Am. J. Trop. Med. Hyg., 89(3), 2013, pp. 482–488
doi:10.4269/ajtmh.13-0102

Phylogeography of *Aedes aegypti* (Yellow Fever Mosquito) in South Florida: mtDNA Evidence for Human-Aided Dispersal

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Abstract. The invasive dengue vector *Aedes aegypti* has persisted for > 200 years in South Florida in the United States. We tested the hypotheses that Florida's landscape creates dispersal barriers and corridors and that long-distance human-aided dispersal structures populations of *Ae. aegypti*. We evaluated the phylogeography of 362 individuals from Florida's East and West Coasts with a 760-bp (418- and 342-bp fragments of *ND5* and *ND4*, respectively) mitochondrial sequence. Populations from these two coasts were not significantly differentiated, suggesting that limited urbanization in central Florida is not a strong barrier to gene flow. Evidence for long-distance dispersal between Ft. Lauderdale and the West and Ft. Myers and the East indicates the importance of human-aided dispersal. West Coast populations showed no genetic differentiation, indicating that West Coast rivers and bays did not significantly impede gene flow. Phylogeographic analysis of haplotypes showed two distinct matrilines with no geographic patterns, suggesting multiple introductions or balancing selection.

INTRODUCTION

Aedes aegypti (yellow fever mosquito) is the major vector of arboviruses causing yellow fever and dengue.[1] Recent *Ae. aegypti*-driven outbreaks of Chikungunya in the Indian Ocean and Italy[2,3] and dengue in South America[4,5] and Key West, Florida[6] emphasize the continuing threat of *Ae. aegypti* to public health. *Ae. aegypti* invaded the Western Hemisphere about three centuries ago and established persistent populations in the states of Florida, Georgia, Louisiana[1] (K. Caillouet, personal communication), Arizona,[7] and Hawaii, despite intense eradication efforts in the 1950s and 1960s.[8]

Ae. aegypti is an urban container-dwelling species[9] that preferentially feeds on human hosts.[10] Human population density, road connectivity, transportation, and urbanization that facilitate human-aided long-distance dispersal (i.e., transport of immatures in water-filled containers like tires or transport of adults in vehicles) likely aided the expansion of *Ae. aegypti* populations.[5,11] In Florida, *Ae. aegypti* is now largely limited to urban areas on the East and West Coasts and some densely populated urban areas in central Florida.[12,13] Although the small-scale genetic structure of *Ae. aegypti* in Florida is likely to be distinct from the structure in Asia and Latin America because of differential dispersal potential associated with greater urban sprawl and the lack of opportunities to breed indoors in Florida,[12,14] the larger-scale population genetic structure is expected to be similar to the structure observed elsewhere, showing panmixia because of the wide dispersal capability of the species.[15] *Ae. aegypti* population history in Florida is complex as a result of founder effects,[16] adaptation,[17] multiple introductions, dispersal, bottlenecks, and expansions.[18,19]

Geographic expansion furthered by dispersal is a key reason for persistence of *Ae. aegypti* in multiple continents.[20] However, barriers to dispersal can place limits on the ultimate range and potential impact of invasive species. A detailed understanding of dispersal barriers may ultimately inform strategies to control this vector.[16,21] In this study, we test the hypothesis that landscape barriers (e.g., rivers, saltwater bays, and non-urban habitat) and dispersal corridors (e.g., roads and contiguous urban habitat) for *Ae. aegypti* affect gene flow patterns at the scale of the Florida peninsula. For example, wide river mouths are evident on the West Coast of Florida, but they are mostly absent on the East Coast of Florida (Figure 1), and human population density is high and evenly distributed along the East Coast, especially in the south, but heterogeneous for much of the West Coast (Figure 1). In Southwestern Florida, we postulate that rural expanses are barriers to *Ae. aegypti* because of insufficient human hosts and oviposition containers, both of which are necessary for dispersal of *Ae. aegypti*. We examined phylogeographic patterns based on mitochondrial DNA (mtDNA) variation of *Ae. aegypti* on the East and West Coasts of South Florida, including the Florida Keys. Specifically, we sampled from urban cemeteries, where water-filled vases offer permanent or semipermanent larval habitats to sustain *Ae. aegypti* populations.[13] We sampled containers within cemeteries, cemeteries within cities, and cities within coasts in a nested sampling pattern to examine the phylogeography and population structure of *Ae. aegypti* across Florida.

