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PHYLOGEOGRAPHY AND POPULATION STRUCURE OF *AEDES AEGYPTI* IN ARIZONA

SAMUEL A. MERRILL, FRANK B. RAMBERG, AND HENRY H. HAGEDORN
Department of Entomology and Center for Insect Science, University of Arizona, Tucson, Arizona

Abstract. *Aedes aegypti*, the mosquito responsible for transmitting dengue, has colonized many cities and towns throughout Arizona. Determining both the migration between, and the origin of, local *Ae. aegypti* populations is important for vector control and disease prevention purposes. Amplified fragment length polymorphism was used to infer geographic structure and local substructure, and effective migration rates (M, migrants per generation) between populations, and to determine genetic differentiation between populations (Φ_{PT}). Three geographically and genetically differentiated groups of populations were identified. Population substructure was only detected in the border town of Nogales. Reliable estimates of M between regions ranged from 1.02 to 3.41 and between cities within regions from 1.66 to 4.44. In general, pairwise Φ_{PT} were lowest between cities within regions. The observed patterns of genetic differentiation suggest infrequent migration between populations and are compatible with the idea of human transport facilitating dispersal between regions.

INTRODUCTION

The mosquito *Aedes aegypti* is the principal vector of the dengue viruses. Fifty to 100 million dengue cases are reported each year.[1,2] Limiting the spread of dengue is contingent upon understanding vector migration and population structure.[3,4] *Aedes aegypti* was first found in Tucson, Arizona in 1946.[5,6] The disappearance of *Ae. aegypti* from Tucson was noted in 1969 through arbovirus surveillance, but reemergence occurred in 1994, with Nogales, Arizona becoming colonized by *Ae. aegypti* the following year.[7] Further investigation showed that *Ae. aegypti* had also colonized the southern Arizonan towns of Naco, Douglas, and Benson.[8]

Vector movement and transport are important factors influencing dengue transmission.[9] Dengue is a health concern in Arizona due to the existence of endemic and epidemic dengue activity in the neighboring state of Sonora, Mexico and along the Texas-Mexican border.[7,10] In addition to the importation of infected adult females and virus introduction from traveling viremic humans, dengue viruses could become introduced to naive *Ae. aegypti* populations through the importation of infected eggs. Multigenerational, transovarial passage and horizontal transmission of dengue from vertically infected progeny has been observed with *Ae. aegypti*.[11,12] Previous work determined that the Tucson population of *Ae. aegypti* could transmit dengue and was most closely related to the Pacific population of Mexico, but other Arizonan populations were not analyzed.[13–16]

Amplified fragment length polymorphism (AFLP) has been well documented, and is aptly suited for analysis of *Ae. aegypti* populations.[17–21] Other molecular marker systems are not well suited for use with *Ae. aegypti*. Microsatellites are not abundant in *Ae. aegypti* and the number of variable loci used in microsatellite studies has been low.[22–24] Mitochondrial sequencing requires prior sequence knowledge and variation may be limited due to colonization events. Random amplified polymorphic DNA loci were not useful for comparisons of distant populations and their reliability has been questioned.[15,25–27] Restriction fragment length polymorphism loci were informative, co-dominant markers, but successful digestion necessitates substantial amounts of genomic DNA and hybridization would be time-consuming.[25]

The aims of this study were to determine genetic differentiation and migration between *Ae. aegypti* populations, detect population substructure, and identify possible embarking populations that may have begun the colonization of Arizona. These aims serve to evaluate the possibility of dengue introduction in Arizona through mosquito transport and to predict the spread of insecticide resistance resulting from control measures. This can inform future policy decisions regarding mosquito control and disease prevention measures. Global positioning system coordinates of positive traps are included to aid future studies because we observed areas within infested cities consistently negative for *Ae. aegypti* (Merrill SA, unpublished data).

Cladistic analysis using genetic distance was performed to detect population structure within geographic populations by allocating individuals into genetic populations. Pairwise Φ_{PT}, an estimator of F_{ST},[28–30] was computed on genetic populations and further cladistic analysis of linearized $\Phi_{PT}/(1 - \Phi_{PT})$ was used to assign these genetic populations to genetic regions.[31] Distant, well-established *Ae. aegypti* geographic populations corresponding to the genetic populations found within the subsequent genetic regions were identified as putative embarking cities of colonization. To analyze migration between cities in the study area, pairwise Φ_{PT} values were again calculated for discrete geographic populations. Effective migration (M) between geographic populations was then estimated from these pairwise Φ_{PT} values.[30,31] Cladistic analysis of linearized $\Phi_{PT}/(1 - \Phi_{PT})$ from geographic populations was used to assign geographic populations into geographic regions. Analysis of molecular variance (AMOVA) was conducted using these geographic regions.

MATERIALS AND METHODS

Mosquito collections. Oviposition traps using hay infusion were modeled after those described previously.[16] Oviposition traps were placed on transect lines through small towns, and in multiple neighborhoods in larger cities (Figure 1 and Table 1). Egg papers were collected at four-day intervals, and trapping was continuous in Benson, Willcox, St. David, Douglas, Naco, Tempe, and Sierra Vista during the dates indicated. Traps were deployed for one week each month in Tucson and Nogales. Egg papers from individual traps were dried overnight, eggs were counted, and the papers were immersed in water to induce hatching. Larvae were fed a sieved homogenate of high-protein rabbit pellets and soy meal. Individual

