Abstract. This study evaluated if the Aedes aegypti population in the city of Cali, Colombia was composed of genetically distinct local populations with different levels of insecticide resistance and dengue vector competence. Insecticide resistance was assayed biochemically and was associated with varying levels of mixed-function oxidases and non-specific esterases. The genes encoding those enzymes were under selective pressure from insecticides used to suppress Ae. aegypti populations. Vector competence showed heterogeneity among the vector populations ranging from 19% to 60%. Population genetic analysis of random amplified polymorphic DNA–polymerase chain reaction products, expressed as genetic distance, Wright’s Fst, and migration rate (Nm), demonstrated moderate genetic differentiation among Ae. aegypti from four sites (Fst = 0.085). The results from all characteristics evaluated in the study demonstrated spatial and temporal variation between Ae. aegypti populations. At any specific time, the local populations of Ae. aegypti were genetically differentiated and unique with respect to insecticide resistance and vector competence. Both characteristics changed independently.

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are human diseases caused by dengue viruses (serotypes D1, D2, D3, and D4). Dengue viruses are transmitted principally by Aedes aegypti, and to a lesser extent by Ae. albopictus. Both species are urban mosquitoes of widespread distribution in tropical areas. Although dengue viruses are the most important arboviruses affecting humans, there is no effective treatment other than supportive care with fluid management. Since a protective vaccine is still in the experimental phase, the only method of prevention is mosquito avoidance.

In Colombia, Ae. aegypti is the main mosquito vector transmitting dengue viruses. The incidence of DF and DHF in Colombia has increased dramatically since the re-invasion of Ae. aegypti following cessation of the eradication campaigns in the 1960s. Cali currently has hyperendemic dengue transmission, and dengue prevention is based on vector control directed principally at mosquito larvae using chemical and biologic insecticides. Adult mosquito control occurs primarily by chemical insecticides (organophosphates and pyrethroids) and is used only during epidemic situations. Additional efforts to control mosquito larvae use biologic control with copepods and fish. Educational programs are carried out principally in schools and by the local newspapers and television channels (Secretary of Health of Cali, Epidemiology Unit, Internal Annual Reports 1995, 1999, 2000, unpublished data). Although there is a standardized program in place, vector control activities change depending on the budget, availability of insecticides, and the political situation. There are no ongoing programs designed to evaluate the impact of these mosquito control activities.

The use of insecticides by mosquito control programs and by the general public has resulted in the emergence of insecticide resistance. Resistance to temephos, an organophosphate insecticide extensively used for larval control, was first identified by a bioassay in 1998 (Suarez MF and others, unpublished data). Later biochemical studies of Ae. aegypti larvae from Cali showed increasing frequencies of resistance-associated enzymes (non-specific esterases [NSE] and mixed function oxidases [MFO]), suggesting the emergence of more than one insecticide resistance mechanism (Ocampo CB and others, unpublished data). In this study, larvae from different locations in Cali showed significant differences in the types of enzyme-based resistance mechanism present and the percentage of mosquitoes showing resistance. In addition, resistance varied from one year to the next at the same location (Ocampo CB and others, unpublished data).

A potential explanation for the intersite variability observed in enzyme levels associated with insecticide resistance is the presence of genetically distinct Ae. aegypti populations at the different study sites. This could lead to the emergence of insecticide resistance at any one site that was dependent on the genetic structure of the population and the extent of local vector control activities (evolutionary selection by insecticide pressure). If a genetically subdivided population were present, an understanding of population dynamics would be essential to achieve effective vector control and could deter the emergence of insecticide resistance. Likewise, it is possible that such population level changes could affect other genetically controlled traits such as vector competence for dengue transmission. Genetic variation and variation in vector competence between closely situated populations of Ae. aegypti has been observed in other parts of the world. These studies showed genetic variability and differences in vector competence in Ae. aegypti populations at a single point in time, but did not explain the dynamics of how these differences could occur. These studies suggested that insecticide pressure is a major selective force contributing to genetic variability. However, insecticide susceptibility, dengue vector competence, and population genetic variation were not examined concomitantly or in relation to temporal change.

