GENETIC STRUCTURE OF AEDES AEGYPTI POPULATIONS IN THAILAND USING MITOCHONDRIAL DNA

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Abstract. A hierarchical population genetic study was conducted among 19 Aedes aegypti populations in Thailand from Chiang Mai in the north to Songkhla province in the south. Single-strand conformation polymorphism analysis was used to examine variation in a 359-basepair region of the NADH dehydrogenase subunit 4 mitochondrial DNA gene (ND4). Seven haplotypes were detected in two lineages previously identified in ND4 haplotypes from North America. Gene flow estimates and highly significant variation among populations within 25 kilometers implicated genetic drift and vector control efforts as major factors in genetic structure. Mantel regression analysis demonstrated no isolation by distance. Urban areas were relatively panmictic, while suburban/rural sites exhibited more restricted gene flow. Significant genetic structure among groups of collections > 100 kilometers apart is consistent with recent (~50 year) expansion of Aedes aegypti from highly populated areas accompanied by founder effects, but could also reflect the overall low genetic diversity in ND4 in Thailand.

INTRODUCTION

Dengue is a major public health problem in tropical regions of the world, causing millions of dengue fever and hundreds of thousands of dengue hemorrhagic fever cases annually. In endemic areas, the annual number of cases has risen steeply since the 1950s. With multiple serotypes circulating in endemic areas, the annual number of cases has risen steeply. Because there is no licensed vaccine for dengue and dengue shock syndrome (DHF/DSS) have increased dramatically since the 1950s. With multiple serotypes circulating in endemic areas, the annual number of cases has risen steeply.

Aedes aegypti is the primary urban vector of the four serotypes of dengue (DEN) viruses. It is unknown when Aedes aegypti first colonized southeast Asia. It was first reported in Thailand by Theobold in 1907, but may have been present for many years prior to that. Causey found Aedes aegypti in most villages along the coast of the Gulf of Thailand and < 5 kilometers from the railway between Bangkok and Chiang Mai, but not in areas further from major lines of transport. Extensive surveys reported by Scanlon in 1965 indicated further penetration of Aedes aegypti from major routes of travel throughout much of Thailand, extending into the eastern and southern regions of Thailand. Based on previous studies that failed to find Aedes aegypti in areas more than a few kilometers from lines of commerce, sylvan and more isolated rural areas were targeted to determine if this species had colonized such areas. These surveys were all negative for Aedes aegypti, but yielded a diverse mosquito fauna. Scanlon concluded that Aedes aegypti was primarily an urban mosquito that could be found in smaller human settlements along major routes of commerce and travel. Today, Aedes aegypti is well established in urban centers and most rural villages in Thailand. Therefore, much of its colonization of Thailand outside urban areas and major commercial routes has likely occurred during the past 50 years.

Aedes aegypti was introduced into many tropical and sub-tropical regions of the world from Africa, largely through human commerce. As it became established in new areas, it retained its close association with humans. There are substantial differences among regions it inhabits, however, with regard to climate, the type and availability of oviposition sites, contiguous areas of suitable habitat, the nature of vector control efforts, and the role of human-aided dispersal. Variation in these kinds of ecological parameters can profoundly affect the population genetic structure at the regional level of Aedes aegypti or any other mosquito species. Understanding patterns of gene flow among Aedes aegypti populations is useful for tracking and even predicting the movement of important genetic traits such as vector competence and insecticide resistance.

Early population genetic studies of Aedes aegypti using allozymes indicated that populations cluster by continent or country of collection. Subsequent researchers examined variation at smaller geographic scales using allozymes, random amplified polymorphic DNA, microsatellite, restriction fragment length polymorphism, and mitochondrial DNA sequence haplotype markers. Although it is difficult to compare studies using different classes of genetic markers, results from the above research lead to different conclusions about the genetic structure of Aedes aegypti populations in different regions of the world. Explanations for these disparities are based on local variation in colonization history, exposure to and methodology of vector control, and Aedes aegypti ecology. An extensive study of populations throughout Mexico using mitochondrial DNA sequence haplotypes of a segment of the NADH dehydrogenase subunit 4 (ND4) gene demonstrated that in that part of the world, Aedes aegypti breeding structure varies geographically. Populations from the Yucatan and Pacific coastal region showed genetic isolation by distance. In the northeastern region, genetic diversity was lower and there was no relationship between genetic and geographic distances. Collections within 130 kilometers of each other in the northeast and within 180 kilometers in the Yucatan showed no barrier to gene flow. Extensive gene flow was observed across the entire Pacific coastal region.

