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LARGE GENETIC DISTANCES AMONG *AEDES AEGYPTI* POPULATIONS ALONG THE SOUTH PACIFIC COAST OF MEXICO

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Abstract. A population genetic analysis was conducted among 20 *Aedes aegypti* collections from 19 cities along the south Pacific coast in the Mexican states of Guerrero, Oaxaca, and Chiapas and in Coatepeque, Guatemala. Genetic variation was scored at 131 random amplified polymorphic DNA loci. The amount of genetic differentiation among collections was ~3 times as great as detected among collections in an earlier study in northeastern Mexico. Regression analysis of linear or road distances on linearized F_{ST} indicated that collections are genetically isolated by distance. Cluster analysis failed to group collections in geographic proximity, and there was as much genetic variation among collections 60 km apart as there was among all collections (~900-km range). The large genetic differentiation in southern Mexico reflects reduced gene flow among mosquitoes arising in a greater diversity of habitats and altitudes than exists among northeastern collections. It is likely that dispersal via human commerce in the northeast confounds patterns of natural gene flow.

INTRODUCTION

Aedes aegypti is the primary urban vector of dengue and yellow fever viruses.[1] Dengue is a major health problem around the world, and thousand of cases of dengue fever and dengue hemorrhagic fever are reported annually.[2,3] We have been determining genetic relationships among *Ae. aegypti* populations in different regions of Mexico to assess the amount of gene flow among breeding sites and therefore potentially the degree of trafficking of dengue virus among sites.[4,5] In related studies, we have assessed variation in vector competence for dengue virus among these sites and have detected large amounts of variation in midgut and disseminated infection rates.[6]

Population genetic studies of *Ae. aegypti* conducted over the last 30 years have defined genetic relationships among collections worldwide[1,7–11] and more recently have focused on local patterns of dispersal.[12,13] We have shown[4] using random amplified polymorphic DNA (RAPD) markers that collections of *Ae. aegypti* from northeastern Mexico are genetically isolated by distance and genetically homogeneous within a range of 90–250 km. This suggested that gene flow among populations decreases with increasing geographic distances but that within ~150 km, mosquitoes exchange genes continuously. More recently, we analyzed variation in mitochondrial ND4 haplotypes in collections from throughout northeastern Mexico, Yucatán, and western Pacific coastal regions of Mexico.[5] Northeastern collections were genetically differentiated from and had lower genetic diversity than Yucatán and western Pacific coastal collections. Yucatán and Pacific collections were genetically homogeneous. Collections in all 3 regions were genetically isolated by distance. Free gene flow occurred among all collections within ~130 km of one another in the northeast and within ~180 km in the Yucatán. F_{ST} values were never large among Pacific collections, suggesting extensive gene flow along the western Pacific coast.

Genetic variation among *Ae. aegypti* collections from southern Pacific Mexico locations have yet to be examined. In the present study, we analyze local patterns of gene flow in *Ae. aegypti* over a total distance of 914 km and among 5 cities within a distance of 62 km of one another (Figure 1). Genetic variation was examined in 60 mosquitoes per collection, and variation was determined at 131 RAPD loci. In addition, we incorporated minimal distances by roads among pairs of collection sites into our regression analysis to test for genetic isolation by distance. Our hypothesis was that if mosquitoes disperse via flight, there should be a positive correlation between geographic and genetic distances. In other words, mosquitoes within flight range of one another should mate more often than those that are further apart. Alternatively, if mosquitoes are transported, even occasionally, through human commerce, then distant populations could be genetically similar and proximal populations could be genetically distinct. In either case, this would disrupt expectations of an isolation-by-distance model based on natural migration.

MATERIALS AND METHODS

Mosquito collections and extraction of DNA. The locations and sample sizes of *Ae. aegypti* larvae collected from natural oviposition sites in each city are listed in Table 1, and the geographic locations of all sampling sites are shown in Figure 1. Collections were obtained from 20 cities distributed in the Mexican states of Oaxaca, Chiapas, and Guerrero and one from Coatepeque, Guatemala. We collected samples at 5 different sites in each city. One was located in the center of each city, and 4 were located at sites due north, south, east, and west of center. At each site, we collected larvae from at least 7 different locations. From 200 to 800 larvae were obtained from each site. These were reared to adults in the laboratory; adults were then stored at –70°C. DNA was isolated from individual mosquitoes by salt extraction[14] and suspended in 500 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0). The DNA was divided into 5–100-μL aliquots and stored at –70°C.