The objective of this study was to examine the dynamics of Ae. aegypti populations in an urban environment (Cali) by investigating epidemiologically relevant biologic variables, insecticide resistance, and vector competence in conjunction with population genetic analyses. This information provides a basis for understanding patterns of transmission of dengue viruses, and for the interpretation and use of information obtained through vector surveillance.
MATERIALS AND METHODS

Rationale and design. This study was conducted in the urban area of Cali, Colombia, which is classified as hyperendemic for dengue based on the transmission of multiple dengue virus serotypes. This city of two million inhabitants was selected on the basis of dengue endemcity, a variation in the risk of acquiring dengue infection in various localities (based on numbers of cases), and preliminary studies using bioassay and biochemical methods indicating the presence of insecticide resistance in \textit{Ae. aegypti} from Cali (Ocampo CB and others, unpublished data).

The objective of the study was to characterize the population dynamics of \textit{Ae. aegypti} in a dengue endemic urban environment using epidemiologically relevant phenotypic characters (insecticide resistance and vector competence) in association with analysis of genotypic markers.

Collections of \textit{Ae. aegypti} were carried out in 2000 and 2001 at five different study sites separated by a minimum distance of 4 km, which exceeds the documented flight distances of this species.\textsuperscript{22-24} The rationale for this collection strategy was to increase the probability of obtaining genetically distinct populations of \textit{Ae. aegypti}. The sites included the Cali residential areas of Salomia, Siloe, Agua Blanca, Puerto Mallarino, and Melendez (Figure 1). Collections were performed during the rainy season (March–April) when mosquito abundance was highest and when the greatest number of genotypes should have been represented. A minimum of 10 larval habitats was sampled per site. These samples were obtained from different types of containers and on different weeks at each site to avoid collections of descendents from small numbers of females and to avoid the direct effects of any vector control activities in the area. For the genetic and biochemical analyses, field-collected larvae reared to five-day-old adults (F\textsubscript{0}) were used. A maximum of 10 adults were analyzed per larval habitat sampled. The bodies of the adult mosquitoes were used to evaluate insecticide resistance attributes and the legs were used to evaluate genotypic variation. Extraction of DNA from the legs rather than the whole insect decreased potential contamination by bacteria, gut contents, and symbionts, and allowed for the biochemical analyses and genetic characterization of each individual mosquito. For the vector competence studies, F\textsubscript{1} and F\textsubscript{2} generation progeny of F\textsubscript{0} mosquitoes were reared under identical conditions and exposed to identical dengue infective blood meals.

Biochemical tests. The bodies of \textit{Ae. aegypti} adults reared from larvae collected at the different urban sites in Cali (\(\geq\) 5 days of age) were stored at \(-70^\circ\)C until the assays were performed. These were used for detection of biochemical mechanisms of resistance to the principal insecticides applied for vector control (organophosphates, carbamates, and pyrethroids). The mechanisms evaluated included NSE, MFO, and acetylcholinesterases as previously described.\textsuperscript{25-30} These biochemical techniques have been compared with bioassay methods and shown to identify mechanisms underlying resistance detected with bioassays.\textsuperscript{31-35} The biochemical tests can detect resistance, determine resistance mechanism(s), and be performed on single field-collected specimens.\textsuperscript{36} Protein concentration was determined for each specimen using the Bradford method to adjust for differences in body mass of individuals that might require correction factors for the enzyme assays.\textsuperscript{25,26,37} Approximately 80–100 mosquitoes were analyzed from each study site (this sample size is sufficient to identify differences \(\geq\) 20% in the frequency of resistance between populations with a power of \([1 - \beta]\) \(\geq\) 0.80).

Individual adult mosquitoes were homogenized in 100 \(\mu\)L of 0.01 M potassium phosphate buffer, pH 7.2, and then suspended in 1 mL of the same buffer. Aliquots of 100 \(\mu\)L were transferred to microtitrte plate (Dynatech Laboratories, Alexandria, VA) wells. Thirty samples were analyzed in triplicate per plate for each assay. Absorbance was measured using an enzyme-linked immunosorbent assay reader (MRX; Dynatech Laboratories). Replicates with a variability coefficient (SD/mean) greater than 0.15 were discarded to avoid differences produced due to handling errors. Three positive controls were present on all test microtitrte plates. Because of the lack of highly resistant \textit{Ae. aegypti} strains, the assay control was developed by constructing standard curves for each enzyme using the corresponding commercially available enzymes. The reference value for susceptibility was defined using the average absorbance value obtained from known susceptible mosquitoes (Rockefeller strain). The average/mean absorbance value of the blank control (reagents without mosquitoes) was subtracted from the values of all sampled mosquitoes to correct for background absorbance. Finally, each absorbance value was standardized as activity per unit protein using the results obtained in the protein assays.

