GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Population genetic structure of *Aedes albopictus* in Penang, Malaysia

M.K.N. Zawani[1], H.A. Abu[1], A.B. Sazaly[2], S.Y. Zary[1] and M.N. Darlina[1]

[1]School of Biological Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia
[2]Tropical Infectious Diseases Research and Education Centre, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia

Corresponding author: M.N. Darlina
E-mail: darlinamdn@usm.my

Genet. Mol. Res. 13 (4): 8184-8196 (2014)
Received September 20, 2013
Accepted August 14, 2014
Published October 7, 2014
DOI http://dx.doi.org/10.4238/2014.October.7.13

ABSTRACT. The mosquito *Aedes albopictus* is indigenous to Southeast Asian and is a vector for arbovirus diseases. Studies examining the population genetics structure of *A. albopictus* have been conducted worldwide; however, there are no documented reports on the population genetic structure of *A. albopictus* in Malaysia, particularly in Penang. We examined the population genetics of *A. albopictus* based on a 445-base pair segment of the mitochondrial DNA cytochrome oxidase 1 gene among 77 individuals from 9 localities representing 4 regions (Seberang Perai Utara, Seberang Perai Tengah, Northeast, and Southwest) of Penang. A total of 37 haplotypes were detected, including 28 unique haplotypes. The other 9 haplotypes were shared among various populations. These shared haplotypes reflect the weak population genetic structure of *A. albopictus*. The phylogenetic tree showed a low bootstrap value with no genetic structure, which was supported by minimum spanning network analysis. Analysis of mismatch distribution showed poor fit of equilibrium distribution. The genetic distance showed low genetic variation, while pairwise F_{ST} values showed no significant difference between all regions in Penang except for some localities.

High haplotype diversity and low nucleotide diversity was observed for cytochrome oxidase 1 mtDNA. We conclude that there is no population genetic structure of *A. albopictus* mosquitoes in the Penang area.

Key words: *Aedes albopictus*; Arbovirus; COI gene; Penang; Population structure

INTRODUCTION

Dengue is an emerging infectious disease affecting more than 50 million individuals each year in tropical and subtropical areas (Rezza, 2012); the number of cases has increased since the initial outbreak in 1989 (Herrera et al., 2006). Some scientists speculated that dengue virus originated in Africa (Ehrenkranz et al., 1971), while others proposed that the virus evolved from a jungle cycle involving lower primates and canopy-dwelling mosquitoes in the Malay peninsula (Smith, 1956). Southeast Asia recorded the highest dengue outbreak worldwide, and cycles of the epidemics have affected the region since 1950s (Shepard et al., 2013). The control of dengue vectors has mainly been achieved through source reduction such as eliminating containers that are favorable sites for oviposition and development of aquatic stages. This can be accomplished by fitting lids on containers or by killing the insects during aquatic stages using insecticides (World Health Organization, 2012). In hot-spot or epidemic areas, insecticide space-spraying is recommended; however, its efficacy in other situations has not been well-documented (Esu et al., 2010). Indoor space-spraying is highly labor-intensive and impractical in the event of an outbreak in most locations. The World Health Organization published a manual regarding the global strategy for dengue prevention and control in 2012. Strategies include the implementation of timely, appropriate clinical management, which involves early clinical and laboratory diagnosis, intravenous rehydration, staff training, and hospital reorganization, and integrated surveillance.

Malaysia reported 35,533 dengue cases that resulted in 107 deaths by September 2010 (Aida et al., 2011). Penang reported 268 cases of dengue fever through May 26. 2013, 98 fewer cases compared to year 2012 (Ministry of Health Malaysia, 2013). In addition to *Aedes aegypti*, *A. albopictus* is an important vector of dengue virus (Nelder et al., 2010). This mosquito is native to Asia and is also known as the Asian tiger mosquito (Usmani-Brown et al., 2009). *Aedes albopictus* is a daytime-biting mosquito that transmits a variety of viruses through a female bite (Aida et al., 2011). This species can breed in artificial container habitats such as tires, buckets, vases, and ornamental ponds, resulting in its rapid and successful widespread distribution (Novak, 1992; Birungi and Munstermann, 2002). *A. albopictus* was found in the Americas in 1985 and is currently present in 8 American countries (Pinheiro and Nelson, 1997). In China, Peng et al. (2012) reported the re-emergence of dengue in a city in southern China 2 decades after a traveler from Southeast Asia introduced *A. albopictus*. Thus, examining the population genetic structure is important for understanding the evolution of this species (Hewitt, 1983), which may lead to novel methods of controlling disease transmission (Ayres et al., 2003).

