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Genetic Structure of *Aedes aegypti* in Australia and Vietnam Revealed by Microsatellite and Exon Primed Intron Crossing Markers Suggests Feasibility of Local Control Options

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Abstract

The distribution of *Aedes aegypti* (L.) in Australia is currently restricted to northern Queensland, but it has been more extensive in the past. In this study, we evaluate the genetic structure of *Ae. aegypti* populations in Australia and Vietnam and consider genetic differentiation between mosquitoes from these areas and those from a population in Thailand. Six microsatellites and two exon primed intron crossing markers were used to assess isolation by distance across all populations and also within the Australian sample. Investigations of founder effects, amount of molecular variation between and within regions and comparison of F_{ST} values among Australian and Vietnamese populations were made to assess the scale of movement of *Ae. aegypti*. Genetic control methods are under development for mosquito vector populations including the dengue vector *Ae. aegypti*. The success of these control methods will depend on the population structure of the target species including population size and rates of movement among populations. Releases of modified mosquitoes could target local populations that show a high degree of isolation from surrounding populations, potentially allowing new variants to become established in one region with eventual dispersal to other regions.

Keywords

Aedes aegypti; dengue; population structure; gene flow

New methods of controlling mosquito-borne diseases are being developed and include genetic manipulation to alter the transmission of mosquito-borne pathogens (James 2005). Attempts to modify transmission of the dengue virus in the vectors, *Aedes aegypti* (L.) and *Aedes*

albopictus (Skuse) are being made. Transgenic *Ae. aegypti* with reduced vector competence for DENV-2 viruses have been developed using RNA interference techniques (Franz et al. 2006). *Ae. aegypti* also is being engineered to contain repressible dominant lethal genes for use in areawide population control based on the sterile insect technique (Yakob et al. 2008).

In another approach, the widely distributed insect endosymbiont *Wolbachia* is being used to modify *Aedes* species. So far, *Wolbachia* has been introduced into both *Ae. aegypti* and *Ae. albopictus* (Xi et al. 2005, Ruang-areerate and Kittayapong 2006, Xi et al. 2006) and establishment of life span-reducing strains of *Wolbachia* that were originally isolated from *Drosophila* (Sinkins and Gould 2006) is being attempted. Life span-reducing strains of *Wolbachia* are expected to decrease transmission of dengue, which only occurs in older females (Watts et al. 1987, Cook et al. 2006).

The success of *Wolbachia*-based control measures will depend on the size of the target population as well as levels of immigration of local mosquitoes. Therefore, it is critical that estimates of population structure are obtained in areas where releases are planned. Studies on the population structure of target mosquitoes including *Ae. aegypti* have been made, initially, to determine the appropriate size of the insecticide treatment area around dengue fever cases (Mousson et al. 2002), but they are now being intensified to address the requirements of new control methods for mosquitoes.

Initial studies of population structure of *Ae. aegypti* used allozymes (Pashley and Rai 1983, Failloux et al. 1995, DinardoMiranda and Contel 1996, Failloux et al. 2002). There are also a few studies in which amplified fragment length polymorphism markers are used (Ravel et al. 2001, Paupy et al. 2004a, Merrill et al. 2005) and a mitochondrial DNA study of *Ae. aegypti* in Thailand (Bosio et al. 2005). A set of microsatellite markers for *Ae. aegypti* was initially isolated by Huber et al. (2001) and used (sometimes in conjunction with isoenzymes) to demonstrate genetic differences among populations in Vietnam (Huber et al. 2002a,b) and other countries in Southeast Asia (Huber et al. 2004). However, a need for a larger set of robust microsatellite loci was identified and two other sets of microsatellite primers have been isolated but not yet used in extensive population surveys (Chambers et al. 2007, Slotman et al. 2007). These other sets of markers should provide an extensive resource for population genetic surveys.

Low rates of movement are expected in populations of *Ae. aegypti* because this species has a very close association with humans and shelters in indoor habitats (Reiter 2007). Some local dispersal occurs as females seek a bloodmeal and as they engage in “skip oviposition” (Reiter 2007)—distributing a small number of eggs among many containers. Significant genetic differentiation at the local scale in Cambodian populations of *Ae. aegypti* detected by both microsatellites and allozymes (Paupy et al. 2003, 2004a,b) is evidence of low rates of movement in this species. Genetic differentiation over a few kilometers was identified in North Cameroon (Paupy et al. 2008). Longer distance movement, although not as common, also can take place. It is thought to be the cause of genetic similarity between populations of *Ae. aegypti* that are separated by hundreds of kilometers, as occurs in Ho Chi Minh City and Phnom Penh (Huber et al. 2004). Long-distance movement probably arises by translocation of desiccation resistant mosquito eggs, aquatic stages, or both in containers moved by humans.

Here, we examine the population genetic structure of *Ae. aegypti* in Australia to determine the degree of isolation of populations and also to compare variability in Australian samples with those from Vietnam and Thailand. *Ae. aegypti* probably arrived in Australia during the mid-nineteenth century. Currently, it maintains a stronghold in Queensland with southern and western limits to its distribution, although it was more widely distributed in the past (O’Gower 1956). It is currently present year-round in urban areas of northern Queensland, Australia

(Sinclair 1992). Outbreaks of dengue fever have occurred regularly in northern Queensland since 1990 (Ritchie et al. 2004, Hanna et al. 2006).