The goal of the present study was to characterize the pattern of Aedes aegypti gene flow and genetic differentiation across a broad geographic area of Thailand. Only a single formal population genetic study of Aedes aegypti has been done in Thailand, and it was limited to Chiang Mai province. We made 19 collections from the city of Chiang Mai in the north
to the Malaysian border in Songkhla province in the south covering a distance of just under 1,400 kilometers. A nested spatial sampling design was used where 3–4 collections ≤ 25 kilometers apart were made within areas that were separated by greater distances. This allowed analysis of variation at three levels: among mosquitoes within individual collections, among collections ≤ 25 kilometers apart, and among groups of collections > 100 kilometers apart. We also tested the hypothesis of isolation by distance. If there are no barriers or artificial enhancements to gene flow, Wright’s island model predicts that populations will reach an equilibrium where genetic distance between populations is positively correlated with geographic distance.18 Departure from the null hypothesis of isolation by distance can suggest other perturbations that may be influencing genetic structure, such as genetic drift, vector control activities, and human-aided dispersal/transport.

MATERIALS AND METHODS

Mosquito collections and extraction of DNA. Mosquitoes were collected during August 2002. Locations and sample sizes are listed in Table 1 and sample locations are denoted in Figure 1. Four collections were made from suburban neighborhoods in Chiang Mai. Collections from around Mae Sot and Kamphaeng Phet were from rural villages. We made three collections each from Bangkok and Hat Yai in urban neighborhoods. We also took single collections from a small town approximately 16 km south of Sadao on the Malaysian border and from a village approximately 30 km northeast of Phang-nga straddling road 415. At all sites we collected fourth-instar larvae and pupae from a variety of artificial containers in and around homes. At most sites, 30 or more containers from throughout the collection area were sampled, and with one exception, at least 20 larval sites were sampled. At the Sadao site, we collected from five heavily infested containers holding mosquitoes of all immature stages. Generally no more than 12 larvae or pupae were taken from a single container, and no greater than two containers sampled from a single premise. Mosquitoes were allowed to emerge and identified to species with a hand lens. Adult males and females were preserved in 95% ethanol until DNA extraction. For most collections, a subset of the total number collected was randomly chosen for processing and approximately equal numbers of males and females were used. The DNA was extracted from individual mosquitoes by salt extraction, resuspended in 250 L of 0.1 × SSC buffer (15 mM NaCl, 0.15 mM sodium citrate), and stored at −80°C.

Mitochondrial gene amplification. We amplified a 359-basepair region of the ND4 gene using published primer sequences.16 Amplifications were carried out in 20-L reactions using 0.5 L of template DNA; all runs included a negative control (all reagents except template). The program consisted of an initial incubation at 95°C for 3 minutes, 10 cycles at 92°C for 30 seconds, 48°C for 1 minute, and 72° for 40 seconds, followed by 40 cycles at 92°C for 30 seconds, 52°C for 35

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* $k$ = the average number of nucleotide differences; $\pi_1$ = nucleotide diversity; $\pi_2$ = nucleotide diversity with Jukes and Cantor correction.
RESULTS

ND4 haplotype identification and phylogenetic analysis. From a total of 1,346 individuals (672 females, 645 males, and 29 larvae), seven distinct haplotypes were identified. Haplotypes 29 and 30 were unique to single individuals from the BK2 collection. Haplotypes 31 and 32 were rare and confined to populations from the southern peninsula of Thailand. Haplotype 28 was more common, but also largely confined to the south: all but one individual carrying this haplotype were sampled in this study.

Phylogenetic analysis among ND4 haplotypes. Phylogenetic relationships among haplotypes were estimated with PAUP4B10 using maximum parsimony and distance/neighbor joining analyses. Branch support in the resolved phylogeny was estimated by bootstrap analysis with 1,000 replicates. Two ND4 sequences of *Aedes albopictus*, one individual from Lao Bao and one from Mae Kasa, were used as outgroups. A haplotype network was constructed using statistical parsimony analysis with the program TCS 1.13.