Population genetics studies of *A. albopictus* have been carried out worldwide as the species continues to spread and displace *A. aegypti* in some areas (Gratz, 2004). Currently, mosquito populations are being investigated at the molecular and behavioral levels using multi-markers (Ravel et al., 2002; Ayres et al., 2003; Herrera et al., 2006) to understand the

epidemiology of disease transmission and to develop innovative strategies for controlling the vector (Bresford and Dobson, 2012). Different genetic markers have also been used to study the population genetic structure of *A. albopictus*, such as microsatellite loci (Kamgang et al., 2011) and mitochondrial DNA regions, including NAD dehydrogenase subunit 5 (Birungi and Munstermann, 2002; Usmani-Brown et al., 2009; Kamgang et al., 2011) and cytochrome oxidase subunit 1 (COI) (Kamgang et al., 2011). Mitochondrial DNA (mtDNA) is sensitive to genetic drift, resulting in greater genetic differentiation; it may also be used as a population-specific marker (Birungi and Munstermann, 2002). Additionally, mitochondrial DNA is maternally inherited and thus genetic relationships among individuals can be determined using recombinant individuals (Lansman et al., 1981).

Previous studies examining the population genetics structure of *A. albopictus* in Cameroon revealed moderate genetic diversity and revealed that the genetic structure of natural populations suggests that multiple introductions occurred in tropical regions (Kamgang et al., 2011). Similarly, polymorphisms in the COI gene have been reported by Shaikevich and Talbalaghi (2013), who proposed that *A. albopictus* originated from multiple ancestor populations. However, there have been no documented studies on the population genetic structure of *A. albopictus* in Malaysia, particularly in Penang, which is located in the northern region of Peninsular Malaysia.

The aim of this study was to investigate the population genetic structure of *A. albopictus* in different regions of Penang, Malaysia using a COI mitochondrial DNA marker. The population structure is relevant to evolution of *A. albopictus* and understanding the migration of this mosquito is useful for controlling the spread of the dengue vector in Malaysia.

MATERIAL AND METHODS

Larvae collection

Mosquitoes were collected as larvae (fourth instar) from 4 regions of Penang, which include Seberang Perai Utara (Bagan Dalam, Kepala Batas), Seberang Perai Tengah (Padang Lalang), Northeast Penang (Universiti Sains Malaysia, Taman Tun Sardon, Tingkat Sungai Gelugor, Teluk Awak), and Southwest part Penang (Permatang Damar Laut, Balik Pulau) (Figure 1, **Table S1**). The latitude and longitude where each sample collected was recorded and is listed in **Table S1**. The sample ID, haplotype, and accession numbers are listed in **Table S2**. Larvae were collected using ovitrap and empty cans from June 2012 to January 2013, where they were left for a week before collection. The larvae were preserved in 70% ethanol and stored at -20°C until DNA extraction.

DNA extraction and species identification

Genomic DNA was extracted from individual larvae specimens using the salt extraction method, resuspended in 100 µL Tris-EDTA buffer, and stored at -20°C. DNA purity was determined based on the A_{260}/A_{280} ratio ranging from 1.8 to 2.0 for all samples (Aljanabi and Martinez, 1997). Larvae of *A. albopictus* and *A. aegypti* were differentiated based morphological characteristics, including differences in the head and abdomen (siphon, setae, and comb scales) (Chung, 1997). However, we did not identify the species based on taxonomy as the

larvae were very small and the body parts may have disassociated when preserved in alcohol. Because of the difficulty in distinguishing the mosquito morphology, polymerase chain reaction (PCR) was used to determine species' identity.

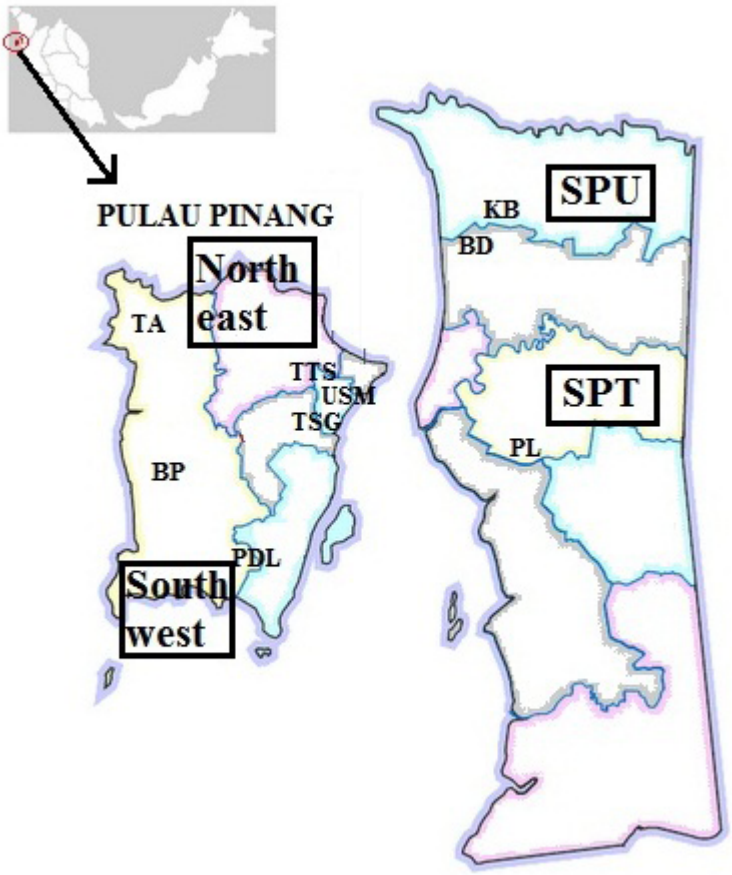

Figure 1. Sampling locations of *Aedes albopictus* in Penang. SPU = Seberang Perai Utara; SPT = Seberang Perai Tengah; TA = Teluk Awak; TTS = Taman Tun Sardon; USM = Universiti Sains Malaysia; TSG = Tingkat Sungai Gelugor; BP = Balik Pulau; PDL = Permatang Damar Laut; KB = Kepala Batas; BD = Bagan Dalam; PL = Padang Lalang.