After evaluation of the available microsatellite markers, we chose to use those markers that showed optimal performance under conditions in our laboratory. The final selection of markers was made from those of Chambers et al. (2007) and Slotman et al. (2007), and we also isolated three new markers from the *Ae. aegypti* genome (<http://aegypti.vectorbase.org>), namely, another microsatellite marker and two exon primed intron crossing (EPIC) markers. EPIC markers (Exon-Primed Intron Crossing markers) flank the coding regions of single copy genes and are used for detection of polymorphisms across noncoding regions. This is the first study to use EPIC markers in mosquito population work although they have now been applied successfully to investigate population structure in some other insects (He and Haymer 1997, Mun et al. 2003). Implications of the results are discussed in light of attempts to introduce new control options for mosquitoes to suppress transmission of pathogens.

Materials and Methods

Mosquito Collections

Ae. aegypti were sampled from 12 locations in far north Queensland in Australia. These locations were made up of three inland and nine coastal locations, including two suburbs (Parramatta Park and Machans Beach) within Cairns (Table 1; Fig. 1). The greatest distance between Australian sample locations was 441 km between Charters Towers and Mossman.

The inland sites were located in the dry tropics of north Queensland, with annual precipitation ranging from 600 to 900 mm and daily minimal/maximal temperatures of $\approx 17\text{--}31^\circ\text{C}$; annual rainfall in the wet tropics sites ranges from 2,000 to 4,500 mm, with average daily minimal/maximal temperature of $19\text{--}29^\circ\text{C}$ (data from Australian Bureau of Meteorology). Mosquitoes were sampled during the wet season, from March to May 2006. Samples were collected using BG-Sentinel traps (run with or without dry-ice) (Williams et al. 2006), ovitraps (Ritchie et al. 2004), or by pipetting larvae from flooded containers. Attempts were made to collect at least 50 live individuals per location. To minimize the number of siblings in the analysis, only five adults per BG-Sentinel trap and one larva per ovitrap or container were used. Live specimens (larvae or adults) were killed by freezing or were submerged directly in absolute ethanol. Samples were preserved in absolute ethanol and stored at -20°C before analysis.

Female *Ae. aegypti* adults were collected from six locations in Vietnam: Cat Hai, Hai Phong Province (Northern Vietnam); Kim Bang, Ha Nam Province (Northern Vietnam); Buon Me Thuat, Dak Lak Province (Central Highlands), Tri Nguyen, Khanh Hoa Province (Central Vietnam); Thanh Thoi, Vinh Long Province (Southern Vietnam); Thanh Phu Dong, Ben Tre Province (Southern Vietnam). Collections were made inside houses using mechanical aspirators (Clarke Mosquito Control, Roselle, IL) between May and August 2006. Individual female mosquitoes were killed by freezing at -20°C , identified to species, and then stored in absolute ethanol at -60°C until processed. A sample of adult *Ae. aegypti* was taken from Plaeng Yao District, Chachoengsao Province, Thailand, from inside houses by using mechanical aspirators.

Genetic Markers

Eight genetic markers were used to screen for variation in populations of *Ae. aegypti* (Table 1). One microsatellite marker (Gyp8) and two ribosomal protein EPIC markers (Rps20b and RpL30a) were developed for this study. The ribosomal protein markers were developed by identifying proteins from the unannotated genomic sequence of *Ae. aegypti* and developing primers for those sequences that had an intron that was within ≈ 300 bp of exons. Rps20b

(78148872 gb AAGE02016649.1 *Aedes aegypti* strain Liverpool cont1.16649, whole genome shotgun sequence) spans position 681–930. RpL30a (78157656 gb AAGE02008136.1 *Aedes aegypti* strain Liverpool cont1.8136) spans position 271–468. The Gyp8 microsatellite marker also was developed from sequences taken from the unannotated genomic sequence of *Ae. aegypti* (contig 1.12955, GenBank accession 78152577, position 29289–29470). A further two microsatellite loci developed by Slotman et al. (2007) (AC1 and AG5) and three loci developed by Chambers et al. (2007) (BbA10, BbH08, and BbB07) also were used to screen samples.

Amplification of microsatellites by polymerase chain reaction (PCR) took place in a volume of 10 μ l with 2 μ l of genomic DNA extracted using either a cetyltrimethylammonium bromide/chloroform method (Weeks et al. 2002) or a Chelex 100 Resin (Bio-Rad Laboratories, Hercules, CA) method (Endersby et al. 2005). Primer concentrations of 0.03 μ M (forward primer end labeled with [γ^{33} -P]ATP), 0.1 μ M (unlabeled forward primer), and 0.4 μ M (reverse primer) were used. The reaction mix contained 2.0 mM MgCl₂, 0.1 mM dNTPs, 0.5 mg/ml purified bovine serum albumin (New England Biolabs, Ipswich, MA), 1 μ l of 10 \times PCR amplification buffer, and 0.4 U of *Taq* polymerase (New England Biolabs). PCR cycling conditions were as follows: AC1, AG5: denaturation (10 min, 94°C), 35 cycles of 94°C (30 s), annealing (30 s) 55°C and extension at 72°C (30 s), with final extension at 72°C (5 min); BbA10, BbH08, BbB07: denaturation (5 min, 94°C), 30 cycles of 94°C (1 min), annealing (1 min) at 60°C and extension at 72°C (2 min), with final extension at 72°C (10 min); and Rps20b, RpL30a, Gyp8: denaturation (5 min, 94°C), 35 cycles of 94°C (30 s), annealing (30 s) at 58°C and extension at 72°C (45 s), with final extension at 72°C (5 min). Fragments derived from PCR were separated through 5% denaturing polyacrylamide gels at 65 W for 2.5–3.5 h and exposed for >12 h to autoradiographic film.