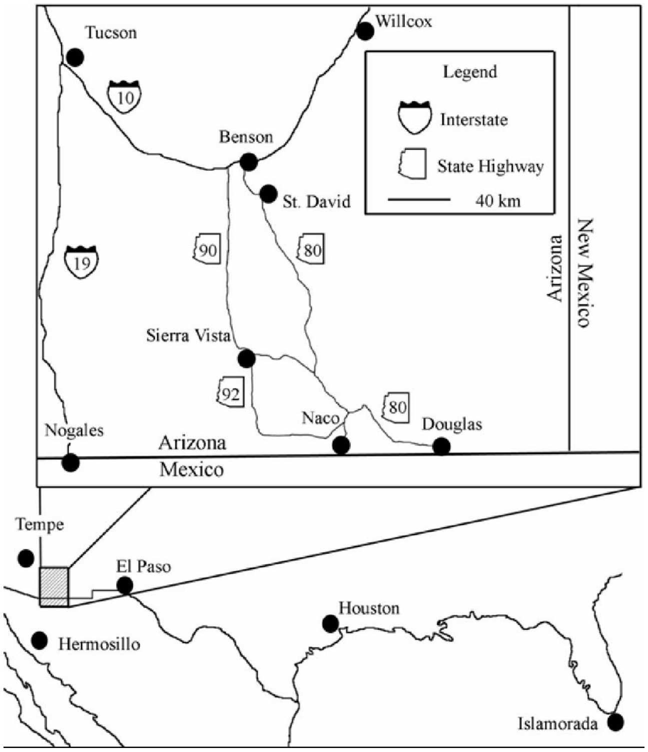

FIGURE 1. Map of *Aedes aegypti* collection locations in Arizona. **Inset** details roadway relationships between *Ae. aegypti*-colonized cities in southern Arizona.

pupae were harvested and stored at −80°C prior to DNA extraction. Larval exuviae for each site were examined to confirm that the specimens were *Ae. aegypti* and to rule out possible contamination by other container-breeding mosquitoes known to inhabit the study area.

Gravid and carbon dioxide traps were placed at the locations indicated in Table 1, and collected mosquitoes were stored at −80°C prior to identification. All collected adults were examined to ascertain species identity, females were dissected to insure that they did not have a blood meal, and spermathecae were removed from females to prevent genomic contamination from mating.

Samples from Islamorada, Florida were provided from the Florida Medical Entomology Laboratory (Vero Beach, FL) as pupae reared from eggs collected in Monroe County, Florida, and were processed as above. Samples from Tempe were provided as eggs from the Arizona Department of Health Services Vector Borne and Zoonotic Disease Section (Phoenix, AZ) and were reared and processed as above. Houston field-collected adults were provided by Adilelkhidir Bala of the Harris County Health Department, Mosquito Control Division (Houston, TX). Samples from Hermosillo, Mexico were provided by Suzanne Hammer (University of Arizona) as DNAs from adults collected in 1998 using carbon dioxide traps.

Extraction of DNA. Individuals were chosen from all traps and collection dates per location and assigned a unique identification code. The extraction protocol followed that of Goldberg and others, with minor modifications.[32] Individual specimens were homogenized using plastic pestles in 1.5-mL centrifuge tubes containing 350 μL of lysis buffer (100 mM Tris-HCl, pH 8.0, 2% sodium dodecyl sulfate, 100 mM NaCl, 50 mM EDTA, 100 mM sucrose) and 12 μL of proteinase K (10 mg/mL), and Phase Lock tubes (Eppendorf AG, Hamburg, Germany) were used in phase separation as per the manufacturer's instructions. Samples were then dried under vacuum and suspended in 50 μL of TE$_{0.1}$. Suspended samples were stored at −20°C. Genomic DNA was quantified with PicoGreen (Molecular Probes, Inc., Eugene, OR) as per the manufacturer's instructions. DNA aliquots were standardized to a concentration of 15 ng/μl with TE$_{0.1}$ (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA).

Amplified fragment length polymorphism analysis. The AFLP was conducted with adapter and primer sequences described by Yan and others (Qiagen, Inc., Valencia, CA).[21] Digestion and ligation reactions were coupled to drive the formation of ligated genomic DNA. The 11-μL digestion/ligation reactions contained 3 μL of genomic DNA standardized at 15 ng/μl. One unit of *Mse* I endonuclease, five units of *Eco* RI endonuclease, and 0.15 Weiss units of T4 DNA ligase (New England Biolabs, Inc., Beverly, MA) were used in each reaction containing 50 μmoles of *Mse* I adapter, 5 μmoles of *Eco* RI adapter, 0.05 M NaCl, 0.55 μg of bovine serum albumin (BSA), and 10× T4 ligase buffer (1× T4 ligase buffer = 50 mM Tris-HCl, pH 7.5, 10 mM MgCl$_2$, 10 mM dithiothreitol, 1 mM ATP, 25 μg/mL of BSA). The *Eco* RI and *Mse* I adapters were constructed by combining and annealing equimolar amounts of the corresponding forward and reverse oligonucleotides. Reactions were covered with 13 μL of mineral oil to prevent evaporation, and incubated at 37°C for 2.5 hours. Reactions were then diluted with 90 μL of TE$_{0.1}$.

Primary polymerase chain reaction (PCR) amplifications were performed in a volume of 30 μL containing 3 μL of dilute digestion/ligation product, 0.5 units of *Taq* polymerase (Eppendorf AG), 0.2 mM deoxynucleotide triphosphates (dNTPs) (Fermentas Inc., Hanover, MD), 0.2 μM *Eco*-A primer, 0.2 μM *Mse*-C primer, and 10× PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3, 20 mM MgCl$_2$). After incubation at 72°C for 2 minutes, amplification consisted of 22 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1 minute. Amplifications were verified by subjecting 8 μL of each reaction to electrophoresis on a 3% agarose gel. Primary amplification products were diluted in 189 μL of TE $_{0.1}$.