Mitochondrial gene amplification

PCR was used to amplify the target region of the COI gene in the mtDNA genome of *A. albopictus.* The 445-base pair (bp) region of the COI gene was amplified using the primer pair designed by Bonacum et al. (2001): C1-J-1718-5'-GGAGGATTTGGAAATTGATTAGT TC-3' and C1-N-2191-5'-CCCGGTAAAATTAAAATATAAACTTC-3'. PCR amplification was carried out in a Bio-Rad (Hercules, CA, USA) thermal cycler. The reaction mixture contained 2.25 µL 10X buffer, 0.5 dNTP mixture (2.5 mM/µL), 0.1 µL 5 U *i-Taq plus* DNA polymerase (Intron, Gyeonggi-do, Korea), 3.0 µL $MgCl_2$ (25 mM/µL), 1.0 µL of each primer,

1.0 µL DNA template, and ultra-pure water to a final volume of 20 µL. Negative controls (all reagents except template) were included in the reactions. The thermal regime consisted of initial denaturation for 2 min at 95°C, followed by 35 cycles at 94°C for 45 s, 46°C for 45 s, 72°C for 1 min, and final incubation at 72°C for 10 min. The amplified products were visualized by electrophoresis in a 2% agarose gel and stained with ethidium bromide.

Purification and sequencing

The samples were purified following the PCR Purification protocol from Genomics Bioscience and Technology (Taiwan). First, 25 µL purified PCR products with single clear bands on 2% electrophoresed agarose gels were selected for automated DNA sequencing and sequenced by Genomics Bioscience and Technology. Products were electrophoresed on an ABI3730*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) following standard protocols.

Sequence analysis

The program MEGA version 5.1 (Tamura et al., 2011) was used to visualize and align all sequences. Sequences were aligned using ClustalW (Thompson et al., 1994), and alignments were subsequently revised manually to maximize the positional homology.

Geographical distribution of genetic diversity

The number of unique haplotypes, haplotype diversity (h), and nucleotide diversity (π) within populations and sampling regions were analyzed using DnaSP version 5.10.1 (Librado and Rozas, 2009). To measure the extent of genetic structuring among samples, we performed analysis of molecular variance (AMOVA) (Excoffier et al., 1992) using 1000 permutations implemented in the ARLEQUIN version 3.5 software (Excoffier and Lischer, 2010). Analysis was performed within populations and among populations within each region. Using the haplotypic frequencies, F_{ST} values were computed by permutation tests from 1000 random permutations of haplotypes between populations. Significant levels of pairwise F_{ST} were obtained under the null hypothesis of no differentiation between populations. Genetic distance was also computed to determine the genetic variance between population using MEGA version 5.1 (Tamura et al., 2011). We also tested for a signature of historical demographic expansion by calculating Tajima's D and Fu's F_S statistics using ARLEQUIN version 3.5 (Excoffier and Lischer, 2010); 1000 simulations used to evaluate the significance level. Negative values of these statistics indicated excess rare alleles in the genealogy, indicating either population expansion or genetic hitchhiking (in response to selection). We also performed mismatch distribution analysis using the DnaSP version 5.10.1 software (Librado and Rozas, 2009) to identify patterns in nucleotide site differences between haplotype pairs.

Phylogenetic analysis

Phylogenetic relationships among haplotypes were reconstructed using the neighbor-joining (Saitou and Nei, 1987) method implemented in MEGA version 5.1 (Tamura et al.,

2011). The reliability of branches was estimated using non-parametric bootstrap re-sampling procedure with 1000 replicates. The tree was rooted using *Aedes aegypti* (GenBank accession No. HE820718.1) as an outgroup.

The relationships among haplotypes were also established by creating a minimum spanning tree using Network version 4.6.1.1 (fluxus-engineering.com), connecting all haplotype as nodes in a network connected by the least number of substitutions. Data for the minimum spanning tree was computed in the ARLEQUIN version 3.5 program (Excoffier and Lischer, 2010) as a matrix of pairwise substitutions between all putative haplotypes. In addition to providing a schematic representation of haplotype relationships, the network allowed inferences of the coalescent history among haplotypes relative to their common ancestry.

RESULTS

Sampling data

A total of 81 *A. albopictus* individuals were captured in 9 different localities, representing 4 regions in Penang. Of these, 53 samples from the island (Penang) were successfully genotyped (Northeast, N = 40; Southwest, N = 13). For the main island, 24 samples were genotyped successfully (Bagan Dalam, N = 7; Kepala Batas, N = 9; Padang Lalang, N = 8) (**Table S1**).

