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BREEDING STRUCTURE OF *AEDES AEGYPTI* POPULATIONS IN MEXICO VARIES BY REGION

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Abstract. A population genetic analysis of *Aedes aegypti* was conducted among 38 collections from throughout coastal regions of Mexico. Multiple collections were made within 5 cities to examine local patterns of gene flow. Single-strand conformation polymorphism analysis was used to screen for variation in a 387-bp region of the Nicotinamide Adenine Dinucleotide Dehydrogenase subunit 4 mitochondrial gene (*ND4*) and 25 haplotypes were detected. Northeastern Mexico collections were genetically differentiated from and had lower genetic diversity than Yucatan and Pacific coastal collections. Yucatan and Pacific collections were genetically homogeneous. Regression analysis of geographic distances and F_{ST} values indicated that collections were genetically isolated by distance in the Pacific and the Yucatan, but not among collections in the northeast. Free gene flow occurred among all collections within 130 km of one another in the northeast and within 180 km in the Yucatan. F_{ST} values were never large among Pacific collections, suggesting extensive gene flow along the Pacific coast.

INTRODUCTION

The mosquito *Aedes aegypti* is the primary urban vector of dengue (DEN) and yellow fever flaviviruses. Understanding the dispersal patterns of this mosquito is important for the development of effective control strategies and predicting DEN and yellow fever outbreaks. *Ae. aegypti* dispersal occurs through adult flight[1] and through transport of eggs, larvae, and adults in discarded bottles, cans, appliances, tires, and cargo containers along commercial routes.[2] Control strategies for *Ae. aegypti* during urban outbreaks of DEN or yellow fever assume that mosquitoes have a lifetime flight range of 50–100 m, and this belief has dictated focal applications of insecticides to disrupt vector-borne transmission of disease.[3]

Early population genetic studies defined genetic relationships throughout the world's range of *Ae. aegypti,*[4–9] whereas more recent studies have focused on local patterns of dispersal.[8,10,11] We recently showed by means of random amplified polymorphic DNA (RAPD) markers that collections in northeastern Mexico are genetically isolated by distance[12] but are genetically homogeneous within a range of 90–250 km. This pattern suggested that gene flow among populations decreases with increasing geographic distances, but that within $\sim$ 250 km, mosquitoes exchange genes continuously. Similar but statistically insignificant patterns were seen when we used a mitochondrial DNA (mtDNA) marker.

In the present study, we have expanded our analysis of *Ae. aegypti* breeding structure in Mexico to include 38 locations distributed throughout most of the areas in Mexico where *Ae. aegypti* is endemic. The distribution of *Ae. aegypti* in Mexico is limited to elevations $<$ 610 m ($<$ 2,000 feet) above sea level.[13] These areas are indicated in Figure 1. All collections were made in the shaded regions. Multiple collections were made from the cities of Monterrey, Merida, Chetumal, Cancun, and Tapachula. This design allowed us to analyze gene flow at 2 levels: among collections within a city and among cities. As in our earlier study, we examine genetic similarity among collections, test for isolation by distance and report the effective migration rate (*Nm*) (the number of migrating reproductive individuals among populations) and the variance effective population size (N_e) (the harmonic average of a successfully reproducing adult population over a unit area) for each of the 38 collections.

We did not use RAPD markers in the present study because we observed[12] that at distances $>$ 150–200 km, it became difficult to compare distant populations. The RAPD bands either became fixed or extinct among distant populations. However, we continue analysis of the same mtDNA region as examined earlier.[12] Following earlier studies in our laboratory,[14] we use single-strand conformation polymorphism (SSCP) analysis[15,16] as a quick, sensitive, and inexpensive means to screen for variation among mitochondrial genes amplified from individual mosquitoes. Intrastrand interactions during SSCP analysis are highly sensitive to the primary sequence of the molecule.[15] In an extensive study of the sensitivity of the technique, SSCP detected 99% of point mutations in DNA molecules 100–300 bp in length and 89% of mutations in molecules of 300–450 bp in length.[17] Most (95–100%) of point mutations in genes $<$ 700 bp were detected by SSCP.[18] In the present study, we sequenced at least 2 copies of all novel SSCP haplotypes to test the sensitivity and reproducibility of the SSCP technique and to gather data with which to assess phylogenetic relationships among haplotypes.

MATERIALS AND METHODS

Mosquito collection and genetic analyses. The locations of *Ae. aegypti* collections are listed in Table 1, as are the number of larvae analyzed in each collection. The geographic locations of all sampling sites appear in Figure 1. Mosquito larvae were hatched and reared to adults in the laboratory. Adults were individually examined to confirm that they were *Ae. aegypti* and then were stored at -70°C awaiting analysis. DNA was obtained from individual mosquitoes.[12] Primers used to amplify the Nicotinamide Adenine