MATERIALS AND METHODS

Between June and October of 2006, we sampled cemeteries (hereafter called sites) in South Florida and collected larvae from all water-filled vases at each site where *Ae. aegypti* was present (Figure 1 and Supplemental Table 1). Because many of these sites harbored *Ae. aegypti*, *Ae. albopictus*, and *Ae. triseriatus*, we brought all larvae to the laboratory, identified them individually, and reared only *Ae. aegypti* to adulthood. Individual adults were placed in 95% ethanol on the day of eclosion.

DNA extraction and sequencing. DNA was extracted from individual mosquitoes using a well-established DNAzol protocol.[22] Published primers were used to amplify a 452-bp region of the *ND5* mtDNA sequence (forward: 5′-TCCTTAGAAT AAAATCCCGC-3′; reverse: 5′-GTTTCTGCTTTAGTTCA TTCTTC-3′)[23] and a 394-bp region of the *ND4* mitochondrial sequence (forward: 5′-GTD YAT TTA TGA TTR CCT AA-3′; reverse: 5′-CTT CGD CTT CCW ADW CGT TC-3′).[24]

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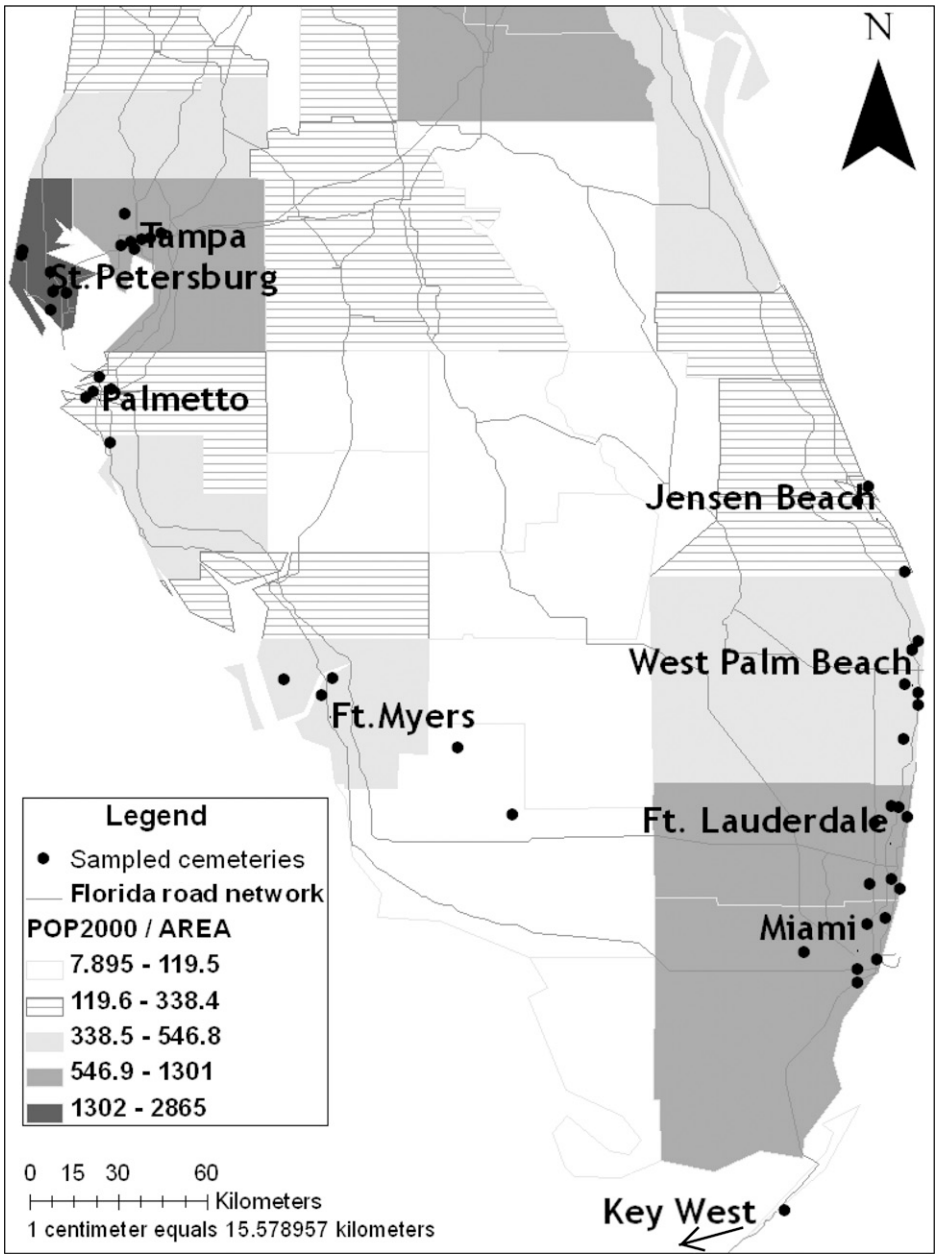

