygosity. Conformance with HWE was tested using the probability test for each locus and each population, using the GENEPOP computer program (Raymond and Rousset 1995). Because this test is robust to allele frequencies, rare alleles were not pooled. We further tested whether distortion from HWE resulted from deficient or excessive heterozygosity, using the F_{IS} statistics (Weir 1990; Rousset and Raymond 1995). F_{IS} is defined as [1 − (observed heterozygosity/expected heterozygosity from HWE)]. Because F_{IS} estimates at individual loci may be unduly influenced by rare alleles, we tested the significance of the average F_{IS} over all loci using the method of Robertson and Hill (1984). Variations in heterozygosity among the populations were analyzed following the method of Weir (1990), using the analysis of variance (ANOVA) with subpopulations, individuals, loci and interactions of loci and individuals as factors. All factors were treated as random effects except loci.

Population genetic structure, gene flow and genetic distance: Population genetic structure was examined with Wright's F-statistics, based on the procedure of Weir and Cockerham (1984) and using the FSTAT computer program (Goudet 1995). Standard deviations (SD) of F-statistics were obtained for each locus by a jackknife procedure over the alleles, and were used to test the significance of the F statistics. We first tested whether the three populations from Trinidad were significantly substructured, then included the Tobago population data in the analysis.

Gene flow (*Nm*) was estimated from the standardized-among-population genetic variance (F_{ST}) estimate of each locus using the relationship $Nm = (1/F_{ST} - 1)/4$, where N is the effective population size of a deme, and m is the rate of gene flow (Wright 1943). This equation assumes the infinite-island model of population structure and gene flow. Few populations probably conform to this assumption, but it provides a useful approximation of the relative magnitude of gene flow. Gene flow was also estimated using the private-alleles method for the appropriate loci (Slatkin and Barton 1989). Private alleles are the alleles unique to a given deme. Nei's unbaised genetic distance for all pairs of populations was calculated based on population allele frequency for all loci (Nei 1987).

RESULTS

DNA polymorphisms and HWE tests: Fifteen cDNA markers examined in this study were all polymorphic. The RFLP patterns of one marker (LF250) indicate that this marker represents a gene duplication (data not shown), and therefore, the 15 markers represented a total of 16 loci. A total of 91 unique alleles were identified, 68 alleles (74.7%) were common to all four populations. The average number of alleles was about five per locus (Table 1). Six loci (LF198, ARC1, LF250a, *para*, LF168 and *Mal*I) exhibited private alleles, and five private alleles were in the Tobago population. An excess of rare alleles was found: 16 alleles (18%) had a frequency less than 0.05. Under the infinite alleles model (equation 8.24; Kimura 1983), we expected to find only four alleles in this frequency class with our sample size (n = 870). The mean sizes of restriction fragments detected by the cDNA clones weighted by their frequencies ranged from 0.73 kb at locus LF250b to 12.90 kb at locus LF352, and exhibited an overall mean of 5.13 kb (95% confidence interval: 3.61–6.64 kb).

In general, high heterozygosity was observed in all four mosquito populations, except at the LF90 locus. The LF90 locus showed significantly lower heterozygosity than the other 15 loci examined (Table 1; ANOVA, t = 8.37, d.f. = 1, $P < 0.0001$). The most heterozygous loci were LF178 on chromosome 1 and LF282 on chromosome 2. The high heterozygosity at the LF178 locus does not seem to be a result of sex linkage (see Figure 2), because males and females showed similar heterozygosity (data not shown). Population average heterozygosity over all 16 loci varied little among populations (ranged from 0.582 for Couva to 0.627 for Tobago), and such variations were not statistically significant (Table 1; ANOVA, F = 1.08, d.f. = 3, 49, $P > 0.05$). Heterozygosity is not correlated with the mean size of restriction fragments weighted by frequencies at a locus (r = 0.22, d.f. = 15, $P > 0.05$), but seems to correlate with the number of observed alleles (r = 0.49, d.f. =15, $P = 0.052$).