![Figure 1](image_url)
Statistical analysis. To compare *Ae. aegypti* populations for biochemical mechanisms of resistance, the mean absorbance values obtained for each individual population were compared with those of other populations using analysis of variance (ANOVA). Multivariate analyses were performed to compare variation in resistance values by site, sex, and year of collection using SPSS version 8.0 (SPSS, Inc., Chicago, IL). Results of the *Ae. aegypti* enzyme activity from the field samples from Cali were compared with those of the insecticide-susceptible Rockefeller strain and the most susceptible strain from Cali.

Vector competence. The oral susceptibility to dengue 2 virus was evaluated in collaboration with the Arthropod Infectious Disease Laboratory at Colorado State University (Fort Collins, CO). Two dengue 2 (D2) strains (New Guinea C [New Guinea, 1944] and JAM1409 [Jamaica, 1983]) were used to evaluate vector competence. The JAM1409 D2 strain was used in the evaluations performed at Colorado State University in 2000 and the New Guinea C D2 strain was used for those conducted at Tulane University (New Orleans, LA) in 2001. Both strains were passaged in C6/36 mosquito cells prior to oral infection. For this study, the assumption was made that both strains had similar infectivity and would otherwise behave similarly in *Ae. aegypti*. All infections were conducted in a biosafety level-3 insectary at Colorado State University and Tulane University.

Adult mosquitoes collected from the Cali field sites were fed on live guinea pigs to obtain eggs (F1). Individuals from F1-F2 generations of *Ae. aegypti* from each study site were later exposed to D2 virus by artificial membrane feeding of virus in rhesus monkey blood. Within a given experiment, all mosquito colonies originating from different sites were exposed to the same D2 virus-blood mixture. Samples from all collection sites were included in each experiment. Blood-fed females were maintained in an insectary maintained at 85°F and a relative humidity of 80%, with a daily light-dark cycle of 16 hours:8 hours for 14 days (the extrinsic incubation period of the virus). Heads of the mosquitoes were tested for viral antigen by immunofluorescent antibody (IFA). Viral titers in the blood meal were quantified using the endpoint method described by Schoepp and Beaty to verify that the virus concentration was similar among blood meals. All specimens used in this analysis were collected and reared, and the progeny were obtained under similar conditions at similar sites. The New Orleans strain of *Ae. aegypti* was used to standardize the methodology and as the control strain for the analysis of vector competence. Mosquitoes were infected by intrathoracic inoculations to provide positive controls for the IFA.

Crosstab analyses were performed within and among the populations collected during 2000 and 2001. The chi-square test was used for the statistical analysis.

Population genetics. *Aedes aegypti* from four different sites in Cali were analyzed using a random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR). This technique was chosen for these studies because it detects large numbers of polymorphisms, and because it is possible to work with small amounts of DNA that can be obtained from parts of the mosquitoes (e.g., legs). From 33 to 40 mosquitoes (principally females) per site per year were analyzed. The mosquitoes were randomly selected from numerous larval habitats sampled at each site to minimize the possibility of collecting descendents of just a few females.

Random amplification of polymorphic DNA. The DNA was isolated by homogenizing the legs of the mosquitoes using a Kontes pestle (Kimble/Kontes, Vineland, NJ) following the procedure described by Black and DuTeau. The samples were then stored at -70°C until amplification.