Secondary PCR amplifications used two primer combinations: *Eco*-AGG plus *Mse*-CTT and *Eco*-ACA plus *Mse*-CAC. The *Eco*-AGG and *Eco*-ACA primers were 5′ labeled with 6-carboxyfluorescein (6-FAM) for fluorescent detection (Applied Biosystems, Foster City, CA). The 15-μL reactions volumes contained 3 μL of dilute primary amplification product as template, 0.5 units of HotMaster Taq polymerase (Eppendorf AG), 0.2 mM dNTPs, 0.167 μM *Eco* primer, 0.194 μM *Mse* primer, and 10× HotMaster PCR buffer. A touchdown protocol (9 cycles at 94°C for 30 seconds, 65°C with −1°C /cycle, 72°C for 1 minute) was used to prevent spurious primer annealing, followed by 30 cycles of regular amplification at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1 minute. Amplifications were verified by subjecting 8 μL of each reaction to electrophoresis on a 3% agarose gel. Secondary amplification products were diluted with 40 μL of molecular biology grade water and submitted for capillary electrophoresis on an ABI 3100 apparatus (Applied Biosystems) at the University of Arizona Genomic Analysis and Technology Core. Negative controls, lacking only template

TABLE 1

Cities, dates, coordinates, trap type used, and sample sizes per trap of *Aedes aegypti* collected in Mexico and the United States

		North latitude			West longitude				
City	Trap	Deg	Min	Sec	Deg	Min	Sec	Type*	Number
Tucson, AZ	170	32	14	50.47	110	56	14.98	Ovi	16
8/02–9/02	177	32	13	12.2	110	54	11.3	Ovi	8
	179	32	16	21.2	110	47	51.3	Ovi	7
	186	32	13	25.6	110	48	44.8	Ovi	11
	187	32	14	39.5	110	48	33.6	Ovi	7
	189	32	22	35.9	111	2	44.1	Ovi	6
	190	32	14	2.7	110	49	56.3	Ovi	8
	192	32	21	24.2	111	0	6.9	Ovi	9
	194	32	10	55.5	110	49	18	Ovi	4
	196	32	15	58.6	110	54	47	Ovi	10
	197	32	13	6.1	110	55	41.8	Ovi	14
	198	32	11	19.7	110	49	42.5	Ovi	12
Nogales, Mexico	144	31	17	15.4	110	55	51.2	Ovi	4
8/02–9/02	147	31	19	49.5	110	57	7.6	Ovi	7
	148	31	16	14.2	110	55	37.5	Ovi	8
	151	31	15	49.9	110	57	23.6	Ovi	7
	156	31	15	46.9	110	56	39.2	Ovi	11
	162	31	19	42.3	110	55	28.7	Ovi	4
	169	31	19	39.79	110	57	5.8	Ovi	4
	171	31	17	1.2	110	57	48.4	Ovi	3
	172	31	16	13.7	110	57	53.3	Ovi	4
	188	31	20	30	110	56	27.4	Ovi	3
Benson, AZ	A	31	57	14.5	110	17	7.8	Ovi	14
8/1/03–9/23/03	B	31	57	14.58	110	17	0.08	Ovi	5
	C	31	57	14.85	110	17	9.63	Ovi	13
	D	31	54	5.07	110	13	4.52	Ovi	2
	E	31	57	15.67	110	17	8.28	Ovi	2
	G	31	57	15.45	110	17	7.22	Ovi	18
St. David, AZ	B	31	53	7.42	110	12	11.95	Ovi	10
8/1/03–9/23/03	D	31	54	5.52	110	13	0.7	Ovi	2
	E	31	54	5.07	110	13	4.52	Ovi	13
	G	31	54	5.2	110	13	6.97	Ovi	13
	H	31	54	5.07	110	13	4.17	Ovi	15
Sierra Vista, AZ	B	31	32	1.47	110	17	13.47	Ovi	17
8/1/03–9/23/03	C	31	32	16.12	110	17	14.22	Ovi	7
	D	31	32	15.53	110	17	13.65	Ovi	10
	E	31	32	15.32	110	18	3.68	Ovi	12
	F	31	32	15.37	110	18	0.52	Ovi	10
	G	31	32	14.72	110	18	1.23	Ovi	9
	H	31	32	14.75	110	18	2.87	Ovi	7
Naco, AZ	A	31	32	14.72	109	57	1.73	Ovi	9
8/1/03–9/23/03	C	31	20	3.37	109	56	15.75	Ovi	9
	D	31	20	2.7	109	56	15.77	Ovi	16
	E	31	20	2.52	109	56	14.38	Ovi	20
Willcox, AZ	P	32	15	12.13	109	50	2.88	Ovi	2
8/1/03–9/23/03	M	32	15	11.88	109	50	1.93	Ovi	†
Douglas, AZ	B	31	20	5.62	109	33	5.22	Ovi	13
8/1/03–9/23/03	C	31	20	4.57	109	33	3.58	Ovi	17
	D	31	20	5.65	109	33	3.55	Ovi	4
	E	31	20	9.58	109	32	4.17	Ovi	4
	G	31	20	9.53	109	32	7.9	Ovi	9
	H	31	20	9.55	109	32	8.88	Ovi	17
	K	31	21	4.17	109	32	12.85	Ovi	9
	L	31	20	11.7	109	32	14.05	Ovi	8
	M	31	20	11.73	109	32	15.58	Ovi	5
Houston, TX	111	29	42	4.82	95	22	21.08	CO_2	2
9/9/03–9/17/03	114	29	42	8.98	95	20	39.64	CO_2	4
	115	29	42	51.82	95	19	16.06	CO_2	8
	116	29	44	27.7	95	21	45.47	CO_2	2
	117	29	43	28.71	95	22	12.64	CO_2	12
	120	29	42	21.53	95	21	54.56	CO_2	2
	123	29	41	31.73	95	20	12.92	GV	1
	125	29	42	51.82	95	19	16.06	GV	1
	212	29	46	40.5	95	18	15.89	CO_2	23
	214	29	46	26.65	95	19	59.05	CO_2	2
	224	29	46	26.65	95	19	59.05	GV	1
	320	29	49	37.91	95	22	23.06	CO_2	4
	412	29	48	12.43	95	30	55.77	CO_2	1
	419	29	49	6.52	95	32	28.93	CO_2	3
El Paso, TX	11	31	45	1.2	106	28	11.28	Ovi	6
8/30/03–9/1/03	21	31	46	4.22	106	29	1.1	CO_2	1
	24	31	46	13.5	106	29	10.18	CO_2	2
Tempe, AZ	16	33	23	13.3	111	55	12.92	Ovi	31
6/01–8/01	19	33	23	13.3	111	55	13.93	Ovi	6
	35	33	23	14.77	111	55	13.17	Ovi	9
	39	33	23	14.75	111	55	10.23	Ovi	4
	40	33	23	14.85	111	55	10	Ovi	6
	44	33	23	16.1	111	55	15.27	Ovi	3
	53	33	40	3.95	111	93	9.53	Ovi	3