FIGURE 1. Sampled cemeteries in south Florida. Counties are differentiated based on human population density (population census 2000) compared with land area (square mile); road connectivity in south Florida is displayed. Black dots represent the cemeteries sampled within each city. <— = Key West is ~ 100 km south west.

We chose *ND4* and *ND5*, because previous studies on *Ae. aegypti*[11,24–30] and other mosquitoes[23,31,32] showed variability in the *ND4* and *ND5* sequences that yielded reliable gene genealogies suitable for inferring population structure and contributions of historical and current demographic processes. Both forward and reverse strands were sequenced using the ABI 3100 automated sequencer at the Keck Center, University of Illinois, Urbana-Champaign, IL. When singleton mutations were detected, those fragments were resequenced in both directions to exclude the probability of *Taq* polymerase amplification error.[33] Sequences were edited using Sequencher (ver. 3.0) and aligned using ClustalW option in SeaView.[34] The *ND5* and *ND4* gene fragments were trimmed to 418 and 342 bp, respectively, and they were then concatenated for a final 760-bp-long gene sequence (Hudson, Kreitman & Aguade test, $P = 0.8$); final analyses were based on the edited 760-bp fragment.

Statistical analysis. Haplotype diversity, number of haplotypes, and average number of nucleotide differences (π) for cemeteries within each city and among cities along the two coasts were calculated using DnaSP 5.0.[35] Identification of haplotypes, calculation of pairwise F_{ST}, analysis of molecular variance (AMOVA), and testing hypotheses of differentiation between the East and West Coasts and between cities within each coast were conducted using ARLEQUIN 3.1.[36] Effective migration rates were calculated as $N_em = (1 - F_{ST})/4F_{ST}$.[37] We used Tajima's D statistic[38] to assess deviations from neutrality that can indicate population history using DnaSP 5.0.[35]

For example, Tajima's *D* values below zero suggest increasing population size or purifying selection, whereas values above zero are consistent with decreasing population size and balancing selection; migration can result in a range of values.[39,40] As a rule of thumb, values greater than +2 or less than –2 are likely to be biologically significant.[40] An unrooted Unweighted Pair Group Method with Arithmetic mean (UPGMA) tree based on pairwise F_{ST} estimates was constructed to display genetic distances within and between East and West Coasts using MEGA 3.[41]

We used one-tailed Mantel tests with genetic (F_{ST}) and geographic distances as well as log-transformed genetic distance ($F_{ST}/1 - F_{ST}$) and log-transformed geographic distance across all sites[42] to test for isolation by distance (IBD).[37] We also used a Mantel test with restricted randomization,[43,44] stratified the data into East and West Coast groups, and thus, tested for IBD within coasts for the entire set of nine sites. Finally, we performed a Mantel test with restricted randomization, stratifying the data into East Coast, West Coast, and Florida Keys, to determine if the population at Key West had a disproportionate effect on patterns of IBD. In all cases, we used 999,000 permutations with the program RT v. 2.1[43] for Mantel tests. Euclidean distances between pairs of sites were calculated using ArcGIS. We implemented the spatial analysis of molecular variance (SAMOVA)[45] algorithm to identify groups of sampled populations (i.e., *K* groups) that are maximally differentiated from one another without any *a priori* assumptions about population structure. We performed 1,000 annealing processes for two to four groups ($K = 2$–4).