The PCR was set up as follows. Each 40-μL PCR mixture contained 5 μL of sample DNA, 200 μM of each dNTP, 1.5 mM MgCl2, 40 pmol of primer (final concentration = 1 μM), and 1.5 units of Taq polymerase (Platinum Taq DNA polymerase; Gibco-BRL, Gaithersburg, MD) in 1× buffer (10× buffer = 200 mM Tris-HCl, pH 8.4, 50 mM KCl). The four primers used included R07: 5′-AAG CCT CGT C-3′, R32: 5′-CTT TCT ACC C-3′, R22: 5′-GAT CAT AGC C-3′, and R08: 5′-CAC ACT CCA G-3′. The DNA was then amplified in a thermal cycler (GeneAmp PDR System 2700; Applied Biosystems, Foster City, CA) using the following conditions: 95°C for two minutes (to activate the Platinum Taq DNA polymerase); then two cycles at 94°C for one minute, 30°C for two minutes, and 72°C for one minute; followed by 40 cycles at 94°C for 30 seconds, 40°C for two minutes, and 72°C for one minute; and one final cycle of 95°C for 30 seconds, 40°C for two minutes, and 72°C for five minutes. Samples were then held at 4°C. Negative controls were included in all runs. These constituted all components listed except the DNA template, which was replaced by 5 μL of sterile water.

Randomly amplified polymorphic DNA was evaluated by agarose and polyacrylamide gel electrophoresis as described by Black and DuTeau. The molecular weights of the scored bands were confirmed using molecular weight standards, and comparability between experiments was assured by analyzing products from different PCRs on the same polyacrylamide gels.

Analysis by RAPD. Analysis of the results was based on the following assumptions: 1) the genomic regions amplified by RAPD-PCR segregate as dominant alleles; 2) each subpopulation is in Hardy-Weinberg equilibrium; 3) alleles in homozygous recessive individuals are identical; and 4) dominant amplified alleles are similar. Since it is assumed that each subpopulation is in Hardy-Weinberg equilibrium, the frequency of recessive alleles (q) was estimated from the square root of the frequency of homozygous recessive (blank) individuals (q²). Population genetic analyses were carried out using the Wright’s Fst estimate assuming the island model. The migration rate (Nm) was determined from Wright’s Fst [Fst = 1/(4Nm + 1)]. For the analysis, gels of the RAPD products were scored for the presence or absence of the chosen bands (loci) and all data were stored in a Microsoft (Redmond, WA) Excel spreadsheet. From here the data were imported into the FORTRAN programs RAPDBIOS, BIOSYS-2, RAPPDIST, and RAPDFST (Black WC IV, 1997, unpublished computer programs). RAPDBIOS and BIOSYS-2 were used to estimate the allelic frequencies, and those frequencies were used to determine which alleles would be included in further population analysis. RAPPDIST was used to compute the genetic distance among the populations. The diploid option was selected along with the Lynch and Milligan correction for small sample sizes and Nei’s genetic distance was calculated. RAPDFST was used with the original presence/absence matrix (excluding low variation alleles) to compute the inbreeding coefficient (Fst) and Nm.
RESULTS

This study documented spatial and temporal genetic variations among *Ae. aegypti* populations, and identified similar variation in the epidemiologically relevant biologic attributes of insecticide susceptibility and dengue vector competence.

**Resistance to insecticides.** Variation in enzymes that confer resistance to commonly used insecticides provided evidence of selective pressure for NSE and MFO among the *Ae. aegypti* populations studied (Figures 2 and 3). These enzymes are associated with resistance to organophosphates, pyrethroids, and insect growth regulators, all of which were being used by vector control authorities and the general public in the municipality of Cali. In contrast, there was no evidence of increases in acetylcholinesterase (which acts at nerve synapses and leads to resistance to organophosphates and carbamates) as a mechanism of insecticide resistance.

Levels of NSE in *Ae. aegypti* from all sites in Cali were below those of the reference susceptible strain (Rockefeller) for both males and females (Figure 2). Similar outcomes have been reported in other studies, and reinforce the suggestion that the most susceptible local strain should be used as a baseline. The rationale for this suggestion is that both strains (one in colony and the other in nature) are under unknown selective pressures that will result in varying levels of enzymes that may or may not be related to past insecticide exposure. In addition, using the most susceptible local strain to determine the resistance baseline allows for tracking enzymatic changes in samples from these populations and will allow early detection of resistance. Although overall NSE levels were considered as representing susceptible populations relative to the control strain, there was a general trend toward increasing NSE levels from 2000 to 2001. Mosquitoes from four of five sites showed increased MFO levels from 2000 to 2001 at time 0 (Figure 3) and time 10 in both females and males. The greatest amount of change of the mean values (3.9-fold) was seen in females from the Puerto Mallarino site at time 0. In contrast to the NSE results, the Rockefeller strain was about equal in MFO levels with Cali strains in 2000. In 2001, MFO levels in female mosquitoes in Cali were generally higher than in the control strain at three sites: Puerto Mallarino, Siloe, and Melendez.