Statistical analysis of haplotype frequencies. Variation among and within collection groups was partitioned by analysis of molecular variance (AMOVA) using the computer program Arlequin 2.0. Significance of variance components was computed using a non-parametric permutation test with 10,100 permutations.

The frequency and nucleotide sequence of haplotypes for each collection were analyzed using DnaSP version 3.99. We calculated the number of polymorphic sites and the average number of nucleotide differences (k). We estimated the nucleotide diversity (π), and nucleotide diversity with Jukes and Cantor correction (πc). Pairwise genetic differences among populations were estimated as NST that is similar to FST but incorporates sequence divergence among haplotypes into the distance estimate. This is equivalent to the infinite-sites model of FST based on DNA sequence data with the Jukes and Cantor correction applied. Pairwise effective migration rates (Nm, expressed as the number of reproductive migrants per generation) were calculated from NST. Pairwise NST values were transformed to NST/(1 − NST) and regressed on pairwise geographic distances and the natural logarithm transformation of geographic distances to test the hypothesis of isolation by distance. Regressions were performed using the Mantel test calculated by the Fortran program MANTEL (written by William C. Black IV, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO). Pairwise NST/(1 − NST) values were used to construct a dendrogram among all collections using unweighted pair-group method with arithmetic averaging analysis in the NEIGHBOR procedure of PHYLIP 3.5C.

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Sequencing confirmed the ability of the SSCP technique to

(seconds, and 72°C for 40 seconds. This was followed by a final extension at 72°C for 5 minutes after which the polymerase chain reaction (PCR) products were stored overnight at 4°C. The PCR products were confirmed by agrose gel electrophoresis. Any runs that showed products in the negative control were discarded.

Single-strand conformation polymorphism (SSCP) analysis and haplotype sequencing. The PCR product (2 μL) was mixed with 10 μL of loading buffer (95% formamide, 10 mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol) and heated to 95°C for 5–6 minutes, then transferred immediately to a wet ice bath. Samples were loaded onto 18 × 16 cm, thin (0.4 mm) 8% polyacrylamide gels. Gels were run for 4–5 hours at a constant 25 milliamps. The running buffer was cooled to a constant 4°C and the buffer tank was packed in ice to preserve single-strand conformations. Gels were stained with SybrGreen (Molecular Probes, Eugene, OR) to visualize DNA fragments.

Haplotypes were identified by SSCP banding pattern. This technique has been shown to be highly sensitive in detection of even single point mutations in fragments ≤ 450 basepairs in length. To evaluate the sensitivity of SSCP in this study, ND4 PCR products from 3–12 individuals of each haplotype (except in the case of unique haplotypes) were reamplified and sequenced along both strands using the same primers used in the PCR amplification. All sequencing was performed at the DNA Analysis Facility at the Johns Hopkins University School of Medicine (Baltimore, MD). Haplotypes were sequentially numbered following the numbering of ND4 sequences for *Ae. aegypti* already present in Genbank.

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Sequencing confirmed the ability of the SSCP technique to
distinguish haplotypes. A total of 41 individuals were sequenced, representing all 7 haplotypes, each city group, and at least one individual from 14 of the collections in this study. In all cases, individuals with the same banding pattern had the same nucleotide sequence. Sequencing also demonstrated the ability of the SSCP method to detect even single nucleotide differences. Haplotypes 28 and 32 differed from haplotype 5 and haplotype 29 differed from haplotype 31 by single base substitutions.

Phylogenetic analysis of the seven haplotypes supports two distinct clades (Figure 2). The bifurcating trees produced by these analyses may not accurately reflect the evolution of nucleotide variants. The haplotype network (Figure 3) shows a more realistic evolutionary pathway based on statistical parsimony, and also supports two clades. Within the first clade (haplotypes 5, 28, and 32) there were two polymorphic sites; within the second clade (haplotypes 27, 29, 30, and 31) there were eight polymorphic sites. In contrast to this low level of variation within a clade, there were 17 polymorphic sites between the two clades. This is reflected in the $\pi_1$ and $\pi_2$ values for the individual clades, which were much lower compared with those of the whole data set (Table 1). The first clade is more common than the second, and accounted for approximately 75% of the samples. These represent two main maternal lineages in Thailand. These two lineages do not cluster geographically.