Random amplified polymorphic DNA–polymerase chain reaction. Random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) was completed in 50-μL reactions using 1 μL of template DNA. Amplification conditions and oligonucleotide primers were as described previously.[4] Amplification was carried out in a Perkin-Elmer DNA Ther-

TABLE 1

Locations, dates of collections, and global positioning system coordinates of *Aedes aegypti* collections (n = 60 mosquitoes per collection) along the south Pacific coast of Mexico

State or country	Cities	Date	Latitude north	Longitude west	Altitude (m)*	Individuals collected (n)
Guatemala	Coatepeque	02/08/96	14.40°	91.52°	560	805
Chiapas	Ciudad Hidalgo (CH)	19/07/96	14.41°	92.09°	10	545
	Huixtla (HX)	22/07/96	15.09°	92.28°	50	657
	Tapachula (TP)	23/07/96	14.54°	92.16°	160	485
	Cacahoatan (CA)	24/07/96	14.59°	92.10°	480	360
	Pijijiapan (PJ)	25/07/96	15.41°	93.13°	50	858
	Puerto Madero (PM)	29/07/96	14.43°	92.25°	1	218
	20 de Noviembre (VN)	31/07/96	14.42°	92.15°	10	719
	Tonalá (TO)	01/08/96	16.05°	93.45°	60	649
	Barra Zacapulco (BZ)	08/08/96	15.11°	92.53°	2	303
Oaxaca	Tapanatepec (TP)	01/08/96	16.22°	94.12°	40	219
	Zanatapec (ZC)	01/08/96	16.28°	94.21°	60	622
	Oaxaca (OA)	13/09/96	17.04°	96.43°	1530	116
	Pochutla (PO)	14/09/96	15.45°	96.28°	150	144
	Puerto Escondido (PE)	15/09/96	15.51°	97.04°	40	100
	Pinotepa Nacional (PN)	15/09/96	16.20°	98.03°	200	247
Guerrero	Juchitan (JT)	15/09/96	16.37°	98.38°	155	167
	Acapulco (AP)	15/09/96	16.52°	99.54°	20	182
	Chilpancingo (CG)	16/09/96	17.33°	99.30°	1300	269
	Iguala (IA)	16/09/96	18.21°	99.32°	720	74

mal Cycler 480 (Perkin-Elmer Applied Biosystems, Foster City, CA). Each reaction set was checked for contamination using a negative control (all reagents included except template DNA). When any PCR product was detected in the negative control, the whole reaction set was discarded and repeated. PCR products were size-fractionated via electrophoresis on large (38 by 50 cm), thin (0.4 mm) polyacrylamide (5%, 0.2% cross-linking) gels containing 7% glycerol. Sharktooth combs (4 mm) were used to load 5–6 μL of sample. Electrophoresis proceeded at room temperature for 16 hours (overnight) at constant voltage (350 V), and the gels were silver stained to visualize DNA fragments.[14]

Statistical analysis. Fragments for RAPD-PCR were analyzed as genetic markers under the following 4 assumptions[13]:

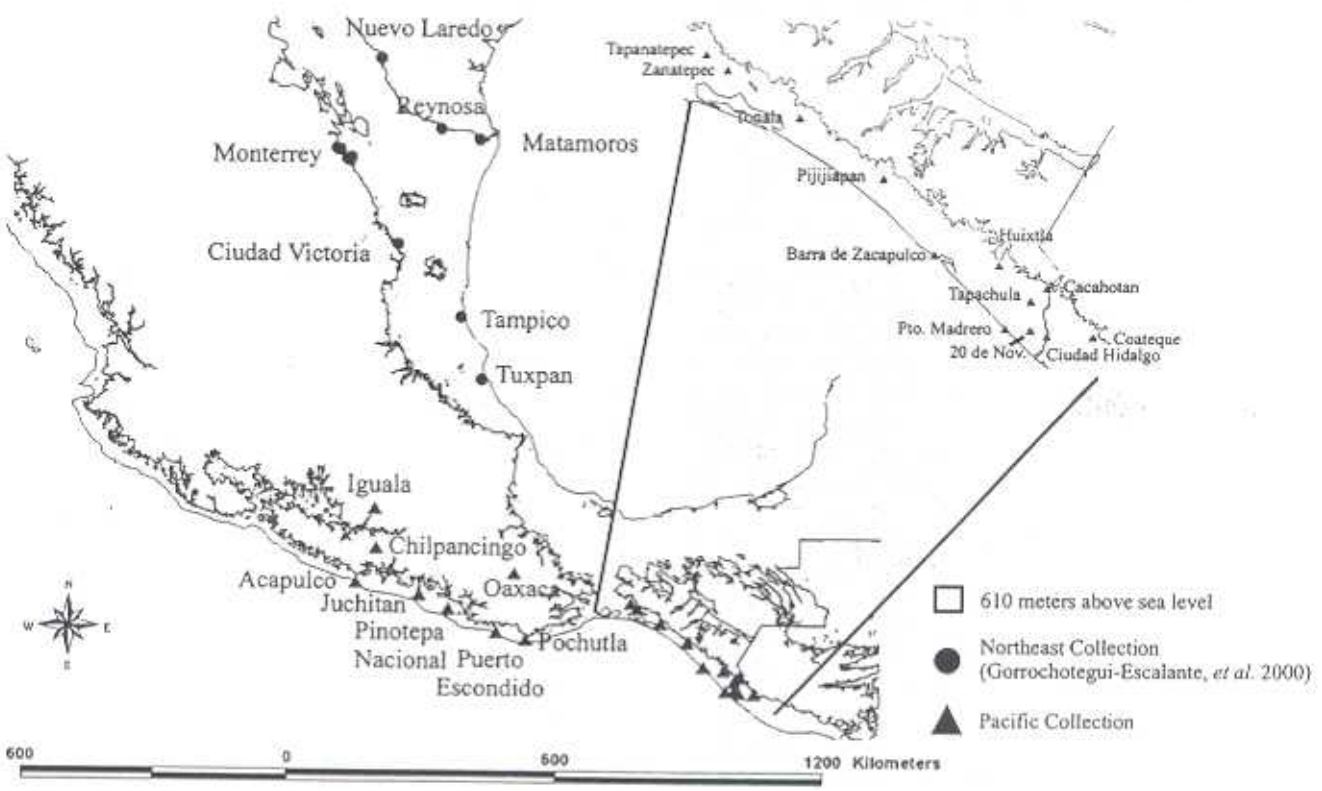

FIGURE 1. Map of the northwest Pacific Ocean coast of Mexico showing the locations of *Aedes aegypti* collections.

(1) RAPD markers segregate in a Mendelian fashion; (2) genotype frequencies at RAPD loci are in Hardy-Weinberg proportions; (3) recessive 'band absent' alleles are identical in state (*iis*) among and within individuals; and (4) dominant 'band present' alleles are *iis* among and within individuals. The statistical methods and equations we used have been described previously and were programmed into the statistical computer program RAPDPLOT (available from slozano@colostate.edu).

Variation in RAPD allele frequencies from cities was analyzed by RAPDFST and RAPDDIST. RAPDFST was used to compute pairwise F_{ST} estimates, a standardized measure of variation in allele frequencies among all populations. Effective migration rates (Nm) were estimated from F_{ST}. Pairwise F_{ST} values were used to construct a dendrogram among all collections using unweighted pair-group method with arithmetic averaging analysis (UPGMA)[15] in the NEIGHBOR procedure in PHYLIP 3.5.[16] RAPDDIST tested the consistency with which the RAPD data set supported each cluster using a bootstrap analysis with 1,000 replications. RAPDBIOS was used to transform a RAPDPLOT data set into a data type 3 BIOSYS-2[17] data set for hierarchical analysis of variance[18] with WRIGHT78.[17] To assess local patterns of gene flow, variation was assessed among collections within a 60-km radius (Cacahoatan, Ciudad Hidalgo, Tapachula, Puerto Madero, and 20 de Noviembre; Figure 1) and among the remaining collections.