* Ovi = oviposition; CO_2 = carbon dioxide; GV = gravid.
† Samples not viable.

DNA, were included in each phase of analysis to detect possible contamination.

Electropherograms were analyzed using Genotyper 3.7 software (Applied Biosystems). Amplification was verified by inspecting individual electropherograms. Successfully amplified samples for population analysis were scored at 254 polymorphic loci for the *Eco*-AGG plus *Mse*-CTT primer pair and at 254 polymorphic loci for the Eco-ACA plus *Mse*-CTT primer pair.

Robustness of the AFLP. Reproducibility and discriminatory power of the AFLP were first investigated by analyzing controlled outcrosses. Field-collected (Nogales, AZ) and colony mosquito eggs (F_{32}, origin: Tucson, AZ) were reared to pupae and isolated individually before emergence. After emergence, colony and field-collected mosquitoes were individually mated and females were fed bovine blood using a feeder. Progeny from the crosses, reared to pupae, and parents were analyzed as described earlier. Using PAUP, siblings from one cross were distinguishable from siblings from other crosses on a phylogram constructed using 421 AFLP loci, minimum evolution optimality, total genetic distance, and a heuristic search using neighbor joining.[33] In one randomly selected family of 42 individuals, maternal inheritance was not seen in any of the 11 loci for which the male was null (Merrill SA, unpublished data). This was consistent with prior inheritance studies of AFLP that indicated low numbers of maternal mitochondrial loci.[34]

Identification of genetic populations through cladistic analysis. The AFLP markers were used under the assumptions of Hardy-Weinberg equilibrium, independent assortment, and identity by descent of both fragment presence and absence. Five hundred eight polymorphic AFLP loci were used to analyze individuals and characterize the populations in the study area.

PAUP was used to generate an individual-based cladogram to detect substructure in geographic populations, thus defining genetic populations. Using minimum evolution, total character difference from 508 polymorphic AFLP loci the midpoint rooted tree was constructed using neighbor-joining and tree-bisection-reconnection.[33]

Cladistic and statistical analyses of genetic populations. Pairwise Φ_{PT} and analysis of molecular variance were computed with 999 permutations using GenAlEx.[18,35] Linearized $\Phi_{PT}/(1 - \Phi_{PT})$ data from genetic populations were examined with PAUP through a minimum evolution, total character difference, unrooted cladogram created using a heuristic search with neighbor-joining and tree-bisection-reconnection branch swapping.[33]

Cladistic and statistical analyses of geographic populations. Pairwise Φ_{PT} and analysis of molecular variance were computed for geographic populations, i.e., all individuals collected in one city, as described earlier. Categories of population differentiation were determined from these pairwise Φ_{PT} values.[36] Pairwise Φ_{PT} values were used to estimate effective migration rates (*M*) between populations.[19,20] Linear $\Phi_{PT}/(1 - \Phi_{PT})$ data were examined with PAUP as described earlier to define geographic population regions for use with AMOVA.

RESULTS

Genetic populations determined through cladistic analysis. The PAUP cladogram is included in Appendix 1 due to size constraints. Appendix 1 can be accessed at www.ajtmh.org. Clades representing Hermosillo, Tempe, and Islamorada did not contain samples from other locations. Other clades, formed from multiple traps and collection dates in one city, contained individuals from other cities (Table 2).

In general, individuals collected from opposite ends of each city clustered together and population substructure based on geography was not observed. However, substructure was detected in Nogales, Sonora. The majority of Nogales, Sonora individuals were in a clade containing individuals from Hermosillo, Tempe, and Tucson, but individuals from traps 147 (July and August), 151 (August), and 169 (August) clustered with individuals from St. David and Sierra Vista. These traps were located in western Nogales, near the border with Arizona, and represented the putative Nogales Minor population (Figure 2).

Cladistic and statistical analyses of genetic populations. For cladistic representation, the Φ_{PT} /(1 – Φ_{PT}) values were calculated with Nogales, Sonora divided into two genetic populations (Nogales and Nogales Minor) as illustrated in the above analysis. Three population regions were observed in the study area, and clear cladistic division of the Nogales and Nogales Minor populations was found (Figure 2). The Western region incorporated Tucson, Hermosillo, Tempe, and Nogales; the Eastern region included Willcox, Houston, El Paso, and Islamorada; the Cochise region contained Douglas, Naco, Sierra Vista, Benson, St. David, and the Nogales Minor population.

