Agriculture and Natural Resources 52 (2018) 596–602

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

# Genetic variation of *Aedes aegypti* mosquitoes across Thailand based on nuclear DNA sequences

Pimnapat Parimittr,[a] Theeraphap Chareonviriyaphap,[c] Michael J. Bangs,[c, d] Uraiwan Arunyawat[a, b, *]

[a] Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand
[b] Center for Advanced Studies in Tropical Natural Resources, Kasetsart University, Bangkok, 10900, Thailand
[c] Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand
[d] Public Health & Malaria Control Dept., PT Freeport Indonesia/International SOS, Kuala Kencana, 99920, Papua, Indonesia

ARTICLE INFO

*Article history:*
Received 22 June 2018
Accepted 12 September 2018
Available online 26 November 2018

*Keywords:*
*Aedes aegypti*
Genetic variation
Nuclear DNA sequence
Single nucleotide polymorphisms

ABSTRACT

The *Aedes aegypti* L. mosquito is the primary vector of dengue viruses in Thailand, where dengue disease is a major public health problem in both urban and rural areas. Understanding the genetic variation of *Ae. aegypti* populations can help to understand the distribution, population structure and gene flow of this species. Single nucleotide polymorphism (SNP) markers were used to analyze the genetic variation of 21 *Ae. aegypti* populations collected across six geographic locations in Thailand. Nuclear DNA sequences of four putative neutral fragments located on different chromosomes were examined. An average of 14 SNPs per kb was detected per population. Tajima's D statistical test showed no significant deviation from the neutral equilibrium model in the majority of populations, suggesting that the detected patterns of variation were under random mutation and genetic drift equilibrium. Relatively low genetic differentiation was detected between all mosquito populations.



## Introduction

Dengue is the most common and widespread mosquito-borne viral infection in the world (Gubler, 2002; Bhatt et al., 2013). Dengue viruses, the cause of dengue fever and more severe disease, are transmitted to humans by the infective bite of the female *Aedes aegypti* (L.) mosquito, the most important species among potential natural vectors in the subgenus *Stegomyia* and moreover, this mosquito species is also responsible for transmitting chikungunya and Zika viruses throughout Southeast Asia, Africa and the Americas (Higgs and Vanlandingham, 2015; Paixão et al., 2016). In Thailand, *Ae. aegypti* was first reported in 1907 and is believed to have spread from Southeast Asia into the Pacific Region during World War II (Gubler, 1998). This mosquito species is widespread in urban and rural areas of Thailand and is a major public health threat and contributor to disease burden in communities (Bureau of Epidemiology, Department of Disease Control, 2014).

The study of population genetics involves the comparison and estimation of temporal heredity changes for describing patterns of genetic diversity in natural populations and developing genotypic maps. Data from population genetic studies of *Ae. aegypti* are useful for a better understanding the epidemiology of dengue transmission and improving vector control. Genetic structure analysis allows identification of genetic differentiation between individuals in subpopulations across areas (Brown et al., 2011; Gloria-Soria et al., 2016).

Mitochondrial DNA (mtDNA) is a commonly used genetic maker for studying molecular diversity in animals. However, the appearance of nuclear mitochondrial pseudogenes (Numt) in the nuclear genome of *Ae. aegypti* may result in over amplification of mtDNA, or even the targeting of actual mtDNA sequences (Hlaing et al., 2009), potentially causing serious complications when analyzing population genetic studies using mtDNA alone (Hazkani-Covo et al., 2010). The use of nuclear DNA is intended to overcome this potential problem with *Ae. aegypti*. As others have demonstrated, the use of nuclear DNA for studying *Ae. aegypti* population genetics appears an acceptable alternative for avoiding some inherent limitations with using mtDNA (Crawford et al., 2017; Pless et al., 2017).

* *Corresponding author.* Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
*E-mail address:* uraiwan.a@ku.ac.th (U. Arunyawat).

https://doi.org/10.1016/j.anres.2018.11.021

In this study, single nucleotide polymorphism (SNP) markers were used to analyze patterns of genetic variation and population differentiation in four putative neutral fragments (*Tsf*, *AelMUC1*, *ApoLp-2* and *CPA*) of *Ae. aegypti*. The *Tsf* (Transferrin) gene is located on chromosome I and is involved in iron transport (Harizanova et al., 2005). The *AelMUC1* (*Mucin-like protein*) gene, is located on chromosome II and involved in immune responses. The *ApoLp-2* (*Apolipophorin II*) gene on chromosome III is involved in neutral fat biosynthesis and transporting lipoprotein (Paduan and Ribolla, 2009). Lastly, the *CPA* (*Carboxypeptidase A*) gene is located on chromosome II and is involved in the degradation of proteins in the digestive tract (Isoe et al., 2009). The four analyzed genes were chosen based on previous study (Paduan and Ribolla, 2009) that represented a suitable marker for *Ae. aegypti* genetic study. Moreover, the reliability of polymerase chain reaction (PCR) amplification was also a concern for the markers selected in this study.