Sequence analysis

Overall, 77 partial sequences (for GenBank accession numbers, see **Table S2**) of a 445-bp segment of the mtDNA COI gene were obtained, representing 9 populations of *A. albopictus*. All showed 99% similarity with *A. albopictus* sequences obtained from GenBank. There were no insertions, deletions, or stop codons present in the sequences obtained. A total of 37 haplotypes were discovered in the nucleotide dataset (Table 1). Of these, 28 were unique, while the other 9 haplotypes were shared among 2 or more populations. Twenty-six variable sites were identified, and 15 were parsimonious informative; 31 mutations were detected in the total sequences.

Table 1. Haplotype distribution among all populations of *Aedes albopictus* collected in Penang.

Locations	Haplotype																																					Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
SPU																																						
KB	2					1				1	1	1	1	1	1																							9
BD	5	1	1																																			7
SPT																																						
PL	2					1															1	1	2	1														8
Northeast																																						
USM	6		1	1					1																													9
TTS					1		1												3											1	1	1	1	1	1	1	1	13
TSG						3																			1	3	1	1	1									10
TA	3	2	3																																			8
Southwest																																						
PDL		3																																				8
BP		1			1	1	1	1								1	1	1	1	1																		5
Total	18	7	5	1	2	6	2	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	2	1	1	3	1	1	1	1	1	1	1	1	1	1	1	77

For abbreviations, see legend to Figure 1.

mtDNA diversity

A pattern of low nucleotide diversity and high haplotype diversity were characterized for COI mtDNA in our datasets (Table 2). Overall, nucleotide diversity per site (π) was the highest in Taman Tun Sardon samples (π = 0.009 ± 0.001 SD), followed by Kepala Batas (π = 0.009 ± 0.002 SD), Balik Pulau (π = 0.008 ± 0.001 SD), Padang Lalang (π = 0.006 ± 0.002 SD), Permatang Damar Laut (π = 0.005 ± 0.001 SD), Tingkat Sungai Gelugor (π = 0.005 ± 0.001 SD), Universiti Sains Malaysia (π = 0.003 ± 0.001 SD), Teluk Awak (π = 0.002 ± 0.0004 SD), and Bagan Dalam (π = 0.002 ± 0.001 SD) (Table 2). Fu's F_S showed non-significant negative values in all populations (except from Teluk Awak, which showed a non-significant positive value) and Tajima's *D* test for neutrality showed a non-significant negative values in Kepala Batas, Bagan Dalam, Padang Lalang, USM, and Permatang Damar Laut. Analysis of the mismatch distribution showed a poor fit of equilibrium distribution (Figure 2).

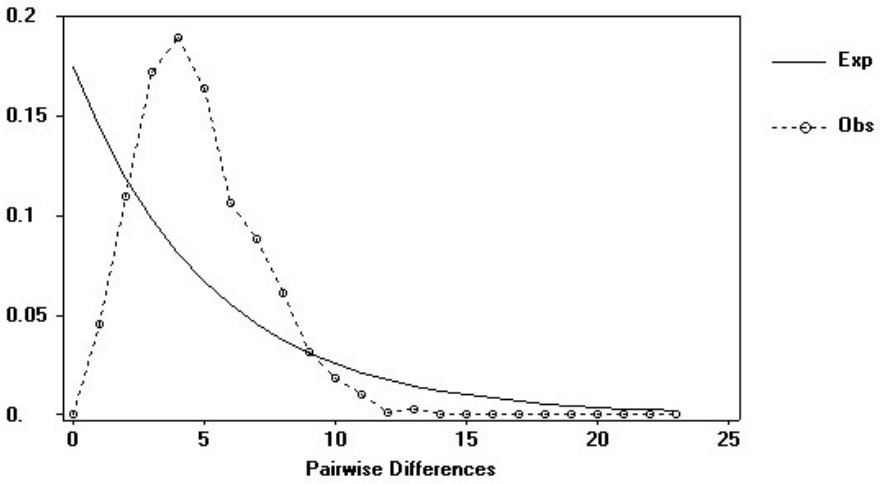

Figure 2. Graph of pairwise distribution showing poor fit of equilibrium distribution. The dashed line shows the empirical pairwise-difference distribution, while the solid line shows the equilibrium distribution with the same mean.

Table 2. Summary of number of haplotypes, nucleotide diversity (π), and haplotype diversity (*h)*, Fu's F_S and Tajima's *D* statistics. N = sample size.

Locations	N	No. of haplotypes	π ± SD	*h* ± SD	F_S	*D*
SPU						
KB	9	8	0.009 ± 0.002	0.972 ± 0.064	-3.471	-0.184
BD	7	3	0.002 ± 0.001	0.524 ± 0.209	-0.438	-0.275
SPT						
PL	8	6	0.006 ± 0.002	0.929 ± 0.084	-1.848	-0.582
Northeast						
USM	9	4	0.003 ± 0.001	0.583 ± 0.183	-0.536	-1.294
TTS	13	11	0.009 ± 0.001	0.962 ± 0.050	-5.543	0.195
TSG	10	6	0.005 ± 0.001	0.867 ± 0.085	-1.746	0.679
TA	8	3	0.002 ± 0.0004	0.750 ± 0.096	0.330	1.449
Southwest						
PDL	8	6	0.005 ± 0.001	0.893 ± 0.111	-2.359	-2.010
BP	5	5	0.008 ± 0.001	1.000 ± 0.126	-2.004	1.241

For abbreviations, see legend to Figure 1.

