GENE FLOW AMONG *Aedes aegypti* (L.) POPULATIONS IN THAILAND

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are the most common and important mosquito-borne viral diseases syndromes in the world. Epidemics of this disease commonly occur throughout the warm tropical and subtropical regions. Over 30 million cases are reported and tens of thousands die from this disease annually. During the last two decades, explosive dengue outbreaks in Thailand have occurred periodically. In the last decade, the number of dengue cases in Thailand has shown a steady increase and disease transmission remains prevalent all over the country. Each year, 50,000 to 200,000 dengue cases are reported in the country. No commercially available vaccine is yet available for the prevention of dengue infection and there are no specific antiviral drugs for treatments. Thus, the control of the mosquito vectors remains the most important method to prevent dengue virus transmission and averting dengue epidemics.

A significant growth in the human population, demographic movement from rural to more crowded urban areas and an increase in tourism-based facilities are seen as major contributors to an increasing trend in dengue incidence. The disease is caused by four dengue virus subtypes (1-4), belonging in the genus *Flavivirus*. The viruses are maintained in a natural cycle that involves humans and susceptible Aedine vector mosquitoes. *Aedes aegypti* (L.), a predominately indoor, human-biting mosquito, is the primary vector worldwide and is a common species throughout Thailand.

Aedes aegypti (L.) is considered to be a polytypic species worldwide because of various morphological, physiological and geographical variations seen (Craig and Van de Hey, 1962; Gouck, 1972; McClelland, 1974; Trpis and Hausermann, 1975; Leahy *et al*.,1978; Tabachnick *et al*.,1979; Tabachnick and Powell,1979; Trpis *et al*.,1995). As a means to develop the most effective vector control measures possible, the taxonomic status and population genetics of *Ae.*

aegypti are extremely important because of the wide involvement of this species in the transmission of human viral pathogens.

In this study, the technique of isozyme electrophoresis was used to compare genetic profiles among *Ae. aegypti* populations in Thailand. This technique and analysis is commonly used in the study of phylogenetic relationships, measuring species homologies and for characterizing ecological-genetic relationships between mosquito populations. Results from these types of studies can be used to estimate the level of natural gene flow between and/or among populations. This information is useful and helps forecast how rapidly different populations can be genetically integrated with one another.

In recent years, *Ae. aegypti* has developed resistance to some currently used insecticides to control this species in Thailand (Chareonviriyaphap *et al.*, 2003). The resistant genes in mosquito populations can be spread via mating to insecticide susceptible populations, either by active or passive migration. Analyses of genetic profiles by comparing isozyme variability and levels of gene flow between and within *Ae. aegypti* populations can provide an estimation of the natural spatial-temporal movement of resistant genes. This study attempted to measure the geographical relationship of various populations of *Ae. aegypti* in Thailand using information from comparable allele variation frequencies by estimating gene flow among those same populations. To accomplish this, allozyme patterns of 24 sample populations of *Ae. aegypti* were compared using 20 different enzyme systems and electrophoresis. Gene flow information can be used as a basis to anticipate and plan more comprehensive strategies to combat insecticide resistance and for more developing a more effective national program to control *Ae. aegypti* in Thailand.

Objective

The objective of this research is to determine gene flow level and genetic structure among *Aedes aegypti* (Linnaeus) populations from different geographic areas of Thailand to better understand the genetic relationships between and within populations and those biological and environmental factors that may interact and influence them.

LITERATURE REVIEW

1. Overview

Dengue (DF) and dengue hemorrhagic fever (DHF) are the most important mosquito-borne viral diseases affecting humans throughout the tropical and subtropical world (Gubler, 1988). Worldwide, there are estimated 50-100 million cases of DF and 250,000-500,000 cases of DHF per year (Rigau-Pérez *et al*., 1998) and tens of thousands die from this disease. Four serotypes of dengue virus; DEN 1, 2, 3, and 4 cause DF and DHF. These serotypes are commonly maintained in an urban transmission cycle by *Ae. aegypti*, a principal mosquito vector. This urban mosquito is present at high densities in human habitations. In Thailand, between 50,000 and 200,000 dengue-related illnesses are reported each year (Communicable Disease Control, 1995-2004). Furthermore, significant population growth, demographic movement to urban residential areas and an increase in tourism-based facilities are deemed major factors involved in the dengue resurgence in the country. Human population densities and ecotope selection seem to affect mosquito population differentiation and thus, on susceptibility to dengue-2 virus (Paupy *et al*., 2000). *Ae. aegypti* is a vector with an unusually close relationship with humans in terms of their dwellings, and immediate surroundings. This species is found virtually everywhere in Thailand and is the primary day-biting mosquito in urban areas.

Bosio *et al*. (2005) summarized that it was unknown when *Ae. aegypti* first colonized southeast Asia. It was first reported in Thailand by Theobald (1907), but might have been present for many years prior to that. Causey (1937) found *Ae. aegypti* in most villages along the coast of the Gulf of Thailand and < 5 kilometers from the railway between Bangkok and Chiang Mai, but not in areas further from major lines of transport. Extensive surveys reported by Scanlon (1965) indicated further penetration of *Ae. aegypti* from major routes of travel throughout much of Thailand, extending into the eastern and southern regions of Thailand. Based on previous studies that failed to find *Ae. aegypti* in areas more than a few kilometers from lines of commerce, sylvan and more isolated rural areas were targeted to determine if this species had colonized such areas. These surveys were all negative for *Ae. aegypti*, but yielded a diverse mosquito fauna. Scanlon (1965) concluded that *Ae. aegypti* was primarily an urban mosquito that could be found in smaller

human settlements along major routes of commerce and travel. Today, *Ae. aegypti* is well established in urban centers and most rural villages in Thailand. Therefore, much of its colonization of Thailand outside urban areas and major commercial routes has likely occurred during the past 50 years (Bosio *et al*., 2005).

Relatively little is known about the genetic structure and gene flow among *Ae. aegypti* populations from various geographic areas of Thailand. Knowledge of population structure and gene flow can help estimate migration between vector populations, provide insights into the epidemiology and transmission of dengue viruses and help develop more responsive as well as effective vector control.

2. Biology of *Ae. aegypti*

2.1 Eggs

Eggs are deposited singly on damp surfaces just above the water line. Embryonic development is usually completed within 48 hours in a warm and humid environment. Once embryonation development is complete, the eggs can withstand long periods of desiccation for over a year. Eggs hatch once the containers are flooded, but not all eggs hatch at the same time (World Health Organization [WHO], 1986).

2.2 Larvae and pupae

The larvae pass through four developmental stages. The duration of larval development depends on temperature, availability of food and larval density in the receptacle. Under optimal conditions, the time taken from hatching to adult emergence can be as short as seven days, including two days in the pupal stage. At low temperatures, it may take several weeks for adults to emerge (WHO, 1986).

2.3 Adults

Soon after emergence, the adult mosquitoes mate and the inseminated females may take their blood meal within 24-36 hours. Blood is the source of protein essential for the maturation of eggs. *Ae. aegypti* has an average adult survival of only eight days (Sheppard *et al.*, 1969). The dispersal of adult females appears to be often limited to within 100 meters of the site of emergence. Passive transportation can occur via eggs and larvae in containers (WHO, 1986).

2.4 Feeding behavior

Ae. aegypti females have two periods of biting activity, one in the morning for several hours after daybreak and the other in the afternoon for several hours before dark (Nelson *et al.*, 1978; Lumsden, 1957; Sheppard *et al*., 1969). In general, this mosquito does not bite at night, except in lighted rooms (WHO, 1986).

2.5 Resting behavior

Ae. aegypti prefers to rest in dark, humid places inside houses or buildings. Less often it can be found outdoors. The preferred indoor resting surfaces are the undersides of furniture, hanging objects such as clothes and curtains and on the walls (WHO, 1986).

2.6 Virus transmission

A vector mosquito may become infected with DHF virus when it feeds on a viraemic human host. After an incubation period, the virus passes through the mosquito's midgut to infect other tissues, including the salivary glands. The infected mosquitoes can transmit dengue virus to persons by injecting the salivary fluid during blood feeding (WHO, 1986).

3. Population genetic studies of *Ae. aegypti*

Population genetic approaches help us estimating genetic exchanges or gene flow between vector populations and provide estimates of their abilities to habor and transmit dengue viruses. This information is crucial prerequisite for a better understanding of dengue transmission and the development of effective control measures. To assess the role of the vector in the changing pattern of dengue transmission, extensive studies on the genetic differentiation of *Ae. aegypti* should be conducted.

In 1966; R.C. Lewontin, J.L. Hubby and H. Harris initiated a new era in the study of the genetic variation in natural populations when they applied the technique of gel electrophoresis to detect amino acid differences in proteins (Snustad and Simmons, 2003). This technique has successfully been used for the study of genetic variation, phylogenetic relationships and evolutionary biology of a wide range of complex organisms, which are difficult to identify by morphological characteristics. Protein gel electrophoresis provides the first estimate of how much of variation exists at the molecular level. In many species, approximately one-third of all genes that encode soluble proteins exhibits an electrophoretic polymorphisms and about 12 to 15 percent of individuals within a population are heterozygotes. These criteria, the proportion of genes that are polymorphic and the proportion of individuals that are heterozygous, are simple to measure and can be used to reflect the amount of the genetic variations within a population (Snustad and Simmons, 2003).

The genetic structure of population is commonly assessed using markers like isozymes. Electrophoretic analyses of protein variation in natural populations have added to the understanding of *Ae. aegypti* genetic profile. The technique uses the ability to detect protein heterogeneity to infer genetic variability. The isozyme gene frequencies show several genetic-geographic groups where population comparisons within a group show lower genetic distances and greater genetic similarity than the between-group comparisons. Genetic variation in *Ae. aegypti* has been well characterized using this approach (Tabachnick and Powell, 1979; Wallis *et al*., 1983). This method is thought to be able to detect low level of gene flow between sympatric populations of *Ae. aegypti* (Tabachnick and Powell, 1978). Thus, *Ae. aegypti* can be divided into

groups on a genetic basis (Powell *et al*., 1980). Mosquito control activities have been shown to introduce genetic heterogeneity into mosquito populations. Tabachnick and Powell (1978) stressed the major impact of human efforts to control mosquitoes on the genetic structure of mosquito populations. Human population density is highly correlated with the intensity of mosquito control undertaken in highly populated areas. The determination of genetic differentiation of vectors showing variation in ability to transmit viruses represents an essential knowledge to better understand dengue epidemiology and to design adapted control strategies.

Ae. aegypti is considered to be a polytypic species worldwide due to morphological, physiological and geographical variations (Tabachnick and Powell, 1979; Tabachnick *et al*., 1979) Sousa *et al*. (2000) reported variation in vector competence among different populations by determining genetic structure that could support in the studies of intraspecific polymorphism in this mosquito. Several studies have described the genetic variation among *Ae. aegypti* populations collected from different regions of the world. Tabachnick *et al*. (1979) analyzed, using electrophoretic zymograms, polymorphism in both domestic and sylvan forms of *Ae. aegypti* populations from Asia, Africa and the Americas. From the analysis, genetic distance between these forms suggested that gene flow was restricted, probably because of the existence of different genotypes related to habitat preferences. Moreover, samples from human housing separated by 1 km or less showed significant differences in allele frequencies, indicating restricted gene flow. Tabachnick and Powell (1979) analyzed 34 populations of *Ae. aegypti* representing the world-wide distribution of the species for genetic variation at 19-22 isozyme loci. The species had an average expected heterozygosity of 0.129 based on 19 loci analyzed in every population. Genetic differences among populations enable inferences on the geographic origin of a sample and would be related to the time of introduction of *Ae. aegypti* into the different continents. Asian populations showed a significant lower level of genetic variation and a fewer number of alleles per locus compared to other populations. This may be related to the time of introduction of *Ae. aegypti* into Asia.