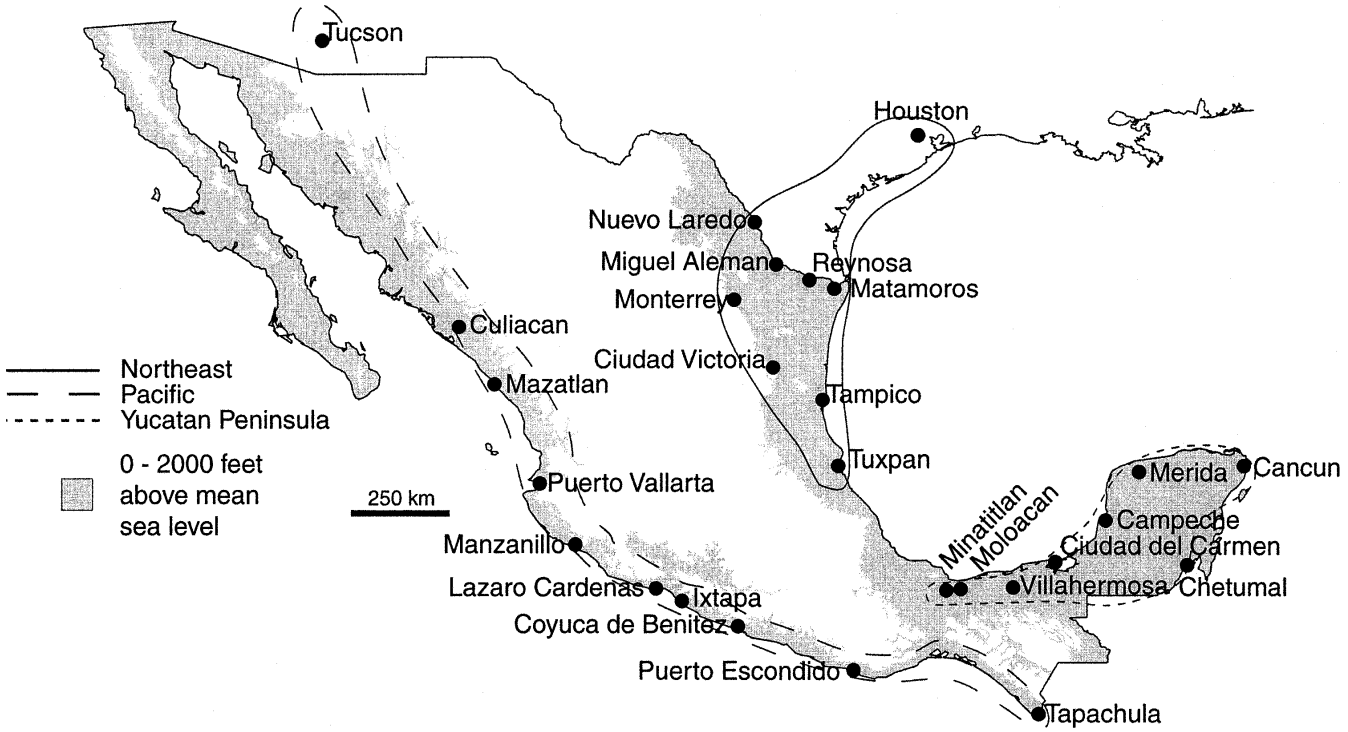

FIGURE 1. Map of *Aedes aegypti* collecting locations in Mexico. The designation of each collection to the northeast, Pacific, or Yucatan Peninsula regions are indicated with lines. Ibanez-Bernal and Gomez-Dantes[13] reported that the distribution of *Aedes aegypti* in Mexico is limited to elevations < 610 m (< 2,000 feet) above sea level. These areas are shaded in gray.

Dinucleotide Dehydrogenase subunit 4 mitochondrial gene (*ND4*) and all the polymerase chain reaction and electrophoresis conditions are as reported earlier.[12] Haplotypes were assigned numbers according to the order of their discovery. The *ND4* polymerase chain reaction products from 56 individuals representing each of the 25 haplotypes were sequenced at least once along both strands on an ABI sequencer (Davis Sequencing, Davis, California). Phylogenetic relationships among haplotypes were estimated as described earlier.[12]

Statistical analysis of mitochondrial haplotype frequencies. Variation in haplotype frequencies within and among cities and regions was examined by means of analysis of molecular variance (AMOVA).[19] Arlequin 2.000 estimated pairwise F_{ST} values and Slatkin's "linearized F_{ST}"[20] [= $F_{ST}/(1 - F_{ST})$] among populations and computed the significance of the variance components associated with each level of genetic structure by a nonparametric permutation test with 100,000 pseudoreplicates.[19] For each collection, the nucleotide sequence and the frequency of each haplotype were also entered into DnaSP.[21] We estimated the number of polymorphic sites, the average number of nucleotide differences (k) among individuals in a collection[22] the nucleotide diversity (π_1)[23] and the nucleotide diversity with Jukes and Cantor correction (π_2).[23] Effective migration rates (Nm) were estimated from F_{ST}.[23] F_{ST} $(1 - F_{ST})$ were used to construct a dendrogram among all collections by means of unweighted pair-group method with arithmetic averaging analysis[24] in the NEIGHBOR procedure in PHYLIP3.5C.[25]

Geographic distances were obtained by Geographic Information Systems on Datum WGS 84 projection (Environmental Systems Research Institute, Redlands, CA). Linearized F_{ST} values were regressed on the natural logarithm of pairwise geographic distances among populations to determine if geographic distance among populations serves as a barrier to gene flow.[20] The Mantel test[26] was performed by MANTEL (available from the corresponding author). The reciprocal of the slope estimated by this regression provides an estimate of the average effective population size.[27]

RESULTS

Mitochondrial haplotype detection and phylogenetic analysis. The *ND4* gene was amplified and surveyed for variation by SSCP among 1,977 mosquitoes. A total of 25 different *ND4* haplotypes were detected with SSCP (Figure 2). The *ND4* gene was sequenced in 56 individual mosquitoes. Sequences of mosquitoes with identical SSCP patterns were identical within each haplotype, and SSCP patterns differed among individuals with even slight sequence differences. For example, haplotypes 1 and 2 differed by a single C↔T transition and differed in the mobility of the denatured single strand band. Haplotypes 21 and 23 differed by a C↔T transition and 2 transversions (A↔C, A↔T). This supports our earlier report[12] that the SSCP technique is specific and reproducible in identifying single nucleotide substitutions in the *ND4* gene in *Ae. aegypti.* The sequences of all 25 novel haplotypes are available in GenBank (accession numbers AF334841–AF334865). The frequencies of each haplotype in a collection are available from the corresponding author.

The 25 haplotypes were manually aligned on the basis of codons with the homologous regions of *Anopheles gambiae* and *An. quadrimaculatus,* and no gaps were required for optimal alignment. Our earlier phylogenetic analysis only

TABLE 1
Locations, dates of collections, coordinates, and sample sizes of *Aedes aegypti* collections in Mexico and the United States