Population structure

Geographical analysis of variation in COI haplotype frequencies was conducted using AMOVA (Table 3). Most variation in the regions (>65%) was observed mosquitoes within populations, while less than 35% was observed among populations. Pairwise F_{ST} values (Table 4) showed no significant difference (P > 0.05) between regions in Penang except for Kepala Batas and Balik Pulau, and Permatang Damar Laut, Teluk Awak, and Bagan Dalam. Genetic distance showed low genetic variation between the samples in all populations (<1%) (Table 4).

Table 3. Analysis of molecular variance (AMOVA) among *Aedes albopictus* collected from Penang.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Fixation index
Southwest					
Among populations	1	3.450	0.346	20.72	F_{ST} = 0.207
Within populations	11	14.550	1.323	79.28	
Northwest					
Among populations	3	21.406	0.603	33.81	F_{ST} = 0.338
Within populations	36	42.469	1.180	66.19	
SPU					
Among populations	1	3.159	0.239	15.81	F_{ST} = 0.158
Within populations	14	17.841	1.274	84.19	

Table 4. Below diagonal: population subdivision (F_{ST}) values for population differentiation based on 1000 permutations of the sequence dataset implemented in the ARLEQUIN ver. 3.5 software. Above diagonal: pairwise Tamura-Nei genetic distances (*D*) among and within 9 populations *of Aedes albopictus* by using the MEGA 5.1 software.

	BD	KB	PL	USM	TTS	TSG	TA	PDL	BP
BD	0.002	0.006	0.004	0.002	0.009	0.007	0.002	0.004	0.007
KB	0.108	0.009	0.009	0.007	0.010	0.009	0.007	0.008	0.008
PL	0.070	0.060	0.006	0.005	0.012	0.009	0.005	0.007	0.010
USM	-0.095	0.073	0.073	0.003	0.010	0.007	0.003	0.005	0.007
TTS	0.018	0.029	0.057	0.144	0.009	0.009	0.009	0.009	0.008
TSG	0.090	0.024	0.133	0.082	0.155	0.005	0.007	0.007	0.008
TA	**0.046**	0.032	0.113	0.099	0.191	0.069	0.002	0.004	0.007
PDL	0.021	0.181	0.152	0.156	0.028	0.022	**0.044**	0.005	0.008
BP	0.028	**-0.031**	0.183	0.127	-0.072	0.034	0.022	0.007	0.008

Bold indicates significant values at P < 0.05. For abbreviations, see legend to Figure 1.

Phylogenetic analysis

A neighbor-joining tree (Figure 3) showed only 1 clade of Penang samples with low bootstrap support. All 37 haplotypes were clearly distinguished from the outgroup *Aedes aegypti*. The network of COI sequences for *A. albopictus* showed genealogical relationships among 37 haplotypes based on the least number of substitutions. The minimum spanning network showed a star-like pattern, with most haplotypes connected by very few mutation steps (Figure 4). There was 1 dominant haplotype (haplotype 1) observed, from which related sequences were separated by 1 mutational step minimally. This indicated a mixture of haplotypes from Seberang Perai Utara (Kepala Batas and Bagan Dalam), Seberang Perai Tengah (Padang Lalang), and the Northeast (USM and Teluk Awak) region of Penang.