Analysis

The following basic statistics were calculated for the genetic marker data using FSTAT, version 2.9.3 (Goudet 1995): allelic richness per population averaged over loci, Weir and Cockerham's measure of F_{IS} , a global estimate of F_{ST} (with 95% confidence limits) (Weir and Cockerham 1984); population pairwise measures of F_{ST} and their significance determined using permutations; and pairs of loci tested for linkage disequilibrium using a log-likelihood ratio test. Measures of F_{ST} over all loci were used to estimate the level of gene flow among populations following the formula $Nm = 1/4 (1/F_{ST} - 1)$ (Slatkin and Barton 1989), where N is the effective population size (the number of individuals contributing to the next generation) and m is the migration rate.

Estimates of observed (H_O) and expected (H_E) heterozygosity were determined using Genetic Data Analysis (GDA) (Lewis and Zaykin 2001) and deviations from Hardy–Weinberg (HW) equilibrium were tested using Genepop, version 3.4 (Raymond and Rousset 1995). Regression and Mantel tests of Slatkin's linearized F_{ST} transformation [$F_{ST}/(1-F_{ST})$] (Rousset 1997), with the natural log of geographical distance were calculated using PopTools, version 2.6 (Hood 2002). Significance of Mantel tests was determined by permutation (10,000 randomizations).

An analysis of molecular variation (AMOVA) was performed in Arlequin, version 3.11 (Schneider et al. 2000) by using pairwise F_{ST} as the distance measure, with 10,000 permutations and missing data for loci set at 10%. The model for analysis partitioned variation among regions (Australia, Thailand, and Vietnam), among populations within regions, and within populations. A factorial correspondence analysis was used to summarize patterns of genetic differentiation between the populations sampled (Genetix, version 4.03) (Belkhir et al. 2004). We plotted the first two underlying factors that explain the majority of the variation in the multilocus genotypes.

A Bayesian analysis to estimate the number of populations within the sample data was made using STRUCTURE, version 2 (Pritchard et al. 2000). A burn-in length of 100,000 was chosen followed by 500,000 iterations and the simulation was run using the admixture model with allele frequencies correlated among populations. The number of populations within the data (K) is estimated by checking the fit of the model for a range of K values. The data consisted of nineteen samples so K values of 1–19 were tested with three runs for each value of K . We used the method of Evanno et al. (2005) to estimate the true K .

We used two indirect methods to estimate N_e for all populations in this study. The first method calculates the effective population size as $N_e = H_E/4v(1 - H_E)$ and follows the assumptions of the Infinite Alleles model (IAM) of evolution (Kimura and Crow 1964). The second method is based on the stepwise mutation model (SMM) (Ohta and Kimura 1973) and calculates the effective population size as $N_e = [(1/(1 - H_E)^2) - 1]/8v$. The methods are based on models for the evolution of microsatellite loci, and we therefore only used data from the six microsatellite loci.

Results

Genetic Markers

Seventy alleles in total were found for the eight genetic markers. The highest number of alleles was found for the microsatellite locus BbB07 (18; Table 2). The EPIC marker RpL30a had the lowest number of alleles (three), whereas the other EPIC marker (RpS20b) showed similar numbers of alleles to the other microsatellite loci. Allelic richness averaged over loci was highest in the samples from Vietnam (Table 3), and the Australian samples consistently had lower numbers of alleles at each locus compared with the Vietnamese samples ($P < 0.05$ for all pairwise population comparisons using a Wilcoxon's signed rank test), although there was no significant difference between allele numbers in Australia and Thailand or Vietnam and Thailand. Observed and expected heterozygosities were moderately high in all populations sampled ($H_O \approx 0.43$ – 0.58 ; $H_E \approx 0.42$ – 0.59). Only one population had a significant F_{IS} , with an excess of homozygotes (Ca Hai, Hai Phong, northern Vietnam). Similarly, this was the only population that was significantly out of HW equilibrium across loci. However, when locus BbB07 was removed from the analyses, this population was no longer out of HW equilibrium and did not show an excess of homozygotes (data not shown). The microsatellite locus BbB07 showed a lack of heterozygotes across all populations (Table 2), and this may reflect the presence of null alleles at this locus. No consistent linkage disequilibrium was found between any loci across all populations, with only three of 532 pairwise tests significant after corrections for multiple comparisons.

Population Differentiation

The estimate of F_{ST} over all populations was 0.069 (0.055–0.085; 99% confidence intervals [CIs]), which indicates that there is significant population differentiation between the samples screened here. An AMOVA showed significant differentiation between samples from Australia, Thailand, and Vietnam, as well as differences within populations from Australia and Vietnam (Table 4). The majority of the variation in the microsatellite loci was explained by variation within populations (90.9%; $P < 0.001$), whereas variation between regions (Australia, Thailand, and Vietnam) explained 5.4% ($P < 0.001$) of the variation and populations within regions explained 3.7% ($P < 0.001$). Population pairwise comparisons of F_{ST} revealed only 20 nonsignificant comparisons out of 171 (Table 5). The Thailand and Vietnam populations were differentiated from all Australian populations; however, the Vietnamese populations showed the least differentiation among themselves with 12 of 15 comparisons for the six populations being nonsignificant.

The first three axes in the factorial correspondence analysis at the population level explained 32.4, 10.7, and 9.1% of the variation. The first two axes are plotted in Fig. 2. Samples from Thailand and Vietnam showed a higher amount of genetic variation than samples from Australia, which seemed more discrete. The Australian samples are clearly differentiated from the Vietnam and Thailand samples, whereas the Vietnam and Thailand samples also seem to be differentiated. STRUCTURE analysis, using the method of Evanno et al. (2005), also indicated that the number of populations within our data set was three (data not shown). However, in the STRUCTURE analysis, samples from Australia were a mixture of two populations, whereas samples from Vietnam and Thailand largely represented one population.