The genotype frequencies at several loci did not con-

TABLE 1

RFLP polymorphisms of four *Aedes aegypti* populations from Trinidad and Tobago, measured by observed heterozygosity and the number of alleles

		Curepe			Couva			San Fernando			Tobago		
Chromosome	Locus	*n*	H_{obs}	F_{IS}[a]	*n*	H_{obs}	F_{IS}	*n*	H_{obs}	F_{IS}	*n*	H_{obs}	F_{IS}
1	LF90	5	0.122	−0.036	3	0.159	−0.076	5	0.256	0.160*	3	0.391	0.244***
	LF230[b]	3	0.250	0.600***	3	0.069	0.880***	3	0.129	0.774***	3	0.296	0.535***
	LF198	6	0.655	0.096	6	0.504	0.100**	6	0.775	0.029	7	0.738	−0.120**
	LF178	6	0.761	0.012	6	0.849	−0.075*	6	0.851	−0.100***	6	0.828	−0.120**
	TY7	5	0.503	0.160*	5	0.546	0.151*	5	0.543	−0.001	5	0.656	0.060*
Average over chromosome *1*		5.5	0.510	0.059	5.0	0.515	0.025	5.5	0.606	0.022	5.3	0.654	0.016
2	ARC1	5	0.750	−0.046	5	0.724	0.015	5	0.702	−0.038	6	0.725	0.010
	LF138	4	0.729	−0.083	4	0.714	−0.201**	4	0.492	−0.075	4	0.487	0.145**
	LF282	9	0.838	−0.009	7	0.795	0.025	8	0.864	−0.060*	7	0.793	−0.102*
	LF98	5	0.642	0.092	5	0.654	−0.035	6	0.682	−0.073	6	0.878	−0.068*
	LF250a	4	0.615	0.060	3	0.596	−0.032	4	0.800	−0.154*	3	0.562	−0.183***
	LF250b	3	0.644	−0.009	3	0.687	−0.038	3	0.662	−0.238***	3	0.557	−0.160*
	LF115	5	0.514	−0.031	5	0.366	−0.016	5	0.566	−0.101	4	0.562	−0.077
Average over chromosome *2*		5.0	0.676	−0.003	4.6	0.648	−0.040	5.0	0.681	−0.105	4.7	0.652	−0.062
3	LF352	6	0.548	0.268**	6	0.727	0.113**	6	0.441	0.292***	6	0.550	0.263***
	LF261	4	0.483	0.035	4	0.279	−0.061	4	0.476	−0.058	3	0.592	0.008
	para	4	0.469	0.152	4	0.563	0.024	5	0.598	0.081	6	0.555	0.103
	LF168	6	0.667	0.042**	7	0.516	0.145**	7	0.667	0.109	6	0.582	−0.043
	*Mal*I	4	0.627	0.103**	4	0.637	0.009	3	0.640	0.034	4	0.577	0.137*
Average over chromosome *3*		4.8	0.559	0.119	5.0	0.544	0.046	5.0	0.564	0.092	5.0	0.571	0.093
Average over all loci		5.1	0.598	0.050	4.8	0.582	0.003	5.1	0.626	−0.012	4.9	0.627	0.006

*$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$. H_{obs}, observed heterozygosity; *n*, number of alleles.

[a] Significant F_{IS} also indicates distortion from HWE. Positive F_{IS} indicates heterozygosity deficit from HWE expectation; negative F_{IS} indicates excess of heterozygosity.

[b] A large proportion of individuals show an apparent gene deletion at the LF230 locus, therefore, this locus was not used for chromosomal average heterozygosity calculation. The heterozygosity and F_{IS} were based on the individuals without deletions. The percentage of individuals showing the gene deletion at the LF230 locus was 41.4 for the Curepe population, 58.6 for Couva, 53.7 for San Fernando, and 46.0 for Tobago.

form to HWE. Loci on chromosome *2* generally exhibited a heterozygote excess, but loci on chromosome *3* that showed HWE distortion exhibited a heterozygote deficit (Table 1). The F_{IS} values varied greatly among the loci, suggesting no systematic inbreeding occurred in these populations. The average F_{IS} over all loci was not significantly different from 0 for each population (Table 1). Departure from HWE probably reflects either the effect of insecticide selection on some loci linked to the resistance loci, or simply sampling error.

Population genetic structure, gene flow and genetic distance: Analysis of F statistics for the three Trinidad populations found small, but statistically significant F_{ST} estimates for all loci (Table 2), suggesting that these populations are genetically differentiated. F_{ST} estimates showed a six-fold difference among loci, with an average F_{ST} over all loci of 0.043. When the Tobago population was included in the analysis, the basic pattern of F_{ST} estimation among the loci was not altered (Table 2). As expected, slightly larger F_{ST} values were obtained for most loci, and the average F_{ST} over all loci was 0.056. The *para* locus exhibited similar polymorphism and genetic differentiation as other random loci.