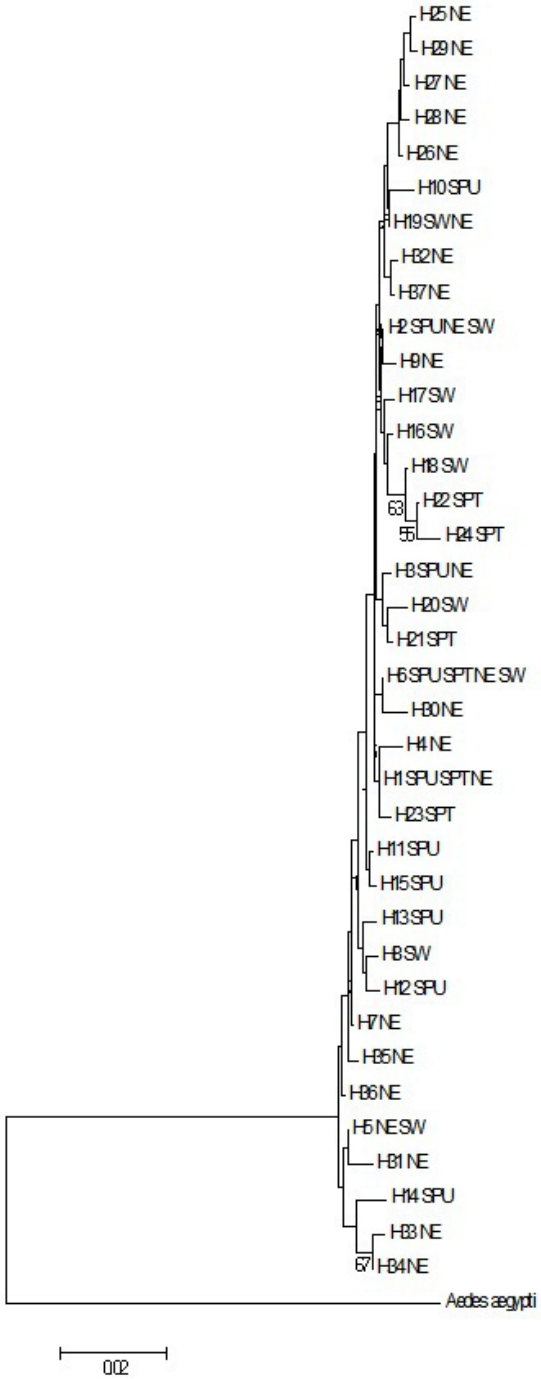

Figure 3. Neighbor-joining tree showing phylogenetic relationships among individual haplotypes. SPU = Seberang Perai Utara; SPT = Seberang Perai Tengah; NE = Northeast; SW = Southwest.

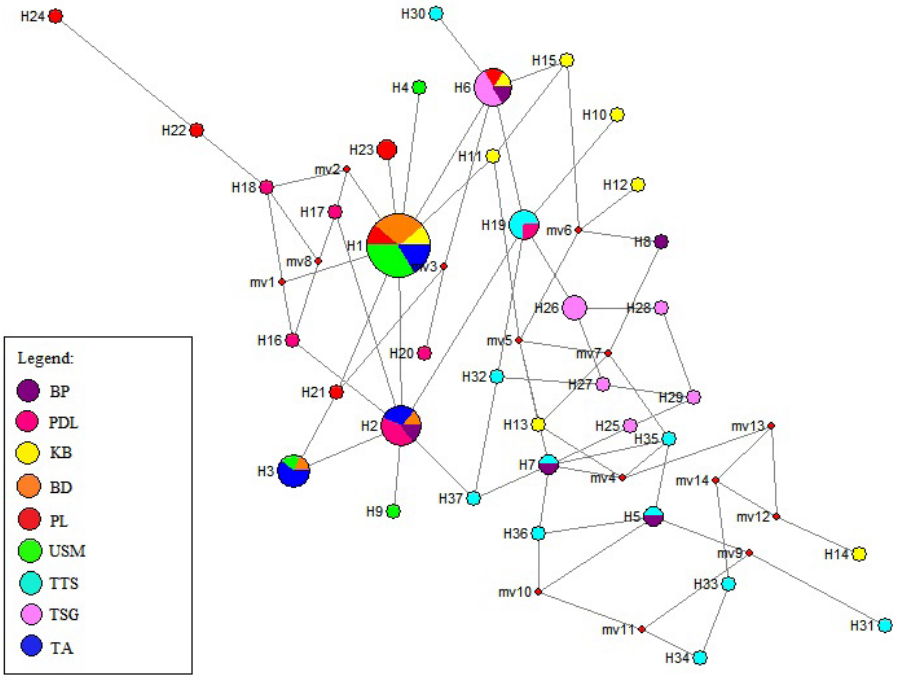

Figure 4. Minimum spanning network among haplotypes of *Aedes albopictus* in 9 locations. TA = Teluk Awak; BD = Bagan Dalam; TTS = Taman Tun Sardon; BP = Balik Pulau; PL = Padang Lalang; PDL = Permatang Damar Laut; TSG = Tingkat Sungai Gelugor; USM = Universiti Sains Malaysia; KB = Kepala Batas.

DISCUSSION

This current study revealed extremely low levels of genetic variation within *A. albopictus* sequences in Penang, Malaysia (Table 2). Other studies of *A. albopictus* mitochondrial diversity also showed that native populations of this species harbor very low or no mitochondrial genetic diversity (Poretta et al., 2012). The pattern observed of genetic variation in populations of *A. albopictus* may be attributed to severe, repeated, or long periods of population bottlenecks that resulted in genetic variation losses because of random genetic drift (Nei et al., 1975; Birungi and Munstermann, 2002). In most areas in Penang, extensive and repeated insect control activities have involved source reduction and insecticide application, leading to the reduction and/or eradication of *A. albopictus* populations (Birungi and Munstermann, 2002; Žitko et al., 2011). As a result, reduced levels of genetic variation were observed in the current study. Another plausible explanation is that the Penang populations of *A. albopictus* were established by a small founding population with low mtDNA variability, and the populations successively expanded and established a few founder females in new geographic areas (Birungi and Munstermann, 2002; Žitko et al., 2011).

We found that *A. albopictus* populations obtained from Penang are characterized by homogenous population structure based on sequences variation in the COI mtDNA gene. Analysis of the mtDNA sequence data in this study showed that 9 haplotypes were shared by 2 or more populations (Table 1). For instance, haplotype 1 (H1) was shared among the SPU,

SPT, and Northeast regions while H6 was shared among the SPU, SPT, Southwest and Northeast regions. H3 was found in SPU and Northeast Penang, H2 was found in SPU and Northeast Penang, and H8 was found in Southwest and Northeast of Penang. H1 was the most dominant, as it was identified in 5 locations (Bagan Dalam, Kepala Batas, Padang Lalang, USM, and Teluk Awak) (Table 1). The presence of shared haplotypes indicate that extensive gene flow and migration between distant populations occurred on a relatively recent evolutionary time scale (Horne et al., 2008).