Isolation by Distance

There was a significant correlation between genetic distance and geographic distance with the Mantel test showing a strong relationship between Slatkin's linearized F_{ST} and the natural log of geographic distance (Mantel $r = 0.662$, $P < 0.001$). Linear regression showed this relationship to be positive (Fig. 3a; $R^2 = 0.422$, $F = 123.462$, $P < 0.001$). Within the Australian samples, there was also a positive correlation between genetic and geographic distance, although this relationship was somewhat weaker (Fig. 3b; Mantel $r = 0.371$, $P = 0.026$; $R^2 = 0.208$, $F = 16.806$, $P < 0.001$).

Effective Population Size and Migration Rate

We used two indirect methods to calculate the effective population size based on marker heterozygosity and mutation rate. The effective population size did not differ greatly between populations using these methods and was generally quite high (Table 3). If we assume a mutation rate of 6.3×10^{-6} (Schug et al. 1997), then Chillagoe in northern Queensland had the lowest population size estimate (IAM = 45051, SMM = 53229) and Thanh, Phu Dong, in southern Vietnam had the highest population estimate (IAM = 102077, SMM = 168672).

Estimates of Nm were generally low between populations with an overall estimate of 3.37 (95% CIs, 2.69–4.29). The lowest Nm was 1.65 and found between Machans Beach in Australia and Kim Bang, Ha Nam, in northern Vietnam. The highest Nm was 89.54 and found between Buon Me Thuat, Dak Lak, and Thanh Phu Dong, Ben Tre in Vietnam. Within Australia, values ranged between 1.99 (Chillagoe and Machans Beach) and 26.63 (Gordonvale and Mareeba), with an average Nm of 7.40.

Discussion

Screening with microsatellite and EPIC markers has identified that there is population structure within *Ae. aegypti* in Australia and in Vietnam. F_{ST} values among Australian populations vary significantly and an AMOVA indicates that significant variation (3.7%) exists between populations within regions. Linearized F_{ST} values (Slatkin 1995) also show isolation by geographic distance to some extent although this relationship is relatively weak. This weaker relationship is highlighted by some disjunct population structure in which widely spaced samples are not differentiated genetically (e.g., Townsville and Machans Beach, Townsville and Mossman, Tully and Mossman). This could reflect the translocation of mosquito eggs and aquatic stages in containers as a result of human travel throughout the region. Inland populations are, however, separated clearly from those on the coast (e.g., Chillagoe and Charters Towers). The F_{ST} values are consistent with those obtained by other researchers working with microsatellites and allozymes in *Ae. aegypti* (Paupy et al. 2004b, 2008).

Ae. aegypti shows greater allelic variation in Vietnam than in Australia and, although not significant, tends to have a higher number of alleles than the Thailand sample at each locus. Previous studies of *Ae. aegypti* in Vietnam showed lower levels of genetic differentiation in

the wet season compared with the dry (Huber et al. 2002b). The collections from Vietnam analyzed in our study were all made during the wet season and few pairwise comparisons showed genetic differentiation. However, the samples of Huber et al. (2002b) were taken from Ho Chi Minh City and surrounding districts, whereas those of the current study were taken in north, south and central Vietnam so further comparisons cannot be made.

The AMOVA showed highly significant variation at the regional level, differentiating populations from Vietnam, Australia and Thailand. This variation is best shown by the correspondence analysis (Fig. 2), in which populations from each region seem separate. Therefore, it is likely that very limited movement occurs between these regions. Allelic richness was lower in Australia compared with Vietnam and possibly Thailand. Further samples would need to be taken from Thailand and other parts of Asia to confirm whether variation in allelic richness is lower in Australia, but the data suggest that *Ae. aegypti* may have gone through a bottleneck during colonization of Australia.

These data are consistent with observations of movement patterns of *Ae. aegypti* that suggest limited directional movement of 100 m within 8 d in Queensland (Russell et al. 2005). The domestic form of *Ae. aegypti* is closely associated with human habitation and, in Asia and the South Pacific, is not found in sylvan habitats (Failloux et al. 2002) because it may have difficulty crossing uninhabited areas (Maciel-De-Freitas et al. 2006). *Ae. aegypti* also has limited resistance to some climatic stresses that can further limit movement (Kearney et al. 2009).

The similar genetic constitution of disparate populations (like Mossman/ Townsville in Queensland and Ha Nam/Vinh Long in Vietnam) may be a consequence of human assisted movement. Recently, an incursion of *Ae. aegypti* occurred in isolated Tennant Creek in the Northern Territory and was attributed to human introduction. However, the presence of population genetic structure suggests that this form of movement is limited. Estimates of Nm between populations varied, but in most instances were low and indicated that generally only a small fraction of a local population consisted of migrants.

We also obtained some long-term indirect estimates of N_e from the microsatellite data. With a mutation rate of 6.3×10^{-6} (Schug et al. 1997), these varied from 45051 to 102077 for the infinite alleles model (Kimura and Crow 1964) and from 53229 to 168671 for the stepwise mutation model (Ohta and Kimura 1973). These estimates will generally have large confidence intervals and could be an order of magnitude greater, depending on the mutation rate. Here, we used the *Drosophila* microsatellite mutation rate (Schug et al. 1997), however, to obtain more accurate short-term estimates of N_e , temporal sampling spanning numerous field generations is required (Waples 1989).