Geographic distances were obtained by the Geographic Information Systems in ArcView software (Environmental Systems Research Institute, Inc., Redlands, CA). Road distances were calculated using the ArcView distance operator and road coverage of Mexico obtained from the Middle America Atlas of Animal Disease Information (USDA/APHIS/VS/CEAH, version 2.0). Pairwise F_{ST} values were linearly transformed ($F_{ST}/1 - F_{ST}$)[19] and regressed on pairwise linear geographic distances and on the shortest road distances among collection sites to determine whether distance via natural dispersal or human commerce account for different amounts of variation in the regression model. These regressions were repeated by a natural logarithm transformation of geographic distances.[20] This transformation was made because populations are not distributed along a linear transect and the reciprocal of the transformed slope provides an estimate of the average effective population size (N_e).[20] Transformations, regression analysis, and the Mantel test[21] were performed by MANTEL (available from slozano@colostate.edu).

RESULTS

Analysis of RAPD allele frequencies. The frequencies of the dominant RAPD allele were estimated at each of the 131 loci (frequency data matrix available from slozano@colostate.edu). Genetic variation among collections was partitioned among the 5 collections within a 60-km radius and among the remaining collections (Table 2). The hierarchical analysis[18] indicated that there was as much variation among collections within 60 km (F_{ST} = 0.095) as there was among all collection sites in the study (F_{ST} = 0.091). An analysis of 0[22] and Lynch and Milligan's F_{ST}[23] yielded similar results. In contrast, among collections within and surrounding the city of Monterrey in northeastern Mexico, F_{ST} = 0.027 and F_{ST} = 0.040 among all northeastern locations. N_em among local populations ranged 2.4–3.8 migrants per generation (Table 2) but was ~9 among collections at the same geographic scale in Monterrey.[4]

Cluster analysis of pairwise F_{ST} was performed among south Pacific and among northeastern collections (Figure 2). The amount of bootstrap support was low, suggesting abundant gene flow among south Pacific collections. The branch lengths among collections from the northeast were generally shorter than branch lengths among south Pacific collections. This again suggests decreased gene flow among *Ae. aegypti* populations in the south Pacific regions of Mexico.

Pairwise $F_{ST}/(1 - F_{ST})$ among collections were regressed against geographic linear distances among sites (Figure 3A) and among the natural logarithm of linear distances (Figure 3B) to determine whether gene flow among collections is correlated with geographic distance (i.e., to test for genetic isolation by distance). Both regression analyses indicated a positive correlation between genetic and geographic distances (Table 3). Both regression analyses were repeated using road distances, and estimated regression parameters changed only slightly. This outcome is not surprising, given the high correlation between minimal linear and road distances (r = 0.99).

N_e remained approximately the same (74 versus 81 mosquitoes per kilometer) between the northeastern and southern Pacific collections. Among northeastern populations, visual inspection of the natural log-transformed plot indicated a finite geographic distance at which $F_{ST}/(1 - F_{ST})$ values increased.[4] This cutoff is nowhere apparent in Figure 3B. This observation is again consistent with our finding large genetic variation among even local collections (Table 2). Regression analyses of road distances were repeated among sites in northeastern Mexico, and the amount of variance accounted for by a linear model (R^2) was half that found among southern Pacific populations. Furthermore, Mantel probabilities were barely significant among northeastern collections but highly significant among southern Pacific collections. The pattern of increased gene flow among northeastern populations, roughly equivalent N_e among the 2 regions, and the absence of a strong pattern of isolation by distance among northeast-

TABLE 2

Partition of variation in the frequency of random amplified polymorphic DNA markers among *Aedes aegypti* along the south Pacific coast of Mexico

Source of variation	Variance component*	% Variation	F_{ST}[18]		F_{ST}(LM)[23]
Among collection within ~60 km	2.031	100%	0.095	0.076	0.095
			Nm = 3.8	3.0	2.4
Among remainder of collections	0.106	0%	0.004	0.020	0.044
Among all collections	1.925	100%	0.091	0.096	0.139