Similarly, tests of population expansion (originally used to test for selective neutrality) indicated qualitative support for population expansion (i.e., negative *D* and F_S; Table 2). This shows that *A. albopictus* populations in Penang may have experienced population growth in the past, but the expansion may have been restricted to separate local areas, resulting in the non-significant negative Fu's F_S and Tajima's *D* value for most populations studied (Liao et al., 2010). This result was further corroborated by mismatch distribution analysis, which showed unimodal population differentiation (Figure 2), couple with star-like phylogenies of minimum spanning network analysis and a geographically localized distribution (Figure 4). These results indicate that *A. albopictus* populations in Penang have passed through recent demographic expansions. The mechanisms behind this expansion are not fully understood; however, demographic parameters of humans may influence the successful distribution and increasing population size of *A. albopictus* in Penang. Additionally, *A. albopictus* showed increase egg hatch, a short aquatic life, increased immature survival, and fecundity under uncontrolled conditions of temperature and humidity, which may contribute to the increased adult population size (Aida et al., 2011).

Comparison of mtDNA COI sequences among all populations of *A. albopictus* from all regions in Penang showed limited phylogeographic partitioning of haplotypes, as demonstrated by the absence of genealogical divergence (Figure 3) and the significant low values of F_{ST} and *D*, respectively (Table 4). This was further supported by AMOVA, which showed that most total mtDNA sequence variation occurred among samples within a population (Table 3).

These results indicate highly effective migration and gene flow of *A. albopictus* between regions in Penang. The normal flight ranges of *A. albopictus* are limited, and these mosquitoes have not been observed to fly in strong winds (Novak, 1992). However, a recent study by Delatte et al. (2013) reported that the flight ranges of the species might increase when females fail to find a suitable site for oviposition or blood-meals, which is likely assisted by wind. Movement and migration of *A. albopictus* may also be assisted by human activities, such as through the transport of used and waste tires as well as from the movement of other water-holding containers (Novak, 1992). Study by Minakawa et al. (2002) found *Anopheles gambiae* larvae at the bottom of a wooden fishing boat, implying that boats may transport mosquito larvae between islands and main lands (Seberang Perai, also known as Province Wellesley) by an approximately 13.5-km bridge, and the main land *A. albopictus* larvae may be moved to the island by vehicle transportation. We showed that *A. albopictus* populations from Penang are characterized by low genetic diversity and homogenous population structure, likely because of recent range expansion through both active (mosquito movement) and passive (dispersal assisted by human activities) modes of dispersal. Further studies including more extensive sampling and different markers (for example, nuclear genes) are needed to confirm this hypothesis.

ACKNOWLEDGMENTS

The authors would like to thank the staff of the Molecular Ecology and Population Genetics Laboratory, School of Biological Sciences, Universiti Sains Malaysia. Research supported by LRGS grant (LRGS/TD/2011/UM/Penyakit-Berjangkit) (UM) of the Ministry of Higher Education, Malaysia (MOHE) and #304/PBIOLOGI/650576 (USM).

Supplementary material

REFERENCES

Aida HN, Dieng H, Ahmad AH, Satho T, et al. (2011). The biology and demographic parameters of *Aedes albopictus* in northern peninsular Malaysia. *Asian Pac. J. Trop. Biomed.* 1: 472-477.

Aljanabi SM and Martinez I (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.* 25: 4692-4693.

Ayres CF, Melo-Santos MA, Sole-Cava AM and Furtado AF (2003). Genetic differentiation of *Aedes aegypti* (Diptera: Culicidae), the major dengue vector in Brazil. *J. Med. Entomol.* 40: 430-435.

Birungi J and Munstermann LE (2002). Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial ND5 sequences: evidence for an independent invasion into Brazil and United States. *Ann. Entomol. Soc. Am.* 95: 125-132.

Bonacum J, DeSalle R, O'Grady P, Olivera D, et al. (2001). New nuclear and mitochondrial primers for systematics and comparative genomics in *DrosophilidA. Drosophila Inf. Serv.* 84: 201-204.

Bresford CL and Dobson SL (2012). Population structure of *Aedes polynesiensis* in the Society Islands of French Polynesia: implications for control using a *Wolbachia*-based autocidal strategy. *Parasites Vectors* 5: 80-92.

Chung CL (1997). A simple method for determining the identity of the eggs of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) by dissection (Diptera: Culicidae). *Chin. J. Entomol.* 17: 86-91.

Delatte H, Toty C, Boyer S, Bouetard A, et al. (2013). Evidence of habitat structuring *Aedes albopictus* populations in Reunion Island. *PLoS Neglected Trop. Dis.* 7: e2111.