State	City	Region	Date	Latitude	Longitude	No. individuals
Nuevo Leon	Monterrey	North	7/09/96	N25°40′00.120″	W100°18′00.000″	57
		South	7/17/96	N25°28′00.120″	W100°10′01.200″	58
		West	7/24/96	N25°30′00.000″	W100°04′58.800″	58
		East	7/24/96	N25°40′59.880″	W100°22′01.200″	58
Tamaulipas	Ciudad Victoria		8/03/96	N23°40′00.120″	W099°15′00.000″	59
	Miguel Aleman		6/16/98	N26°23′30.000″	W099°03′39.001″	50
	Matamoros		7/29/96	N26°15′00.000″	W097°28′00.120″	59
	Nuevo Laredo		8/10/97	N27°30′00.000″	W099°28′00.121″	48
	Reynosa		7/20/97	N26°10′00.120″	W098°10′00.121″	59
	Tampico		8/04/96	N23°40′00.120″	W097°49′59.881″	59
Veracruz	Moloacan		9/21/98	N17°59′09.000″	W094°20′46.001″	55
	Minatitlan		9/16/96	N17°58′47.000″	W094°32′27.000″	56
	Tuxpan		8/24/96	N21°10′00.120″	W097°25′00.120″	59
Tabasco	Villahermosa		9/10/98	N17°59′59.986″	W092°54′00.007″	58
Campeche	Campeche		8/10/98	N19°53′59.988″	W090°36′00.016″	53
	Ciudad del Carmen		8/15/98	N18°35′59.994″	W091°47′59.998″	52
Yucatan	Merida		7/1/99	N20°57′00.013″	W089°38′23.994″	57
		North	7/10/99	N21°00′44.640″	W089°37′51.600″	49
		South	7/10/99	N20°57′06.840″	W089°38′26.881″	35
		Central	7/10/99	N20°57′58.680″	W089°39′57.240″	46
		East	7/10/99	N20°59′28.320″	W089°35′00.600″	53
		West	7/10/99	N20°58′39.000″	W089°39′28.801″	60
Quintana Roo	Cancun	Central	6/11/99	N21°08′24.012″	W086°52′47.992″	32
		North	6/11/99	N21°09′03.613″	W086°52′38.974″	53
	Chetumal	Central	6/12/99	N18°29′59.996″	W088°18′00.004″	38
		North	6/12/99	N18°30′29.307″	W088°17′49.967″	54
Chiapas	Tapachula I		8/15/98	N14°48′14.760″	W088°18′44.640″	57
	Tapachula II		8/16/98	N14°54′49.243″	W092°14′28.172″	42
Oaxaca	Puerto Escondido		6/12/99	N15°51′04.000″	W097°04′00.000″	57
Guerrero	Coyuca de Benitez		11/13/99	N17°00″27.121″	W100°05′20.419″	50
	Ixtapa-Zihuatanejo		11/14/99	N17°39′05.351″	W101°31′55.577″	59
Michoacan	Lazaro Cardenas		11/14/99	N17°57′08.512″	W102°11′41.074″	42
Colima	Manzanillo		11/15/99	N19°05′13.819″	W104°17′52.573″	49
Jalisco	Puerto Vallarta		11/15/99	N20°41′41.846″	W105°14′29.804″	52
Sinaloa	Mazatlan		11/16/99	N23°14′48.111″	W106°25′59.430″	49
	Culiacan		7/27/98	N24°47′36.000″	W107°22′20.001″	36
Arizona	Tucson		9/10/98	N32°13′18.000″	W110°55′33.001″	59
Texas	Houston		10/5/98	N29°45′04.000″	W095°21′47.001″	50
Total (38 collections)						1,977

involved the first 7 haplotypes and estimated that they arose as 2 separate clades, one containing haplotypes 1, 2, and 6 and the other containing haplotypes 3, 4, 5, and 7 (Figure 3).[12] In contrast, the current phylogenetic analysis (Figure 4) indicates a single monophyletic lineage with the original 3, 4, 5, 7 haplotype lineage being basal to the 1, 2, 6 haplotype lineage.

Genetic relationships among populations. Cluster analysis of pairwise linearized F_{ST} values among collections (Figure 5) indicates several genetic clusters and a number of outliers. All collections along the northeast coast, with the exceptions of Houston and Miguel Aleman, fall within a single cluster, separate from the collections from the Yucatan and the Pacific coast. The majority of Pacific collections fall within a single cluster. However, this cluster also contains Gulf coastal collections from Campeche and Villahermosa. The 2 clusters above this mostly contain Yucatan collections but also contain the Lazaro Cardenas collection from the Pacific coast. The ''Yucatan cluster'' contains the remaining Yucatan collections but also contains the Ixtapa collection from the Pacific coast.

Culiacan, Tucson, Moloacan/Minatitlan, and Nuevo Laredo all appear to be distantly related to one another and to the other collections. Tucson is geographically the most distant of all of our collections. Moloacan and Minatitlan are in close geographic proximity to one another but are also in proximity to Villahermosa. However, they are genetically distant from Villahermosa mosquitoes. The extreme genetic isolation of Nuevo Laredo was reported for both RAPD and mtDNA markers in our earlier study.[12]

Genetic isolation by distance. Pairwise linearized F_{ST} values were regressed against the natural logarithm of the pairwise geographic distances among collections (Figure 3, Table 2) to determine if gene flow among collections is correlated with geographic distance (i.e., to test for isolation by distance). This analysis indicated a significant correlation between genetic and geographic distances among collections from the Yucatan and the Pacific but no correlation was detected among collections from northeast Mexico (Table 2).

Genetic distances remained small below geographic distances of 90 ($\sim e^{4.5}$) km and became large at distances $>$ 150 ($\sim e^{5.0}$) km (Figure 3). In contrast, linearized F_{ST} estimates only gradually increased and never became large