RESULTS

Within cemeteries, the number of individual vases that held *Ae. aegypti* varied between 1 and 19. A total of 68 mitochondrial haplotypes was identified from 362 sequenced *Ae. aegypti* individuals (Supplemental Table 2). A single haplotype (AEF6) was shared across all of nine cities sampled, whereas AEF4 was present in all of the inland cities (Supplemental Table 2). A total of 20 haplotypes was shared in at least two of the sampled cities; a majority of the haplotypes (48), however, were found only within a single city (Tampa = 11, St. Petersburg = 5, Palmetto = 4, Ft. Myers = 2, Miami = 9, Ft. Lauderdale = 8, West Palm Beach = 5, and Jensen Beach = 4). The nucleotide sequences were characterized by 35 polymorphic sites, of which 33 sites were parsimony-informative. Nucleotide substitutions were identified at 35 of 760 sites, of which 91.42% were transitions.

Haplotype and nucleotide diversities were comparable on the West Coast (0.870 and 0.01362, respectively) and the East Coast (0.901 and 0.01329, respectively) (Supplemental Table 3). There was a moderate level of genetic differentiation when considering all of the cemeteries sampled across South Florida ($F_{ST} = 0.05708$, $Nm = 8.26$). Within cities, across cemetery sites, values of F_{ST} ranged between 0 (Ft. Myers, Jensen Beach, and West Palm Beach) and 0.17430 (St. Petersburg). The hierarchical AMOVA of all sites, except Key West, did not detect any significant differentiation between the East and West Coasts ($F_{CT} = -0.00159$, $P > 0.05$); however, the differentiations among cities within each coast ($F_{SC} = 0.02835$) and within cities ($F_{ST} = 0.02681$; both $P < 0.01$) were significant (Supplemental Table 4). In a separate AMOVA comparing three groups (East Coast, West Coast, and Key West), results were similar: the coasts and Key West were not differentiated from one another ($F_{CT} = 0.0067$, $P > 0.2$) (Table 1). An AMOVA of the West Coast sites alone confirmed extensive gene flow among cities along this coast ($F_{CT} = -0.00240$, $P > 0.05$) and to a lesser extent, cemeteries within cities ($F_{SC} = 0.05128$, $P > 0.05$) but significant differentiation within cemeteries ($F_{ST} = 0.04901$, $P < 0.0430$). In contrast, a similar analysis for the East Coast sites alone detected significant differentiation only among cities ($F_{CT} = 0.05577$, $P = 0.0146$) (Supplemental Table 4).

Pairwise comparison of genetic differentiation between cities indicated significant differences between Jensen Beach and both Ft. Lauderdale and Key West after Bonferroni correction ($P < 0.0013$) (Supplemental Table 5). The F_{ST}-based UPGMA tree (Figure 2) highlights likely long-distance dispersal between Ft. Lauderdale and the West as well as Ft. Myers and the East, a close connection between Tampa and Palmetto separate from other sites, and the genetic distinctness of the Key West and Jensen Beach populations. The haplotype network identified AEF6 (the haplotype shared across all sampled cities) as most ancestral (Supplemental Figure 1 and Supplemental Table 2). Analyses of mismatch frequencies of pairwise sequence differences and segregating sites display bimodal distributions (Supplemental Figures 2 and 3). Although this result is consistent with two distinct clades, these clades are less obvious in the network, which had no clear spatial patterns in haplotype distribution. Tajima's *D* tests for deviations from neutrality were significant for all cities in the West Coast but none of the East Coast cities (Supplemental Table 6). Although not always significant, Tajima's *D* values were greater than two for many sites (Supplemental Table 6), which can be indicative of a high degree of dispersal.

Unrestricted analysis of all nine sites yielded a significant positive correlation between genetic and geographic distances (one-tailed Mantel test, $R_M^2 = 0.093$, $P = 0.0399$) (Figure 3). Correlation of log–log distances approached significance (one-tailed Mantel test, $R_M^2 = 0.077$, $P = 0.0635$).[42] Because

TABLE 1

Partitioning of variance components and F_{ST} of partial *ND4* and *ND5* mitochondrial haplotypes identified from cities in south Florida, including Key West

Source of variation	df	Sum of squares	Variance components	Percent variation	Φ Statistics
East Coast, West Coast, and Key West					
Among groups	2	25.243	0.03443	0.67	0.00670
Among cities within groups	6	67.042	0.14769	2.88	0.02895*
Within cities	354	1,753.599	4.95367	96.45	0.03546†
Total	362	1,845.884	5.13580		

* $P < 0.05$.
† $P < 0.001$.
df = degrees of freedom.