## Materials and methods

### *Mosquito samples and DNA extraction*

Immature and adult stage mosquitoes were collected across Thailand between 2012 and 2014 (Fig. 1; Table 1). Larvae were collected in artificial containers and adults were trapped using sweep nets in and around homes. In total, 21 mosquito populations were collected from six geographic locations of Thailand, including two insular populations (Phuket and Chang islands). Immature mosquitoes were reared to adults. All adult specimens were identified to the species level using standard morphological characters (Rueda, 2004), and stored at −20 °C until genomic DNA extraction using GF-1 Tissue DNA Extraction kits (Vivantis; USA). Ten adult female mosquitoes from each location were used for DNA extraction and further analysis.

### *DNA amplification and sequencing*

DNA samples were amplified using PCR with four primer pairs of the coding regions described (*Tsf*, *AelMUC1*, *ApoLp-2* and *CPA* genes), and PCR product sizes of 1095, 429, 320 and 261 bp, respectively. Primer pairs of the *Tsf*, *AelMUC1* and *ApoLp-2* genes were obtained from previous work (Paduan and Ribolla, 2009); whereas, primers for the *CPA* gene (forward: 5′ TGAGCACGCCTCGAATCAC 3′ and reverse: 5′CAGTCTCAACAGCCTTCACAG 3′) were newly designed using the exon-primed, intron-crossing method (He and Haymer, 1997). A total volume of 25 μl comprised the PCR mixture, containing approximately 100 ng of DNA template, 10X buffer, 50 mM $MgCl_2$, 10 mM dNTPs, 10 μM forward primer, 10 μM reverse primer and 1 Unit *Taq* polymerase (Vivantis; USA). The PCR products were purified and sent to Macrogen Inc. (Seoul, Korea) for sequencing. Sequences were aligned using the BioEdit 7.2.5 software (Hall, 1999) and edited manually afterwards.

### *Population genetic analyses*

Genetic diversity parameters number of SNPs, haplotype ratio, nucleotide diversity, and statistical tests of neutrality (Tajima's D test) (Tajima, 1989), and Fu and Li's D test (Fu and Li, 1993) were estimated using the DnaSP 5.1 software (Librado and Rozas, 2009).

The total fixation index ($F_{ST}$) value based on the method of Hudson et al. (1992) and pairwise $F_{ST}$ values based on Slatkin (1995) were used to measure population differentiation. The levels of genetic differentiation within and among the 21 populations, and within and among the six geographical regions were estimated using the analysis of molecular variance (AMOVA), with the Arlequin 3.5 program (Excoffier and Lischer, 2010). Population clustering of the 21 populations was done using Bayesian analysis of population structure using the BAPS 6.0 software (Corander et al., 2008).

**Table 1**
Twenty-one sample locations for *Ae. aegypti* in Thailand.

| Province | Geographic region | Geo-coordinates |
|---|---|---|
| Nakhon Ratchasima | Northeastern | 1466′59.69″N<br>10143′53.94″E |
| Roi Et | Northeastern | 1605′76.91″N<br>10365′32.59″E |
| Sisaket | Northeastern | 1461′68.07″N<br>10470′24.54″E |
| Ubon Ratchathani | Northeastern | 1535′70.32″N<br>10450′74.46″E |
| Yasothon | Northeastern | 1580′41.46″N<br>10414′48.97″E |
| Chiang Rai | Northern | 19°61′54.24″N<br>100°08′69.94″E |
| Lampang | Northern | 1818′33.86″N<br>9928′11.27″E |
| Lamphun | Northern | 1865′38.69″N<br>9901′26.94″E |
| Chiang Mai | Northern | 1841′0.62″N<br>9859′38.99″E |
| Phuket (Phuket Island) | Southern | 785′12.02″N<br>9837′72.94″E |
| Pattani | Southern | 687′46.2″N<br>10124′96.57″E |
| Songkhla | Southern | 718′87.82″N<br>10060′79.86″E |
| Surat Thani | Southern | 915′09.09″N<br>9932′18.99″E |
| Satun | Southern | 664′14.19″N<br>10006′75.96″E |
| Trang | Southern | 757′01.18″N<br>9961′30.37″E |
| Yala | Southern | 583′73.49″N<br>10120′74.28″E |
| Chachoengsao | Eastern | 1338′56.12″N<br>10179′93.16″E |
| Chanthaburi | Eastern | 1244′35.4″N<br>0152′26.24″E |
| Trat (Chang island) | Eastern | 1205′14.08″N<br>0232′32.27″E |
| Bangkok | Central | 1370′93.68″N<br>10058′41.26″E |
| Tak | Western | 1578′64.35″N<br>9884′41.97″E |

### *Ethics statement*

Animal care or biosafety and all experimental procedures were approved by the Animal Experiment Committee, Biosafety Committee Kasetsart University, Bangkok, Thailand (Animal Ethics Permission number ACKU03558).