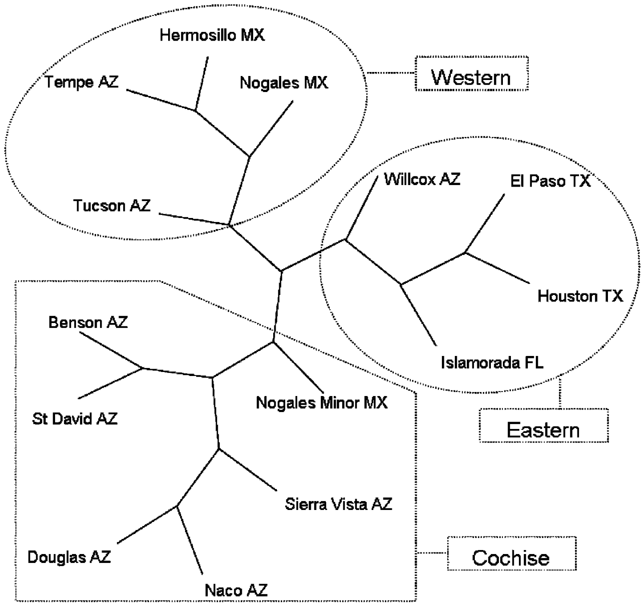

FIGURE 2. Unrooted cladogram of pairwise $\Phi_{PT}/(1 - \Phi_{PT})$ between *Aedes aegypti* genetic populations. Benson, St. David, Douglas, Naco, Sierra Vista, and Nogales Minor represent the Cochise region. Hermosillo, Tempe, Tucson, and Nogales, Sonora represent the Western region. Willcox, Islamorada, Houston, and El Paso represent the Eastern region. Φ_{PT} = genetic differentiation between populations.

TABLE 2

Cladistic analysis of population substructure

Clade*	Individual outliers in clade
Tucson	El Paso Tempe
Cochise County†	Nogales Minor Tucson
Douglas	Naco Sierra Vista
Naco	Douglas Sierra Vista
Benson	Douglas
St. David	Benson Douglas
Sierra Vista	Douglas
Houston	Willcox El Paso

* Clades formed from samples collected at multiple trap locations and dates.

† The Cochise County clade represented the Cochise region. It contained the putative Nogales minor population and the populations of Douglas, Naco, Benson, St. David, and Sierra Vista.

All pairwise Φ_{PT} comparisons were statistically significant at the $P < 0.001$ level as determined by a permutation test (see Materials and Methods), except for Willcox and Houston, El Paso, Hermosillo, Benson, and Nogales Minor, (Merrill SA, unpublished data). The Nogales Minor population was found to be significantly differentiated from the Nogales population at the $P < 0.001$ level.

Cladistic and statistical analysis of geographic populations. Pairwise population differentiation, Φ_{PT}, and effective migration (*M*) between geographic populations are shown in Table 3. In these calculations, the genetic populations of Nogales and Nogales Minor are included as the geographic population of Nogales. Cladistic analysis of Φ_{PT} /(1 – Φ_{PT}) values indicated the same regional structure found in Figure 2, with the geographic population of Nogales in the Western region (Merrill SA, unpublished data). With the exception of the Cochise genetic region containing the Nogales Minor genetic population, each genetic region and its corresponding geographic region were found to be one in the same.

Most populations were moderately differentiated ($0.15 > \Phi_{PT} > 0.05$), and none exhibited little differentiation ($\Phi_{PT} < 0.05$). Tempe was greatly differentiated ($0.16 < \Phi_{PT}$) from all populations except Tucson, Nogales, and Hermosillo. Great differentiation ($0.20 < \Phi_{PT}$) was also observed between St. David and Tempe. El Paso was greatly differentiated ($0.16 < \Phi_{PT}$) from Tempe, Willcox, and St. David. Willcox was greatly differentiated ($0.16 < \Phi_{PT}$) from all populations except Houston and Benson. Permutations of Φ_{PT} between all

TABLE 3

Pairwise population differentiation (Φ_{PT}) below the diagonal, and effective migration (M), in individuals per generation, between geographic *Aedes aegypti* populations above the diagonal

	Nogales	Tucson	Hermosillo	Tempe	Islamorada	Houston	Benson	St. David	Sierra Vista	Naco	Douglas	Willcox*
Nogales	–	3.21	3.02	1.66	1.91	1.91	2.72	2.03	2.66	2.57	2.99	
Tucson	0.072	–	1.99	1.79	1.86	2.35	3.38	2.16	3.41	2.2	2.62	
Hermosillo	0.076	0.112	–	2.86	1.91	1.66	2.05	1.42	1.75	1.86	2.03	
Tempe	0.131	0.122	0.08	–	1.21	1.19	1.33	1.02	1.21	1.24	1.32	
Islamorada	0.116	0.119	0.12	0.17	–	2.32	1.94	1.57	1.64	1.73	1.76	
Houston	0.116	0.096	0.13	0.17	0.1	–	3.2	2.23	2.3	2.23	2.41	
Benson	0.084	0.069	0.11	0.16	0.11	0.07	–	3.68	3.31	2.91	4.05	
St. David	0.11	0.104	0.15	0.2	0.14	0.1	0.06	–	2.63	2.28	2.48	
Sierra Vista	0.086	0.068	0.12	0.17	0.13	0.1	0.07	0.09	–	3.18	3.93	
Naco	0.089	0.102	0.12	0.17	0.13	0.1	0.08	0.1	0.07	–	4.44	
Douglas	0.077	0.087	0.11	0.16	0.12	0.09	0.06	0.09	0.06	0.05†	–	
Willcox*	0.163	0.194	0.16	0.25	0.2	0.14	0.14	0.23	0.18	0.24	0.18	–
El Paso*	0.125	0.117	0.11	0.16	0.12	0.08	0.1	0.16	0.14	0.14	0.14	0.19

* Willcox (n = 2) and El Paso (n = 10) migration not displayed due to possible Φ_{PT} inflation from small sample sizes.