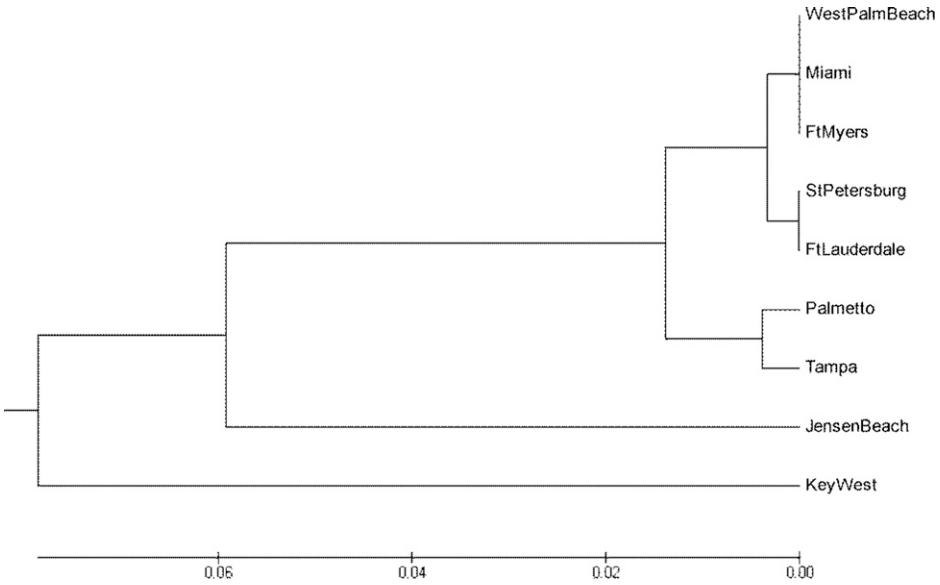

FIGURE 2. F_{ST}-based UPGMA tree showing the relationship between sampled cities in south Florida.

our analyses also showed differentiation of East versus West Coast populations and the Key West population, this apparently significant correlation in unrestricted analysis may be misleading. Our restricted randomization Mantel test, stratifying populations into East and West Coasts, yielded a marginally non-significant relationship between geographic and genetic distances (one-tailed Mantel test, $R_M^2 = 0.103$, $P = 0.0510$). In contrast, a restricted randomization Mantel test, with randomizations stratified into East Coast, West Coast, and Florida Keys, yielded a clearly non-significant relationship between genetic and geographic distances (one-tailed Mantel test, $R_M^2 = 0.019$, $P = 0.2017$). Thus, it seems that most of the apparent relationship of genetic and geographic distances derives from populations on the two coasts (and thus, geographically far apart) being genetically distinct and the population at Key West being both distinct and geographically distant from most others.

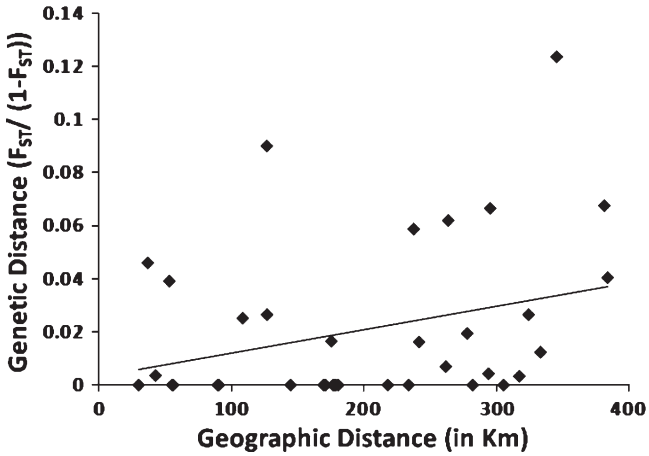

FIGURE 3. Correlation of genetic [$F_{ST}/(1 - F_{ST})$] distance of *A. aegypti* partial *ND4* and *ND5* mtDNA sequences and geographic distance across all sampled cities in South Florida [negative values of $F_{ST}/(1 - F_{ST}) = 0$; $P = 0.2017$].

SAMOVA on the mainland cities alone (excluding Key West) identified two optimal groups (Jensen Beach versus all others) based on the most significant F_{CT} index (Supplemental Table 7). SAMOVA including Key West showed four optimal groups (Table 2), suggesting that there were at least three putative barriers to dispersal or distinct population histories of *Ae. aegypti* in this geographic region. These groupings are consistent with the F_{ST}-based UPGMA tree (Figure 2), which also shows Key West as the most distinct population, with Jensen Beach next and Palmetto as divergent from all remaining populations except Tampa.