## Results

### *Genetic diversity*

The genetic diversity of 21 *Ae. aegypti* mosquito populations across Thailand was estimated based on four putative neutral DNA sequences: *Tsf*, *AelMUC1*, *ApoLp-2* and *CPA* fragments. In total, 106, 27, 10 and 23 SNPs were detected from the sequence lengths of 1,095, 429, 321 and 261 bp sequences, respectively. A mean of 14 SNPs per kilobase ($\pi = 0.014$) was observed from the distribution of SNPs among the four nuclear DNA genes. Estimates of nucleotide diversity ($\pi$) ranged from 0.009 (*ApoLp-2*) to 0.019 (*AelMUC1*), and ($\theta_W$) ranged from 0.005 (*ApoLp-2*) to 0.016 (*Tsf*) (Table 2).

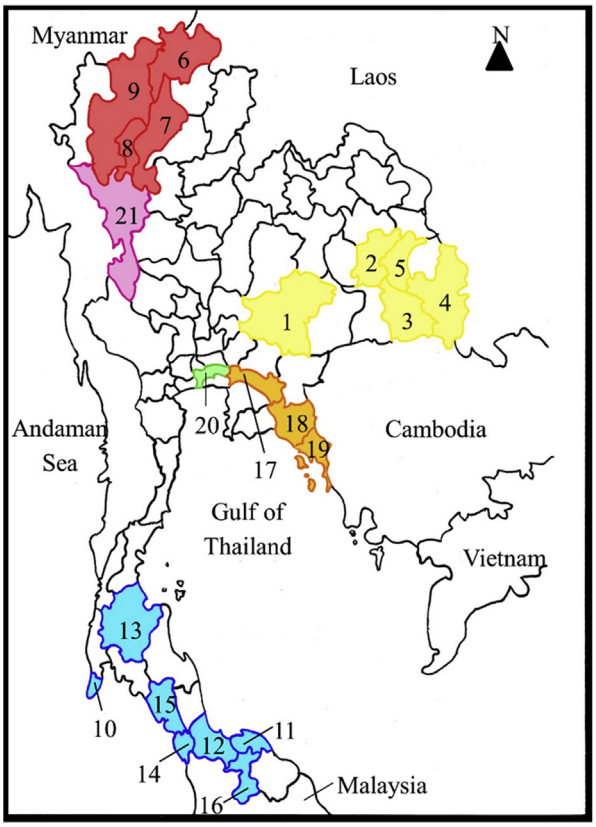


**Fig. 1.** Collection sites (by province) of sampled *Ae. aegypti* populations in Thailand.

Consistently low levels of genetic diversity ($\pi$ and $\theta_W$) were detected from the Bangkok population in all four genes (Fig. 2A–D). Moreover, the lowest levels of nucleotide diversity were also seen in the Bangkok population compared with the estimated diversity of populations from the other six locations in Thailand (Fig. 3).

Statistical neutrality tests (Tajima's D and Fu and Li's D (one-tailed) tests) were used to measure deviation from neutral equilibrium expectation. Fragment sequences of the *Tsf*, *AelMUC1* and *ApoLp-2* genes showed positive D values, while fragment sequences of the *CPA* gene produced a negative value (Table 2). A positive Tajima's D value indicates an excess of intermediate frequency polymorphisms in the population, while a negative Tajima's D value indicates an excess of low frequency polymorphisms. No significant Tajima's D value was found in fragment sequences of the *Tsf*, *ApoLp-2* and *CPA* genes ($p > 0.05$), suggesting that these fragments are under neutral equilibrium control. In contrast, a significant positive Tajima's D value (1.968) was found in a fragment sequence of the *AelMUC1* gene ($p < 0.05$), indicating it is not under neutral equilibrium control. Moreover, it was also found that most of the 21 populations showed no significant deviations from the neutral equilibrium model. Fu and Li's D tests indicated none of the fragments had a significant deviation from the neutral equilibrium model. A positive Fu and Li's value was observed for fragments of the *AelMUC1*, *ApoLp-2* and *CPA* genes, indicating an excess of polymorphisms on internal branches (intermediate frequency polymorphisms). In contrast, a negative Fu and Li's D value was shown in a fragment of the *Tsf* gene, indicating an excess of polymorphisms on external branches of the genealogy (singletons).