Wallis *et al*. (1984) analyzed genetic variation at 11 loci in 18 Caribbean collections of *Ae. aegypti*, using starch gel electrophoresis. The results showed that, while there was some relationship between geographic proximity and genetic distance, the overall picture among islands

was one of gene frequency patchiness, with some collections clearly not conforming to any geographic pattern. This was attributed to the combined effects of high rates of gene flow among islands and with the mainland American continent and the activities of various vector control agencies in the region.

Paupy *et al*. (2000) analyzed genetic differentiation of *Ae. aegypti* in Tahiti and Moorea (French Polynesia) by starch gel electrophoresis and found considerable (i.e. high F_{ST} values) and significant geographic differences. Differences in *Ae. aegypti* genetic structure were related to human population densities and to particularity in mosquito ecotopes. In highly urbanized areas, mosquitoes were highly structured. Recurrent extinction events consecutive to insecticide treatments during dengue outbreaks tend to differentiate mosquito populations. In less populated zones, differences in ecotope characteristics could explain the lack of differentiation among mosquitoes from rural environments. In the lowest populated zones, mosquitoes were less differentiated. The results suggested that the genetic structure of *Ae. aegypti* population depends on human population density (and intensity of insecticidal control) and ecological characteristics of mosquito ecotopes. *Ae. aegypti* lives in urban domestic environments. Its strong anthropophily and its capacity to oviposit in artificial breeding sites limit the species' spontaneous dispersal so enhance population differentiation (Tien *et al*., 1999).

Sousa *et al*. (2000) presented estimates of genetic variability in *Ae. aegypti* populations from central Argentina and determinations of genetic distances among them by analyzing allozymic frequencies at 11 loci in samples from 3 localities. The proportion of polymorphic loci varied between 27.3 and 63.6. Expected mean heterozygosity ranged from 0.090 to 0.161 and Rogers' similarity among samples ranged between 0.909 and 0.958. The lack of relationship between genetic and geographic distances was in agreement with a recent colonization of the studied area. The mean Wright's coefficient F_{ST} value (0.065) indicated low levels of genetic differentiation among populations from different localities. Given the recent reinfestation with this mosquito in Argentina, the high levels of polymorphism found could indicate multiple introductions of representative samples from genetically different subpopulations. Knowledge of geographic patterns of genetic variability can provide markers to identify possible biotypes or

subspecies and their relationship with ecological, behavioral or other attributes of epidemiologic relevance.

Costa Fraga *et al*. (2003) analyzed eighteen enzymatic loci in *Ae. aegypti* populations from four neighborhoods in the city of Manaus in Brazil. The analysis showed that the Downtown population was the most polymorphic (P = 55.6%), with the largest number of alleles per locus (1.7) and highest level of observed and expected heterozygosity ($H_o = 0.152 \pm 0.052$, $H_e = 0.174 \pm 0.052$, respectively).

Presently, a minority of knowledge is known about the rate of spread of *Ae. aegypti* throughout Thailand. Chareonviriyaphap and Lerdthusnee (2002) studied the genetic differentiation of *Ae. aegypti* (L.) mainland and island populations from southern Thailand by using starch gel electrophoresis to compare the differences of isozyme patterns and reported that a large effective migration rate among one collection from the mainland from Don Sak Harbor and the other 4 collections from Mae-Nam, Na-Thon, Ma-Ret and Taling-Ngam districts on Samui Island. As a result, a high rate of gene flow occurred among all 5 collections of *Ae. aegypti* between Koh Samui and mainland Thailand. The percent polymorphic loci (24.2-33%) in the 4 island collections were lower than in the mainland collection (36.4%). No fixed differences in alleles were detected. Also, no significant differentiation was found among the 5 collections from Surat Thani Province.

Another experiment, Netthanomsak (2004) studied genetic structure of *Ae. egypti* along the coastal area of Gulf of Thailand, covering 6 following provinces; Trat, Samut Prakan, Bangkok, Chumphon, Surat Thani and Songkhla; by using starch gel electrophoresis. The results revealed low genetic variations among all six populations. Greatest gene flow rate was found between Bangkok and Chumphon populations and the lowest was from Samut Prakan and Songkhla populations. High gene flow rate between the populations could be due to indirect or passive movement of *Ae. aegypti* eggs via the commercial and business transportations. All 6 populations of *Ae. aegypti* are considered to be conspecific populations with some minor genetic variation.

Lerdthusnee and Chareonviriyaphap (1999) compared isozyme patterns of *Ae. aegypti* populations collected from pre- and post- *Bacillus thuringiensis israelensis* (*B.t.i*) treatment sites in Thailand by using starch gel electrophoresis and reported that the number of polymorphic loci were lower in the *B.t.i.* treated population. Lower genetic variability was found in populations collected from *B.t.i.* treated sites possibly due to a genetic bottleneck produced by the *B.t.i.* treatment. Heterozygosity increased in the months following *B.t.i.* treatment, probably because of immigration when the control program was withdrawn. Also no fixed differences in alleles were detected among the populations collected.

Mousson *et al*. (2002) analyzed the population genetic structure and differentiation regarding vector competence for a dengue virus of 15 *Ae. aegypti* samples collected from Chiang Mai in northern Thailand. Based on polymorphism of 10 isozyme loci, genetic differentiation was confirmed among samples collected in different subdistricts (high F_{ST} values and $P < 0.05$). Based on infection rate for a dengue 2 virus, susceptibilities were similar in mosquitoes collected in San Nuea subdistrict and in Choeng Doi subdistrict and were heterogeneous in populations sampled in other subdistricts. These findings were related to insecticide treatments. Moreover, Huber *et al*. (2004) analysed the genetic differentiation and gene flow of *Ae. aegypti* using microsatellite markers and showed that there was less genetic differentiation between mosquito populations from Ho Chi Minh City and Phnom Penh than from either of them and Thailand, suggesting that passive migrations through human transportation help to explain this pattern of differentiation.

Bosio *et al*. (2005) studied a hierarchical population genetic among 19 *Ae. aegypti* populations in Thailand from Chiang Mai in the North to Songkhla province in the South. Single-strand conformation polymorphism analysis was used to examine variation in a 359-basepair region of the NADH dehydrogenase subunit 4 mitochondrial DNA gene (ND4). Seven haplotypes were detected in two lineages previously identified in ND4 haplotypes from North America. Gene flow estimates and highly significant variation among populations within 25 kilometers implicated genetic drift and vector control efforts as major factors in genetic structure. No isolation by distance was observed. Urban areas were relatively panmictic, while suburban/rural sites exhibited more restricted gene flow. Significant genetic structure among

groups of collections > 100 kilometers apart was consistent with recent (~50 year) expansion of *Ae. aegypti* from highly populated areas accompanied by founder effects, but could also reflect the overall low genetic diversity in ND4 in Thailand. These results had some implications for vector control. In the suburban/rural setting, where populations were more isolated, insecticide use could rapidly select for insecticide resistance if such variation exists in the population. Genetic bottlenecks of this kind and/or genetic drift could radically change the phenotype of the population for other important traits such as vector competence. Because gene flow from these populations appeared to be limited, however, genotype would spread more slowly to surrounding populations. In the urban setting, *Ae. aegypti* populations would be expected to recover quickly from insecticide treatments because they contained larger areas of panmixia. Insecticide resistance may develop more slowly because immigration of susceptible individuals or gene flow from outside the treated area was not impeded. The role of gene flow in impeding movement of insecticide resistance alleles was explored in *Culex pipiens* in southern France (Lenormand *et al*., 1998). However, depending on the frequency of insecticide application, insecticide resistance may spread by gene flow throughout the urban area from the treated population (Pasteur *et al*., 1995). If insecticides with residual activity are used, they could continue to impact gene flow for as long as they persist. This would affect migration of susceptible individuals into the treated area, but would not impede dispersal of resistant individuals out of the treated area. Because the population genetic structure of *Ae. aegypti* varies from one region to another in Thailand, understanding the local population dynamics will lead to more focused, appropriate and hopefully sustainable vector control strategies.

However, in recent years there has been no more advancement in the information concerning the population structure and gene flow which indicate the spread of *Ae. aegypti* throughout Thailand. This valuable information can be used to forecast how quick the populations can be integrated by using the information from gene frequencies. Then, the trend and direction of the movement of resistant genes to current used insecticides throughout Thailand could also be detected. This knowledge could be used as a basis to plan the comprehensive strategies for effective national control program for controlling *Ae. aegypti* , an important dengue virus vector in Thailand.

MATERIALS AND METHODS

1. Mosquito populations

Five leading dengue endemic provinces showed in Appendix Table 1 were served as collection sites. These include Bangkok, Chon Buri, Surat Thani, Nakhon Sawan and Nakhon Ratchasima. Four to five districts were selected within each province. Detailed backgrounds of all collecting sites are summarized in Appendix Table 2. An average of 100-500 larvae was sampled per site.

All *Aedes aegypti* samples were collected as larvae or pupae and reared to adults in a protected insectary at the Department of Entomology, Kasetsart University. Strict segregation of field specimens was maintained in the insectary to prevent potential contamination from other *Ae. aegypti* colonies present. Adult mosquitoes were either tested immediately or shortly frozen (- 20^{o}C) before processing.

2. Starch gel electrophoresis

An average of 30-40 adults with an equal number of males and females from each population was processed and tested by electrophoresis. Starch gel electrophoresis was performed using 20 enzyme systems on three different buffer systems, Tris-citrate (TCss) (Shaw and Prasad, 1970), Morpholine (Morph) and Lithium hydroxide (LiOH) (Pasteur *et al.*, 1988). Details of all enzyme and buffer systems are given in Table 1. Horizontal starch gel electrophoresis was conducted as described in Harris and Hopkinson (1976), Manguin *et al.* (1995) and Chareonviriyaphap and Lerdthusnee (2002).

Electrophoresis was carried out on starch gel using 55 g of Sigma's potato starch (Sigma chemical Co., St. Louis, MO), 25 g of sucrose and 550 ml of the appropriate gel buffer. Each mosquito was ground in 25 µl of grinding buffer (25 µl/3 wicks) and the homogenate was absorbed onto 0.4 x 1.4 cm cellulose polyacetate wicks (Gelman Sciences Inc., Ann Arbor, MI).

Each mosquito was run on 3 different buffers. The Morph, TCss and LiOH were run for ca. 6 hours at a constant power of 16 volts/cm (Manguin *et al*., 1995). Each gel was stained and incubated at 37^{o}C for 15-60 minutes.