† Douglas Φ_{PT} = 0.05 due to rounding.

populations had a $P < 0.001$, except for Willcox comparisons. The Willcox comparisons produced P values ranging from 0.026 to 0.001.

The lowest effective migration[37] (n > 50, $P < 0.001$) was 1.02 individuals per generation between St. David and Tempe, which are members of two different regions (Figures 1 and 2). The highest observed rate (n > 50, $P < 0.001$) was 4.44 individuals per generation between Douglas and Naco, which are geographically proximal members of the Cochise region. Migration rates between populations in Arizona varied according to geography. Average pairwise migration within regions was 3.29, 2.10, and 2.42 individuals per generation in the Cochise, Eastern, and Western regions, respectively. Effective migration for Willcox (n = 2) and El Paso (n = 10) were not computed because small sample sizes are known to artificially inflate F_{ST} estimators and grossly underestimate M.

Table 4 shows the results of AMOVA analyses. Of the total genetic diversity from the two marker sets, the majority (88.5%) can be attributed to variation within geographic populations, 8.4% to variation among geographic populations within regions, and 3.1% to variation among regions. Average statistics compiled from the two marker sets suggested that populations within regions were moderately, yet significantly, differentiated ($\Phi_{PR} = 0.09$, $P < 0.001$), and that regions were little, yet significantly, differentiated ($\Phi_{RT} = 0.03$, $P < 0.001$).

DISCUSSION

The cladistic analyses and patterns of population differentiation suggested that there were three regions of *Ae. aegypti* in Arizona. Populations were moderately and significantly differentiated from each other within regions. Between regions populations were significantly differentiated either greatly or moderately. More significant molecular variance was found between populations than between regions, suggesting weak regional structure possibly due to interregional migration. Our documentation of *Ae. aegypti* distribution and the prior population histories suggest that the examined populations were viable and stable, excluding Willcox. The limited finding of *Ae. aegypti* in Willcox suggested small population range and size, possibly from recent colonization. In addition to providing unreliable estimates of M, the small sample sizes may have influenced the non-significant Φ_{PT} values observed for Willcox.

TABLE 4

Results of analysis of molecular variance

Set*	Level†	d.f.	SS	MS	Est. Var.	Φ	P<
A	R	2	1447.81	723.91	1.97	0.04	0.001
	P	10	2266.15	226.61	3.52	0.08	0.001
	W	721	28271	39.21	39.21	0.12	0.001
B	R	2	750.85	375.43	0.56	0.02	0.001
	P	10	2051.46	205.15	3.25	0.09	0.001
	W	721	23102.2	32.04	32.04	0.11	0.001

* Set A primers; Eco-AGG + Mse-CTT; B primers: Eco-ACA + Mse-CAC.

† Variation was partitioned: R = among regions (Φ_{RT}); P = among populations nested within regions (Φ_{PR}); and W = within populations nested within regions (Φ_{PT}).

The Eastern region encompassed the well-established populations in Islamorada, Florida and Houston, Texas; it also included the city of Willcox, Arizona. The Western region included the well-established Mexican population of Hermosillo, and extended north from Mexico incorporating Nogales (Sonora), Tucson, and Tempe. The extent of the Eastern and Western regions was in agreement with previous work by Gorrochoteguli-Escalante and others, who found that the northeast Mexico genetic population, including the geographic population of Houston, was distinct from the Pacific Mexico population, to which Tucson belonged.[15] The Cochise County Arizona region appeared to represent an interface between the Western and Eastern regions, and included the Douglas, Naco, Sierra Vista, Benson, St. David, and Nogales Minor populations.

Although Nogales was found to contain two separate populations, most populations were more genetically homogenous, but had relationships that spanned regional classifications. Although Benson clustered in the Cochise region and exhibited the lowest differentiation with Douglas, the lowest differentiation observed for Houston was with Benson. Tucson demonstrated the lowest differentiation with Sierra Vista, and second lowest with Benson. These data supported the idea that the Cochise region was produced by mixing between the Western and Eastern regions.

Population substructure was only detected in Nogales, Sonora. Nogales, Sonora and Nogales, Arizona are contiguous sister cities divided by the international border. In July and August, three traps in northwestern Nogales, Sonora collected individuals that belonged to the Cochise region. Subsequent analysis showed that this Nogales Minor population was distinct from the remaining Nogales population, which belonged to the Western region. Confounding this observed substructure, the three traps that collected Cochise region individuals in July and August all collected individuals from the Western region in September. This suggests that the population substructure in Nogales, Sonora was temporary. Such temporal partitioning could have been due to recent immigration from the nearby Cochise region. The suspected partitioning of St. David was dismissed because all members composing the St. David South population were collected from one trap on one collection date, were likely to have been siblings, and were not distinct from the remainder of the St. David population.

The lowest levels of population differentiation, thus most frequent migration, were found in the Cochise region between Douglas and the cities of Naco, Benson, and Sierra Vista. The most isolated Arizona population was Tempe, which was at the northern extreme of the Western region. These data show that large geographic distances, such as the distance from Houston to Benson, were not insurmountable migratory barriers for *Ae. aegypti*, but that local migration was more common. High rates of local, intra-regional migration found in the Cochise region and lower local migration observed in the Western region suggest that the hypothetical spread of insecticide resistance through transport of mosquitoes is more likely to occur within the Cochise region, but that actual dengue introduction is more likely in the Western region. Compared with populations of the Cochise region, the populations of the Western region were generally more isolated from each other, but exchanged more migrants with Hermosillo, Sonora, a city with endemic dengue.