Ehrenkranz NJ, Ventura AK, Cuadrado RR, Pond WL, et al. (1971). Pandemic dengue in Caribbean countries and the southern United States - past, present and potential problems. *New Engl. J. Med.* 285: 1460-1469.

Esu E, Lenhart A, Smith L and Horstick O (2010). Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. *Trop. Med. Int. Health* 15: 619-631.

Excoffier L and Lischer HEL (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10: 564-567.

Excoffier L, Smouse PE and Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Gratz NG (2004). Critical review of the vector status of *Aedes albopictus*. *Med. Vet. Entomol.* 18: 215-227.

Herrera F, Urdaneta L, Rivero J, Zoghbi N, et al. (2006). Population genetic structure of the dengue mosquito *Aedes aegypti* in Venezuela. *Mem. Inst. Oswaldo Cruz* 101: 625-633.

Hewitt G (1983). The role of population genetics in our understanding of evolution. *Tuatara* 26: 73-74.

Horne JB, van Herwerden L, Choat JH and Robertson DR (2008). High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners. *Mol. Phylogenet. Evol.* 49: 629-638.

Kamgang B, Brengues C, Fontenille D, Njiokou F, et al. (2011). Genetic structure of the tiger mosquito, *Aedes albopictus*, in Cameroon (Central Africa). *PLoS One* 6: e20257.

Lansman RA, Shade RO, Shapira JF and Avise JC (1981). The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. *J. Mol. Evol.* 17: 214-226.

Liao PC, Kuo DC, Lin CC, Ho KC, et al. (2010). Historical spatial range expansion and a very recent bottleneck of *Cinnamomum kanehirae* Hay. (Lauraceae) in Taiwan inferred from nuclear genes. *BMC Evol. Biol.* 10: 124.

Librado P and Rozas J (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452.

Minakawa N, Seda P and Yan G (2002). Influence of host and larval habitat distribution on the abundance of African malaria vectors in western Kenya. *Am. J. Trop. Med. Hyg.* 67: 32-38.

Ministry of Health Malaysia (2013). Dengue Situation in Malaysia for the 21st Week. Available at [http://www.moh.gov.my/press_releases/409]. Accessed June 5, 2013.

Nei M, Maruyama T and Chakraborty R (1975). The bottleneck effect and genetic variability in populations. *Evolution* 29: 1-10.

Nelder M, Kesavaraju B, Farajollahi A, Healy S, et al. (2010). Suppressing *Aedes albopictus*, an emerging vector of dengue and chikungunya viruses, by a novel combination of a monomolecular film and an insect-growth regulator. *Am. J. Trop. Med. Hyg.* 82: 831-837.

Novak R (1992). The Asian Tiger Mosquito, *Aedes albopictus*. *Wing Beats* 3: 5.

Peng HJ, Lai HB, Zhang QL, Xu BY, (2012). A local outbreak of dengue caused by an imported case in Dongguan China. *BMC Public. Health* 12: 83-91.

Pinheiro F and Nelson M (1997). Re-emergence of dengue and emergence of dengue haemorrhagic fever in the Americas. *Dengue Bull.* 21: 16-24.

Poretta D, Mastrantonio V, Bellini R, Somboon P (2012). Glacial history of a modern invader: Phylogeography and species distribution modelling of the Asian tiger mosquito *Aedes albopictus*. *PLoS One* 7: e44515.

Ravel S, Herve JP, Diarrassouba S, Kone A, et al. (2002). Microsatellite markers for population genetic studies in *Aedes aegypti* (Diptera: Culicidae) from Cote d'Ivoire: evidence for a microgeographic genetic differentiation of mosquitoes from Bouake. *Acta Trop.* 82: 39-49.

Rezza G (2012). Aedes albopictus and the reemergence of dengue. *BMC Public. Health* 12: 72.

Saitou N and Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-425.

Shaikevich E and Talbalaghi A (2013). Molecular characterization of the Asian tiger mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae) in Northern Italy. *ISRN Entomol.* 2013: 1-6.

Shepard DS, Lees R, Ng CW, Undurraga EA, et al. (2013). Burden of dengue in Malaysia: report from collaboration between universities and the Ministry of Health of Malaysia. Available at [http://people.brandeis.edu/~shepard/Report_dengue_in_Malaysia_v50.pdf]. Accessed June 5, 2013.

Smith CE (1956). The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. *J. Trop. Med. Hyg.* 59: 243-251.

Tamura K, Peterson D, Peterson N, Stecher G, et al. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731-2739.

Thompson JD, Higgins DG and Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673-4680.

Usmani-Brown S, Cohnstaedt L and Munstermann LE (2009). Population genetics of *Aedes albopictus* (Diptera: Culicidae) invading populations, using mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 5 sequences. *Ann. Entomol. Soc. Am.* 102: 144-150.

World Health Organization (2012). Technical Elements. In: Global Strategy for Dengue Prevention and Control 2010-2020. WHO Press, Geneva, 8-18.

Žitko T, Kovačić A, Desdevises Y and Puizina J (2011). Genetic variation in East-Adriatic populations of the Asian tiger mosquito *Aedes albopictus* (Diptera: Culicidae), inferred from NADH5 and COI sequence variability. *Eur. J. Entomol.* 108: 501-508.