3. <u>Data analysis</u>

Analysis of allele frequencies, mean number of alleles per locus, observed and expected heterozygosity, conformity to the Hardy-Weinberg equilibrium, percentage of polymorphic loci, inbreeding coefficient (F_{IS}), fixation index (F_{ST}) and genetic distances were calculated using BIOSYS-1 (Swofford and Selander, 1989). Differentiation among populations was determined by *F*-statistics (F_{ST}). The number of effective migration rate (N_em) among populations were estimated from the F_{ST} values with equation of $N_em = (1\text{-}F_{ST})/4F_{ST}$ (Wright, 1978), where N_e is the effective population size and *m* is the migration rate between populations. Because *m* is the proportion of migrants (number of migrants/N_e), N_em is actually an estimate of the number of migrants regardless of population size that would be allowed and still permit the observed degree of genetic differentiation between the tested populations. The exchange of genes was estimated from the number of effective migration rate. The design was prepared to analyze gene flow at two levels, among populations within a province and among provinces. GENEPOP-3.1 was used to analyze the isolation by distance between populations (Rousset, 1997). This was measured by the relationship between pairwise estimates of F_{ST} and logarithms of geographical distance to determine whether geographical distance among populations serves as a barrier to gene flow. Cavalli-Sforza & Edwards (1967) chord distance and modified Rogers distance (Wright, 1978) were used for the cluster analysis by unweighted pair group method arithmetic averaging (UPGMA) to produce the phenogram.

RESULTS

Of 20 enzyme systems, 31 putative loci were detected (Table 1). Twelve loci, *AKS-1, AOX-1, ARK-1, G6PD-1, GCD-1, GPI-1, HK-1, HK-3, MPI-1, PGD-1, PK-1* and *XDH-1*, were monomorphic in all populations, whereas nineteen were polymorphic in all populations (Table 2). Among nineteen polymorphic loci, three loci, *IDH-1, MDH-1* and *PGM-1*, were polymorphic in all populations. Eight loci, *AKS-2, ATA-1, ATA-2, FUM-1, HAD-1, IDH-1, MDH-1* and *PGM-1*; showed allelic polymorphism in all populations from Bangkok. Five loci, *HAD-1, IDH-1, MDH-1, MDH-2* and *PGM-1*, showed allelic polymorphism in all populations from Chon Buri. Seven loci, *ATA-1, ATA-2, IDH-1, MDH-1, MDH-2, ME-1* and *PGM-1*, showed allelic polymorphism in all populations from Surat Thani. Six loci, *ACO-2, ATA-2, IDH-1, MDH-1, MDH-2* and *PGM-1*, showed allelic polymorphism in all populations from Nakhon Sawan. Five loci, *IDH-1, MDH-1, MDH-2, ME-1* and *PGM-1*, showed allelic polymorphism in all populations from Nakhon Ratchasima. The allelic frequencies of all polymorphic loci were provided in Table 2. In summarized, *AKS-2* and *FUM-1* loci were polymorphic only in Bangkok populations. *ATA-1* showed allelic polymorphism in Bangkok and Surat Thani populations. *ATA-2* showed allelic polymorphism in Bangkok, Surat Thani and Nakhon Sawan populations. *ACO-2* locus was polymorphic solely in Nakhon Sawan populations. *HAD-1* locus was polymorphic in Bangkok and Chon Buri populations, whereas was monomorphic in the others. *MDH-2* showed allelic polymorphism in Chon Buri, Surat Thani, Nakhon Sawan and Nakhon Ratchasima populations. *ME-1* was observed as a polymorphic locus in Surat Thani and Nakhon Ratchasima populations. Details of polymorphic loci for all populations are summarized in Table 2.

Chi-square test was performed to observe if there are any significant differences between observed and expected allelic frequencies. Of 744 comparisons, there are 39 significant deviations from the Hardy-Weinberg equilibrium ($P < 0.05$), representing a value of approximately 5% of the expected deviations by chance alone (Tables 2 and 3). Seventeen of these significant departures were found from populations of metropolitan areas of Bangkok, 4 from Chon Buri, 9 from Surat Thani, 8 from Nakhon Sawan and only 1 from Nakhon Ratchasima. Details of loci departing from the Hardy-Weinberg equilibrium in all populations are shown in Table 3.

Table 1 Enzyme systems used in electrophoretic studies on adult *Aedes aegypti*

Enzyme system	E.C.Number[1]	Gene symbol	No. loci[2]	Buffer[3]
Aconitase	4.2.1.3	*ACO*	2	TCss
Adenylate kinase	2.7.4.3	*AKS*	3	TCss
Aldehyde oxidase	1.2.3.1	*AOX*	1	LiOH
Arginine kinase	2.7.3.3	*ARK*	3	TCss
Aspartate transaminase	2.6.1.1	*ATA*	2	Morph
Fumarase	4.2.1.2	*FUM*	1	TCss
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PD*	1	TCss
Glycerol dehydrogenase	1.1.1.72	*GCD*	1	TCss
α-glycerophosphate dehydrogenase	1.1.1.8	*GPD*	1	LiOH
Glucose phosphate isomerase	5.3.1.9	*GPI*	1	LiOH
Hydroxy acid dehydrogenase	1.1.1.30	*HAD*	1	Morph
Hexokinase	2.7.1.1	*HK*	3	Morph
Isocitrate dehydrogenase	1.1.1.42	*IDH*	1	Morph
Malate dehydrogenase	1.1.1.37	*MDH*	2	Morph
Malic enzyme	1.1.1.40	*ME*	1	LiOH
Manose-6-phosphate isomerase	5.3.1.8	*MPI*	1	Morph
Phosphogluconate dehydrogenase	1.1.1.43	*PGD*	1	TCss
Phosphoglucomutase	5.4.2.2	*PGM*	1	Morph
Pyruvate kinase	2.7.1.40	*PK*	3	TCss
Xanthine dehydrogenase	1.2.1.37	*XDH*	1	LiOH

[1] Enzyme Commission Number

[2] Number of scorable bands per phenotype

[3] Refers to electrophoresis buffer (see Materials and Methods)

Table 2 Allele frequencies and sample sizes (*n*) at 24 population sites from five provinces in Thailand

Locus / allele	Bangkok population				
	Lak Si	Rat Burana	Lat Krabang	Huai Khwang	Bangkok Noi
ACO-1					
n	16	18	18	18	18
100	1.000	1.000	1.000	1.000	1.000
66	0.000	0.000	0.000	0.000	0.000
ACO-2					
n	16	18	18	18	18
-100	1.000	1.000	1.000	0.889	0.917
-59	0.000	0.000	0.000	0.111	0.083
AKS-1					
n	28	28	28	22	22
100	1.000	1.000	1.000	1.000	1.000
AKS-2					
n	10	12	12	30	30
117	0.200	0.208	0.208	0.200	0.200
100	0.800	0.792	0.792	0.800	0.800
AKS-3					
n	30	32	32	30	30
108	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
83	0.000	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Bangkok population				
	Lak Si	Rat Burana	Lat Krabang	Huai Khwang	Bangkok Noi
AOX-1					
n	20	22	22	30	30
100	1.000	1.000	1.000	1.000	1.000
ARK-1					
n	32	28	28	22	22
100	1.000	1.000	1.000	1.000	1.000
ARK-2					
n	32	32	32	30	30
116	0.000	0.000	0.016	0.000	0.017
100	1.000	1.000	0.984	1.000	0.983
83	0.000	0.000	0.000	0.000	0.000
ARK-3					
n	32	32	32	30	30
104	0.000	0.000	0.000	0.000	0.000
100	0.969	0.969	1.000	0.850	0.983
92	0.031	0.031	0.000	0.150	0.017
ATA-1					
n	32	32	32	30	28
141	0.000	0.000	0.016	0.000	0.000
128	0.016	0.094	0.016	0.017	0.089
100	0.984	0.906	0.938	0.983	0.911
70	0.000	0.000	0.031	0.000	0.000

Table 2 (Continued)

Locus / allele	Bangkok population				
	Lak Si	Rat Burana	Lat Krabang	Huai Khwang	Bangkok Noi
ATA-2					
n	32	32	32	30	28
-100	0.875	0.844	0.844	0.900	0.911
-59	0.125	0.156	0.156	0.100	0.089
FUM-1					
n	18*	18*	18*	18*	20*
100	0.833	0.778	0.750	0.667	0.525
27	0.167	0.222	0.250	0.333	0.475
G6PD-1					
n	20	22	22	30	30
100	1.000	1.000	1.000	1.000	1.000
GCD-1					
n	20	22	22	30	30
100	1.000	1.000	1.000	1.000	1.000
GPD-1					
n	32	32	32	30	30
122	0.000	0.000	0.016	0.000	0.000
100	1.000	1.000	0.984	1.000	1.000
GPI-1					
n	28	28	28	20	20
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Bangkok population				
	Lak Si	Rat Burana	Lat Krabang	Huai Khwang	Bangkok Noi
HAD-1					
n	32	32	32	30	30
100	0.891	0.938	0.969	0.883	0.950
67	0.109	0.063	0.031	0.117	0.050
HK-1					
n	32	32	32	30	30
100	1.000	1.000	1.000	1.000	1.000
HK-2					
n	32	32	32	30	30
100	1.000	1.000	1.000	1.000	0.983
87	0.000	0.000	0.000	0.000	0.017
HK-3					
n	22	22	32	30	30
100	1.000	1.000	1.000	1.000	1.000
IDH-1					
n	32*	32*	32*	30*	26
144	0.000	0.000	0.000	0.000	0.019
127	0.156	0.016	0.047	0.067	0.154
100	0.719	0.891	0.734	0.800	0.827
92	0.109	0.094	0.219	0.133	0.000
73	0.016	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Bangkok population				
	Lak Si	Rat Burana	Lat Krabang	Huai Khwang	Bangkok Noi
MDH-1					
n	32*	32*	32*	30*	30
142	0.000	0.000	0.000	0.000	0.083
132	0.016	0.078	0.031	0.200	0.117
125	0.219	0.281	0.188	0.067	0.267
115	0.078	0.156	0.203	0.000	0.000
100	0.563	0.359	0.406	0.600	0.533
77	0.078	0.125	0.125	0.000	0.000
63	0.047	0.000	0.047	0.133	0.000
MDH-2					
n	28	28	28	12*	20
-100	1.000	1.000	1.000	0.583	0.925
-53	0.000	0.000	0.000	0.417	0.075
ME-1					
n	32	32	32	30	30
100	1.000	1.000	1.000	0.883	1.000
83	0.000	0.000	0.000	0.117	0.000
MPI-1					
n	32	32	32	30	30
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Bangkok population				
	Lak Si	Rat Burana	Lat Krabang	Huai Khwang	Bangkok Noi
PGD-1					
n	32	32	32	30	30
100	1.000	1.000	1.000	1.000	1.000
PGM-1					
n	20*	22	22	30*	28*
185	0.025	0.000	0.000	0.017	0.018
162	0.050	0.000	0.000	0.167	0.179
143	0.075	0.023	0.023	0.033	0.000
126	0.050	0.136	0.045	0.033	0.000
114	0.000	0.023	0.000	0.017	0.000
100	0.700	0.705	0.932	0.700	0.696
89	0.025	0.023	0.000	0.017	0.089
73	0.075	0.091	0.000	0.017	0.018
PK-1					
n	10	10	10	30	30
100	1.000	1.000	1.000	1.000	1.000
PK-2					
n	30	32	32	30	30
114	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Bangkok population				
	Lak Si	Rat Burana	Lat Krabang	Huai Khwang	Bangkok Noi
PK-3					
n	22	22	22	30	30
108	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
91	0.000	0.000	0.000	0.000	0.000
XDH-1					
n	32	32	32	30	30
100	1.000	1.000	1.000	1.000	1.000
H_{exp}**	0.088	0.089	0.082	0.123	0.099
	(0.031)	(0.032)	(0.031)	(0.033)	(0.031)

Table 2 (Continued)