The EPIC markers proved useful in detecting population genetic structure in *Ae. aegypti*. The markers were in Hardy–Weinberg equilibrium suggesting that they reflect population processes rather than strong selection. The mean F_{ST} for these markers among populations was similar to that estimated for the microsatellites (0.073), although mean allele number was somewhat lower. The advantage of these markers is that they are unlikely to suffer from null alleles and can be isolated from genomic studies or expressed sequence tag libraries. EPIC markers are becoming more popular for use in population genetic studies in insects (Berrebi et al. 2006, Atarhouch et al. 2007, Rolland et al. 2007) and do not require assumptions about a particular model of evolution that is often required for microsatellites.

The data have several implications for novel control options when introducing mosquitoes into populations. Under several envisaged scenarios, new mosquito variants need to be established at a threshold exceeding an unstable equilibrium point (Turelli and Hoffmann 1999, Sinkins and Gould 2006, Huang et al. 2007). Once this point is exceeded, the new variants will spread

close to fixation. For instance, in the mechanism involving *Wolbachia*, with nonoverlapping generations and no age structure, the unstable point for a *Wolbachia* variant is $\approx 20\%$ depending on the rate of maternal transmission, fitness costs associated with the infection and incompatibility levels induced by *Wolbachia* (Turelli and Hoffmann 1999). Substantial numbers of new variants would be needed for this strategy to be successful, although there also might be other options such as decreasing numbers of the target population through pesticide applications, dry season targeting, or source reduction programs to remove desiccation resistant eggs, before establishing new variants.

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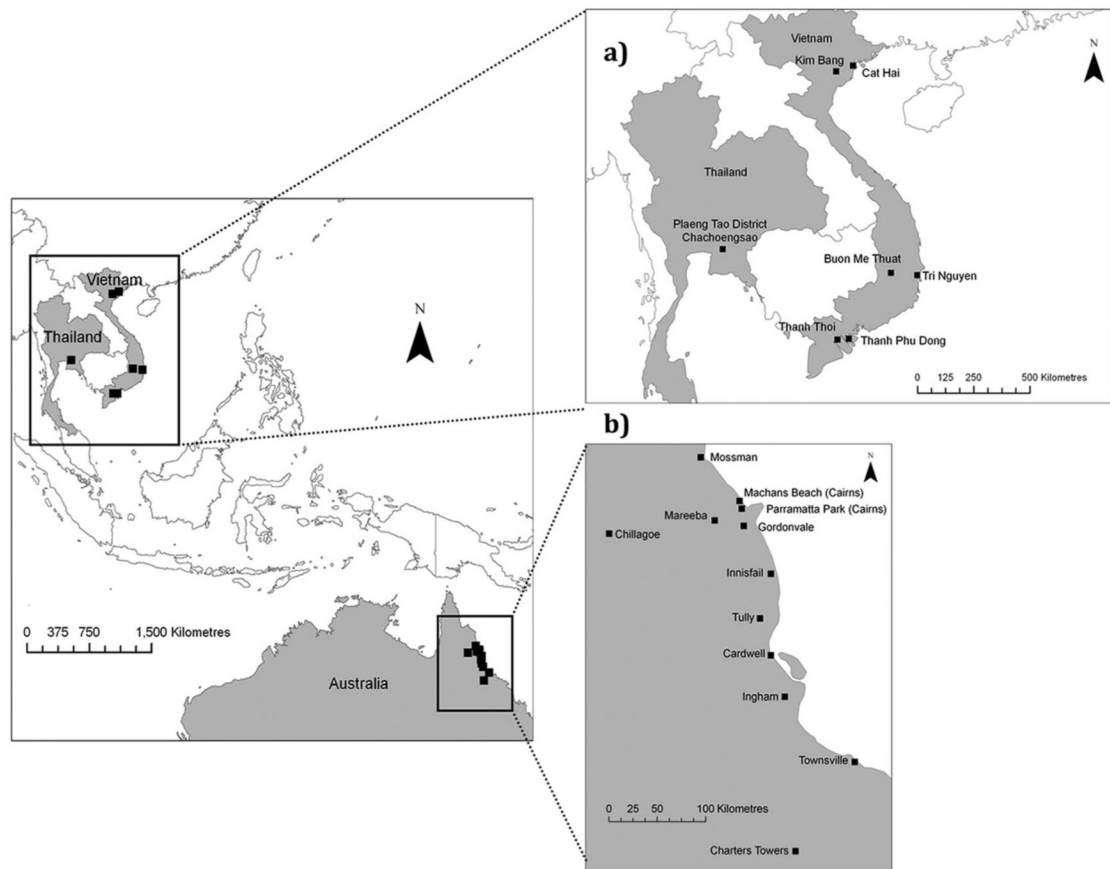


Fig. 1. Map of areas where *Ae. aegypti* was sampled in Australia, Vietnam, and Thailand. Inset figures: (a) collection sites in Thailand and Vietnam and (b) collection sites in north Queensland.