DISCUSSION

Long-distance human-aided dispersal. *Ae. aegypti* dispersal depends on two main factors: the availability and movement of containers and human transport.[7,46–48] If *Ae. aegypti* dispersal within Florida occurs only by flying adults, then numerous, uniformly distributed aquatic container habitats would seem to be necessary to act as stepping stones for gene flow and long-distance dispersal over time. However, unlike many developing countries where *Ae. aegypti* populations have been studied, peridomestic aquatic container habitats, such as domestic water storage containers, seem to be relatively rare in the United States.[14] Consequently, we postulated that relatively low human population density and associated scarcity of peridomestic habitat in Central South Florida as well as the West Coast rivers and water bodies would impede dispersal of *Ae. aegypti* (Figure 1) between the coasts. We find, however, no significant differentiation between the East and West Coast populations, suggesting that practically no large-scale barriers to gene flow exist. This result explains the presence of considerable human-aided long-distance dispersal of *Ae. aegypti* in South Florida (Supplemental Table 4). Such human-aided dispersal may explain both the clustering of Ft. Myers' populations with the populations on the East Coast and the clustering of Ft. Lauderdale's populations with St. Petersburg's population on the West Coast (Figure 2).

TABLE 2

Fixation indices corresponding to the groups of populations inferred by SAMOVA analysis for *Ae. aegypti* populations in south Florida (with Key West) tested for partial *ND4* and *ND5* mitochondrial sequences

No. of groups	Group composition	F_{SC}	F_{ST}	F_{CT}
2	1. Key West; 2. all other populations	0.0233*	0.0974*	0.07592
3	1. Key West; 2. Jensen Beach; 3. all other populations	0.0145*	0.0884*	0.07492*
4	1. Key West; 2. Jensen Beach; 3. Palmetto; 4. all other populations	–0.0003*	0.04486	0.04515†

*$P < 0.05$.
†$P < 0.001$.

The absence of a significant pattern of isolation by distance also suggests a prominent role for human-aided dispersal, a likely mechanism disrupting any population structure based on dispersal by adult flight.

Tajima's *D* values of *Ae. aegypti* populations in Miami and the West Coast are unusually high and positive, and they yield an overall significant positive value across all sampled cities. Although population decrease and balancing selection can result in significant positive Tajima's *D* values, values more than two are rare. Simulation models indicate that extremely high *D* values (i.e., > 2) are six to seven times more likely with an average migration rate of 0.1/generation.[40] Migration rather than local bottlenecking or selection on mtDNA seems the most parsimonious explanation for the high *D* values for West Coast cities and Miami, especially because the UPGMA tree (Figure 2) and the pairwise effective number of migrants also suggest high immigration into those cities, with the exception of Ft. Lauderdale. It is beyond the scope of our study to test alternative hypotheses of bottlenecks versus dispersal to explain these large Tajima's *D* values (R. Nielsen, personal communication).

The Key West population exhibited a negative, albeit nonsignificant Tajima's *D*, suggesting either population expansion or purifying selection. Key West is known for its extensive mosquito control program (http://keysmosquito.org/), and it is a popular tourist destination, which may contribute to human-aided immigration of *Ae. aegypti* into the island. Hence, repeated bottleneck–recolonization events may be common in Key West, and they may contribute to the differentiation of this population evident in SAMOVA (Table 2).

Cryptic barriers to gene flow. In contrast to the West Coast, where gene flow was extensive (Table 2 and Supplemental Tables 4 and 5), the differentiation among East Coast cities was unexpectedly significant given the almost contiguous road network and urban areas that should provide corridors for natural dispersal as well as human-aided dispersal. Although effective vector control in East Coast cities could have created patterns of asynchronous local bottlenecking and recolonization, we found no evidence supporting such population fluctuations, suggesting that there may be cryptic barriers to gene flow (e.g., patchy distribution of peridomestic habitat) in the urban landscape. Study of *Ae. aegypti* populations across the east and west sides of Uriah Butler Highway in Trinidad, West Indies, using mtDNA and microsatellite DNA showed a similar pattern, where cryptic barriers in the urban landscape limited dispersal.[49] Another detailed, fine-scale analysis of local landscapes within and between East Coast cities using nuclear markers would be necessary to identify such barriers.