Locus / allele	Chon Buri population			
	Bang Lamung	Phanat Nikhom	Mueang Chon Buri	Si Racha
ACO-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
66	0.000	0.000	0.000	0.000
ACO-2				
n	40	42	36	36
-100	1.000	1.000	1.000	1.000
-59	0.000	0.000	0.000	0.000
AKS-1				
n	30	42	36	36
100	1.000	1.000	1.000	1.000
AKS-2				
n	20	26	36	36
117	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000
AKS-3				
n	40	42	36	36
108	0.000	0.000	0.000	0.014
100	1.000	1.000	1.000	0.986
83	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Chon Buri population			
	Bang Lamung	Phanat Nikhom	Mueang Chon Buri	Si Racha
AOX-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
ARK-1				
n	30	42	18	18
100	1.000	1.000	1.000	1.000
ARK-2				
n	40	42	36	36
116	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000
83	0.000	0.000	0.000	0.000
ARK-3				
n	40	42	36	36
104	0.000	0.000	0.000	0.000
100	1.000	1.000	0.972	1.000
92	0.000	0.000	0.028	0.000
ATA-1				
n	40	42	36	36
141	0.000	0.000	0.000	0.000
128	0.050	0.000	0.000	0.028
100	0.950	1.000	0.986	0.972
70	0.000	0.000	0.014	0.000

Table 2 (Continued)

Locus / allele	Chon Buri population			
	Bang Lamung	Phanat Nikhom	Mueang Chon Buri	Si Racha
ATA-2				
n	40	42	36	36
-100	1.000	1.000	1.000	1.000
-59	0.000	0.000	0.000	0.000
FUM-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
27	0.000	0.000	0.000	0.000
G6PD-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
GCD-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
GPD-1				
n	40	42	36	36
122	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000
GPI-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Chon Buri population			
	Bang Lamung	Phanat Nikhom	Mueang Chon Buri	Si Racha
HAD-1				
n	40	42	36	36
100	0.875	0.833	0.958	0.958
67	0.125	0.167	0.042	0.042
HK-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
HK-2				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
87	0.000	0.000	0.000	0.000
HK-3				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
IDH-1				
n	40	42	36	36
144	0.000	0.000	0.000	0.000
127	0.150	0.119	0.042	0.111
100	0.850	0.881	0.958	0.889
92	0.000	0.000	0.000	0.000
73	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Chon Buri population			
	Bang Lamung	Phanat Nikhom	Mueang Chon Buri	Si Racha
MDH-1				
n	40	39	36	36
142	0.000	0.000	0.000	0.000
132	0.000	0.000	0.000	0.000
125	0.275	0.487	0.486	0.319
115	0.000	0.000	0.000	0.000
100	0.663	0.410	0.444	0.653
77	0.063	0.103	0.069	0.028
63	0.000	0.000	0.000	0.000
MDH-2				
n	40	42	36	36
-100	0.988	0.988	0.764	0.750
-53	0.013	0.012	0.236	0.250
ME-1				
n	40	42	36	36*
100	1.000	1.000	0.833	0.722
83	0.000	0.000	0.167	0.278
MPI-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Chon Buri population			
	Bang Lamung	Phanat Nikhom	Mueang Chon Buri	Si Racha
PGD-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
PGM-1				
n	40*	42*	36	36*
185	0.000	0.000	0.000	0.000
162	0.075	0.036	0.000	0.000
143	0.087	0.119	0.167	0.208
126	0.000	0.000	0.014	0.028
114	0.038	0.000	0.028	0.028
100	0.788	0.833	0.792	0.736
89	0.013	0.000	0.000	0.000
73	0.000	0.012	0.000	0.000
PK-1				
n	20	16	36	36
100	1.000	1.000	1.000	1.000
PK-2				
n	20	26	36	36
114	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Chon Buri population			
	Bang Lamung	Phanat Nikhom	Mueang Chon Buri	Si Racha
PK-3				
n	40	42	36	36
108	0.000	0.000	0.000	0.014
100	1.000	1.000	1.000	0.986
91	0.000	0.000	0.000	0.000
XDH-1				
n	40	42	36	36
100	1.000	1.000	1.000	1.000
H_{exp}**	0.047	0.045	0.058	0.067
	(0.022)	(0.023)	(0.025)	(0.026)

Table 2 (Continued)

Locus / allele	Surat Thani population				
	Chaiya	Ban Na Doem	Khian Sa	Don Sak	Mueang Surat Thani
ACO-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	0.986	1.000
66	0.000	0.000	0.000	0.014	0.000
ACO-2					
n	35	35	35	36	39
-100	0.957	0.957	1.000	0.986	0.962
-59	0.043	0.043	0.000	0.014	0.038
AKS-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
AKS-2					
n	35	35	35	36	39
117	0.000	0.000	0.000	0.014	0.013
100	1.000	1.000	1.000	0.986	0.987
AKS-3					
n	35	35	35	36	39
108	0.000	0.000	0.000	0.000	0.000
100	1.000	0.986	0.986	0.972	0.974
83	0.000	0.014	0.014	0.028	0.026

Table 2 (Continued)

Locus / allele	Surat Thani population				
	Chaiya	Ban Na Doem	Khian Sa	Don Sak	Mueang Surat Thani
AOX-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
ARK-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
ARK-2					
n	35	35	35	36	39
116	0.000	0.000	0.014	0.000	0.000
100	1.000	1.000	0.986	1.000	1.000
83	0.000	0.000	0.000	0.000	0.000
ARK-3					
n	35	35	35*	36	39
104	0.000	0.000	0.014	0.000	0.000
100	1.000	1.000	0.971	1.000	1.000
92	0.000	0.000	0.014	0.000	0.000
ATA-1					
n	40	40	40	46	49
141	0.000	0.000	0.000	0.000	0.000
128	0.138	0.138	0.100	0.054	0.092
100	0.863	0.863	0.900	0.946	0.908
70	0.000	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Surat Thani population				
	Chaiya	Ban Na Doem	Khian Sa	Don Sak	Mueang Surat Thani
ATA-2					
n	40*	40*	40*	46	49*
-100	0.738	0.738	0.700	0.804	0.765
-59	0.262	0.262	0.300	0.196	0.235
FUM-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
27	0.000	0.000	0.000	0.000	0.000
G6PD-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
GCD-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
GPD-1					
n	35	35	35	36	39
122	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
GPI-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Surat Thani population				
	Chaiya	Ban Na Doem	Khian Sa	Don Sak	Mueang Surat Thani
HAD-1					
n	40	40	40	46	49
100	0.975	0.938	1.000	0.978	0.990
67	0.025	0.063	0.000	0.022	0.010
HK-1					
n	40	40	40	46	49
100	1.000	1.000	1.000	1.000	1.000
HK-2					
n	40	40	40	46	49
100	1.000	1.000	1.000	1.000	1.000
87	0.000	0.000	0.000	0.000	0.000
HK-3					
n	40	40	40	46	49
100	1.000	1.000	1.000	1.000	1.000
IDH-1					
n	40	40	40	46	49
144	0.000	0.000	0.000	0.000	0.000
127	0.100	0.050	0.075	0.120	0.153
100	0.900	0.950	0.925	0.880	0.847
92	0.000	0.000	0.000	0.000	0.000
73	0.000	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Surat Thani population				
	Chaiya	Ban Na Doem	Khian Sa	Don Sak	Mueang Surat Thani
MDH-1					
n	40	40	40*	46*	47
142	0.000	0.000	0.000	0.000	0.000
132	0.000	0.000	0.050	0.000	0.011
125	0.188	0.162	0.262	0.185	0.128
115	0.075	0.075	0.112	0.185	0.117
100	0.538	0.762	0.563	0.565	0.702
77	0.200	0.000	0.013	0.065	0.043
63	0.000	0.000	0.000	0.000	0.000
MDH-2					
n	40	40*	40	46	49
-100	0.850	0.712	0.788	0.783	0.806
-53	0.150	0.287	0.213	0.217	0.194
ME-1					
n	35	35	35	36	39
100	0.957	0.929	0.857	0.958	1.000
83	0.043	0.071	0.143	0.042	0.000
MPI-1					
n	40	40	40	46	49
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Surat Thani population				
	Chaiya	Ban Na Doem	Khian Sa	Don Sak	Mueang Surat Thani
PGD-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
PGM-1					
n	40	40	40*	46	49
185	0.000	0.000	0.000	0.000	0.000
162	0.000	0.000	0.000	0.000	0.000
143	0.038	0.300	0.150	0.152	0.041
126	0.013	0.025	0.063	0.065	0.071
114	0.025	0.175	0.000	0.065	0.061
100	0.813	0.488	0.788	0.685	0.745
89	0.000	0.000	0.000	0.000	0.000
73	0.112	0.013	0.000	0.033	0.082
PK-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
PK-2					
n	35	35	35	36	39
114	0.000	0.000	0.000	0.028	0.013
100	1.000	1.000	1.000	0.972	0.987

Table 2 (Continued)

Locus / allele	Surat Thani population				
	Chaiya	Ban Na Doem	Khian Sa	Don Sak	Mueang Surat Thani
PK-3					
n	35	35	35	36	39
108	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	0.986	1.000
91	0.000	0.000	0.000	0.014	0.000
XDH-1					
n	35	35	35	36	39
100	1.000	1.000	1.000	1.000	1.000
H_{exp}**	0.073	0.082	0.078	0.079	0.072
	(0.027)	(0.029)	(0.028)	(0.028)	(0.025)

Table 2 (Continued)

Locus / allele	Nakhon Sawan population				
	Mae Poen	Mae Wong	Mueang Nakhon Sawan	Krok Phra	Takhli
ACO-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
66	0.000	0.000	0.000	0.000	0.000
ACO-2					
n	35	35	35	35	40
-100	0.757	0.929	0.829	0.943	0.825
-59	0.243	0.071	0.171	0.057	0.175
AKS-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
AKS-2					
n	35	35	35	35	40
117	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
AKS-3					
n	35	35	35	35	40
108	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
83	0.000	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Nakhon Sawan population				
	Mae Poen	Mae Wong	Mueang Nakhon Sawan	Krok Phra	Takhli
AOX-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
ARK-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
ARK-2					
n	35	35	35	35	40
116	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	0.986	1.000	1.000
83	0.000	0.000	0.014	0.000	0.000
ARK-3					
n	35	35	35	35	40
104	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	0.957	0.950
92	0.000	0.000	0.000	0.043	0.050
ATA-1					
n	35	35	35	35	40
141	0.000	0.000	0.000	0.000	0.000
128	0.000	0.000	0.029	0.000	0.000
100	0.986	0.843	0.971	1.000	0.975
70	0.014	0.157	0.000	0.000	0.025

Table 2 (Continued)