### *Genetic structure*

Genetic differentiation among the 21 mosquito populations was estimated using the total $F_{ST}$ values of Hudson et al. (1992). A relatively low level of genetic differentiation was detected from all

**Table 2**
Nucleotide diversity of sampled *Ae. aegypti* populations across Thailand within four selected genes.

| Gene | Sample size | Length (base pair) | Number of SNPs | Haplotype ratio | Nucleotide diversity | | Test of neutrality | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $\pi$[a] | $\theta_W$[b] | Tajima's D | Fu and Li's D |
| *Tsf* | 204 | 1095 | 106 | 0.42 | 0.017 | 0.016 | 0.177 | −0.268 |
| *AelMUC1* | 208 | 429 | 27 | 0.29 | 0.019 | 0.011 | 1.968* | 0.240 |
| *ApoLp-2* | 208 | 320 | 10 | 0.06 | 0.009 | 0.005 | 1.826 | 1.144 |
| *CPA* | 208 | 261 | 23 | 0.19 | 0.011 | 0.015 | −0.701 | 0.509 |

SNP = single nucleotide polymorphism.
*$p < 0.05$.
[a] Nei (1987).
[b] Watterson (1975).

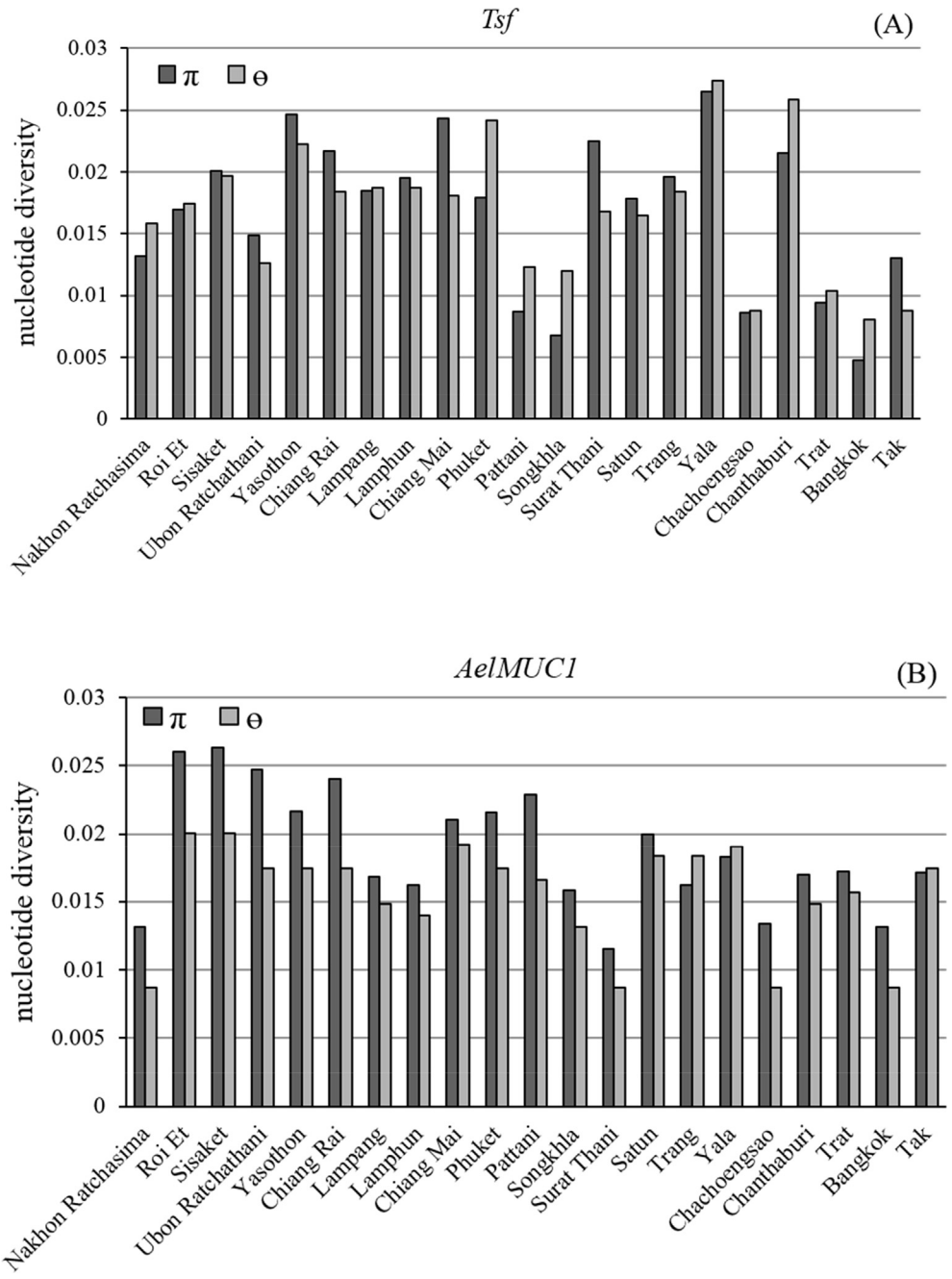


**Fig. 2.** Nucleotide diversity, ($\theta_w$; Watterson, 1975 and $\pi$; Nei, 1987), of 21 *Ae. aegypti* populations across different provinces of Thailand for gene fragments: *Tsf* (A), *AelMUC1* (B), *ApoLp-2* (C) and *CPA* (D).

fragments, suggesting low evolutionary separation between the 21 population samples across Thailand (Table 3).