**Gene flow and genetic relationships among populations.** Gene flow estimates varied widely among populations. Pairwise $N_{ST}$ values ranged from 0 to 0.795, corresponding to an $N_m$ of infinity (identical haplotype frequencies) to 0.13 reproductive migrants per generation. All of the very high $N_{ST}$ values (> 0.600) were contributed by three populations: CM3, KP2, and Phang-nga. All other $N_{ST}$ values were ≤ 0.340. These genetic relationships can be seen graphically in the dendrogram based on linearized $N_{ST}$ values (Figure 4). The populations CM3, KP2, and Phang-nga are basal to the rest of the populations, illustrating their relative genetic distance.

The Mantel test regressing genetic and geographic distances indicated no significant association using either straight line distance (Mantel correlation = −0.005, Mantel $P = 0.60, r^2 = 0$) or the natural logarithm of distance (Mantel correlation = 0.007, Mantel $P = 0.50, r^2 = 0$), with virtually flat slopes (Figure 5). Approximate pairwise road distances between collection sites were also measured and regressed against pairwise $N_{ST}$ values. This regression was also insignificant, with a slope close to zero. This result was expected, considering the straight line and road distance matrices were highly correlated (Mantel correlation = 0.99, Mantel $P =$
Therefore, there is no isolation by distance across the regions of Thailand sampled in this study.

**Nested analysis of haplotype frequencies.** Variation in ND4 sequence and haplotype frequency was partitioned by AMOVA. When grouped by cities in or around which collections came from, a majority (∼57%) of the variation was found among mosquitoes within collections. Among collections ∼25 km apart within groups, a smaller (∼18%) but highly significant proportion of the variation was found. This indicates that there is substantial population structuring among populations at this geographic scale. Among groups of collections (ranging from 105 to 1,380 km apart) ∼25% of the variation was found, demonstrating significant genetic structuring at each of the hierarchical geographic levels.

Since CM3, KP2, and Phang-nga showed high pairwise NST values, there was concern these populations might skew the AMOVA results. Therefore, we conducted another AMOVA with these three populations removed. Again, a majority (∼82%) of the variation was found among mosquitoes within collections. The variation found among collections within groups was only ∼2%, but remained statistically significant (P = 0.01), so that even with CM3, KP2 and Phang-nga removed significant genetic structuring among populations ≤ 25 km apart was found. Among groups of collections still accounted for, ∼16% of the variation and F_CT remained large (∼0.162) and highly statistically significant. The F_CT values are similar to those found in Mexico (0.200–0.217). F_CT values of this magnitude have also been found in Anopheles darlingi (0.349) and the tsetse fly subspecies Glossina morsitans submorsitans (0.338).

Since some haplotypes were found only in populations from the southern peninsula, we conducted another AMOVA dividing the populations into two groups: Central/North and South Thailand (Table 1 shows this subdivision). The variance between these two regions (∼7%) was not statistically significant (Table 2). Using the associated fixation index (F_CT = 0.06954) as Wright’s hierarchical F-statistic, this corresponds to an Nm between regions of 6.70. Comparing the haplotype sequences of the two regions in DnaSP yielded consistent results with NST = 0.07943 and Nm = 5.80. These measures of gene flow indicate a lack of genetic differentiation between the Central/North and South regions.

**DISCUSSION**

The broad population genetic survey reported here leads to several conclusions about both the colonization history and current population dynamics of Ae. aegypti in Thailand. Genetic diversity in this study was much lower than that found in other studies of Ae. aegypti in which ND4 sequences were analyzed. This, together with historical accounts of the distribution of Ae. aegypti, suggests a recent (<60 years) expansion of Ae. aegypti from urban and coastal areas, although the genetic data alone are not conclusive. The lack of isolation by
distance and patterns of pairwise estimates of gene flow between collections indicate that populations may have experienced substantial bottlenecks as they were established in different areas, most probably through human movement of mosquitoes. Genetic similarity between Central/North and South populations may reflect a common ancestry rather than continuing gene flow across such a large region. High $N_{ST}$ estimates between populations in close proximity are likely the result of both genetic drift and past control efforts.