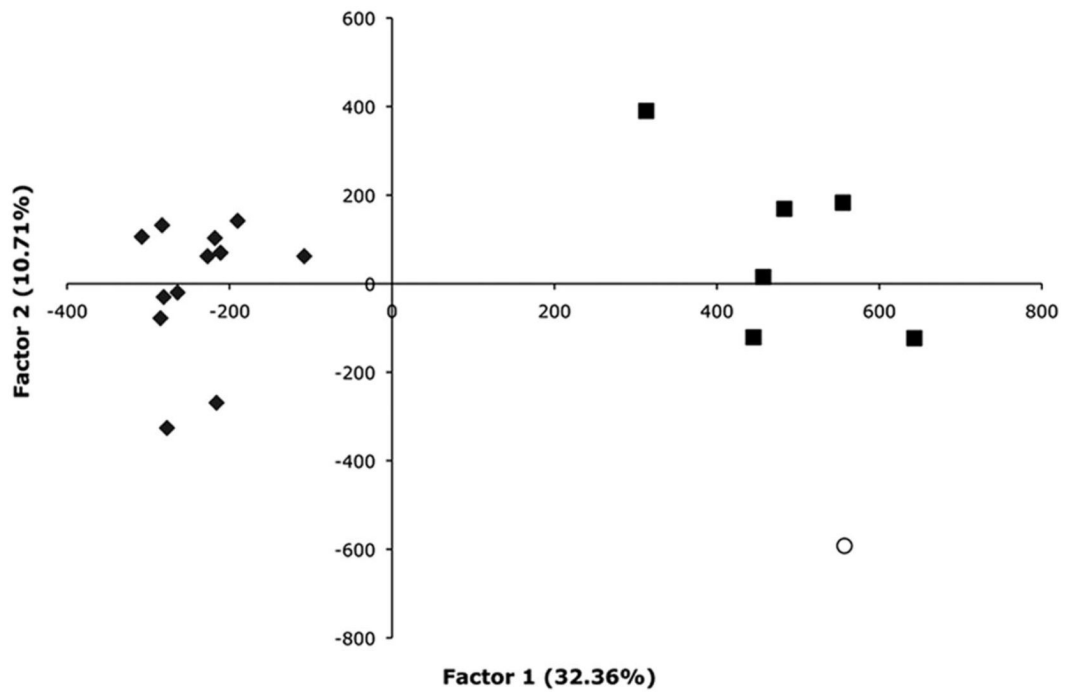


Fig. 2. Factorial correspondence analysis by population for *Ae. aegypti* from Australia, Thailand, and Vietnam. Each point represents a sample region weighted by number of individuals and the sum of alleles present. Closed squares, Vietnam population samples; closed diamonds, Australian population samples; open circle, Thailand sample. Percentage of variation explained by the first factor = 32.36%.

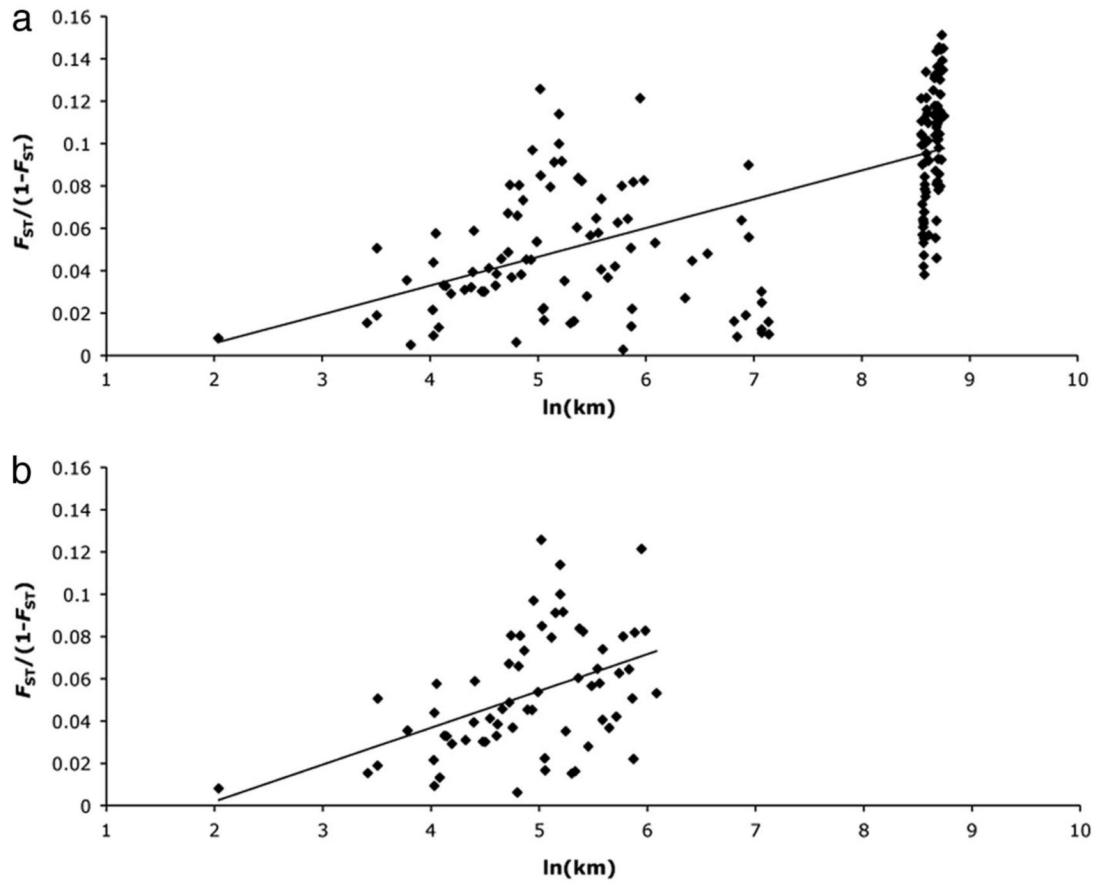


Fig. 3. Regression of Slatkin's linearized F_{ST} ($F_{ST}/1 - F_{ST}$) against the natural logarithm of geographical distance (km) for (a) all pairs of populations from Australia, Vietnam, and Thailand (Mantel $r = 0.662$, $P < 0.001$; $R^2 = 0.422$, $F = 123.462$, $P < 0.001$); and (b) population pairs from Australia only (Mantel $r = 0.371$, $P = 0.026$; $R^2 = 0.208$, $F = 16.806$, $P < 0.001$).