In AMOVA analysis, low variation among all populations (0.37%) demonstrated a low degree of genetic differentiation (Table 4). Additionally, the genetic separation between the mainland and the two island populations was estimated using pairwise $F_{ST}$ values for all four gene fragments. The Chang Island population showed no genetic differentiation from mainland populations ($F_{ST} = 0.000$), while the Phuket Island population showed a non-significant differentiation ($F_{ST} = 0.0059$).

Population clustering of the 21 mosquito populations using the combined data of the four fragment genes was tested using BAPS analysis (Fig. 4). The best cluster pattern for the 21 populations was five ($K = 5$) with the best log of marginal likelihood. Across the majority of populations, the highest proportion cluster was 36%. This cluster indicated the appearance of a common shared haplotype in 85.7% of sampled populations across Thailand, including the northeastern, northern, southern, eastern, central (Bangkok) and western (Tak province) regions. The least common cluster (5%) was found only in southern and eastern populations (also with a relatively low frequency), indicating a unique haplotype in these populations.

## Discussion

Using four nuclear genes, the mean number of SNP sites among 21 populations across Thailand was 14 per kb, corresponding well

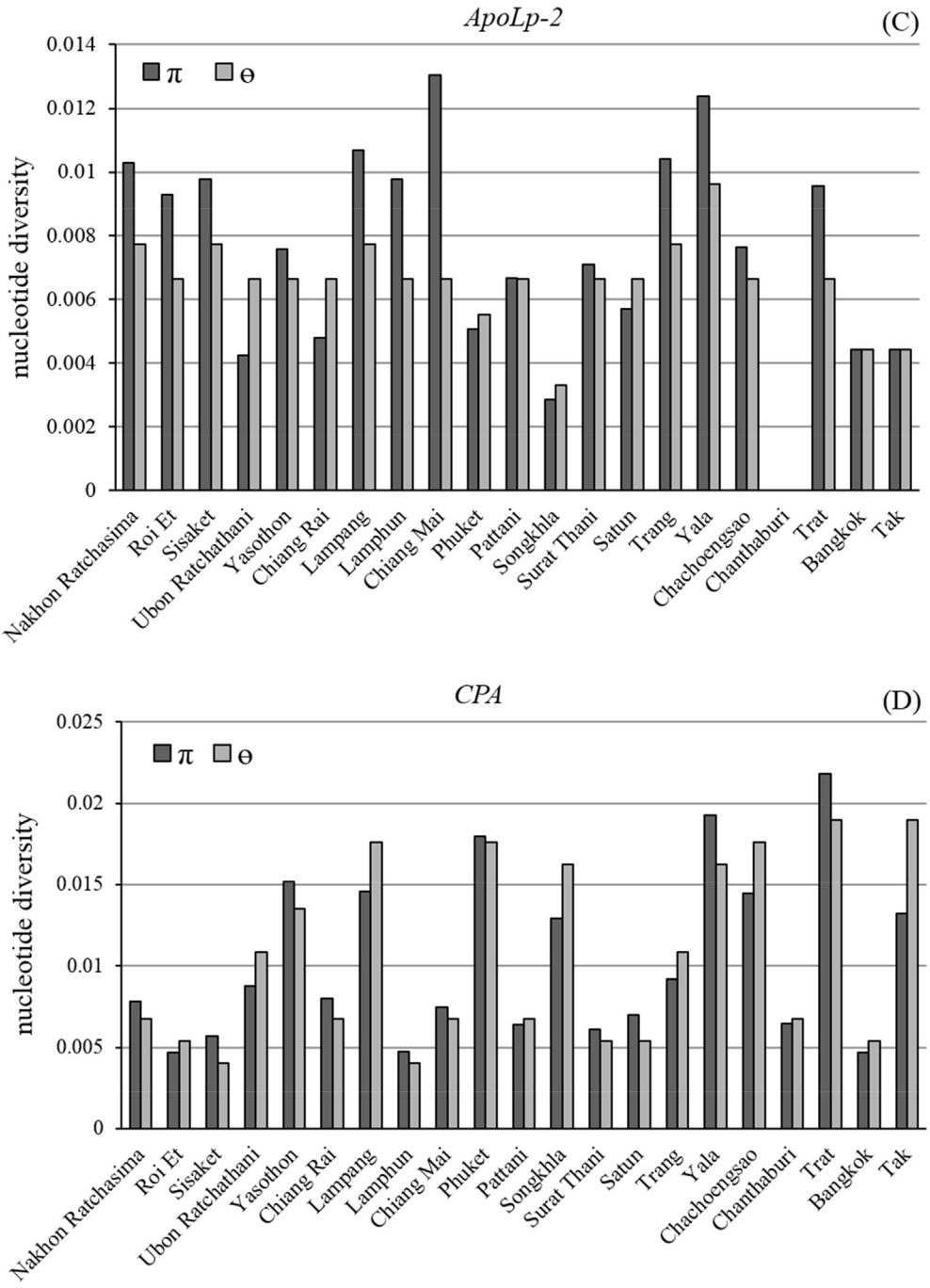


**Fig. 2.** (*continued*).

with the average number of SNP sites (12 per kb) in a previous study examining 25 nuclear genes in *Ae. aegypti* (Morlais and Severson, 2003). The use of specific SNP sites allows the analysis of variation in a single nucleotide that occurs at a specific position in the genome, and where each variation is present to some appreciable degree within a population. Single nucleotide alterations are usually considered to be point mutations that have been evolutionarily successful enough to recur in a significant proportion of the population of a species.