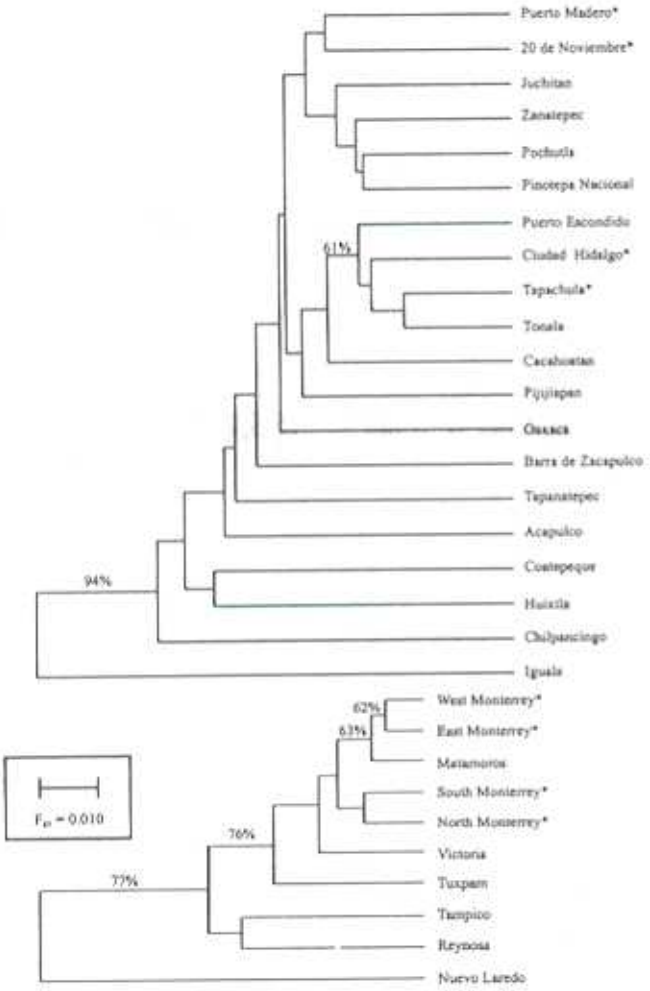

FIGURE 2. Dendrogram arising from a unweighted pair-group method with arithmetic averaging (UPGMA) cluster analysis of pairwise F_{ST} distances between collections. The 5 populations that were located within ~60 km of one another are labeled with an asterisk. The lower graph comes from our previous study[4] but is presented on a common scale here to illustrate the differences in genetic distances. Bootstrap support is indicated above any branches supported in > 50% of the 1000 pseudoreplications.

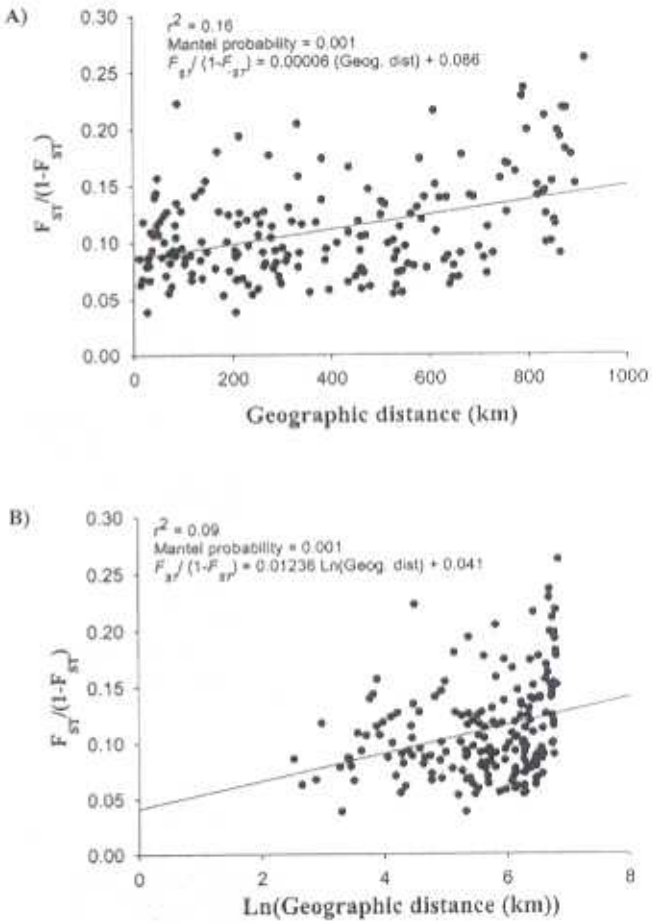

FIGURE 3. Regression analysis of (**A**) pairwise $F_{ST}/(1 - F_{ST})$ regressed on pairwise geographic distances between collections and (**B**) pairwise $F_{ST}/(1 - F_{ST})$ regressed on pairwise natural logarithm-transformed geographic distances.

ern populations are all consistent with a hypothesis of dispersal via human commerce in northeastern Mexico.