The importance of geography and human migration on *Ae. aegypti* population structure is likely, especially because the dry climate and geographic distances in the study region negate *Ae. aegypti* dispersal between cities by flight. The geography of southern Arizona has shaped *Ae. aegypti* population structure by influencing highway routes and human migration. While vast distances typically separate the Cochise and Eastern regions, many mountains separate the Cochise region from the Western region. All cities of the Cochise region are contained in the San Pedro Valley, which runs north from Mexico. Douglas represents a roadway hub in the Cochise region, with connections to Benson, Naco, and Sierra Vista though state highways. Tucson and Nogales reside in the Santa Cruz Valley and are connected by Interstate 19. Interstate 10 connects Tucson to the Phoenix metro area, including Tempe. Interstate 10 also connects Tucson, Benson, Willcox, El Paso, and Houston.

Roadway systems correspond to observed patterns of population differentiation. Douglas is linked to Benson, Naco, and Sierra Vista by Arizona Routes 80 and 92. Route 90 and Interstate 10 connect Tucson and Sierra Vista. Tucson and Houston are joined to Benson by Interstate 10. Although St. David lies between Douglas and Benson on Route 80, the majority of the traffic to St. David is from nearby Benson, accounting for the close genetic relationship of St. David and Benson *Ae. aegypti* populations.

The extent and origin of the Western region showed that *Ae. aegypti* from dengue-endemic areas of western Mexico have penetrated as far north as the Phoenix metro area. Migration from the Eastern region, another area with endemic dengue near the Texas-Mexico border, was detected in Willcox and Benson. The Cochise region represents the interface between the Western and Eastern regions, and received migrants from both.

We and others previously demonstrated that necessary conditions for dengue transmission, i.e., parity, population levels, vector competence, and blood feeding tendencies of *Ae. aegypti*, were met in Tucson.[13–16] The lack of dengue cases in Arizona could be attributable to infrequent migration from dengue areas, resulting in the failure to introduce the virus to the Arizonan vector populations. Infrequent migration supports the observation of moderate differentiation between populations in the study area and could account for the initial colonization of the cities in Arizona. The absence of dengue in Arizona may not be solely attributable to infrequent migration of *Ae. aegypti*. Reiter and others suggested that economic factors such as air conditioning were paramount in limiting dengue transmission in Texas.[38] Given the genetic history of Arizonan *Ae. aegypti* populations, similar factors may be integral in preventing dengue transmission in Arizona.

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Authors' addresses: Samuel A. Merrill, Medical Scientist Training Program, Washington University in St. Louis, 660 S. Euclid Avenue, Box 8226, St. Louis, MO, 63108. Frank B. Ramberg, and Henry H. Hagedorn, Department of Entomology, University of Arizona, 1140 E. South Campus Drive, Forbes Room 410, PO Box 210036, Tucson, AZ, 85721-0036, E-mail: hagedorn@ag.arizona.edu.

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APPENDIX 1

Phylogenetic Analysis Using Parsimony (PAUP) cladogram of all individuals analyzed, as described in the Materials and Methods. Each individual was tracked with a unique identification code. Codes were devised from collection location, trap designation, and collection date, if known, as shown in Table 1. Four different coding schemes exist. For example, "Nogales Sept 172 10903" was an individual collected in trap 172 in Nogales, Mexico during September and 10903 was a unique identifier; "Douglas 1 820h92b" is the second individual (b) harvested on September 2, 2003 from eggs collected on August 20, 2003 from the 1 trap in Douglas, AZ; "FL11" is the 11th individual obtained from Islamorada, FL; and "Houston 111a" is the first individual (a) obtained from trap 111 in Houston, TX.