In terms of nucleotide diversity, both the $\pi$ and $\theta_W$ values for the Bangkok population showed a consistent low level of diversity in all genes, and the lowest level of diversity among the six geographical locations. The relatively low level of genetic diversity in Bangkok may have been the result of more frequent mosquito control activities in large urban areas. Such control, typically involving the application of insecticides to control immature and adult mosquitoes, may have periodically suppressed mosquito numbers sufficiently to decease genetic diversity (intraspecific breeding); a finding coincident with the lower number of reported dengue cases (lower transmission) in the central regions of Thailand (Bureau of EpidemiologyDepartment of Disease ControlMinistry of Public Health, 2014). In Bangkok, *Ae. aegypti* samples were collected from only one site (in Khlong Toei district). This district is a densely populated, economically depressed community, where most

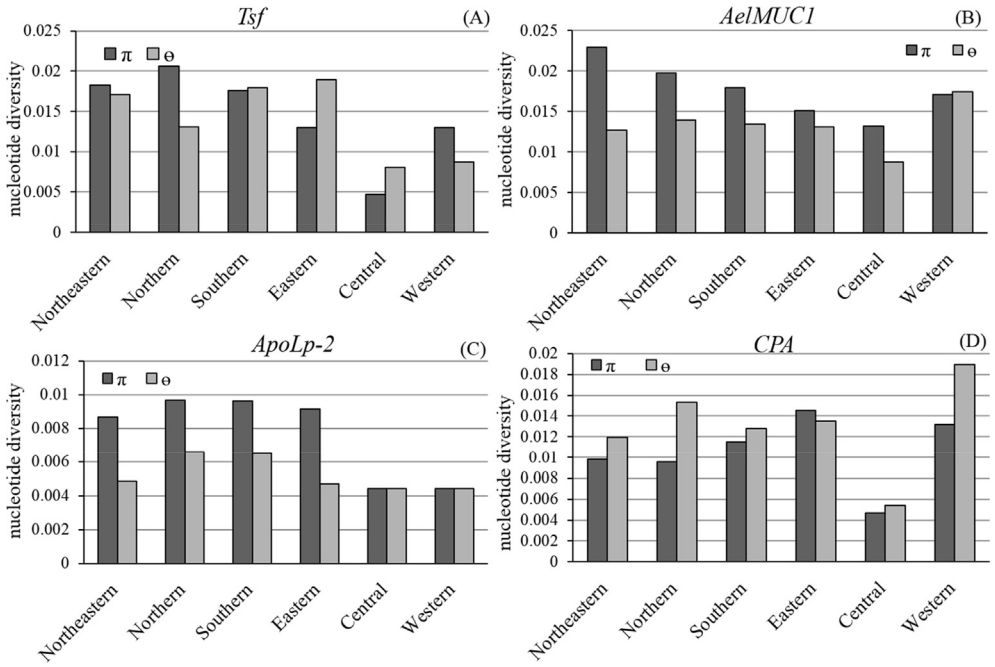


**Fig. 3.** Nucleotide diversity, $\theta_w$ (Watterson, 1975) and $\pi$ (Nei, 1987), of *Ae. aegypti* populations in six geographic regions of Thailand for gene fragments: *Tsf* (A), *AelMUC1* (B), *ApoLp-2* (C) and *CPA* (D).

**Table 3**
Total fixation index ($F_{ST}$) values of sampled *Ae. aegypti* populations across Thailand.

| Gene | Total $F_{ST}$ value[a] |
|---|---|
| *Tsf* | 0.06 |
| *AelMUC1* | 0.02 |
| *ApoLp-2* | 0.24 |
| *CPA* | 0.08 |

[a] Hudson et al. (1992).

houses are of modest-to-poor construction and in close proximity to each other (Tonn et al., 1969). These circumstances allow mosquitoes to move more easily from one house to another. Thus, the samples collected from water containers in this locality could have contained siblings (from the same parent), potentially leading to the lower level of genetic diversity seen. However, it was also possible that the immature stage samples collected from the water containers might have represented multiple parental sources.