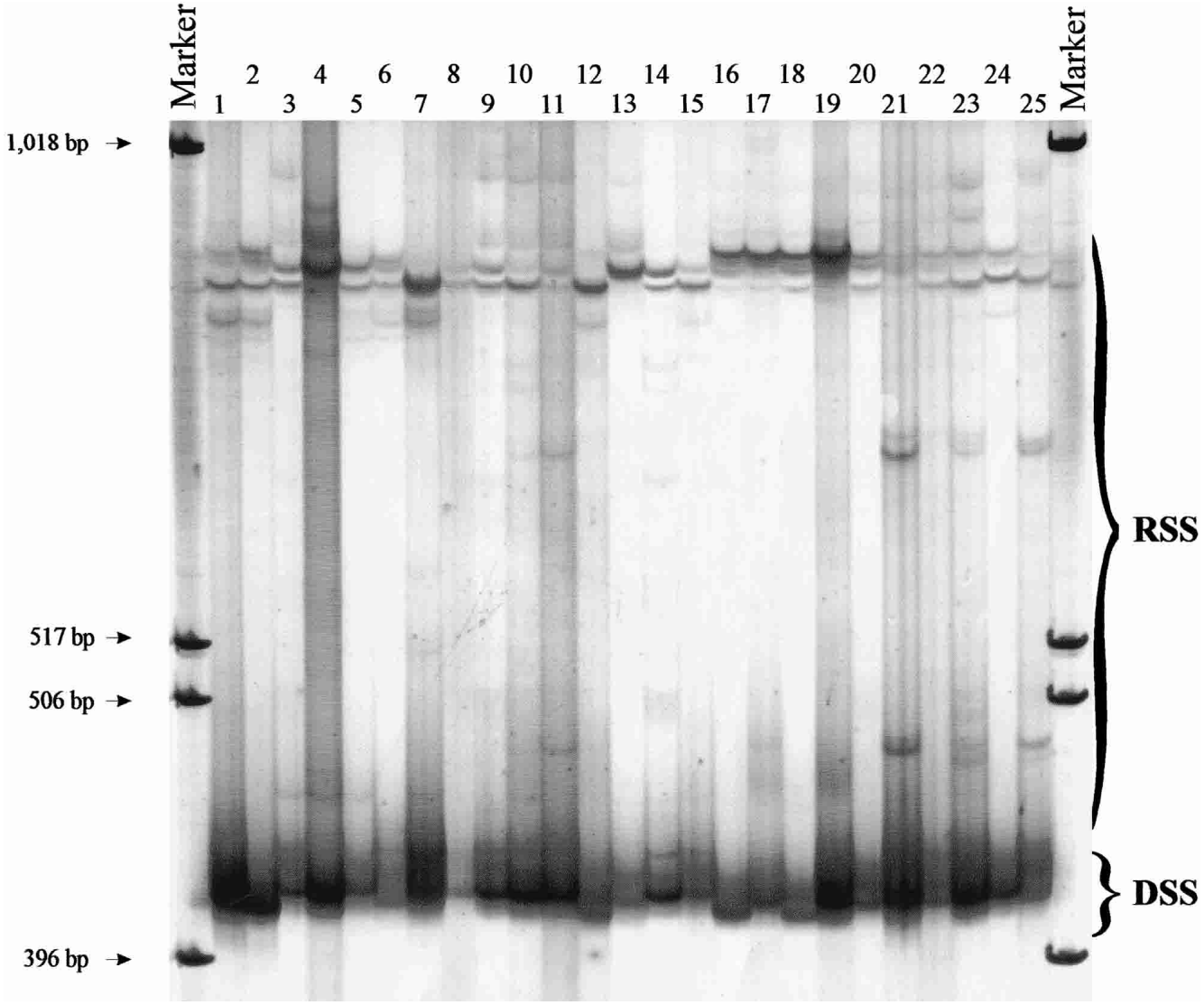

FIGURE 2. Silver-stained single-strand conformation polymorphism showing each of the 25 novel haplotypes identified among *Aedes aegypti*. DSS = denatured single strands; RSS = renatured single strands.

along the Pacific coast. It therefore appears that a great deal of gene flow occurs among Pacific coastal populations.

The average effective population (N_e) size was 16 mosquitoes/km over the entire collection area (Table 2). N_e was the lowest in the northeast (10 mosquitoes/km) and greatest in the Pacific (22 mosquitoes/km).

Haplotype diversity. Collections from the northeast consistently had a lower average number of haplotype differences among individuals (k) and fewer polymorphic sites than collections from the Yucatan and along the Pacific coast (Table 3, Figure 6). Pacific and Yucatan collections had similar number of polymorphic sites, but Pacific collections consistently had greater k values. Thus, mosquitoes in the Pacific collections not only had the greatest number of mtDNA haplotypes, but the sequences within these haplotypes were more diverse than in the other locations.

Nested analysis of haplotype frequencies. Haplotype frequencies were compared among collections within cities and among cities by AMOVA.[19] This partitioning was done among 1) all collections, 2) between northeastern collections and the remaining collections, 3) between the Yucatan and Pacific collections, and within 4) Pacific, 5) Yucatan, and 6) northeastern collections (Tables 4 and 5).

In the first AMOVA, among all collections, 72% of the variation in haplotype frequencies arose among individuals in a collection, whereas ~ 22% of the variation arose among cities and only 6.5% arose among collections within cities (Table 2). In the second AMOVA, ~ 15% of the variation was accounted for by differences between collections in the northeast as compared with those in the Yucatan and Pacific regions. There was as much variation among sites in either of the 2 regions (~ 15%) as there was between the 2 regions. Thus, both observations agree with the clustering patterns shown in Figure 5. In the third AMOVA, only 5% of the variation was accounted for by differences between collections in the Yucatan as compared with those in the Pacific region. There was a great deal more variation (~ 15%) among collections within either of the 2 regions. This ob-