Locus / allele	Nakhon Sawan population				
	Mae Poen	Mae Wong	Mueang Nakhon Sawan	Krok Phra	Takhli
ATA-2					
n	35	35*	35	35	40
-100	0.800	0.586	0.914	0.886	0.925
-59	0.200	0.414	0.086	0.114	0.075
FUM-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
27	0.000	0.000	0.000	0.000	0.000
G6PD-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
GCD-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
GPD-1					
n	35	35	35	35	40
122	0.014	0.000	0.029	0.014	0.000
100	0.986	1.000	0.971	0.986	1.000
GPI-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Nakhon Sawan population				
	Mae Poen	Mae Wong	Mueang Nakhon Sawan	Krok Phra	Takhli
HAD-1					
n	35	35	35	35	40
100	0.971	1.000	0.800	0.900	0.925
67	0.029	0.000	0.200	0.100	0.075
HK-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
HK-2					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
87	0.000	0.000	0.000	0.000	0.000
HK-3					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
IDH-1					
n	35	35	35	35*	40
144	0.000	0.000	0.000	0.000	0.000
127	0.171	0.014	0.243	0.143	0.150
100	0.829	0.986	0.757	0.857	0.850
92	0.000	0.000	0.000	0.000	0.000
73	0.000	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Nakhon Sawan population				
	Mae Poen	Mae Wong	Mueang Nakhon Sawan	Krok Phra	Takhli
MDH-1					
n	35	35*	35	35	40
142	0.000	0.000	0.000	0.000	0.000
132	0.000	0.000	0.000	0.000	0.000
125	0.471	0.329	0.357	0.486	0.300
115	0.000	0.000	0.000	0.000	0.000
100	0.514	0.671	0.471	0.443	0.625
77	0.000	0.000	0.143	0.029	0.075
63	0.014	0.000	0.029	0.043	0.000
MDH-2					
n	35*	35	35*	35	40*
-100	0.600	0.757	0.700	0.829	0.637
-53	0.400	0.243	0.300	0.171	0.363
ME-1					
n	35	35	35	35	40
100	1.000	1.000	0.986	1.000	1.000
83	0.000	0.000	0.014	0.000	0.000
MPI-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Nakhon Sawan population				
	Mae Poen	Mae Wong	Mueang Nakhon Sawan	Krok Phra	Takhli
PGD-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
PGM-1					
n	35*	35	35	35	40*
185	0.000	0.000	0.000	0.000	0.000
162	0.057	0.014	0.143	0.029	0.013
143	0.171	0.100	0.157	0.086	0.150
126	0.014	0.000	0.014	0.029	0.013
114	0.000	0.000	0.000	0.000	0.000
100	0.743	0.886	0.657	0.857	0.800
89	0.014	0.000	0.000	0.000	0.013
73	0.000	0.000	0.029	0.000	0.013
PK-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
PK-2					
n	35	35	35	35	40
114	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Nakhon Sawan population				
	Mae Poen	Mae Wong	Mueang Nakhon Sawan	Krok Phra	Takhli
PK-3					
n	35	35	35	35	40
108	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
91	0.000	0.000	0.000	0.000	0.000
XDH-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
H_{exp}**	0.082	0.063	0.094	0.064	0.074
	(0.030)	(0.025)	(0.032)	(0.023)	(0.026)

Table 2 (Continued)

Locus / allele	Nakhon Ratchasima population				
	Soeng Sang	Prathai	Kaeng Sanam Nang	Sikhio	Dan Khun Thot
ACO-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
66	0.000	0.000	0.000	0.000	0.000
ACO-2					
n	35	35	35	35	40
-100	1.000	1.000	1.000	1.000	1.000
-59	0.000	0.000	0.000	0.000	0.000
AKS-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
AKS-2					
n	35	35	35	35	40
117	0.000	0.000	0.000	0.029	0.000
100	1.000	1.000	1.000	0.971	1.000
AKS-3					
n	35	35	35	35	40
108	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
83	0.000	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Nakhon Ratchasima population				
	Soeng Sang	Prathai	Kaeng Sanam Nang	Sikhio	Dan Khun Thot
AOX-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
ARK-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
ARK-2					
n	35	35	35	35	40
116	0.071	0.000	0.057	0.014	0.075
100	0.929	1.000	0.943	0.986	0.925
83	0.000	0.000	0.000	0.000	0.000
ARK-3					
n	35	35	35	35	40
104	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
92	0.000	0.000	0.000	0.000	0.000
ATA-1					
n	35	35	35	35	40
141	0.000	0.000	0.000	0.000	0.000
128	0.014	0.014	0.014	0.029	0.000
100	0.986	0.986	0.986	0.971	1.000
70	0.000	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Nakhon Ratchasima population				
	Soeng Sang	Prathai	Kaeng Sanam Nang	Sikhio	Dan Khun Thot
ATA-2					
n	35	35	35	35	40
-100	1.000	0.986	0.943	1.000	1.000
-59	0.000	0.014	0.057	0.000	0.000
FUM-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
27	0.000	0.000	0.000	0.000	0.000
G6PD-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
GCD-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
GPD-1					
n	35	35	35	35	40
122	0.000	0.000	0.029	0.000	0.000
100	1.000	1.000	0.971	1.000	1.000
GPI-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Nakhon Ratchasima population				
	Soeng Sang	Prathai	Kaeng Sanam Nang	Sikhio	Dan Khun Thot
HAD-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
67	0.000	0.000	0.000	0.000	0.000
HK-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
HK-2					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
87	0.000	0.000	0.000	0.000	0.000
HK-3					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
IDH-1					
n	35*	35	35	35	40
144	0.000	0.000	0.000	0.000	0.000
127	0.057	0.014	0.043	0.029	0.150
100	0.943	0.986	0.957	0.971	0.850
92	0.000	0.000	0.000	0.000	0.000
73	0.000	0.000	0.000	0.000	0.000

Table 2 (Continued)

Locus / allele	Nakhon Ratchasima population				
	Soeng Sang	Prathai	Kaeng Sanam Nang	Sikhio	Dan Khun Thot
MDH-1					
n	35	35	35	35	40
142	0.000	0.000	0.000	0.000	0.000
132	0.000	0.000	0.000	0.000	0.000
125	0.500	0.457	0.386	0.214	0.038
115	0.000	0.000	0.000	0.000	0.000
100	0.500	0.529	0.586	0.771	0.875
77	0.000	0.014	0.029	0.014	0.087
63	0.000	0.000	0.000	0.000	0.000
MDH-2					
n	35	35	35	35	40
-100	0.857	0.914	0.829	0.857	0.913
-53	0.143	0.086	0.171	0.143	0.087
ME-1					
n	35	35	35	35	40
100	0.914	0.843	0.743	0.871	0.913
83	0.086	0.157	0.257	0.129	0.087
MPI-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000

Table 2 (Continued)

Locus / allele	Nakhon Ratchasima population				
	Soeng Sang	Prathai	Kaeng Sanam Nang	Sikhio	Dan Khun Thot
PGD-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
PGM-1					
n	35	35	35	35	40
185	0.000	0.000	0.000	0.000	0.000
162	0.000	0.000	0.000	0.000	0.000
143	0.229	0.257	0.186	0.257	0.100
126	0.043	0.014	0.000	0.086	0.000
114	0.000	0.057	0.043	0.000	0.000
100	0.729	0.657	0.743	0.657	0.900
89	0.000	0.014	0.000	0.000	0.000
73	0.000	0.000	0.029	0.000	0.000
PK-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
PK-2					
n	35	35	35	35	40
114	0.000	0.000	0.000	0.043	0.000
100	1.000	1.000	1.000	0.957	1.000

Table 2 (Continued)

Locus / allele	Nakhon Ratchasima population				
	Soeng Sang	Prathai	Kaeng Sanam Nang	Sikhio	Dan Khun Thot
PK-3					
n	35	35	35	35	40
108	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000
91	0.000	0.000	0.000	0.000	0.000
XDH-1					
n	35	35	35	35	40
100	1.000	1.000	1.000	1.000	1.000
H_{exp}**	0.052	0.050	0.064	0.052	0.037
	(0.022)	(0.024)	(0.025)	(0.022)	(0.014)

* Deviation from the Hardy-Weinberg equilibrium ($P < 0.05$)

** Average expected genetic heterozygosity and the standard error in parenthesis

Table 3 Loci departing from the Hardy-Weinberg equilibrium of 24 populations of *Aedes aegypti* from 5 provinces in Thailand

Province	District	Departure loci [1]	
		Heterozygote deficiency	Heterozygote excess
Bangkok	Lak Si	*FUM-1, IDH-1, PGM-1*	*MDH-1*
	Rat Burana	*FUM-1, IDH-1, MDH-1*	-
	Lat Krabang	*FUM-1, IDH-1, MDH-1*	-
	Huai Khwang	*FUM-1, IDH-1, MDH-1, PGM-1*	*MDH-2*
	Bangkok Noi	*FUM-1, PGM-1*	-
Chon Buri	Bang Lamung	*PGM-1*	-
	Phanat Nikhom	*PGM-1*	-
	Mueang Chon Buri	-	-
	Si Racha	*PGM-1*	*ME-1*
Surat Thani	Chaiya	-	*ATA-2*
	Ban Na Doem	-	*ATA-2, MDH-2*
	Khian Sa	*ARK-3, MDH-1, PGM-1*	*ATA-2*
	Don Sak	*MDH-1*	-
	Mueang Surat Thani	-	*ATA-2*
Nakhon Sawan	Mae Poen	-	*MDH-2, PGM-1*
	Mae Wong	-	*ATA-2, MDH-1*
	Mueang Nakhon Sawan	-	*MDH-2*
	Krok Phra	*IDH-1*	-
	Takhli	-	*MDH-2, PGM-1*
Nakhon Ratchasima	Soeng Sang	*IDH-1*	-
	Prathai	-	-
	Kaeng Sanam Nang	-	-
	Sikhio	-	-
	Dan Khun Thot	-	-

[1] Significant departure from the Hardy-Weinberg equilibrium ($P < 0.05$)

In Bangkok populations, departures at *FUM-1* locus were observed from Laksi, Rat Burana, Lat Krabang, Huai Khwang and Bangkok Noi; at *IDH-1* and *MDH-1* loci from Laksi, Rat Burana, Lat Krabang and Huai Khwang; at *MDH-2* locus from Huai Khwang; at *PGM-1* locus from Laksi, Huai Khwang and Bangkok Noi (Tables 2 and 3). Departures from the Hardy-Weinberg equilibrium for *FUM-1* in all collections in Bangkok were due to a deficiency of heterozygotes. In contrast, departure from the Hardy-Weinberg equilibrium at the *MDH-2* locus in Huai Khwang was due to excessive heterozygotes (Table 3).

In Chon Buri populations, departures at *PGM-1* locus were observed from Bang Lamung, Phanat Nikhom, and Si Racha; as well as at *ME-1* locus from Si Racha (Tables 2 and 3). Departure from the Hardy-Weinberg equilibrium at the *ME-1* locus in Si Racha was due to excessive heterozygotes (Table 3).

In Surat Thani populations, departures at *ATA-2* locus were observed from Chaiya, Ban Na Doem, Khian Sa and Mueang Surat Thani, at *ARK-3* locus from Khian Sa, at *MDH-1* locus from Khian Sa and Don Sak, at *MDH-2* locus from Ban Na Doem, at *PGM-1* locus from Khian Sa (Tables 2 and 3). Departures from the Hardy-Weinberg equilibrium at the *ATA-2* locus in Chaiya, Ban Na Doem, Khian Sa and Mueang Surat Thani, as well as at the *MDH-2* locus in Ban Na Doem were due to excessive heterozygotes. In contrast, departure from the Hardy-Weinberg equilibrium at the *PGM-1* locus in Khian Sa was due to a deficiency of heterozygotes (Table 3).