Most of the 21 *Ae. aegypti* populations sampled showed no significant deviation from the neutral equilibrium model, suggesting that the pattern of genetic variation was most likely explained by the influence of mutation and random genetic drift interaction, rather than influenced by forces of natural selection. Comparing the mainland populations and the two island populations, mosquitoes from Chang Island showed no genetic difference from mainland samples, although the geographic distance between Chang Island and the mainland is greater than between Phuket Island and the mainland. Phuket Island showed slight, but no significant genetic differentiation from mainland populations either. This was not unexpected, since both islands have large and frequent interaction with the mainland and so the opportunity for regular reintroduction of outside populations of *Ae. aegypti* is substantial. The present data suggested that the 21 *Ae. aegypti* populations sampled across Thailand presented no significant genetic differences between populations, the capacity to transmit dengue and other viruses, as well as strategies to control this important mosquito species could likely be uniform throughout the country.

The BAPS analysis of the fragment sequence data showed the appearance of a common haplotype among a majority (85.7%) of populations using the clustering model, indicating that samples shared similar genetic patterns. Five clusters appeared best for population clustering among the 21 locations across Thailand. A common haplotype appeared in most of the populations and was present in the northeastern, northern, southern, and eastern locations, as well as in the central (Bangkok) and western (Tak

**Table 4**
Analysis of molecular variance with combined four genes and sampled *Ae. aegypti* populations.

| Source of variance | Degrees of freedom | Variance component | Variation (%) | Fixation index |
|---|---|---|---|---|
| Among 6 geographic locations | 5 | 0.066 | 0.37 | $F_{CT}$[a] = 0.004 |
| Among populations within 6 geographic locations | 15 | 1.950 | 10.77 | $F_{SC}$[b] = 0.108* |
| Within populations | 187 | 16.092 | 88.87 | $F_{ST}$[c] = 0.111* |
| Total | 207 | 18.108 | | |

*$p < 0.05$.
[a] Fixation index among groups.
[b] Fixation index among populations within groups.
[c] Fixation index within populations.

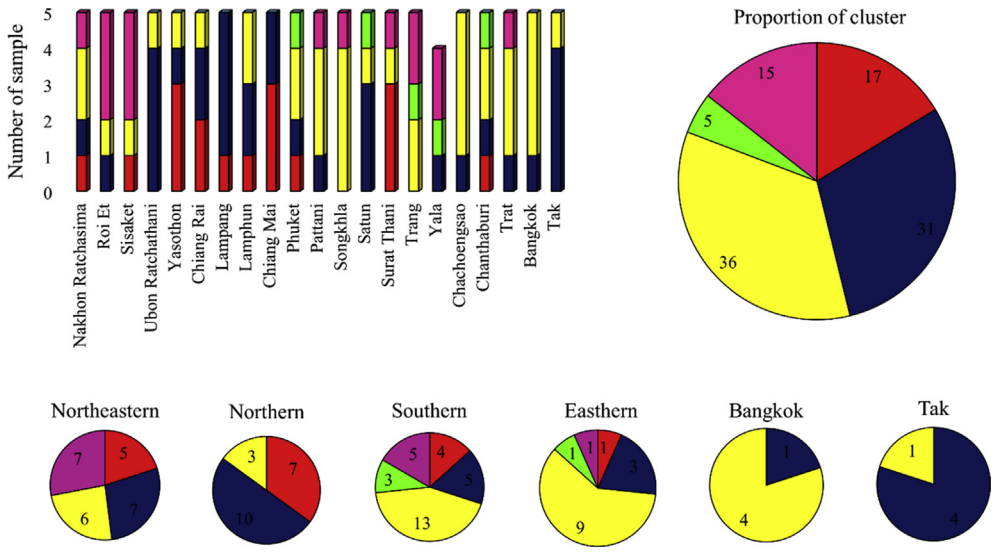


**Fig. 4.** Bayesian analysis of population structure based on 21 *Ae. aegypti* populations across Thailand using combined four fragment genes, where numbers represent number of samples.

province) populations, indicating clear genetic similarity among populations. A unique haplotype only appeared in some southern and eastern populations.

This study assists in understanding the contemporary genetic structure of *Ae. aegypti* populations in Thailand. However, the small sample size was a possible limitation to the conclusions and that future studies increasing the number of fragments and samples analyzed should provide. The strong similarity of mosquito populations between geographically separated regions in the country has possible epidemiological as well as vector control and pest management implications (such as genetic manipulation and insecticide resistance) for combating virus transmission by *Ae. aegypti*.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgements

The last author was supported by the Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Bangkok, Thailand.