Table 1

Collection details of *Ae. aegypti* samples screened with eight nuclear markers from Australia, Thailand, and Vietnam (wet season 2006)

Country	Location	Life stage	Sample size	
Australia	Parramatta Park, Cairns, Queensland	Adult/larval	32	
	Townsville, Queensland	Adult	35	
	Charters Towers, Queensland	Adult/larval	29	
	Ingham, Queensland	Adult/larval	48	
	Machans Beach, Cairns, Queensland	Adult	48	
	Innisfail, Queensland	Larval	46	
	Chillagoe, Queensland	Adult/larval	43	
	Tully, Queensland	Larval	44	
	Gordonvale, Queensland	Larval	44	
	Mareeba, Queensland	Larval	36	
	Cardwell, Queensland	Adult/larval	44	
	Mossman, Queensland	Larval	31	
	Thailand	Plaeng Yao District, Chachoengsao Province	Adult	48
	Vietnam	Cat Hai, Hai Phong, Northern	Adult	22
Kim Bang, Ha Nam, Northern		Adult	19	
Tri Nguyen, Khanh Hoa, Central		Adult	52	
Buon Me Thuat, Dak Lak, Highland		Adult	38	
Thanh Phu Dong, Ben Tre, Southern		Adult	30	
	Thanh Thoi, Vinh Long, Southern	Adult	19	

Genetic markers used to screen samples of *Ae. aegypti* from Australia, Thailand, and Vietnam collected during the wet season, 2006

Table 2

Locus	Type	Alleles/Marker origin	H_E	H_O	HW P value
Rps20b	EPIC	6Present study	0.4991	0.4930	0.2691
RpL30a	EPIC	3Present study	0.1042	0.0979	0.9999
Gyp8	Msat	7Present study	0.7592	0.7120	0.0510
AC1	Msat	8Slotman <i>et al.</i> (2007)	0.6832	0.5991	0.4597
AG5	Msat	11Slotman <i>et al.</i> (2007)	0.7018	0.6213	0.1864
BbA10	Msat	12Chambers <i>et al.</i> (2007)	0.5327	0.4812	0.0417
BbH08	Msat	5Chambers <i>et al.</i> (2007)	0.2722	0.2702	0.9999*
BbH07	Msat	18Chambers <i>et al.</i> (2007)	0.8329	0.7269	<0.001

Expected (H_E) and observed heterozygosity (H_O) and HW P values are indicated over all populations.

* Asterisk denotes significance after correcting for multiple comparisons using the Dunn-Sidak method (Zar 1996).

Table 3

Ae. aegypti population statistics from screening with eight genetic markers

Pop	Location	<i>r</i>	<i>F_{IS}</i>	<i>H_E</i>	<i>H_O</i>	HW- <i>P</i>	<i>N_e^{IAM}</i> (<i>N_e^{SMM}</i>)
Australia	Parramatta Park, Cairns, Queensland	3.75	-0.005	0.5534	0.5563	0.9804	95989 (153786)
	Townsville, Queensland	3.62	0.003	0.4707	0.4679	0.2988	58785 (76122)
	Charters Towers, Queensland	4.11	-0.016	0.5402	0.5489	0.1127	77382 (112087)
	Ingham, Queensland	3.48	0.001	0.4422	0.4417	0.2826	52195 (64749)
	Machans Beach, Cairns, Queensland	3.70	0.009	0.5375	0.5326	0.1712	85160 (128822)
	Innisfail, Queensland	3.92	0.034	0.5237	0.5062	0.9754	75352 (107883)
	Chillagoe, Queensland	3.01	-0.075	0.4214	0.4526	0.5083	45051 (53229)
	Tully, Queensland	3.66	0.062	0.5307	0.4980	0.0097	80992 (119731)
	Gordonvale, Queensland	3.42	-0.053	0.4979	0.5238	0.3670	68679 (94546)
	Mareeba, Queensland	3.46	-0.033	0.4503	0.4652	0.9999	54361 (68407)
	Cardwell, Queensland	3.59	-0.021	0.4699	0.4795	0.2135	58062 (74839)
	Mossman, Queensland	3.50	0.078	0.4681	0.4321	0.3378	57973 (74682)
	Plaeng Yao District, Chachoengsao Province	4.09	0.044	0.5756	0.5507	0.3745	81848 (121573)
	Cat Hai, Hai Phong, Northern Vietnam	4.53	0.164*	0.5488	0.4606	0.0004	83457 (125072)
Thailand	Kim Bang, Ha Nam, Northern	4.87	0.114	0.5785	0.5114	0.0259	84667 (127731)
	Tri Nguyen, Khanh Hoa, Central	4.33	0.045	0.5366	0.5125	0.0187	74929 (107016)
	Buon Me Thuat, Dak Lak, Highland	4.41	0.096	0.5308	0.4803	0.3876	76729 (110727)
	Thanh Phu Dong, Ben Tre, Southern	5.06	0.036	0.5758	0.5553	0.8587	102077 (168672)
	Thanh Thoi, Vinh Long, Southern	4.81	0.023	0.5884	0.5754	0.8783	97966 (158552)

Mean values over loci are indicated for allelic richness (*r*), inbreeding (*F_{IS}*), expected (*H_E*) and observed (*H_O*) heterozygosities, Hardy-Weinberg equilibrium *P* values. Indirect estimates of *N_e* were calculated under the assumptions of an IAM and SMM by using a mutation rate of 6.3×10^{-6} (Shug et al. 1997).