In Nakhon Sawan populations, departure at *ATA-2* locus was observed from Mae Wong, at *IDH-1* locus from Krok Phra, at *MDH-1* locus from Mae Wong, at *MDH-2* locus from Mae Poen, Mueang Nakhon Sawan and Takli, at *PGM-1* locus from Mae Poen and Takhli (Tables 2 and 3). Departures from the Hardy-Weinberg equilibrium at the *ATA-2* and *MDH-1* loci in Mae Wong, as well as at the *MDH-2* locus in Mae Poen, Mueang Nakhon Sawan and Takhli were due to excessive heterozygotes. In contrast, departure from the Hardy-Weinberg equilibrium at the *IDH-1* locus in Krok Phra was due to a deficiency of heterozygotes (Table 3).

In Nakhon Ratchasima populations, only one locus, *IDH-1* from Soeng Sang, deviated from the Hardy-Weinberg equilibrium (Tables 2 and 3). The departure was due to a deficiency of heterozygotes (Table 3).

In general, the populations from Bangkok showed a higher polymorphism than populations from the other four provinces (Table 2). *ACO-1* (allele 66) was present only in population of Don Sak district of Surat Thani in low frequency (0.014), whereas the *ACO-2* (allele -59) was absent in populations of Chon Buri and Nakhon Ratchasima. The *AKS*-2 (allele 117) was not found in Chon Buri and Nakhon Sawan populations. The *AKS-3* (allele 108) was observed exclusively in Si Racha district of Chon Buri in low frequency (0.014), while *AKS-3* (allele 83) was observed only in Ban Na Doem, Khian Sa, Don Sak and Mueang Surat Thani districts of Surat Thani province in low frequencies (0.014-0.028). The *ARK-2* (allele 116) was not found in Chon Buri and Nakhon Sawan populations and allele 83 was present only in Mueang Nakhon Sawan district of Nakhon Sawan in low frequency (0.014). The *ARK-3* (allele 104) was solely found in Khian Sa district of Surat Thani in low frequency (0.014) and allele 92 was totally absent from Nakhon Ratchasima. The *ATA-1* (allele141) was observed exclusively in Lat Krabang district of Bangkok in low frequency (0.016) and allele70 was not found in Surat Thani and Nakhon Ratchasima populations. The *ATA-2* (allele-59) was completely absent from Chon Buri, but present in low frequency (0.014-0.300) in Bangkok, Surat Thani and Nakhon Ratchasima as well as in four districts of Nakhon Sawan. This allele was present in high frequency (0.414) in Mae Wong population in Nakhon Sawan. The *FUM-1* (allele 27) was found specifically in Bangkok populations in rather high frequency (0.475) in Bangkok Noi district, however, it was found in low frequencies (0.167-0.333) in the other four populations in Bangkok. The *GPD-1* (allele122) was completely absent from Chon Buri and Surat Thani populations. *HAD-1* (allele 67) was completely absent from Nakhon Ratchasima populations. The *HK-2* (allele 87) and *IDH-1* (allele 144) were exclusively found in Bangkok Noi district of Bangkok in low frequencies (0.017 and 0.019, respectively). The *IDH-1* (allele 127) was generally found in all populations in low frequencies (0.014-0.243), allele 92 was only present in Lak Si, Rat Burana, Lat Kra Bang and Huai Khwang districts of Bangkok in low frequencies (0.094-0.219) and allele 73 was found only in Laksi district of Bangkok in low frequency (0.016). The *MDH-1* (allele 142) was observed exclusively in Bangkok Noi district of Bangkok in low frequency (0.083) and

alleles 132 and 115 were not found in Chon Buri, Nakhon Sawan and Nakhon Ratchasima. The *MDH-1* (allele 125) was observed in rather high frequencies (0.457-0.500) in Phanat Nikhom and Mueang Chon Buri districts of Chon Buri, in Mae Poen and Krok Phra districts of Nakhon Sawan, as well as in Soeng Sang and Prathai districts of Nakhon Ratchasima, whereas it was observed in low frequencies (0.038-0.386) in other populations. The *MDH-1* (allele 63) was entirely absent from Chon Buri, Surat Thani and Nakhon Ratchasima. The *MDH-2* (allele -53) was found in rather high frequencies in Huai Khwang district of Bangkok (0.417) and in Mae Poen district of Nakhon Sawan (0.400).This allele was found in low frequencies (0.000-0.363) in all other populations. The *PGM-1* (allele 185) was specifically found in Lak Si, Huai Khwang and Bangkok Noi in low frequencies (0.017-0.025), whereas allele 162 was not found in Surat Thani and Nakhon Ratchasima. The *PGM-1* (alleles 114 and 89) were also not found in Nakhon Sawan and Surat Thani populations, respectively. The *PK-2* (allele 114) was exclusively found in Don Sak and Mueang Surat Thani districts of Surat Thani as well as in Sikhio district of Nakhon Ratchasima in low frequencies (0.013-0.043). The *PK-3* (allele 108) was observed exclusively in Si Racha district of Chon Buri and allele 91 was specifically found in Don Sak district of Surat Thani, both in equally low frequencies (0.014) (Table 2).

Percent polymorphic loci of all 24 populations were quite variable, ranging from 12.9 to 35.5 % (Table 4). The greatest percent polymorphism was found in Huai Khwang district (35.5%) of Bangkok, with the greatest number of alleles per locus (1.7) and highest level of heterozygosity ($H_o = 0.116$). The lowest percent polymorphism was observed from Phanat Nikhom and Mueang Chon Buri districts (12.9%) of Chon Buri, as well as from Prathai and Sikhio districts (12.9%) of Nakhon Ratchasima with allele per locus of 1.3-1.4 and the average level of observed heterozygosity of 0.044-0.059. The lowest heterozygosity was found in Dan Khun Thot district (0.039) of Nakhon Ratchasima. The highest heterozygosity was found in Huai Khwang district (0.116) of Bangkok (Table 4). The observed heterozygosities from all populations were not significantly different from all Hardy-Weinberg expected heterozygosity ($t_{0.025} = 0.902^{ns}$, df = 23, $P > 0.05$).

Table 4 Genetic variability at 31 loci of 24 populations of *Aedes aegypti* from 5 provinces in Thailand

Population	Average alleles per locus	% polymorphic loci [1]	Mean heterozygosity	
			H_{obs}	H_{exp} [2]
Bangkok				
-Lak Si	1.6 ± 0.3	22.6	0.082 ± 0.031	0.088 ± 0.031
-Rat Burana	1.5 ± 0.2	25.8	0.071 ± 0.029	0.089 ± 0.032
-Lat Krabang	1.6 ± 0.2	22.6	0.060 ± 0.025	0.082 ± 0.031
-Huai Khwang	1.7 ± 0.2	35.5	0.116 ± 0.035	0.123 ± 0.033
-Bangkok Noi	1.6 ± 0.2	32.3	0.088 ± 0.030	0.099 ± 0.031
Chon Buri				
-Bang Lamung	1.3 ± 0.1	16.1	0.052 ± 0.025	0.047 ± 0.022
-Phanat Nikhom	1.3 ± 0.1	12.9	0.044 ± 0.023	0.045 ± 0.023
-Mueang Chon Buri	1.4 ± 0.1	12.9	0.059 ± 0.025	0.058 ± 0.025
-Si Racha	1.4 ± 0.1	16.1	0.069 ± 0.030	0.067 ± 0.026
Surat Thani				
-Chaiya	1.5 ± 0.2	19.4	0.084 ± 0.032	0.073 ± 0.027
-Ban Na Doem	1.5 ± 0.2	25.8	0.094 ± 0.034	0.082 ± 0.029
-Khian Sa	1.5 ± 0.2	22.6	0.077 ± 0.028	0.078 ± 0.028
-Don Sak	1.6 ± 0.2	19.4	0.076 ± 0.026	0.079 ± 0.028
-Mueang Surat Thani	1.5 ± 0.2	19.4	0.081 ± 0.029	0.072 ± 0.025
Nakhon Sawan				
-Mae Poen	1.4 ± 0.2	19.4	0.095 ± 0.036	0.082 ± 0.030
-Mae Wong	1.3 ± 0.1	19.4	0.085 ± 0.037	0.063 ± 0.025
-Mueang Nakhon Sawan	1.5 ± 0.2	22.6	0.100 ± 0.035	0.094 ± 0.032
-Krok Phra	1.4 ± 0.1	22.6	0.063 ± 0.024	0.064 ± 0.023
-Takhli	1.5 ± 0.2	25.8	0.086 ± 0.032	0.074 ± 0.026

Table 4 (Continued)

Population	Average alleles per locus	% polymorphic loci [1]	Mean heterozygosity	
			H_{obs}	H_{exp} [2]
Nakhon Ratchasima				
-Soeng Sang	1.3 ± 0.1	19.4	0.052 ± 0.023	0.052 ± 0.022
-Prathai	1.4 ± 0.2	12.9	0.057 ± 0.029	0.050 ± 0.024
-Kaeng Sanam Nang	1.4 ± 0.1	19.4	0.069 ± 0.027	0.064 ± 0.025
-Sikhio	1.4 ± 0.1	12.9	0.059 ± 0.025	0.052 ± 0.022
-Dan Khun Thot	1.2 ± 0.1	19.4	0.039 ± 0.015	0.037 ± 0.014
			$t_{0.025} = 0.902^{ns}$	

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

[2] Unbiased estimate and standard error (Nei, 1978)

In all 24 populations, mean value of F_{ST} calculated from all polymorphic loci was 0.091. The greatest F_{ST} value (0.286) was found at *FUM-1*, while the moderate F_{ST} value (0.156) was found at *AKS-2*. All other loci demonstrated only small F_{ST} values or negligible genetic differences (Table 5). Mean value of F_{IS} calculated from all polymorphic loci was -0.042. High positive F_{IS} value was obtained from *FUM-1* (0.774), whereas the greatest negative value was obtained from *MDH-2* (-0.344).

In Bangkok populations, mean value of F_{ST} from all polymorphic loci was 0.052. The greatest F_{ST} value (0.295) was found at *MDH-2*. All other loci demonstrated small F_{ST} values or negligible genetic differences (Table 6). Mean value of F_{IS} calculated from all polymorphic loci was 0.115. High positive F_{IS} value was obtained from *FUM-1* (0.774), whereas the greatest negative value was obtained from *MDH-2* (-0.574).

In Chon Buri populations, mean value of F_{ST} from all polymorphic loci was 0.053. All polymorphic loci demonstrated only small F_{ST} values or negligible genetic differences (Table 7). Mean value of F_{IS} calculated from all polymorphic loci was -0.044. High positive F_{IS} value was obtained from *PGM-1* (0.269), whereas the greatest negative value was obtained from *ME-1* (-0.309).

In Surat Thani populations, mean value of F_{ST} calculated from all polymorphic loci was 0.026. All polymorphic loci demonstrated only small F_{ST} values or negligible genetic differences (Table 8). Mean value of F_{IS} calculated from all polymorphic loci was -0.087. High positive F_{IS} value was obtained from *ARK-3* (0.489), whereas the greatest negative value was obtained from *ATA-2* (-0.344).

In Nakhon Sawan populations, mean value of F_{ST} calculated from all polymorphic loci showed negligible differentiation (0.046). All polymorphic loci demonstrated only small F_{ST} values or negligible genetic differences (Table 9). Mean value of F_{IS} from all polymorphic loci was -0.156. High positive F_{IS} value was obtained from *IDH-1* (0.019), whereas the greatest negative value was obtained from *MDH-2* (-0.438).