Nogales Sept 172 10903
Nogales Sept 169 11131
Nogales Sept 169 11132
Nogales Sept 147 12211
Nogales Sept 151 12171
Nogales Sept 151 12174
Nogales Sept 148 12782
Nogales Sept 148 12786
Nogales Sept 144 12561
Nogales Aug 188 9051
Tucson Aug 170 8672
Nogales Sept 148 12784
Nogales Sept 172 10905
Nogales Sept 172 11071
Nogales Sept 172 11072
Nogales Sept 144 12562
Nogales Sept 144 10991
Nogales Sept 144 10992
Nogales Sept 171 12441
Nogales Sept 171 12443
Nogales Sept 171 12442
Nogales Sept 156 12353
Nogales Sept 188 12421
Nogales Sept 188 12422
Nogales Sept 151 12172
Nogales Sept 151 12173
Nogales Sept 162 12302
Nogales Sept 156 12161
Nogales Sept 156 12355
Nogales Sept 156 12502
Nogales Sept 156 12164
Nogales Sept 156 12503
Nogales Sept 156 12163
Nogales Sept 156 12357
Nogales Sept 156 12352
Nogales Sept 156 12356
Nogales Sept 156 12501
Nogales Sept 156 12354
Nogales Sept 162 12301
Nogales Sept 162 12722
Nogales Sept 162 12721
Nogales Sept 148 12785
Nogales Sept 148 12787
Nogales Sept 148 12789
Nogales July 147 113
Nogales July 147 115
Nogales July 147 116
Nogales July 147 114
Nogales Aug 169 8431
Nogales Aug 169 8432
Tucson Aug 198 8551
Tucson Aug 198 8554
Nogales Aug 151 8401
Nogales Aug 151 8403
Nogales Aug 147 8411
Nogales Aug 147 8412
StDavid b 91h911a
StDavid b 91h911c
StDavid b 91h913
StDavid b 91h911b
StDavid b 91h911d
StDavid b 91h911e
StDavid b 91h912
StDavid b 91h914b
StDavid b 91h914a
StDavid b 91h915
SierraVista b 95h915a
SierraVista b 95h924a
SierraVista b 95h924b
Douglas c 91h98e
Douglas c 91h98h
Douglas l 824h910a
Douglas l 824h910b
Douglas e 91h910a
Douglas e 91h910b
Douglas g 824h97b
SierraVista d 820h96a
SierraVista d 820h97b
SierraVista d 820h96b
SierraVista d 820h98c
SierraVista h 95h917
SierraVista d 820h96c
SierraVista d 820h97a
SierraVista d 820h98a
SierraVista d 820h98b
SierraVista b 95h915b
SierraVista f 91h924a
SierraVista f 91h924b
SierraVista f 91h924d
SierraVista e 91h914a
SierraVista e 91h914c
SierraVista e 91h914b
SierraVista e 91h914d
SierraVista e 91h923c
SierraVista e 91h918a
SierraVista e 91h923a
SierraVista e 91h914e
SierraVista e 91h918b
SierraVista e 91h918c
SierraVista e 91h918d
SierraVista e 91h923b
SierraVista c 91h910a
SierraVista c 91h910c
SierraVista c 91h919a
SierraVista c 91h910d
SierraVista c 91h919b
SierraVista c 91h910b
SierraVista c 91h910e
SierraVista g 91h923a
SierraVista g 91h923b
SierraVista g 91h923c
SierraVista d 95h919a
SierraVista g 827h95a
SierraVista g 827h95c
SierraVista g 827h95b
SierraVista g 827h95e
SierraVista g 827h95d
SierraVista g 827h97
SierraVista f 824h95c
SierraVista f 824h96a
SierraVista f 827h97a
SierraVista d 95h919b
SierraVista h 95h925b
SierraVista h 95h928d
SierraVista f 91h924c
SierraVista h 95h923b
SierraVista h 95h925a
SierraVista h 95h925c
Tucson Aug 189 9021
SierraVista h 95h923a
Houston 412 1
Benson g 824h919
Benson a 95h921b
Benson b 95h916e
Benson b 95h916a
Benson b 95h916d
Benson b 95h916f
Benson b 95h916b
Benson b 95h916c
Benson c 95h916a
Benson c 95h916c
Benson c 95h916d
Benson b 824h831c
Benson g 824h95b
Benson b 824h831d
Benson b 824h831e
Benson g 824h831b
Benson c 824h92a
Benson d 820h91a
Benson g 824h831a
Benson g 824h831e
Benson g 824h95a
Benson g 824h831d
Benson g 824h96e
Benson g 824h831f
Benson g 824h831c
Benson g 824h96a
Benson g 824h96f
Benson c 827h912a
Benson c 827h912b
Benson g 91h918
Douglas b 820h830
Benson c 824h92b
Benson d 820h831
Benson e 95h916a
Benson e 95h916b
Benson c 827h95a
Benson c 827h95c
Benson c 827h95b
Benson c 95h916b
Benson a 827h916
Benson a 91h916f
Benson a 91h916a
Benson a 95h921a
Benson a 91h916c
Benson a 91h916b
Benson a 91h916d
Benson a 95h915b
Benson a 91h916e
Benson a 95h915a
Benson b 91h918a
Benson b 91h918b
Benson g 95h920
StDavid e 91h98a
StDavid d 824h95
StDavid d 824h97
StDavid e 95h921
StDavid g 814h829a
StDavid g 814h831a
StDavid g 814h831c
StDavid g 814h829b
StDavid g 814h831d
StDavid g 814h831b
StDavid e 95h916a
StDavid e 95h924a
StDavid e 95h919a
StDavid e 95h919c
StDavid e 95h923a
StDavid e 95h916b
StDavid e 95h919b
StDavid e 95h923d
StDavid e 95h924b
StDavid e 95h923b
StDavid e 95h923c
StDavid h 95h912a
StDavid h 95h912b
StDavid h 95h919b
StDavid h 95h924a
StDavid h 95h916a
StDavid h 95h921b
StDavid h 95h916b
StDavid h 95h925c
StDavid h 95h919a
StDavid h 95h921c
StDavid h 95h921a
StDavid h 95h923a
StDavid h 95h919c
StDavid h 95h923b
StDavid h 95h925b
StDavid g 814h829c
StDavid g 814h829e
StDavid g 814h829g
StDavid g 814h829h
StDavid g 814h829d
StDavid g 814h829f
StDavid g 814h829i
Douglas h 824h92a
SierraVista b 824h915
SierraVista b 824h919a
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SierraVista b 824h97c
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SierraVista b 824h94a
SierraVista b 824h97b
SierraVista b 824h97d
SierraVista b 824h95a
Naco e 820h911a
Naco e 820h95a
Naco e 820h99
Naco e 820h912c
Naco e 820h98a
Naco e 820h98e
Naco e 820h98b
Naco e 820h911b
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Naco e 820h912b
Naco e 820h98c
Naco e 820h95b
Naco e 820h98d
Douglas g 91h98a
Douglas g 91h98b
Naco e 817h831a
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Douglas g 820h92a
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Douglas g 820h92b
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Douglas g 820h98a
Douglas g 820h98b
Naco d 820h912
Naco d 820h918
Douglas c 91h98b
Douglas c 91h98i
Naco d 827h98
Douglas l 820h92b
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Douglas l 820h97c
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Douglas k 824h910a
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