Assuming that the populations are at an equilibrium between migration and random drift, the average number of migrants exchanged per generation can be calculated. Average gene flow (*Nm*) among the four populations, based on the F_{ST} method, was 4.2 migrants per generation (95% confidence interval: 3.2–5.7). This estimate was similar to the estimate based on the average frequency of six private alleles present in the populations ($Nm = 4.5$). Table 3 shows genetic distances and gene flow between each pair of populations calculated from the pair-wise average F_{ST}. A large gene flow between the Tobago and Trinidad populations was detected. There was no significant correlation between genetic distance and geographic distance ($r^2 = 0.5$, d.f. = 5, $P > 0.05$).

TABLE 2

F_{ST} statistics and *Nm* estimates of four *Aedes aegypti* populations of Trinidad and Tobago

Chromosome	Locus	F_{ST}		*Nm* estimates of all populations[a]	
		Trinidad populations[b]	Trinidad and Tobago populations[a]	Based on FST	Based on the private-alleles method
1	LF90	0.053 ± 0.022	0.107 ± 0.102	2.1	—[c]
	LF198	0.121 ± 0.062	0.109 ± 0.046	2.0	3.5
	LF178	0.011 ± 0.006	0.025 ± 0.013	9.8	—
	TY7	0.033 ± 0.022	0.034 ± 0.011	7.1	—
2	ARC1	0.064 ± 0.046	0.049 ± 0.034	4.9	177.6
	LF138	0.044 ± 0.032	0.030 ± 0.022	8.1	—
	LF282	0.019 ± 0.011	0.041 ± 0.027	5.9	—
	LF98	0.042 ± 0.012	0.061 ± 0.021	3.9	—
	LF250a	0.081 ± 0.053	0.099 ± 0.045	2.3	0.8
	LF250b	0.106 ± 0.067	0.110 ± 0.041	2.0	—
	LF115	0.021 ± 0.012	0.055 ± 0.039	4.3	—
3	LF352	0.119 ± 0.059	0.079 ± 0.037	2.9	—
	LF261	0.038 ± 0.034	0.102 ± 0.060	2.2	—
	para	0.039 ± 0.025	0.035 ± 0.017	6.9	70.9
	LF168	0.020 ± 0.013	0.040 ± 0.021	6.0	1.7
	*Mal*I	0.034 ± 0.019	0.020 ± 0.015	12.3	9.2
Summary over 16 loci		0.043 ± 0.007	0.056 ± 0.008	4.2	4.5

Values are ± SD.

All F_{ST} values were significantly larger than 0 at $P < 0.001$. The test was performed using a jackknifing procedure over samples.

[a] $n = 4$.

[b] $n = 3$.

[c] The estimate was not available because no private alleles existed for the locus.

Hitchhiking effect on DNA polymorphisms: Genetic hitchhiking occurs when a (neutral) mutation changes frequency through genetic linkage to a mutation that is selected, resulting in reduced genetic variation surrounding the target site of selection. Low DNA polymorphism at the LF90 locus suggests that hitchhiking has probably occurred in the genome region of the *EST*-4 locus. We collected additional evidence to test for this hypothesis by examining genetic polymorphism at the LF230 locus, which also is in the general genomic region of *EST*-4 (Figure 2). An apparent chromosomal deletion event occurred around this locus in 42–59% of the individuals (Figure 3), and low heterozygosity (0.07–0.29) was observed among those individuals without the apparent deletion (Table 1). However, substantial reduction of heterozygosity for the *para* locus and other loci in the vicinity of *para* was not observed (Table 1).

DISCUSSION

DNA polymorphisms of four *A. aegypti* mosquito populations were examined using RFLP markers. The Trinidad populations have been exposed to OPs every 3–4 months for about two decades. These populations have therefore experienced intense selection by insecticides, that probably resulted in periodic population bottlenecks. A population bottleneck maintains a long-term effect on population heterozygosity, even for species with a large intrinsic rate of growth such as *A. aegypti* (Nei *et al.* 1975). Thus, genetic polymorphisms are expected to decline rapidly during insecticide use for any locus in the mosquito genome. Loci conferring OP resistance are expected to maintain lower genetic variability than other random loci in the genome, and genetic variation of other closely-linked neutral loci may be reduced through genetic linkage.

If recurrent population bottlenecks have occurred in the mosquito populations, low polymorphism for all loci in the genome would be expected. In contrast to the expectation, we observed high polymorphisms for most loci. For the same loci, average heterozygosities of

TABLE 3

***Nm* matrix based on pairwise F_{ST} estimates and Nei's unbiased genetic distance matrix**

	Curepe	Couva	San Fernando	Tobago
Curepe		0.041	0.057	0.113
Couva	11.4		0.112	0.166
San Fernando	8.6	3.7		0.124
Tobago	4.3	2.6	3.7	

Numbers below diagonal line are F_{ST} estimates, above the diagonal line are Nei's unbiased genetic distance.