Enzyme levels associated with the two biochemical mechanisms of resistance that had increased varied among populations by site and year of collection (*P* < 0.001, by ANOVA). Female *Ae. aegypti* showed significantly higher levels of enzyme activity than males (*P* < 0.001). However, the overall pattern of enzyme activity was similar in males and females from a given site.

**Vector competence.** The *Ae. aegypti* infection rate with D2 virus ranged from 19% to 60% infectious, as determined by IFA detection of virus antigen in head tissues (Table 1). Vector competence varied among populations collected from different sites and by year of collection. In 2001, significant differences (*P* = 0.01) in vector competence were found among the populations from the five sites. Within-site variation in vector competence between 2000 and 2001 was significant only for Siloe (Table 1).

The mixture of virus and monkey blood used to simulta-

![](image1.png)

**FIGURE 2.** Mean ± SD absorbances of non-specific esterases (NSE) in female *Aedes aegypti* study populations for the years 2000 and 2001.

![](image2.png)

**FIGURE 3.** Mean ± SD absorbances of mixed function oxidases (MFO) (time zero) in female *Aedes aegypti* study populations for the years 2000 and 2001.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Year</th>
<th>Positive % (n)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Salomia (North)</td>
<td>2000</td>
<td>45.1 (51)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>19 (21)</td>
<td></td>
</tr>
<tr>
<td>Siloe (West)</td>
<td>2000</td>
<td>56.5 (56)</td>
<td>0.046†</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>35.1 (57)</td>
<td></td>
</tr>
<tr>
<td>Melendez (South)</td>
<td>2000</td>
<td>55.1 (49)</td>
<td>0.712</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>59.7 (77)</td>
<td></td>
</tr>
<tr>
<td>Agua Blanca (East)</td>
<td>2000</td>
<td>33.3 (3)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>29 (31)</td>
<td></td>
</tr>
<tr>
<td>Pto Mallarino (Northeast)</td>
<td>2000</td>
<td>42.2 (45)</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>35.3 (17)</td>
<td></td>
</tr>
<tr>
<td>New Orleans</td>
<td>2001</td>
<td>42.9 (35)</td>
<td>0.337</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>30.8 (39)</td>
<td></td>
</tr>
</tbody>
</table>

* Chi-square analysis was done comparing temporal variation in vector competence at each site.
† Statistically significant result (*P* < 0.05).

TABLE 1
Comparative dengue vector competence of *Aedes aegypti* collected at different sites in years 2000 and 2001, as measured by a positive head squash immunofluorescent antibody result*
neously evaluate vector competence was identical within a given experiment for the populations from different sites. Titters of virus between experiments ranged from $10^{6.8}$ to $10^{7.8}$. 50% tissue culture infective doses/mL (TCID$_{50}$/mL). Virus titer in the blood did not change during the period of exposure to feeding.

**Genetic markers.** Genetic variability analyses included the *Ae. aegypti* populations from Agua Blanca (east), Salomia (north), Melendez (south), and Siloe (west). The population obtained in Puerto Mallarino (northeast) was excluded because it was derived from a single yard with a large number of tires serving as breeding sites. Mosquitoes used in this analysis came from multiple larval habitats per site (as described in the Materials and Methods).

Only alleles with an average population frequency between 0.1 < $P$ > 0.6 were used for the population analysis.44 Eleven of 21 alleles fulfilled this criterion. To determine differences between populations in different years, the following parameters were calculated: Nei’s genetic distance, Wright’s $F_{st}$ (estimates the degree to which *Ae. aegypti* is divided into subpopulations), and Nm. Two levels of organization were considered in conducting the analysis. First, spatial and temporal population variation was evaluated considering that all collections were made of independent populations (Table 2, Analysis Parameter [AP] A and B). Second, the populations were grouped to analyze spatial and temporal variation in the overall population of *Ae. aegypti* sampled (Table 2, AP C and D). Differences by location and time of collection are evident when the samples were analyzed as independent populations (AP A and B), but not when they are grouped by year or location (AP C and D). The values obtained for Nei’s genetic distance and the inbreeding coefficient ($F_{st}$) demonstrated a greater degree of differentiation among populations when they were considered independently. Similarly, gene flow was lower between populations (Table 2). The population dynamics shown by this analysis is schematically represented in Figure 4, which illustrates the spatial and temporal changes that could be occurring in *Ae. aegypti* populations from Cali or any other urban setting.