Compared with studies of ND4 in *Ae. aegypti* from Mexico, estimates of genetic variability in Thailand were consistent with the hypothesis of a small introduction of mosquitoes and relatively recent expansion throughout the country. In Mexico, nucleotide diversity ($\pi_1$ and $\pi_2$) and $k$ were $2\sim3$ times greater than in Thailand. Over a similar geographic distance, 25 haplotypes were detected compared with only 7 in Thailand. The number of polymorphic sites was similar to that in Mexico (16–24). This represents a much lower genetic diversity in Thailand than in Mexico.

We added the sequences of the haplotypes identified in Thailand to the 25 haplotypes from Mexico and the southern United States.\textsuperscript{32} Haplotype 5, the most common in Thailand, was also found in Mexico, where it was very rare. All other haplotypes are so far unique to Thailand. Phylogenetic relationships among all haplotypes were estimated in PAUP4B10 using neighbor-joining, maximum parsimony, and maximum likelihood analyses. In all phylogenies, the Thailand haplotypes did not cluster together. Instead, members of Clade 1 fell into the basal lineage identified in the Mexico study, and Clade 2 members were grouped in the distinct, derived lineage. This supports the hypothesis that the two lineages, present in both regions, evolved before the exportation of *Ae. aegypti* from its ancestral source, presumably in North Africa.\textsuperscript{6}

It would be necessary to expand sampling to other parts of the world, especially regions of Africa, to test this hypothesis.

The Mantel analysis showed no isolation by distance in Thailand, in contrast to studies done in Mexico. This result is also consistent with recent range expansion of *Ae. aegypti* in Thailand because under Wright’s island model sufficient time has not passed for populations to approach equilibrium where isolation by distance could be detected.\textsuperscript{29} There are at least three explanations for this observation (none mutually exclusive). First, human movement of mosquitoes could transport mosquitoes long distances, increasing genetic similarity between geographically distant populations. Second, genetic drift, a stochastic process, could cause populations to become more similar or more distinct independent of their geographic relationship. Third, vector control efforts could impose severe genetic bottlenecks on local populations, profoundly affecting their genetic similarity with other populations.

Our AMOVA results were consistent with genetic drift playing a large role in Thailand, with 57% of the variation found among mosquitoes within collections. More importantly, variation among collections within cities (18%, all collections ≤ 25 km apart) was also highly significant, demonstrating a high degree of genetic differentiation at this small spatial scale, something that is indicative of genetic drift. Removing three populations (CM3, KP2, and Phang-nga) from the analysis did not qualitatively affect the results. Genetic structure at this level, while smaller, remained statistically significant ($P = 0.01$). Haplotypes 28–32 differed from the common form in their respective clade by one or a few nucleotide differences. These variants likely arose through mutation in local populations in Thailand. Under the influence of genetic drift, we would expect such variants to be rare and restricted to single populations or populations in close proximity connected by suitable habitat. Haplotype diversity is kept low because of local extinction of rare variants.\textsuperscript{40} The ecologic situation of *Ae. aegypti* in urban areas such as Bangkok and Hat Yai, with populations occurring over large areas of continuous habitat, would be most likely to harbor rare variants. While we sampled separate neighborhoods in city habitats, *Ae. aegypti* is present throughout most of the cities from which we sampled. Consistent with this scenario, the unique haplotypes 29 and 30 were found in a single population in Bangkok, and the rare haplotypes 31 and 32 were found in collections from Hat Yai. Haplotype 28 was found in all collections from Hat Yai, was the most common haplotype in the Phang-nga collection, and was also found in the Sadao and KP2 collections. It is possible this haplotype is becoming more widespread.

Effective mosquito control efforts, which typically occur at the neighborhood/village level, can impose severe genetic bottlenecks on local populations. This can be manifested as
very high $N_{ST}$ values between collections regardless of their geographic proximity. We did not have information on the recent history of control efforts at the specific sites we sampled, although a reduction in genetic diversity following insecticide treatment has been documented in villages in Mae Sot County. However, the ecology of the mosquito can again lead to predictions about these effects. In contrast to the urban situation, the suburban/rural situation seen in Chiang Mai, Kamphaeng Phet, and Mae Sot indicates that those populations may be reasonably isolated. Neighborhoods sampled in Chiang Mai were separated by open space, industrial, or agricultural areas which represent a dispersal barrier for $Ae. aegypti$. Likewise, rural villages were separated by scrub or agricultural land that we would expect to be a hostile environment to $Ae. aegypti$, especially during the dry season. The movement of $Ae. aegypti$ has been shown to be influenced by the availability of oviposition sites in Puerto Rico. Dispersing between villages could therefore be a risky prospect for gravid females in Thailand, especially when oviposition containers are plentiful within sites as is typically the case. Reduced dispersal would tend to preserve the genetic effects of severe bottlenecks. In urban settings, the widespread availability of oviposition containers between sampling sites would constitute continuous suitable habitat and could lead to unimpeded dispersal. In this setting, local effects of vector control would quickly be broken down by gene flow.