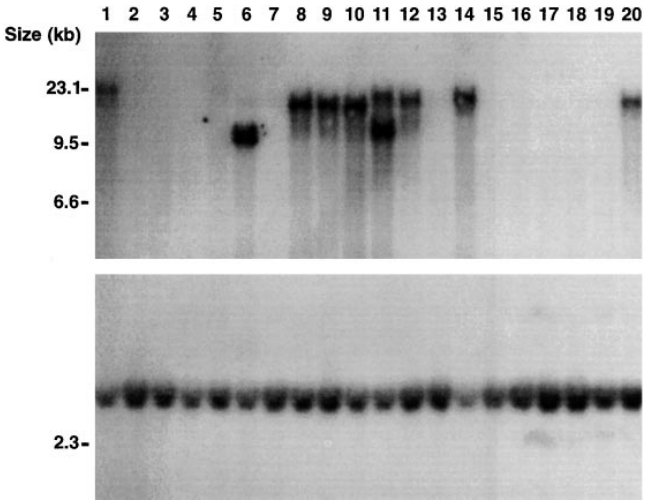

Figure 3.—Southern blot analysis of natural *Aedes aegypti* populations probed with cDNA clones LF230 (top) and LF90 (bottom). The mosquito genomic DNA was digested with *Eco*RI. Each lane is for a single mosquito. (Top) Apparent gene deletion around the LF230 locus in 55% of individuals (11 out of 20). (Bottom) Probe LF90 was used as a control to demonstrate that absence of hybridization of mosquito genomic DNA to LF230 was not due to incomplete DNA digestion, or to poor probe conditions. DNA hybridization was observed for the same individuals with all other markers tested. See Table 1 for heterozygosity and percentage of gene deletion at the LF230 locus.

the populations studied here are substantially higher than a laboratory population, which has not been exposed to insecticides for more than 20 years and has not experienced population bottleneck (Yan *et al.* 1997). The highest heterozygosity was observed for loci at two chromosomal regions (LF198-LF178 on chromosome *1*, and LF282-LF98 on chromosome *2*). Coincidentally, these two chromosomal regions in *A. aegypti* harbor genes determining vector competence for filarial worms and malaria parasites (Severson *et al.* 1994b, 1995). The high levels of heterozygosity observed may be explained by two mechanisms. The first is that the effective size of population bottlenecks has never been small, because heterogeneous habitats may provide effective shelters for the field populations. The second is that genetic polymorphisms are introduced and maintained by large gene flow among populations. The gene flow estimates seem to support the second hypothesis.

The LF90 locus consistently exhibited lower heterozygosity than other loci in the genome for the four populations used in this study. The heterozygosity of a RFLP locus may be influenced by several factors, including the size of the probes, the size of the regions being probed by the probes, reduced mutation or recombination rates in these genome regions, natural or artificial selection on a particular locus, and hitchhiking (selective sweep) of a selectively neutral locus by selectively favored substitutions at linked loci. We argue that the polymorphism pattern of the LF90 locus likely reflects the result of a hitchhiking effect. First, the putative function of the LF90 clone is coding for ribosomal protein S14 (Severson and Zhang 1996), and thus the RFLP fragments of LF90 themselves are presumably neutral. Second, LF90 is located in the general chromosomal region of *EST*-4, and gene amplification at an esterase locus is a common mechanism of OP resistance. Our populations have been under selective pressure by OP insecticides for decades. Third, low heterozygosity of the LF90 locus is likely not related to the size of the genome region being probed by the LF90 marker, because we found no significant correlation between heterozygosity and RFLP fragment sizes. Fourth, the observed heterozygosity of the LF90 locus in laboratory colonies of *A. aegypti* that have not been exposed to insecticide selection was similar to other random loci across the genome (Yan *et al.* 1997).

Our argument for a hitchhiking effect is strengthened by the RFLP data of the LF230 locus, which is linked to LF90 and also is in the general genomic region of *EST*-4. We found very low DNA polymorphism and an apparent gene deletion for many individuals at this locus, a phenomenon which has not been observed in other laboratory colonies of *A. aegypti* (Yan *et al.* 1997). Gene deletions may be the result of unequal recombination in this chromosomal region, associated with the esterase gene amplification. For example, OP resistant Culex mosquitoes possess 250–500 copies of a 30-kb esterase B1 gene, compared to a single copy in susceptible individuals (Mouchès *et al.* 1990). Given the genome size of *A. aegypti* of about 320 Mb (Zaitlin and Severson, unpublished results), if the magnitude of esterase gene amplification in the Aedes mosquitoes is similar to Culex mosquitoes, then the hitchhiking effect associated with OP insecticide selection could affect meiotic pairing across a large genome region of chromosome *1*, and could lead to chromosomal abnormalities (*i.e.*, deletions or duplications) within this region. In contrast, a hitchhiking effect associated with DDT usage is not evident for the *para* locus, as indicated by the fact that genetic heterozygosity at the *para* locus and loci closely linked to *para* was similar to other random loci in the genome. This result is consistent with the hypothesis that in the years since DDT was abandoned, the populations have had time to re-equilibrate.