Table 5 *F*-statistic analysis of polymorphic loci in 24 populations of *Aedes aegypti* in Thailand

Locus	F_{IS} [1]	F_{ST} [2]
ACO-1	-0.014	0.013
ACO-2	-0.169	0.106
AKS-2	-0.241	0.156
AKS-3	-0.022	0.018
ARK-2	-0.057	0.044
ARK-3	-0.046	0.066
ATA-1	-0.103	0.054
ATA-2	-0.293	0.123
FUM-1	0.774	0.286
GPD-1	-0.023	0.018
HAD-1	-0.075	0.062
HK-2	-0.017	0.016
IDH-1	0.181	0.055
MDH-1	0.011	0.076
MDH-2	-0.344	0.105
ME-1	-0.183	0.111
PGM-1	0.037	0.054
PK-2	-0.034	0.030
PK-3	-0.014	0.013
Mean	-0.042	0.091
		$N_em = 2.50$

[1] F_{IS} = Inbreeding coefficient

[2] $F_{ST} > 0.25$ Great genetic differentiation among the subpopulations

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 6 *F*-statistic analysis of polymorphic loci in 5 populations of *Aedes aegypti* within Bangkok

Locus	F_{IS} [1]	F_{ST} [2]
ACO-2	-0.110	0.063
AKS-2	-0.255	0.000
ARK-2	-0.016	0.010
ARK-3	-0.121	0.065
ATA-1	-0.077	0.026
ATA-2	-0.151	0.007
FUM-1	0.774	0.056
GPD-1	-0.016	0.013
HAD-1	-0.098	0.016
HK-2	-0.017	0.013
IDH-1	0.506	0.035
MDH-1	0.032	0.046
MDH-2	-0.574	0.295
ME-1	-0.132	0.096
PGM-1	0.185	0.046
Mean	0.115	0.052
		N_em = 4.54

[1] F_{IS} = Inbreeding coefficient

[2] $F_{ST} > 0.25$ Great genetic differentiation among the subpopulations

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 7 *F*-statistic analysis of polymorphic loci in 4 populations of *Aedes aegypti* within Chon Buri

Locus	F_{IS} [1]	F_{ST} [2]
AKS-3	-0.014	0.010
ARK-3	-0.029	0.021
ATA-1	-0.039	0.018
HAD-1	-0.143	0.034
IDH-1	0.070	0.017
MDH-1	-0.082	0.043
MDH-2	-0.302	0.120
ME-1	-0.309	0.141
PGM-1	0.269	0.013
PK-3	-0.014	0.010
Mean	-0.044	0.053
		N_em = 4.47

[1] F_{IS} = Inbreeding coefficient

[2] $F_{ST} > 0.25$ Great genetic differentiation among the subpopulations

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 8 *F*-statistic analysis of polymorphic loci in 5 populations of *Aedes aegypti* within Surat Thani

Locus	F_{IS} [1]	F_{ST} [2]
ACO-1	-0.014	0.011
ACO-2	-0.040	0.011
AKS-2	-0.014	0.008
AKS-3	-0.023	0.006
ARK-2	-0.014	0.011
ARK-3	0.489	0.017
ATA-1	-0.128	0.010
ATA-2	-0.344	0.006
HAD-1	-0.045	0.019
IDH-1	-0.077	0.014
MDH-1	0.102	0.031
MDH-2	-0.285	0.012
ME-1	-0.108	0.040
PGM-1	0.029	0.060
PK-2	-0.024	0.015
PK-3	-0.014	0.011
Mean	-0.087	0.026
		N_em = 9.37

[1] F_{IS} = Inbreeding coefficient

[2] $F_{ST} > 0.25$ Great genetic differentiation among the subpopulations

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 9 *F*-statistic analysis of polymorphic loci in 5 populations of *Aedes aegypti* within Nakhon Sawan

Locus	F_{IS} [1]	F_{ST} [2]
ACO-2	-0.216	0.040
ARK-2	-0.014	0.011
ARK-3	-0.049	0.029
ATA-1	-0.133	0.080
ATA-2	-0.365	0.109
GPD-1	-0.022	0.010
HAD-1	0.002	0.064
IDH-1	0.019	0.044
MDH-1	0.018	0.031
MDH-2	-0.438	0.032
ME-1	-0.014	0.011
PGM-1	-0.107	0.029
Mean	-0.156	0.046
		N_em = 5.18

[1] F_{IS} = Inbreeding coefficient

[2] $F_{ST} > 0.25$ Great genetic differentiation among the subpopulations

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

In Nakhon Ratchasima populations, mean value of F_{ST} calculated from all polymorphic loci showed small differentiation (0.051). All polymorphic loci demonstrated only small F_{ST} values or negligible genetic differences (Table 10). Mean value of F_{IS} calculated from all polymorphic loci was -0.097. High positive F_{IS} value was obtained from *IDH-1* (0.094), whereas the greatest negative value was obtained from *ME-1* (-0.158).

Gene flow between collections was calculated by estimating the number of effective migration rate (N_em) The N_em among all populations from different provinces was quite low (2.50) (Table 5). Within populations from provinces, the N_em of Surat Thani population was the highest (9.37) (Table 8). In populations of Bangkok, Chon Buri, Nakhon Sawan and Nakhon Ratchasima the N_em were 4.54, 4.47, 5.18 and 4.65, respectively (Tables 6-7 and 9-10).

Paired analyses were conducted using an *F*-statistics by comparing all combinations of populations when all 31 loci were considered together (Tables 11-15). In Bangkok populations, small differentiations were found between both Rat Burana and Huai Khwang (F_{ST} = 0.052) and Lat Krabang and Huai Khwang (F_{ST} = 0.055). All others between populations demonstrated only negligible differentiation (Table 11). The N_em values between populations were generally high among pairwise comparison (4.30-17.61). The greatest N_em value reflecting the highest migration rate was observed between both Lak Si and Rat Burana and Rat Burana and Lat Krabang (17.61), whereas the smallest rate was observed between Lat Krabang and Huai Khwang (4.30) (Table 11).

In Chon Buri populations, small differentiation was found between Phanat Nikhom and Si Racha (F_{ST} = 0.057). All others between populations demonstrated only negligible differentiations (Table 12). The N_em values between populations were generally high among pairwise comparison (4.14-16.42). The greatest N_em value reflecting the highest migration rate was observed between Mueang Chon Buri and Si Racha (16.42). The smallest rate was observed between Phanat Nikhom and Si Racha (4.14) (Table 12).

Table 10 *F*-statistic analysis of polymorphic loci in 5 populations of *Aedes aegypti* within Nakhon Ratchasima

Locus	F_{IS} [1]	F_{ST} [2]
AKS-2	-0.029	0.023
ARK-2	-0.070	0.023
ATA-1	-0.020	0.006
ATA-2	-0.051	0.035
GPD-1	-0.029	0.023
IDH-1	0.094	0.042
MDH-1	-0.056	0.109
MDH-2	-0.156	0.010
ME-1	-0.158	0.032
PGM-1	-0.143	0.032
PK-2	-0.045	0.035
Mean	-0.097	0.051
		$N_em = 4.65$

[1] F_{IS} = Inbreeding coefficient

[2] $F_{ST} > 0.25$ Great genetic differentiation among the subpopulations

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 11 Pairwise *F*-statistics at all loci within 5 populations of *Aedes aegypti* from Bangkok

Populations compared	F_{ST} [1]	Effective migration rate ($N_e m$)	Distance (Km)
Lak Si : Rat Burana	0.014	17.61	23.9
Lak Si : Lat Krabang	0.016	15.36	23.8
Lak Si : Huai Khwang	0.045	5.31	10.2
Lak Si : Bangkok Noi	0.028	8.68	14.6
Rat Burana : Lat Krabang	0.014	17.61	40.2
Rat Burana : Huai Khwang	0.052	4.56	16.0
Rat Burana : Bangkok Noi	0.029	8.37	7.1
Lat Krabang : Huai Khwang	0.055	4.30	24.2
Lat Krabang : Bangkok Noi	0.036	6.69	33.1
Huai Khwang : Bangkok Noi	0.034	7.10	9.2
Coefficient of determination of isolation by distance between populations, $r^2 = 0.08^{ns}$			

[1] $F_{ST} > 0.25$ Great differentiation

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 12 Pairwise *F*-statistics at all loci within 4 populations of *Aedes aegypti* from Chon Buri

Populations compared	F_{ST} [1]	Effective migration rate ($N_e m$)	Distance (Km)
Bang Lamung : Phanat Nikhom	0.022	11.11	73.5
Bang Lamung : Mueang Chon Buri	0.045	5.31	47.3
Bang Lamung : Si Racha	0.043	5.56	24.3
Phanat Nikhom: Mueang Chon Buri	0.032	7.56	26.3
Phanat Nikhom : Si Racha	0.057	4.14	50.3
Mueang Chon Buri : Si Racha	0.015	16.42	24.0
Coefficient of determination of isolation by distance between populations, $r^2 = 0.03^{ns}$			

[1] $F_{ST} > 0.25$ Great differentiation

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

In Surat Thani populations, negligible differentiations were found in all combinations (Table 13). The N_em values between populations were generally high among pairwise comparison (6.89-35.46). The greatest N_em value reflecting the highest migration rate was observed between Don Sak and Mueang Surat Thani (35.46). The smallest rate was observed between Chaiya and Ban Na Doem (6.89) (Table 13).

In Nakhon Sawan populations, small differentiation was found between Mae Wong and Mueang Nakhon Sawan (F_{ST} = 0.060). All other between populations demonstrated only negligible differentiations (Table 14). The N_em values between populations were generally high among pairwise comparison (3.92-22.48). The greatest N_em value reflecting the highest migration rate was observed between Mae Poen and Takhli (22.48). The smallest rate was observed between Mae Wong and Mueang Nakhon Sawan (3.92) (Table 14).

In Nakhon Ratchasima populations, small differentiations were found between Soeng Sang and Dan Khun Thot, Prathai and Dan Khun Thot as well as Kaeng Sanam Nang and Dan Khun Thot (F_{ST} = 0.074, 0.078 and 0.053, respectively). All others between populations demonstrated only negligible differentiations (Table 15). The N_em values between populations were generally high among pairwise comparison (2.96-35.46). The greatest N_em value was observed between Soeng Sang and Prathai (35.46), whereas the smallest rate was observed between Prathai and Dan Khun Thot (2.96) (Table 15).

Analyses of isolation by distance on pairwise within and among populations were tested using pairwise estimates of F_{ST} and logarithm of geographic distance to determine the correlation between gene flow and geographic distance. The analyses indicated that there were no correlations ($P > 0.05$) between genetic and geographic distance within populations (Tables 11-15).