**DISCUSSION**

This study demonstrated spatial and temporal population differences with respect to expression of enzymes that confer resistance to insecticides, vector competence, and genetic markers in *Ae. aegypti* in Cali. However, the results suggest that for the overall population there is considerable gene flow and that the population is panmictic. Recent studies in urban environments with high human population densities in Asia showed that this type of environment promoted population differentiation in *Ae. aegypti*, resulting in heterogeneous patterns of vector competence.11,12,13,15 The investigators suggested that local vector control activities could be one of the major causes of heterogeneity in the populations. The results from the present study are in agreement with those observations. However, the other studies did not evaluate temporal genetic variation in urban populations of *Ae. aegypti*. Temporal studies such as this one allow observation of the rapidity of changes at the population level. In addition, they illustrate how selection pressure and gene flow can influence important vector characteristics relevant to pathogen transmission. This study addresses questions about the forces driving modern evolutionary selection on urban mosquito populations, and can help us gain insight into what might happen when genetic variation changes according to selective pressures (e.g., insecticides) permitting or eliminating zones of genetic exchange (gene flow). A decrease in genetic exchange and a reduction in population size (bottleneck) can produce a reduction in available genetic variability, thereby leading to local populations differentiated from one another at a given point in time. The picture of genetic variability depends on the time sampled. The letters identify the populations at different times.

**TABLE 2**

*Summary of population genetics analyses of *Aedes aegypti* collected at different sites in 2000 and 2001*.

<table>
<thead>
<tr>
<th>Analysis parameters</th>
<th>Genetic distance (Nei’s)</th>
<th>Inbreeding coefficient ($F_{st}$)</th>
<th>Migration rate (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Spatial variation among samples (each population sampled in different years was considered unique [n = 8 populations])</td>
<td>0.012–0.016</td>
<td>0.085</td>
<td>2.7</td>
</tr>
<tr>
<td>B. Temporal variation within collection site between years (Each population at a given site was compared from one year to the next [n = 4 sites])</td>
<td>0.031–0.046</td>
<td>0.048 to 0.073</td>
<td>3.2–4.9</td>
</tr>
<tr>
<td>C. Total temporal variation between years. (All populations from year 2000 were combined and compared to those from 2001 [n = 2 years])</td>
<td>0.0244</td>
<td>0.033</td>
<td>7.3</td>
</tr>
<tr>
<td>D. Total spatial variation among sites. (Data for each population [site] from both years were combined and compared [n = 4 sites])</td>
<td>0.0036–0.014</td>
<td>0.021</td>
<td>11.5</td>
</tr>
</tbody>
</table>

* Analysis parameters A–D describe if and how data were combined to examine different spatial and temporal levels of genetic variation.
cally transformed mosquitoes are released into such an environment.

In this study of the population dynamics of *Ae. aegypti* from an urban setting, enzyme levels associated with insecticide resistance and vector competence varied independently at the different sites and times evaluated. As suggested in other studies, the variability in levels of MFO and NSE observed in this study is likely to be the result of selective pressure from insecticides used by vector control programs and the general public. In 2001, government-sponsored vector control programs were almost non-existent in Cali because of a lack of resources. Insecticide use by the general public should be considered as one of the major factors inducing selection pressures on *Ae. aegypti* populations during that year. Most commercially available insecticides have pyrethroids as their active ingredients. These compounds induce selection primarily on MFO enzymes; the enzymatic mechanism expressed at higher levels in *Ae. aegypti* from Cali in 2001. The observed variation in MFO levels in the *Ae. aegypti* from Cali could be explained by differing levels of insecticide selection pressure at the locations where collections were made. This suggests that in mosquito populations in Cali, and in other urban areas, insecticide resistance can vary from site to site at different times of the year. These results demonstrate that the *Ae. aegypti* population in an urban area is in continuous flux and can be genetically responsive to focal selection pressures. The population structure in turn can affect local vector control success and potential for pathogen transmission. However, high gene flow in association with decreased or varying selection pressures can also affect population genetic structure. Insecticide pressure is likely to constitute the major selective force affecting these populations, and as a result induces genetic changes through bottleneck and genetic drift effects.