Ideally, the hitchhiking effect should be demonstrated at the nucleotide diversity level (Kaplan *et al.* 1989). Unfortunately, nucleotide diversity cannot be appropriately estimated for the present data, because our RFLP data is based on one restriction enzyme. To statistically rule out the possibility of low heterozygosity caused by reduced mutation rates and/or increased functional constraints in the LF90 gene region, one needs to examine intraspecific variation and interspecific divergence over several gene regions for closely-related species. This method has been elegantly applied

to Drosophila studies (Begun and Aquadro 1991, 1992). The rationale is that, if reduced mutation rate in a gene region leads to low intraspecific variation, then interspecific divergence is expected to be smaller than in other gene regions. However, hitchhiking effect only reduces intraspecific polymorphism, but will not affect interspecific divergence (Begun and Aquadro 1991). The magnitude of the hitchhiking effect should be inversely proportional to the recombination distance. In addition, laboratory experiments should be conducted to investigate the hypothesis that gene deletions in the genome region containing LF230 result from unequal recombination between susceptible individuals and resistant individuals with an amplified esterase gene.

Gene flow estimates among the four mosquito populations were very high compared to other animal species (Slatkin 1985). Our gene flow estimates are, however, consistent with other studies of natural *A. aegypti* (Apostol *et al.* 1996) and *C. pipiens* (Chevillon *et al.* 1995) populations. Extinction and recolonization may constitute an important and powerful form of gene flow for the mosquito populations in small geographic areas. Suitable niches may often be vacated by insecticide applications, and then subsequently recolonized by mosquitoes. Direct estimates of *A. aegypti*'s natural dispersal ability, however, found limited flight ranges in urban areas, usually within 1 km when there are no geographic barriers (Reiter *et al.* 1995). Therefore, gene flow between the Trinidad and Tobago populations must be assisted by human activities, because a 35-km strait is far beyond *A. aegypti*'s flight ability. *A. aegypti*-infested water containers transported from Trinidad to Tobago have occasionally been detected since 1983 (Chadee 1990).

Slatkin and Barton (1989) showed that, in a subdivided population that is at a demographic equilibrium, both F_{ST} and private-alleles methods can provide reasonably accurate estimates of *Nm* under a variety of conditions. Our results indicate that *Nm* estimates based on F_{ST} were more consistent among loci and are probably more reliable than the private-alleles method. However, two factors may lead to biased estimates of gene flow for both methods. First, strong selection by insecticides may have significant effects on population allele frequencies, and produce local differentiation. Thus, gene flow based on F_{ST} would be underestimated. Second, the assumption that the populations are in genetic equilibrium may not be true for mosquitoes. Assuming that mutation is small relative to migration, the half time to equilibrium ($t_{1/2}$) between gene flow and genetic drift is calculated as $t_{1/2} = Ln2/(2m + 1/N)$ (Crow and Aoki 1984). If the generation time of mosquitoes is 1 month, and $Nm = 4.2$ (from the present study), $t_{1/2}$ is about 3 years for a population with $N = 500$. The interval between insecticide sprayings is typically only 3–4 months, far less than the time required to reach genetic equilibrium.

In this study, we applied classical population genetic theory and molecular techniques to study the evolutionary consequences of insecticide utilization in medically-important field pest populations. We made specific predictions concerning gene polymorphisms and spatial variations based on the history of insecticide application. These predictions were then tested by RFLP analysis of loci representative of the mosquito genome. Our data were generally consistent with the predictions. We observed evidence for a hitchhiking effect in the general chromosomal region containing genes presumed to confer resistance to OPs. The hitchhiking effect was reflected by low DNA polymorphisms and gene deletions for loci surrounding the *EST*-4 locus gene region. Gene deletions and reduced genetic variability in genome regions of chromosome *1* may be the result of the hitchhiking effect associated with the spread of the amplified *EST*-4 gene, which increases the fitness of the mosquitoes in the OP environment (Wood and Bishop 1981). Large gene flow among the mosquito populations likely resulted from human-assisted migration, and may explain the rapid spread of insecticide resistant genes (Raymond *et al.* 1991).

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