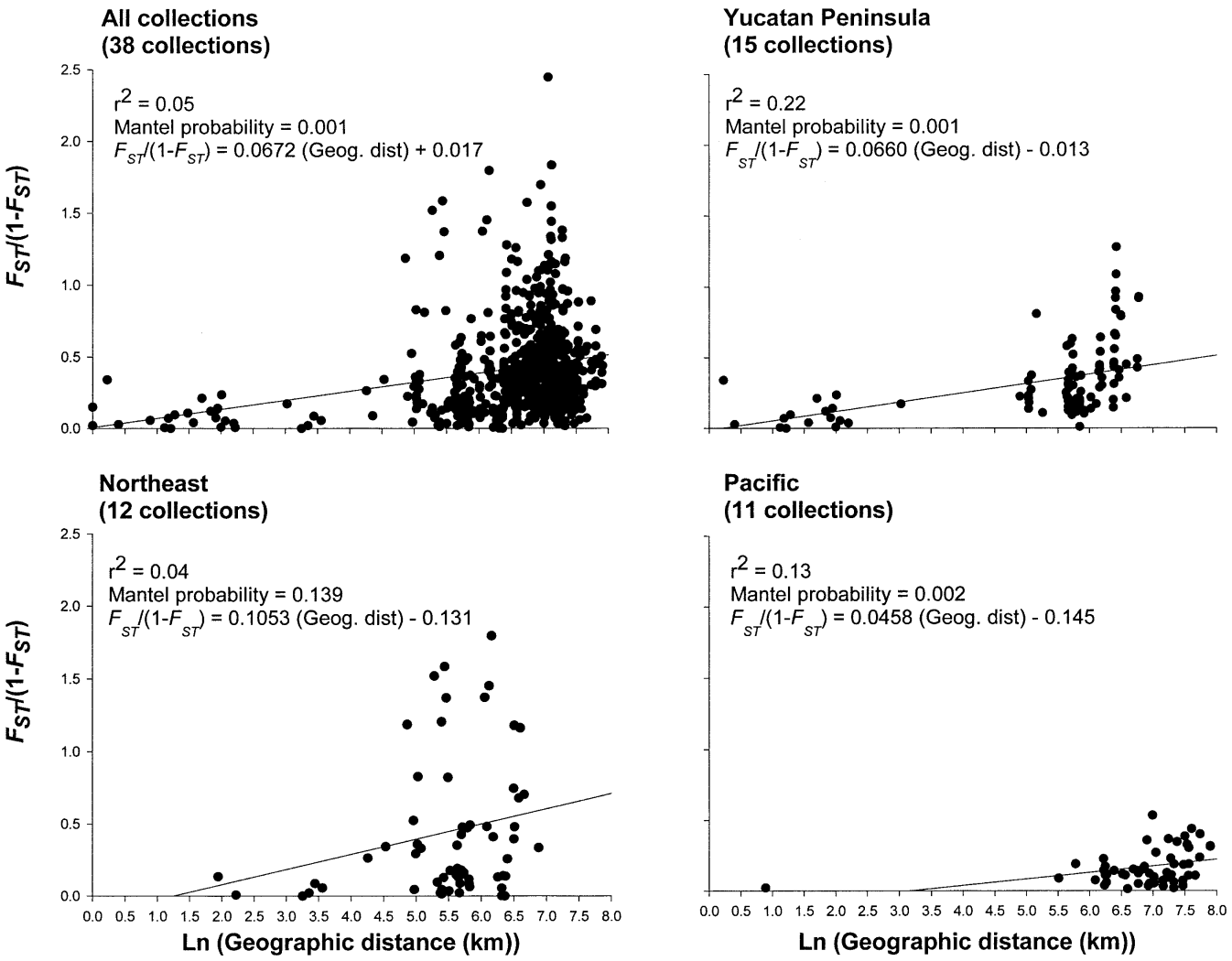

FIGURE 3. Regression analysis of pairwise $F_{ST}/(1 - F_{ST})$ regressed on pairwise ln(geographic distances) between collections.

servations also supports the lack of distinct clustering among Pacific and Yucatan collections (Figure 5). Within the Pacific region (AMOVA 4), variation among collections within Tapachula only accounted for 2% of the overall variance, and differences among Pacific collections sites accounted for ~ 12% of the overall variance. Within the Yucatan region (AMOVA 5), variation among collections within Merida, Chetumal, and Cancun accounted for ~ 9% of the overall variance, almost as much as occurred among collections in the Yucatan (~ 10%). Within the northeast region (AMOVA 6), variation among collections within Monterrey only accounted for ~ 4% of the overall variance, but differences among northeastern collection sites accounted for 16% of the overall variance. These patterns of variation within each region agree with the diversity analysis (Table 3, Figure 6). The greatest diversity occurred among mosquitoes within the Pacific collections (~ 85%), followed by the Yucatan (~ 81%), then the northeastern collections (~ 80%).

Effective migration rates (*Nm*). Estimating effective migration rates (*Nm*) from F_{ST} assumes that populations fit the assumptions of Wright's island model.[28] A major assumption of this model is that migration rates are equal among all populations. Our regression analysis indicated that this assumption was false among collections from different cities in the Yucatan and Pacific but was valid within northeastern collections. Regression analysis was repeated among collections within cities. Genetic and geographic distances were independent (regression analyses available from the corresponding author). By use of Wright's F_{ST} in the hierarchical analysis, *Nm* ranged 2.5–19.5 individuals (Table 5) within cities and averaged 12.7 among all collections in the northeast. *Nm* and N_e (10 individuals, Table 2) are thus in close agreement.

DISCUSSION

The patterns of diversity identified in the present study suggest that gene flow among *Ae. aegypti* in Mexico varies by region. Gene flow is moderate among northeastern Mexico populations, and populations appear not to be strongly isolated by distance. Genetic diversity is low among northeastern *Ae. aegypti.* Gene flow is less and populations are isolated by distance in the Yucatan peninsula. Genetic diversity varies greatly among Yucatan sites. Gene flow is large and genetic diversity is high among Pacific coastal populations.

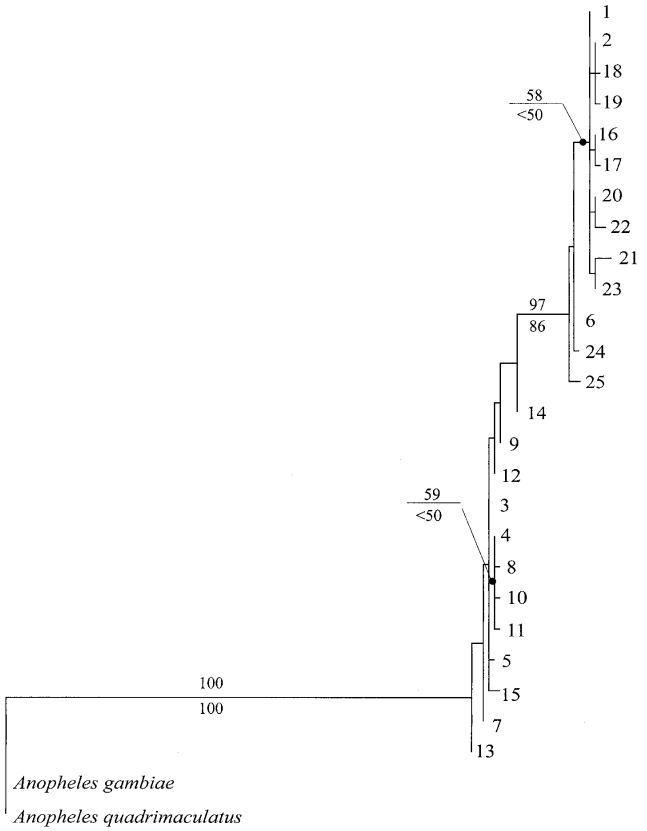

FIGURE 4. Maximum-likelihood tree showing phylogenetic relationships among the 25 individual haplotypes. For branches with > 50% bootstrap support, the percentage support using maximum parsimony analysis appears above each branch; the percentage support using Tamura-Nei genetic distance/neighbor joining (NJ) appears below each branch.

Our earlier study was confined to collections from northeastern Mexico.[12] The same data, in addition to new data from collections in Houston and Miguel Aleman, were included in the present study. Mosquitoes in northeastern Mexico contained only 7 of the 25 haplotypes that we have so far detected. Furthermore, the average nucleotide diversity was lower among mosquitoes in these collections than in the Yucatan or Pacific collections. These observations are consistent with 2 hypotheses. Northeastern Mexico *Ae. aegypti* populations may be maintained by few individuals with repeated bottlenecks. Alternatively, northeastern populations may have been founded by fewer mosquitoes than populations along the Pacific or in the Yucatan. Arguing against the second hypothesis, there is a great deal of commerce and a number of large port cities in northeastern Mexico and the southeastern United States.