The dendrogram of populations based on linearized $N_{ST}$ values (Figure 4) shows no clustering by city, supporting the observation of no isolation by distance. Figure 4 also shows three genetic outliers: Phang-nga, KP2, and CM3. Phang-nga was a single collection, and consequently is not informative here. KP2 and CM3, however, had $N_{ST}$ scores of 0.300–0.771 ($n = 9$) compared with other collections from their respective city group, indicating high genetic differentiation. This could reflect the effect of past control efforts. In the urban collections from Bangkok and Hat Yai, where gene flow would more quickly eliminate the effects of severe local bottlenecks, $N_{ST}$ among sites within cities ranged from 0.000 to 0.0882 ($n = 6$), representing very low genetic differentiation. These observations are consistent with the scenario presented for $Ae. aegypti$ population structure in Ho Chi Minh City, Vietnam, where collections from the heart of the city were less differentiated compared with collections from the outskirts of the city.

The rural villages sampled near Mae Sot showed no genetic diversity in ND4. All individuals carried haplotype 5. This together with the significant among group variance component (25% of total variation) in the AMOVA and the lack of isolation by distance suggests that colonization of much of Thailand has occurred recently (< 60 years) and has been characterized by founder events. It is important to note that this hypothesis, while consistent with historical surveys of $Ae. aegypti$, may be a reflection of the overall low diversity of ND4 in Thailand. Expansion of $Ae. aegypti$ at a more distant time in the past accompanied by founder effects and/or subsequent local genetic drift could have yielded the same pattern. A more thorough test of this hypothesis would require additional collections from within Mae Sot, which is the likely source of mosquitoes that colonized the surrounding rural villages in which we worked. We also do not know if haplotype frequencies change over time. To address these questions would require collections from more than one time point in the Mae Sot area villages and Bangkok.

This study looked at nucleotide sequence in one region of mitochondrial DNA. More detailed information could be gained from multiple nuclear loci. Toward that end we are developing single nucleotide polymorphism markers for population genetic use in $Ae. aegypti$. These markers will be useful for more detailed studies of gene flow at smaller spatial scales, and for examining temporal variation in genetic structure.

Our measures of genetic variability are consistent with the worldwide surveys of allozyme heterozygosity in $Ae. aegypti$. These studies found the lowest rates of genetic variability in Asian collections, predominantly from the Indian subcontinent. Tabachnik speculated that these Asian populations were the source of introduction to Southeast Asia, and from there to Indonesia, Australia, and mainland China. It would be worthwhile to examine a series of collections from these areas both to examine the population genetics of $Ae. aegypti$ in these regions on a finer scale and to test the colonization history hypothesis.

Our results have some implications for vector control. In the suburban/rural setting, where populations are more isolated, insecticide use could rapidly select for insecticide resistance if such variation exists in the population. Genetic bottlenecks of this kind and/or genetic drift could radically change the phenotype of the population for other important traits such as vector competence. Because gene flow from these populations appears limited, however, genotypes will spread more slowly to surrounding populations. In the urban setting, $Ae. aegypti$ populations would be expected to recover quickly from insecticide treatments because they contain larger areas of panmixia. Insecticide resistance may develop more slowly because immigration of susceptible individuals from outside the treated area is not impeded. The role of gene flow in impeding movement of insecticide resistance alleles was explored in $Culex pipiens$ in southern France. However, depending on the frequency of insecticide application, insecticide resistance may spread by gene flow throughout the urban area from the treated population. If insecticides with residual activity are used, they could continue to impact gene flow for as long as they persist. This would affect migration of susceptible individuals into the treated area, but would not impede dispersal of resistant individuals out of the treated area. Because the population genetic structure of $Ae. aegypti$ varies from one region to another in Thailand, understanding the local population dynamics will lead to more focused, appropriate, and hopefully sustainable vector control strategies.

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