DISCUSSION

This is our third study of the population genetics of *Aedes aegypti* in Mexico. The first study[4] encompassed the northeast of Mexico and used RAPD and mitochondrial markers. The second study included collections from the northeast, the Yucatán, and the western Pacific coast of Mexico and used mitochondrial markers.[5] The breeding structure of *Ae. aegypti* varies a great deal according to the region analyzed. Along the western Pacific coast, genetic distances tended to be very small, suggesting extensive gene flow among collections from as far south as Tapachula to as far north as Tucson. Genetic distances were greater among the collections in northeastern Mexico. The comparative analysis we present here suggests that dispersal in the northeast and along the Pacific coast probably occurs through human commerce. In contrast, in the Yucatán[5] and along the southern Pacific coast, genetic distances tended to be larger and genetic isolation by distance was pronounced. The dispersal of *Ae. aegypti* in southern Mexico may therefore occur primarily through flight.

In a related study,[6] we compared midgut infection (MI) and disseminated infection (DI) rates among populations from the northeast, the Yucatán, and the western Pacific coast of Mexico. In general, the largest differences in MI and DI rates were detected among populations from the Yucatán. For example, mosquitoes collected from Ciudad del Carmen had 58% MI and 43% DI, whereas mosquitoes from Chetumal had 87% MI and 82% DI. The largest genetic distances were detected among Yucatán populations. The genetic distances among southern Pacific collections were also very large. Two collections were assessed for MI and DI at nearby sites in Tapachula (Figure 1). One collection demonstrated 78% MI and 72% DI, and the other 55% MI and 50% DI. The results of the present study therefore predict large differences in MI and DI rates among southern Pacific collections. That study is under way at this time.

A general correlation between genetic distances and variation in vector competence phenotypes suggests a general model for the population genetics of vector competence in

Table 3

Regression of F_{ST} on linear or road geographic distances among *Aedes aegypti* collections from northeastern Mexico* and the south Pacific Mexican coast

Region (marker), slope	Intercept	R^2	Mantel probability	$4Nm^2$
South Pacific coast (random amplified polymorphic DNA)				
$F_{ST}/(1 F_{ST}) = 0.0001 \times$ km lin. dst.	0.086	0.162	0.001	
$F_{ST}/(1 F_{ST}) = 0.0001 \times$ km road dst.	0.085	0.166	0.001	
$F_{ST}/(1 F_{ST}) = 0.0124 \times$ ln(km lin. dst.)	0.042	0.090	0.002	81 individuals
$F_{ST}/(1 F_{ST}) = 0.0119 \times$ ln(km road dst.)	0.041	0.080	0.004	
Northeastern Mexico*				
$F_{ST}/(1 F_{ST}) = 0.0001 \times$ km lin. dst.	0.051	0.087	0.043	
$F_{ST}/(1 F_{ST}) = 0.0001 \times$ km road dst.	0.052	0.090	0.114	
$F_{ST}/(1 F_{ST}) = 0.0135 \times$ ln(km lin. dst.)	0.004	0.121	0.002	74 individuals
$F_{ST}/(1 F_{ST}) = 0.0143 \times$ ln(km road dst.)	0.003	0.129	0.069	

lin. dst. = linear distance

Ae. aegypti. At least 2 loci have been demonstrated to control M1 and D1 in *Ae. aegypti*.[23] The same forces that cause large genetic differences among RAPD or mitochondrial markers would presumably also affect genetic distances among vector competence loci.[24] This would in turn predict large differences in vector competence phenotypes among populations.