## References

Bhatt, S., Gething, P.W., Bardy, O.J., et al., 2013. The global distribution and burden of dengue. Nature 496, 504–507.

Brown, J.E., McBride, C.S., Johnson, P., et al., 2011. Worldwide patterns of genetic differentiation imply multiple 'domestications' of *Aedes aegypti*, a major vector of human diseases. Proc. Biol. Sci. 278, 2446–2454.

Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, 2014. Dengue Fever. October 2016. Bangkok, Thailand.

Corander, J., Marttinen, P., Sirén, J., Tang, J., 2008. Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinf. 9, 539. doi.org/10.1186/1471-2105-9-539.

Crawford, J.E., Alves, J.M., Palmer, W.J., et al., 2017. Population genomics reveals t hat an anthropophilic population of *Aedes aegypti* mosquitoes in West Africa recently gave rise to American and Asian populations of this major disease vector. BMC Biol. 15, 16. https://doi.org/10.1186/s12915-017-0351-0.

Excoffier, L., Lischer, H.E., 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.

Fu, Y.X., Li, W.H., 1993. Statistical tests of neutrality of mutations. Genetics 133, 693–709.

Gloria-Soria, A., Ayala, D., Bheecarry, A., et al., 2016. Global genetic diversity of *Aedes aegypti*. Mol. Ecol. 25, 5377–5395.

Gubler, D.J., 1998. Epidemic dengue and dengue hemorrhagic fever: a global public health problem in the 21st century. In: Scheld, W., Armstrong, D., Hughes, J. (Eds.), Emerging Infections 1, vol. 1. ASM Press, Washington, DC, USA, pp. 1–14.

Gubler, D.J., 2002. The global emergence/resurgence of arboviral diseases as public health problems. Arch. Med. Res. 33, 330–342.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Harizanova, N., Georgieva, T., Dunkov, B., Yoshiga, T., Law, J., 2005. *Aedes aegypti* transferrin. Gene structure, expression pattern, and regulation. Insect Mol. Biol. 14, 79–88.

Hazkani-Covo, E., Zeller, R.M., Martin, W., 2010. Molecular poltergeists: mitochondrial DNA copies (numts) in sequenced nuclear genomes. PLoS Genet. 6, e1000834. https://doi.org/10.1371/journal.pgen.1000834.

He, M., Haymer, D.S., 1997. Polymorphic intron sequences detected within and between populations of the oriental fruit fly (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 90, 825–831.

Higgs, S., Vanlandingham, D., 2015. Chikungunya virus and its mosquito vectors. Vector Borne Zoonotic Dis. 15, 231–240.

Hlaing, T., Tun-Lin, W., Somboon, P., et al., 2009. Mitochondrial pseudogenes in the nuclear genome of *Aedes aegypti* mosquitoes: implications for past and future population genetic studies. BMC Genet. 10, 11. https://doi.org/10.1186/1471-2156-10-11.

Hudson, R.R., Slatkin, M., Maddison, W., 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132, 583–589.

Isoe, J., Zamora, J., Miesfeld, R.L., 2009. Molecular analysis of the *Aedes aegypti* carboxypeptidase gene family. Insect Biochem. Mol. Biol. 39, 68–73.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

Morlais, I., Severson, D., 2003. Intraspecific DNA variation in nuclear genes of the mosquito *Aedes aegypti*. Insect Mol. Biol. 12, 631–639.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY, USA.

Paduan, K., Ribolla, P., 2009. Characterization of eight single nucleotide polymorphism markers in *Aedes aegypti*. Mol. Ecol. Resour. 9, 114–116.

Paixão, E.S., Barreto, F., Teixeira, M.G., Costa, M.C.N., Rodrigues, L.C., 2016. History, epidemiology, and clinical manifestations of Zika: a systematic review. Am. J. Public Health 106, 606–612.

Pless, E., Gloria-Soria, A., Evans, B.R., Kramer, V., Bolling, B.G., Tabachnick, W.J., Powell, J.R., 2017. Multiple introductions of the dengue vector, *Aedes aegypti*, into California. PLoS Negl. Trop. Dis. 11, e0005718. https://doi.org/10.1371/journal.pntd.0005718.

Rueda, L.M., 2004. Pictorial Keys for the Identification of Mosquitoes (Diptera: Culicidae) Associated with Dengue Virus Transmission. Magnolia Press, Auckland, New Zealand.

Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139, 457–462.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.

Tonn, R.J., Sheppard, P., Macdonald, W., Bang, Y., 1969. Replicate surveys of larval habitats of *Aedes aegypti* in relation to dengue haemorrhagic fever in Bangkok, Thailand. Bull. World Health Organ. 40, 819–829.

Watterson, G.A., 1975. On the number of segregating sites in genetical models without recombination. Theor. Popul. Biol. 7, 256–276.