Although this study found random changes in all the RAPD alleles evaluated (genetic drift), this does not exclude that hitchhiking events can occur on specific regions of the genome where resistance genes are present. This investigation did not identify specific alleles that influence insecticide susceptibility and vector competence. However, the findings provide a basis for understanding spatial and temporal variation in populations. Ideally, studies to understand the selection processes that are occurring in the populations should include specific molecular markers for insecticide resistance and vector competence. Unfortunately, such markers have not yet been identified, but availability of the genome of *Ae. aegypti* promises to make this possible in the near future.

The biochemical assays used in this study may not always accurately represent biologic resistance to a given insecticide. However, their use did reveal temporal and spatial changes in enzymes that have been linked to insecticide resistance. Biochemical assays were selected to measure the potential for development of or loss of resistance because they can be linked to genetic change, and they allow individual mosquitoes to be examined, which is impossible using most bioassay methods. Ideally, in a vector control setting, the biochemical tests initially would be performed along with bioassays to assess accuracy for predicting real biologic resistance. After the initial comparison, biochemical tests could be used alone to monitor ongoing effectiveness of vector control efforts, with bioassays used to confirm any overt changes in susceptibility.

The vector competence analysis is limited because it is only one of several variables that influence vectorial capacity (overall ability of a vector to transmit a pathogen). Spatial and temporal fluctuations in *Ae. aegypti* abundance and life span, human anti-mosquito behavior, and vector control activities can also influence virus transmission. However, vector competence itself is important in transmission efficiency and can influence epidemiologic situations. Another limitation of the vector competence evaluation was that two different strains of dengue 2 virus were used (New Guinea C in 2001 and JAM1409 in 2000). However, since only one (Silo) of the five sites sampled showed significant differences in vector competence between populations collected in 2000 and 2001 (Table 1), the assumption that both strains behaved in a similar manner in the mosquitoes appears reasonable. If they behaved differently, then all the sites should have shown population differences between collections made in 2000 and 2001.

The use of RAPDs for population genetics allows analysis of small amounts of DNA and the detection of a large number of polymorphisms. Nevertheless, this methodology may be limited by the difficulty to precisely replicate results because of bands associated with contaminants, and by the assumptions made in conducting the data analyses. To decrease potential methodologic limitations, the DNA was extracted from legs to avoid gut contaminants, and at least one sample per PCR set was repeated. In addition, the bands were selected from a representative mosquito population sample and only well defined bands were scored.

The present study demonstrated that *Ae. aegypti* from highly populated urban areas could be considered as a population with multiple local populations that can be monitored to control the emergence and spread of insecticide resistance, especially when focal outbreaks of dengue are detected. Frequent monitoring of insecticide resistance or the biochemical surrogate allows mosquito control programs to determine when insecticides need to be alternated to mitigate the onset of resistance. Evidence of high gene flow between populations within the urban area also indicates that focal insecticide resistance must be rapidly managed by eliminating the insecticide pressure completely, or by changing the class of insecticide used. Because changes in vector competence and enzyme levels associated with resistance can change over relatively short periods of time, the success of vector control activities and the ability to combat dengue epidemics in urban areas depend on frequent surveillance for insecticide resistance in appropriately selected localities. While currently this strategy of intensive surveillance would be difficult for many countries to achieve on a national basis, there are municipalities within countries that do have the resources to initiate this type of surveillance. The relevance of this type of surveillance is highlighted by the fact that insecticide resistance in mosquitoes continues to be reported from many areas around the world. Once the laboratory tools are in place, it should be possible for municipalities to initiate and continue periodic surveillance for insecticide resistance. The results presented in this paper provide a basis for surveillance strategies to take into account the complexity of urban *Ae. aegypti* populations and the dynamics of resistance.

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