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REFERENCES

1. Tabachnick WJ, 1991. The yellow fever mosquito: evolutionary genetics and arthropod-borne disease. *Am Entomol 37:* 14–24.
2. Monath TP, 1994. Dengue: the risk to developed and developing countries. *Proc Natl Acad Sci U S A 91:* 2395–2400.
3. Gubler DJ, 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. Gubler DJ, Kino G, eds. *Dengue and Dengue Hemorrhagic Fever*. New York: CAB International, 1–23.
4. Gorrochotegui-Escalante N, Muñoz ML, Fernandez-Salas I, Beaty BJ, Black WC IV, 2000. Genetic isolation by distance among *Aedes aegypti* populations along the northeastern coast of Mexico. *Am J Trop Med Hyg 62:* 200–209.
5. Gorrochotegui-Escalante N, Gomez-Machorro C, Lozano-Fuentes S, Fernandez-Salas I, Muñoz ML, Farfan-Ale JA, Garcia-Lejo J, Beaty BJ, Black WC IV, 2002. The breeding structure of *Aedes aegypti* populations in Mexico varies by region. *Am J Trop Med Hyg*. In press.
6. Bennett KE, Olson KE, Muñoz ML, Fernandez-Salas I, Farfan JA, Higgs S, Black WC, Beaty BJ, 2002. Variation in vector competence for dengue 2 virus among 24 collections of *Aedes aegypti* from Mexico and the United States. *Am J Trop Med Hyg*. In press.
7. Tabachnick WJ, Powell JR, 1979. A world-wide survey of genetic variation in the yellow fever mosquito, *Aedes aegypti*. *Genet Res 34:* 215–229.
8. Tabachnick WJ, Munstermann LE, Powell JR, 1979. Genetic distinctness of sympatric forms of *Aedes aegypti* in east Africa. *Evolution 33:* 287–295.
9. Powell JR, Tabachnick WJ, Arnold J, 1980. Genetics and the origin of a vector population: *Aedes aegypti*, a case study. *Science 208:* 1385–1387.
10. Wallis GP, Tabachnick WJ, Powell JR, 1984. Genetic heterogeneity among Caribbean populations of *Aedes aegypti*. *Am J Trop Med Hyg 33:* 492–498.
11. Wallis GP, Tabachnick WJ, Powell JR, 1983. Macrogeographic genetic variation in human commensal: *Aedes aegypti*, the yellow fever mosquito. *Genet Res 41:* 241–258.
12. Apostol GL, Black WC IV, Reiter P, Miller BR, 1994. Use of randomly amplified polymorphic DNA amplified by polymerase chain reaction markers to estimate the number of *Aedes aegypti* families at oviposition sites in San Juan, Puerto Rico. *Am J Trop Med Hyg 51:* 89–97.
13. Apostol BL, Black WC IV, Reiter P, Miller BR, 1996. Population genetics with RAPD-PCR markers: the breeding structure of *Aedes aegypti* in Puerto Rico. *Heredity 76:* 325–334.
14. Black WC IV, DuTeau NM, 1997. RAPD-PCR and SSCP analysis for insect population genetic studies. Crampton J, Beard CB, Louis C, eds. *The Molecular Biology of Insect Disease Vectors: A Methods Manual*. New York: Chapman and Hall, 361–373.
15. Sokal RR, Sneath PHA, 1963. *Principles of Numerical Taxonomy*. San Francisco: W.H. Freeman.
16. Felsenstein J, 1993. *Phylogeny Inference Package*. Version 3.5C. Seattle, WA: University of Washington.
17. Swofford DL, Selander RB, 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J Hered 72:* 281–283.
18. Wright S, 1978. *Evolution and the Genetics of Populations IV: Variability Within and Among Natural Populations*. Chicago, IL: University of Chicago Press.
19. Slatkin M, 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution 47:* 264–279.
20. Rousset F, 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics 145:* 1219–1228.
21. Mantel N, 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res 27:* 209–220.
22. Weir BS, 1996. *Genetic Data Analysis II*. Sunderland, MA: Sinauer Associates.
23. Lynch M, Milligan BG, 1994. Analysis of population genetic structure with RAPD markers. *Mol Ecol 3:* 91–99.
24. Bosio CF, Fulton RE, Salasek ML, Beaty BJ, Black WC IV, 2000. Quantitative trait loci that control vector competence for dengue-2 virus in the mosquito *Aedes aegypti*. *Genetics 156:* 687–698.