In support of the first hypothesis, northeastern Mexico is more arid than either the Pacific or Yucatan regions. Earlier population genetic studies also found large genetic distances between *Ae. aegypti* collections from the southeastern United States (including Houston) and northeastern Mexican (including Montemorelos near Monterrey, Ciudad Victoria, and Piedras Negras northwest of Nuevo Laredo).[4,8] Furthermore, southeastern U.S. and Mexican collections arose on separate branches in a cluster analysis of allele frequencies.[2] These earlier studies did not extend into the Yucatan or along the Pacific coast. We have found strong evidence of genetic isolation of the northeastern *Ae. aegypti* populations from those in the Yucatan. Punta Villa Rica is the white area south of Tuxpan extending to the coast in Figure 1. It is an area with elevations > 610 m, few roads, and little commerce. It is possible that Punta Villa Rica may limit north-south gene flow along the Atlantic coast.

Populations in northeastern Mexico in this study did not appear to be strongly genetically isolated by distance from one another. However, this does not contradict our earlier report of isolation by distance among these northeastern collections.[12] In that study, evidence of isolation by distance was detected by RAPD markers but not with mitochondrial markers. We explained that this discrepancy was probably due to high mutation rates in RAPD loci. We observed[12] that at distances > 150–200 km, it became difficult to compare populations because RAPD bands either became fixed or extinct among distant populations. This result is consistent with a hypothesis that frequent point mutations in primer annealing sites caused the independent gain or loss of RAPD bands in different populations. The likelihood of independent gain or loss increases as the distance among populations lengthens or the time because gene flow increases. Therefore, RAPDs can underestimate genetic distances and overestimate rates of gene flow.

Various extrinsic forces could disrupt genetic isolation by distance. With *Ae. aegypti,* human transportation of eggs, larvae, or adults in containers along commercial routes could cause geographically distant populations to become genetically similar. Arid environments or active mosquito abatement practices would cause populations to undergo genetic bottlenecks. Thus, populations in proximity could become genetically distinct. A hypothesis of frequent genetic drift is consistent with the reduced genetic variability among northeast collections. Either genetic drift or human commerce could be reducing genetic isolation by distance among northeastern Mexico populations.

Cluster analysis of collections from the 3 regions suggest that 2 Yucatan collections (Villahermosa and Campeche) were most similar to collections along the Pacific. Conversely, 2 Pacific collections (Ixtapa and Lazaro Cardenas) were most similar to Yucatan collections. All 4 collections have high genetic diversity (Figure 6), suggesting that they have not been subject to recent bottlenecks. These observations are consistent with the hypothesis of long distance east-west transport of the *Ae. aegypti* populations across the Yucatan peninsula via human commerce. But there is a region extending from the Atlantic to the Pacific coasts in the Yucatan Peninsula that never exceeds 610 m (Figure 1). Thus, additional gene flow via flight also remains a possibility. We are in the process of analyzing geographic distances among these populations, correcting for distances along roads connecting the sites, as well as estimating the relative amount of commerce along routes connecting these various sites.

Cluster analysis of collections also revealed the existence of a number of genetically distinct populations. These included the following: Nuevo Laredo, Houston and Miguel Aleman from northeast Mexico, Moloacan and Minatitlan from the Yucatan, and Culiacan and Tucson along the Pacific coast. Mosquitoes from Nuevo Laredo, Houston, and Moloacan all had low genetic variability (Figure 6). This is con-