* Asterisk denotes significantly different from zero after corrections for multiple comparisons at the table-wide $\alpha = 0.05$ level.

Analysis of molecular variation at eight molecular markers among three regions (Australia, Vietnam, and Thailand), among populations within regions and within populations of *Ae. aegypti*

Table 4

Source of variation	df	Sum of squares	Variance components	Fixation indices	P value	% variation
Among regions	2	90.19	0.111	0.054	<0.001	5.40
Among populations within regions	16	117.90	0.075	0.039	<0.001	3.68
Within populations	1,397	2,605.06	1.865	0.091	<0.001	90.93
Total	1,415	2,813.15	2.051			

Table 5

Pairwise population comparisons of *Ae. aegypti* from Australia, Thailand, and Vietnam

	1	2	3	4	5	6	7	8	9	10	11	1213	14	15	16	17	18	19
1. Parramatta	0	0.0547	0.0483	0.0836	0.0081	0.0211	0.0783	0.0301	0.0152	0.0321	0.0433	0.03710	0.0440	0.074	0.0725	0.0452	0.0404	0.0368
2. Townsville	282.2	0	0.0437	0.0294	0.0689	0.0160	0.0741	0.0150	0.0608	0.0590	0.0062	0.02160	0.1015	0.1266	0.1222	0.0921	0.1026	0.0989
3. Charters	354.1	107.5	0	0.0909	0.0757	0.0404	0.1083	0.0355	0.0606	0.0764	0.0570	0.05050	0.0846	0.1016	0.1188	0.0842	0.0846	0.0538
4. Ingham	197.0	95.3	180.4	0	0.1023	0.0619	0.0536	0.0465	0.0737	0.0761	0.0482	0.03900	0.1270	0.1314	0.1261	0.1084	0.1181	0.1040
5. Machans	7.9	267.0	358.7	180.1	0	0.0318	0.1117	0.0556	0.0186	0.0421	0.0510	0.03960	0.0749	0.1019	0.1013	0.0587	0.0596	0.0634
6. Innisfail	72.2	207.0	302.9	122.6	63.2	0	0.084	0.0131	0.0284	0.0356	0.0293	0.01640	0.0973	0.076	0.1034	0.0999	0.0728	0.0698
7. Chillagoe	134.5	322.4	382.2	241.1	151.4	185.2	0	0.0884	0.0744	0.0745	0.0773	0.06830	0.1113	0.1169	0.1054	0.1161	0.0946	0.0941
8. Tully	114.0	200.4	283.3	112.5	81.9	59.2	140.9	0	0.0545	0.0629	0.0379	0.02190	0.0988	0.0922	0.1152	0.1182	0.1006	0.0907
9. Gordonvale	18.8	254.5	340.5	166.3	33.3	66.3	124.5	57.6	0	0.0093	0.0434	0.03200	0.0942	0.0598	0.0789	0.0926	0.0505	0.0571
10. Mareeba	37.2	310.8	395.5	222.6	56.3	116.2	114.5	112.3	56.3	0	0.0340	0.03430	0.1158	0.0526	0.0849	0.0993	0.0540	0.0667
11. Cardwell	151.7	121.2	212.9	33.4	146.8	90.2	215.3	81.1	133.3	189.6	0	0.02720	0.0985	0.0893	0.1184	0.1097	0.0716	0.0837
12. Mossman	65.1	354.6	439.3	266.4	94.2	156.9	129.3	156.2	100.2	44.0	233.4	00.102	0.1054	0.1200	0.1255	0.0904	0.0996	0.0827
13. Thailand	5900.5	6095.3	6121.6	6021.2	5895.0	5951.8	5781.6	5920.6	5885.7	5838.8	5996.8	5805.00	0.0825	0.0529	0.0600	0.0459	0.0428	0.0264
14. Cat Hai, N	5961.6	6186.7	6232.6	6104.6	5955.2	6016.4	5864.5	5995.9	5952.5	5900.2	6076.1	5861.4	0	0.0213	0.0368	0.0159	0.0122	0.0244
15. Kim Bang, N	6164.8	6338.8	6384.1	6256.9	6108.0	6169.1	6016.7	6148.3	6105.1	6052.9	6228.5	6014.2	155.0	0	0.0312	0.0187	0.0099	0.0157
16. Tri Nguyen, C	6058.1	6281.1	6325.5	6199.5	6051.7	6112.7	5959.1	6091.3	6048.5	5996.6	6171.4	5958.1	127.0	79.9	0	0.0088	0.0106	0.0293
17. Buon Me Thuat C	5294.8	5502.4	5536.8	5424.8	5289.0	5347.7	5183.8	5320.5	5282.1	5232.9	5398.7	5197.1	912.4	1017.6	939.2	0	0.0028	0.0136
18. Thanh Phu Dong, S	5282.4	5478.9	5506.6	5404.3	5276.9	5333.9	5164.4	5303.1	5267.8	5220.6	5379.6	5186.7	1178.1	1259.9	1180.0	326.3	0	0.0050
19. Thanh Thoi, S	5321.1	5516.7	5543.8	5442.3	5315.6	5372.4	5202.6	5341.4	5306.3	5259.3	5417.8	5225.5	1179.5	1256.3	1176.5	352.2	45.6	0

Upper diagonal is pairwise *F*_{ST} estimates, below diagonal is pairwise geographic distance (km). Significance of *F*_{ST} estimates (obtained by 171,000 permutations) at the table-wide $\alpha = 0.05$ level are indicated by normal type (bold type indicates nonsignificance).