Invasion history. As an invasive species in South Florida, we might expect that *Ae. aegypti* would show reduced genetic diversity,[16,19] but the evidence is mixed; also, comparable data are limited. For example, we identified 68 haplotypes from 362 individuals (0.886 haplotype diversity [*Hd*]) for the combined *ND4* and *ND5* mtDNA fragments, and we identified 12 haplotypes (*Hd* = 0.713) for *ND5* and 42 haplotypes (*Hd* = 0.817) for *ND4* when analyzing the fragments separately. By comparison, native mosquitoes have greater genetic diversity. For example, *Ae. vexans*[31] displayed 34 *ND5* haplotypes among 54 individuals (0.953 *Hd*), and *Culex tarsalis*[32] displayed 64 *ND4* haplotypes among 170 individuals (0.887 *Hd*). Genetic diversity of *Ae. aegypti* in Southern Florida is consistent with a complex history of long-distance dispersal and extinction–recolonization since its invasion.[50–53]

Comparison of *Ae. aegypti* population structure with other geographic areas. Our sequence and phylogenetic analyses of the *ND4* and *ND5* mtDNA fragments documented two matrilineal clades that had no geographic structure, similar to *Ae. aegypti* studied in several other geographic areas.[11,15,24–30,54] Discovery of two *ND4* mtDNA clusters of *Ae. aegypti* in Northeastern Mexico provided the first evidence of introductions of two independent mitochondrial lineages or a newly introgressed matriline.[24,47] Other *ND4* mtDNA studies came to similar conclusions for *Ae. aegypti* populations in Thailand,[25] Peru,[26] Venezuela,[27] Brazil,[28,54] and several sites in South America and Africa[29,30] and for *Ae. japonicus*, a recent invader into the United States.[55] An alternative explanation could be that two clades exist in the source population (African continent),[30] which could have been introduced into Florida through a single invasion event. To identify the source populations for such introductions into the Americas, comparisons[29] of two clusters of *Ae. aegypti ND4* haplotypes from the Americas with haplotypes from three localities each in Africa and Asia/Polynesia indicated that one cluster was more similar to Asia/Polynesia (Singapore, Cambodia, and Tahiti) and Africa (Uganda and Guinea), whereas the other cluster was more closely related to Senegal in Africa. Although most of the above studies have used the *ND4* partial sequence, our analysis of both *ND4* and *ND5* partial sequences again showed a similar pattern, supporting the multiple introduction hypothesis. Although nuclear copies of mtDNA may explain clustering of haplotypes into two distinct clades,[24–30,54,56,57] we confirmed the absence of nuclear copies in our *Ae. aegypti* mtDNA sequences.

In summary, we have some evidence for two separate introductions of *Ae. aegypti* into Southern Florida, which has been postulated by other authors, and find no evidence for a significant barrier to dispersal between the East and West Coasts of Florida. We interpret our results as evidence for a prominent role of human-aided long-distance dispersal and incidence of panmixia between these coasts, which has been illustrated in the case of *Ae. aegypti* in the Americas using a Bayesian coalescent framework-based gene flow network model analysis.[15] Significant long-distance and human-aided dispersal

in Florida may have implications for vector control efforts directed at this species. Such intercity dispersal is likely to interfere with local vector eradication attempts by contributing to recolonization if local eradication is achieved. For transgenic approaches to vector control or eradication (e.g., sterile male release and release of insects with dominant lethal genes), long-distance dispersal and population connectivity can have important effects on surrounding non-target populations, but the effects depend on the control method used.[21] Intercity dispersal argues for a statewide or regional approach to attempts at vector eradication or control. The evidence presented here for absence of barriers to dispersal and the importance of human-aided dispersal may be important considerations for efforts to combat recent increases in dengue activity in Florida.

Received February 24, 2013. Accepted for publication July 2, 2013.

Published online August 5, 2013.

Note: Supplemental figures and tables appear at www.ajtmh.org.

Acknowledgments: We are grateful to L. P. Lounibos, G. F. O'Meara, and W. J. Tabachnick for their input and laboratory space and B. Kesavaraju, P. Leisnham, and D. Bustamante for help with collecting.

Financial support: This study was funded by Phi Sigma and Graduate Student Association Grants from Illinois State University (to K.D.), National Institutes of Health Grant AI R15-068692-01 (to S.A.J. and S.S.L.), and a grant from Illinois State University (to S.A.J.).

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