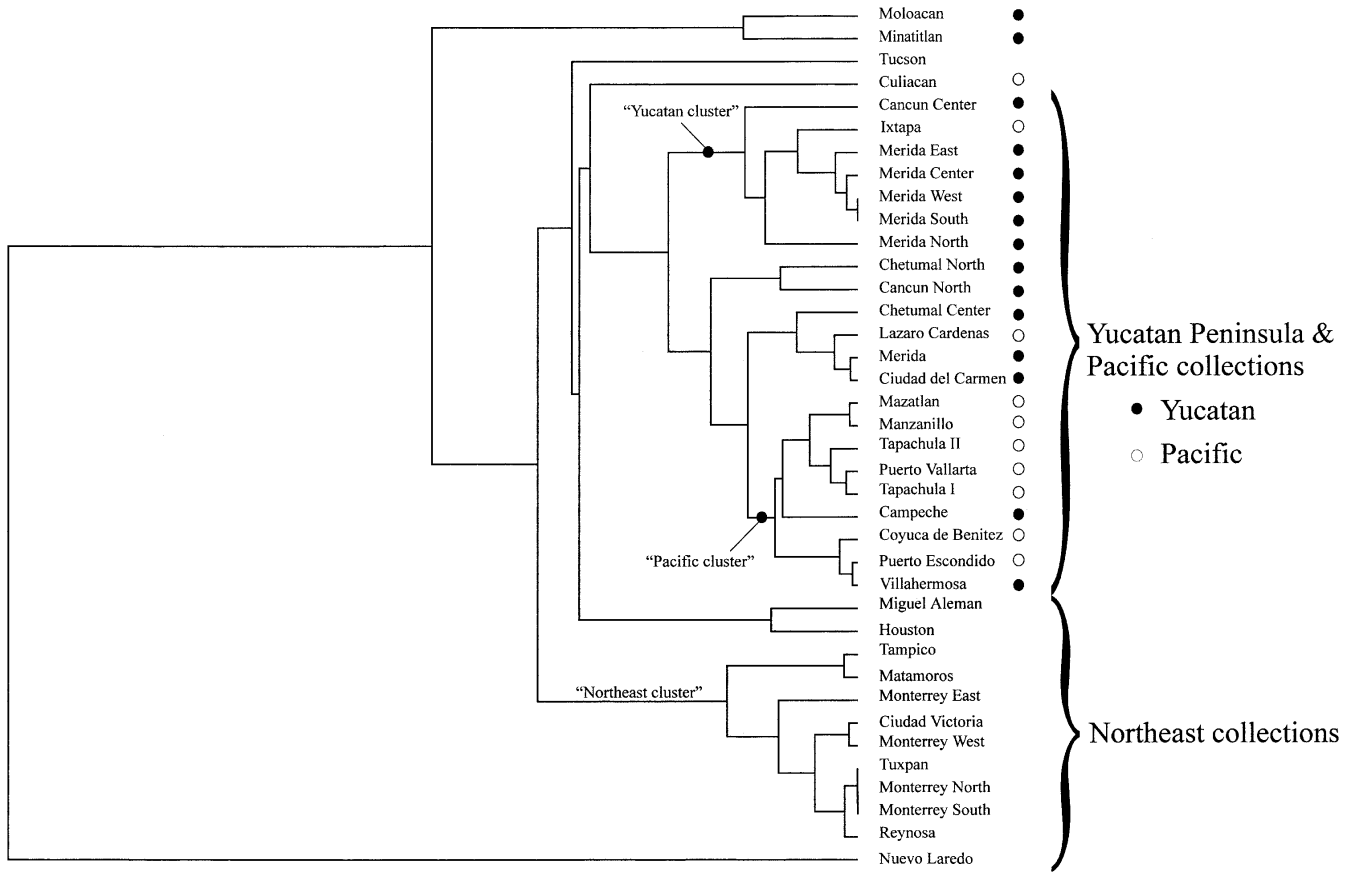

FIGURE 5. Unweighted Pair Group Method using arithmetic Average cluster analysis of pairwise $F_{ST}/(1 - F_{ST})$ relationship between collections.

sistent with a hypothesis that these populations were established by few mosquitoes. The genetic similarity of Houston and Miguel Aleman further suggests that mosquitoes in Houston may have been established through commerce from Miguel Aleman. Similarly, the genetic similarity of Moloacan and Minatitlan further suggests that Moloacan mosquitoes may have been established through commerce from Minatitlan. The fact that these are so genetically distinct from all other mosquitoes in this study also raises the possibility that these may represent an independent introduction through port cities in the Yucatan. The high genetic variability detected among mosquitoes in the Culiacan and Tucson collections argues against these being genetically distinct due to a founder effect. Instead, these may reflect a gradual change in haplotype frequencies along the north-south gradient of our Pacific collection sites.

Genetic isolation by distance was pronounced (Figure 3), and genetic variability tended to be larger (Figure 6) among collections in the Yucatan. It may be that human transport is reduced in the Yucatan and that a more humid, tropical environment may decrease genetic drift. Genetic isolation by distance was significant but without any large F_{ST} values (Figure 3) and genetic variability was consistently large among mosquitoes in Pacific collections (Figure 6). We suggest that *Ae. aegypti* populations along the Pacific coast are genetically homogeneous due to human transport along the coast. As indicated in Figure 1, there is a continuous distribution of sites with elevations < 610 m all along the Pacific

TABLE 2
Regression of F_{ST} for *ND4* mitochondrial markers on geographic distances

Collection	Slope, $F_{ST}/(1 - F_{ST})$ =	Intercept	R^2	Mantel	$4D\pi\sigma^2$ probability
All collections	0.00009 × geographic distance	0.334	0.02	0.002	
	0.06172 × ln(geographic distance)	0.017	0.05	0.001	16 individuals
Northeast	0.00031 × geographic distance	0.342	0.02	0.167	
	0.10525 × ln(geographic distance)	−0.131	0.04	0.139	10 individuals
Yucatan	0.00072 × geographic distance	0.078	0.41	0.001	
	0.06595 × ln(geographic distance)	−0.013	0.22	0.001	15 individuals
Pacific	0.00009 × geographic distance	0.055	0.24	0.026	
	0.04584 × ln(geographic distance)	−0.145	0.13	0.001	22 individuals

TABLE 3
Estimates of variability in the mitochondrial genome among *Aedes aegypti* collections

	N	Polymorphic sites	k	π_1	π_2
Northeast region	674	16	6.332	0.01636	0.01674
Nuevo Laredo	48	14	0.881	0.00228	0.00232
Reynosa	59	13	6.291	0.01626	0.01662
Monterrey South	58	13	4.978	0.01286	0.01314
Monterrey North	57	13	5.569	0.01439	0.01470
Monterrey East	58	13	2.258	0.00583	0.00596
Monterrey West	58	13	2.752	0.00711	0.00725
Cd. Victoria	59	14	2.286	0.00591	0.00600
Matamoros	59	14	5.953	0.01538	0.01572
Tampico	59	14	2.927	0.00756	0.00770
Tuxpan	59	14	6.060	0.01566	0.01599
Miguel Aleman	50	15	6.343	0.01639	0.01673
Houston	50	4	1.151	0.00297	0.00299
Yucatan peninsula	751	24	8.363	0.02161	0.02207
Moloacan	55	4	1.001	0.00259	0.00259
Minatitla	56	15	2.579	0.00666	0.00681
Villahermosa	58	17	6.570	0.01698	0.01735
Campeche	53	18	7.675	0.01983	0.02026

coast. Furthermore, as in the Yucatan, humid, tropical environments may decrease genetic drift.

In contrast to our first study,[12] phylogenetic analysis of the *ND4* haplotypes indicated a single monophyletic lineage, with one lineage being basal to a second, distinct, well-supported lineage. The different phylogenetic patterns in the 2 studies are probably the result of inadequate sampling of haplotypes in our first study. It would be interesting to sample *Ae. aegypti* mtDNA haplotypes from throughout the world to determine if intermediate haplotypes could be detected and possibly to determine the most likely geographic origin of the basal lineages.

In general, these results have a number of implications for reduction of *Ae. aegypti* populations in Mexico. In all regions, under distances of 150 km, populations of *Ae. aegypti* can be expected to remain genetically uniform. This suggests that in the absence of local selection, genes affecting DEN susceptibility or insecticide resistance should remain uni-

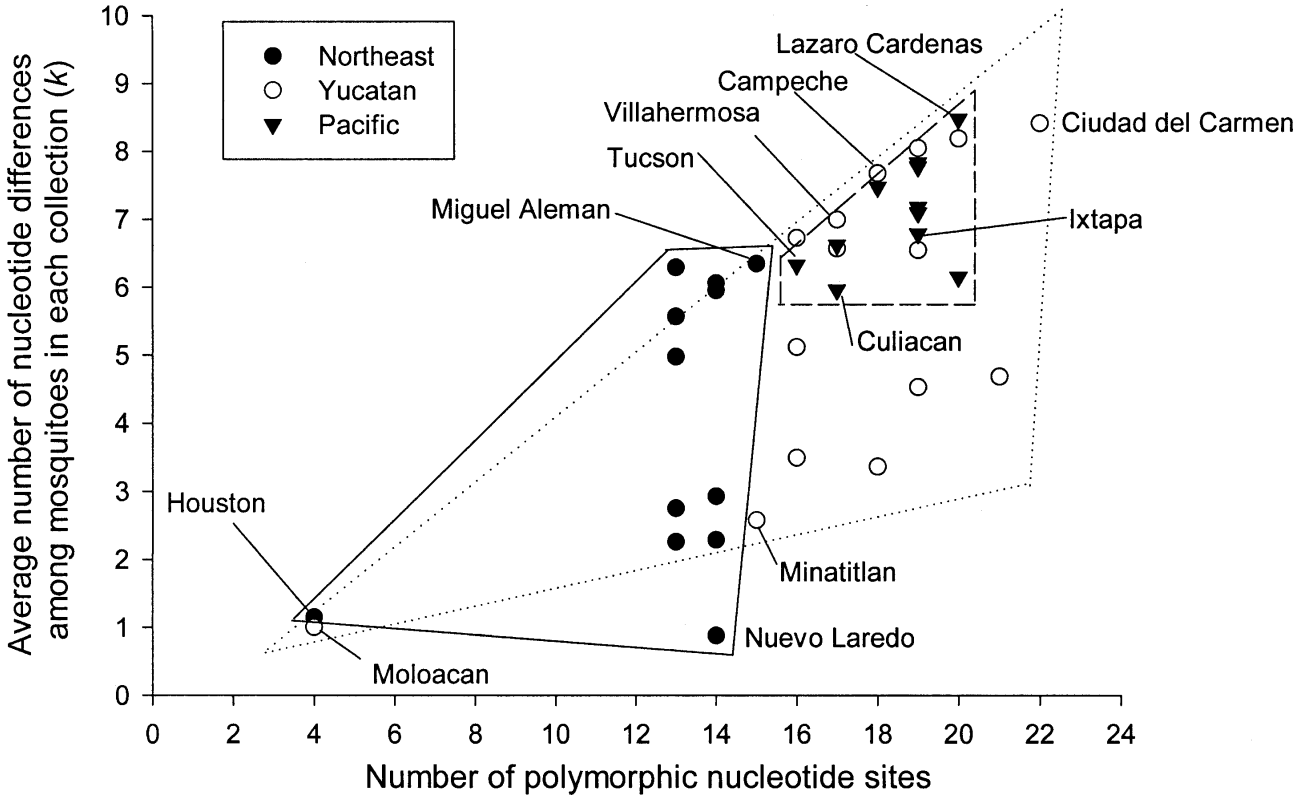

FIGURE 6. The number of polymorphic sites found in each collection, plotted against the average number of nucleotide differences (*k*) among mosquitoes within a collection.

TABLE 4
Partitioning of variation in the frequency of genetic markers among *Aedes aegypti* collections in Mexico

Source of variation	Degree of freedom	Sum of squares	Variance components	Variation (%)
Within cities				
Within Tapachula, Merida, Chetumal, Cancun, Monterrey	11	20.5	0.030*	6.5
Along cities in the northeast and Yucatan/Pacific cities	1	63.1	0.073*	14.8
Among cities in Yucatan and Pacific	1	15.9	0.024†	5.1
Within Tapachula	1	0.8	0.010‡	2.2
Within Merida, Chetumal, Cancun	4	31.6	0.039§	8.6
Within Monterrey	1	7.9	0.014¶	3.8
Among cities				
All cities	26	237.0	0.100*	21.7
Among Northeast versus Yucatan/Pacific collections	32	134.6	0.075*	15.2
Among Yucatan vs. Pacific collections	21	77.5	0.068*	14.7
Among Pacific cities	9	34.9	0.054#	12.4
Among Yucatan cities	10	25.7	0.045*	10.0
Among northeast cities	9	33.4	0.056*	16.0
Within collections				
All collections	1,939	645.7	0.333*	71.8
Northeast, Yucatan, and Pacific collections	1,725	592.5	0.343*	69.9
Yucatan and Pacific collections	1,110	408.0	0.368*	80.1
Pacific collections	541	204.9	0.379*	85.4
Yucatan collections	724	283.9	0.364*	81.2
Northeast collections	615	184.5	0.300*	80.2
Total				
All collections	1,976	903.2	0.464	–
Northeast, Yucatan, and Pacific collections	1,758	790.3	0.491	–
Yucatan and Pacific collections	1,132	501.4	0.459	–
Pacific collections	551	240.6	0.443	–
Yucatan collections	738	321.2	0.449	–
Northeast collections	625	225.7	0.374	–

* 0.00000 ± 0.00000.
† 0.00416 ± 0.00060.
‡ 0.06713 ± 0.00216.
§ 0.00099 ± 0.00036.
¶ 0.12792 ± 0.00300.
# 0.03584 ± 0.00183.

TABLE 5
Fixation indexes between and among collections

Collection	*F*	Index
All collections	*F*(within cities)	0.084* (*Nm* = 5.5)
	F(among cities)	0.217* (*)
	F(all collections)	0.282*†
Northeast vs. Yucatan/Pacific	*F*(within cities)	0.179* (2.3)
	F(between northeast and Yucatan/Pacific)	0.148*†
	F(all collections)	0.301*†
Yucatan versus Pacific	*F*(within cities)	0.155‡ (2.7)
	F(Yucatan versus Pacific)	0.051*†
	F(all collections)	0.199*†
Pacific	*F*(within cities)	0.025* (19.5)
	F(among cities)	0.124†**
	F(all collections)	0.146†§
Yucatan	*F*(within cities)	0.110¶ (4.0)
	F(among cities)	0.087*†
	F(all collections)	0.187*†
Northeast	*F*(within cities)	0.167# (2.5)
	F(among cities)	0.038* (12.7)
	F(all collections)	0.199* (2.0)

* 0.00000 ± 0.00000.
† Collections are genetically isolated by distance; *Nm* estimates are invalid. Probability (random value ≥ observed value) (100,172 permutations).
‡ 0.00416 ± 0.00060.
§ 0.06713 ± 0.00216.
¶ 0.00099 ± 0.00036.
# 0.12792 ± 0.00300.
** 0.03584 ± 0.00183.

form in frequency. Furthermore, transgenic mosquitoes or release of genes into populations within 150 km of one another, or along most locations along the Pacific coast should rapidly spread. At the same time, results from the Nuevo Laredo, Houston, Miguel Aleman, Moloacan, Minatitlan, Culiacan, and Tucson populations indicate that *Ae. aegypti* populations may shift in genetic composition either due to the introduction of foreign populations or due to genetic drift arising due to founders effects. It will be interesting to examine genes affecting DEN susceptibility or insecticide resistance in these populations to determine if loss or abrupt changes in genetic diversity affect these epidemiologically significant phenotypes.

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