Table 13 Pairwise *F*-statistics at all loci within 5 populations of *Aedes aegypti* from Surat Thani

Populations compared	F_{ST} [1]	Effective migration rate (N_em)	Distance (Km)
Chaiya : Ban Na Doem	0.035	6.89	74.8
Chaiya : Khian Sa	0.012	20.58	70.0
Chaiya : Don Sak	0.012	20.58	143.1
Chaiya : Mueang Surat Thani	0.010	24.75	55.9
Ban Na Doem : Khian Sa	0.024	10.17	23.2
Ban Na Doem : Don Sak	0.018	13.64	112.8
Ban Na Doem : Mueang Surat Thani	0.023	10.62	40.9
Khian Sa : Don Sak	0.009	27.53	85.0
Khian Sa : Mueang Surat Thani	0.014	17.61	45.9
Don Sak : Mueang Surat Thani	0.007	35.46	87.4
Coefficient of determination of isolation by distance between populations, $r^2 = 0.12^{ns}$			

[1] $F_{ST} > 0.25$ Great differentiation

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 14 Pairwise *F*-statistics at all loci within 5 populations of *Aedes aegypti* from Nakhon Sawan

Populations compared	F_{ST} [1]	Effective migration rate ($N_e m$)	Distance (Km)
Mae Poen : Mae Wong	0.040	6.00	33.3
Mae Poen : Mueang Nakhon Sawan	0.016	15.38	82.2
Mae Poen : Krok Phra	0.026	9.37	74.0
Mae Poen : Takhli	0.011	22.48	109.5
Mae Wong : Mueang Nakhon Sawan	0.060	3.92	86.7
Mae Wong : Krok Phra	0.047	5.07	90.8
Mae Wong : Takhli	0.044	5.43	139.9
Mueang Nakhon Sawan : Krok Phra	0.020	12.25	22.9
Mueang Nakhon Sawan : Takhli	0.013	18.98	80.5
Krok Phra : Takhli	0.022	11.11	69.4
Coefficient of determination of isolation by distance between populations, $r^2 = 0.02^{ns}$			

[1] $F_{ST} > 0.25$ Great differentiation

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 15 Pairwise *F*-statistics at all loci within 5 populations of *Aedes aegypti* from Nakhon Ratchasima

Populations compared	F_{ST} [1]	Effective migration rate ($N_e m$)	Distance (Km)
Soeng Sang : Prathai	0.007	35.46	162.2
Soeng Sang : Kaeng Sanam Nang	0.013	18.98	198.2
Soeng Sang : Sikhio	0.028	8.68	110.0
Soeng Sang : Dan Khun Thot	0.074	3.13	142.9
Prathai : Kaeng Sanam Nang	0.010	24.75	53.5
Prathai : Sikhio	0.022	11.11	141.9
Prathai : Dan Khun Thot	0.078	2.96	147.2
Kaeng Sanam Nang : Sikhio	0.019	12.91	151.4
Kaeng Sanam Nang : Dan Khun Thot	0.053	4.47	110.4
Sikhio : Dan Khun Thot	0.034	7.10	42.5
Coefficient of determination of isolation by distance between populations, $r^2 = 0.01^{ns}$			

[1] $F_{ST} > 0.25$ Great differentiation

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Average genetic distances between all populations (Table 16) supported the lack of genetic differentiation on *Ae. aegypti* populations in Thailand. The Cavalli-Sforza & Edwards (1967) chord distance and modified Rogers distance (Wright, 1978) of any two among all 24 populations of *Ae. aegypti* varied from 0.027-0.113 and 0.048-0.117, respectively (Table 16). Cavalli-Sforza & Edwards (1967) chord distances were clustered by the unweighted pair group method (UPGMA) to produce the phenogram as shown in Figure 1. The Chon Buri populations were closely related to the Nakhon Ratchasima populations, whereas the Surat Thani populations were distantly related to the Nakhon Sawan populations. The Chon Buri and Nakhon Ratchasima populations as well as the Surat Thani and Nakhon Sawan populations were more distantly related, whereas the Bangkok populations were markedly different from all other populations (Figure 1).

The matrix of genetic distances based on Cavalli-Sforza & Edwards (1967) chord distance between 24 *Ae. aegypti* populations in Thailand showed that the Huai Khwang vs. Phanat Nikhom populations produced the largest genetic difference (0.177), whereas the least were seen between Mueang Surat Thani vs. Don Sak populations (0.048) as well as between Si Racha vs. Mueang Chon Buri populations (0.049) (Table 16).

In Bangkok, Huai Khwang vs. Rat Burana populations produced the largest genetic difference (0.146), whereas the least genetic difference was seen between Rat Burana vs. Lak Si populations (0.072). In Chon Buri, Si Racha vs. Phanat Nikhom showed the largest genetic difference (0.100), on the other hand, the least genetic difference was observed between Si Racha vs. Mueang Chon Buri populations (0.049). In Surat Thani, Chaiya vs. Ban Na Doem produced the largest genetic difference (0.086), on the contrary, the least genetic difference was seen between Mueang Surat Thani vs. Don Sak populations (0.048). In Nakhon Sawan, Mueang Nakhon Sawan vs. Mae Wong showed the largest genetic difference (0.121), conversely, the least genetic differences were observed between Mae Poen vs. Takhli as well as Krok Phra vs. Takhli populations (0.059). In Nakhon Ratchasima, Dan Khun Thot vs. Prathai produced the largest genetic difference (0.092), whereas the least genetic difference was seen between Kaeang Sanam Nang vs. Prathai populations (0.053) (Table 16).

Table 16 Matrix of genetic distance between 24 *Aedes aegypti* populations in Thailand

All populations	Bangkok population				
	Lak Si	Rat Burana	Lat Krabang	Huai Khwang	Bangkok Noi
1. Lak Si	*****	0.049	0.052	0.098	0.073
2. Rat Burana	0.072	*****	0.048	0.107	0.074
3. Lat Krabang	0.079	0.076	*****	0.108	0.081
4. Huai Khwang	0.127	0.146	0.150	*****	0.087
5. Bangkok Noi	0.117	0.129	0.141	0.117	*****
6. Bang Lamung	0.117	0.138	0.143	0.167	0.142
7. Phanat Nikhom	0.118	0.140	0.142	0.177	0.153
8. Mueang Chon Buri	0.145	0.153	0.157	0.160	0.169
9. Si Racha	0.149	0.160	0.164	0.160	0.171
10. Chaiya	0.124	0.121	0.136	0.163	0.156
11. Ban Na Doem	0.149	0.150	0.163	0.159	0.170
12. Khian Sa	0.137	0.134	0.140	0.157	0.161
13. Don Sak	0.123	0.123	0.134	0.159	0.162
14. Mueang Surat Thani	0.120	0.118	0.133	0.155	0.149
15. Mae Poen	0.149	0.171	0.166	0.150	0.150
16. Mae Wong	0.154	0.164	0.155	0.167	0.160
17. Mueang Nakhon Sawan	0.134	0.160	0.162	0.147	0.151
18. Krok Phra	0.119	0.142	0.141	0.145	0.144
19. Takhli	0.135	0.154	0.158	0.145	0.149
20. Soeng Sang	0.146	0.155	0.155	0.171	0.163
21. Prathai	0.142	0.146	0.154	0.169	0.166
22. Kaeng Sanam Nang	0.148	0.154	0.156	0.166	0.167
23. Sikhio	0.139	0.148	0.151	0.162	0.164
24. Dan Khun Thot	0.142	0.162	0.150	0.171	0.166

Table 16 (Continued)

All populations	Chon Buri population			
	Bang Lamung	Phanat Nikhom	Mueang Chon Buri	Si Racha
1. Lak Si	0.062	0.075	0.094	0.095
2. Rat Burana	0.085	0.083	0.096	0.108
3. Lat Krabang	0.091	0.093	0.107	0.115
4. Huai Khwang	0.121	0.133	0.115	0.108
5. Bangkok Noi	0.103	0.112	0.119	0.122
6. Bang Lamung	*****	0.045	0.070	0.071
7. Phanat Nikhom	0.050	*****	0.058	0.082
8. Mueang Chon Buri	0.092	0.084	*****	0.043
9. Si Racha	0.094	0.100	0.049	*****
10. Chaiya	0.107	0.113	0.110	0.108
11. Ban Na Doem	0.121	0.137	0.112	0.099
12. Khian Sa	0.125	0.131	0.103	0.097
13. Don Sak	0.111	0.119	0.103	0.099
14. Mueang Surat Thani	0.111	0.124	0.122	0.117
15. Mae Poen	0.119	0.118	0.112	0.118
16. Mae Wong	0.128	0.129	0.117	0.129
17. Mueang Nakhon Sawan	0.099	0.096	0.108	0.110
18. Krok Phra	0.087	0.077	0.085	0.101
19. Takhli	0.100	0.097	0.089	0.102
20. Soeng Sang	0.096	0.093	0.064	0.065
21. Prathai	0.090	0.094	0.054	0.058
22. Kaeng Sanam Nang	0.105	0.106	0.066	0.063
23. Sikhio	0.100	0.106	0.073	0.063
24. Dan Khun Thot	0.091	0.100	0.091	0.085

Table 16 (Continued)

All populations	Surat Thani population				
	Chaiya	Ban Na Doem	Khian Sa	Don Sak	Mueang Surat Thani
1. Lak Si	0.076	0.103	0.086	0.073	0.074
2. Rat Burana	0.077	0.112	0.088	0.080	0.087
3. Lat Krabang	0.086	0.127	0.096	0.090	0.092
4. Huai Khwang	0.114	0.113	0.110	0.104	0.106
5. Bangkok Noi	0.111	0.128	0.116	0.110	0.109
6. Bang Lamung	0.069	0.093	0.080	0.066	0.066
7. Phanat Nikhom	0.081	0.114	0.089	0.080	0.091
8. Mueang Chon Buri	0.078	0.094	0.069	0.068	0.086
9. Si Racha	0.081	0.080	0.066	0.066	0.078
10. Chaiya	*****	0.075	0.043	0.042	0.038
11. Ban Na Doem	0.086	*****	0.062	0.054	0.060
12. Khian Sa	0.084	0.083	*****	0.038	0.046
13. Don Sak	0.063	0.066	0.072	*****	0.031
14. Mueang Surat Thani	0.056	0.077	0.083	0.048	*****
15. Mae Poen	0.115	0.109	0.115	0.111	0.110
16. Mae Wong	0.110	0.111	0.105	0.113	0.109
17. Mueang Nakhon Sawan	0.099	0.116	0.125	0.108	0.110
18. Krok Phra	0.100	0.113	0.105	0.101	0.102
19. Takhli	0.097	0.109	0.113	0.100	0.098
20. Soeng Sang	0.119	0.114	0.094	0.108	0.120
21. Prathai	0.107	0.097	0.093	0.098	0.115
22. Kaeng Sanam Nang	0.097	0.097	0.087	0.096	0.111
23. Sikhio	0.115	0.108	0.095	0.097	0.111
24. Dan Khun Thot	0.112	0.128	0.112	0.113	0.117

Table 16 (Continued)

All populations	Nakhon Sawan population				
	Mae Poen	Mae Wong	Mueang Nakhon Sawan	Krok Phra	Takhli
1. Lak Si	0.108	0.105	0.088	0.076	0.093
2. Rat Burana	0.116	0.108	0.104	0.084	0.108
3. Lat Krabang	0.123	0.112	0.114	0.092	0.112
4. Huai Khwang	0.109	0.124	0.103	0.114	0.095
5. Bangkok Noi	0.124	0.128	0.114	0.108	0.116
6. Bang Lamung	0.098	0.097	0.075	0.057	0.074
7. Phanat Nikhom	0.097	0.106	0.074	0.042	0.083
8. Mueang Chon Buri	0.076	0.094	0.073	0.048	0.064
9. Si Racha	0.086	0.099	0.079	0.072	0.067
10. Chaiya	0.083	0.062	0.078	0.065	0.071
11. Ban Na Doem	0.087	0.079	0.089	0.096	0.079
12. Khian Sa	0.076	0.053	0.084	0.065	0.072
13. Don Sak	0.073	0.069	0.069	0.062	0.060
14. Mueang Surat Thani	0.080	0.063	0.079	0.071	0.064
15. Mae Poen	*****	0.077	0.054	0.061	0.042
16. Mae Wong	0.077	*****	0.099	0.078	0.079
17. Mueang Nakhon Sawan	0.075	0.121	*****	0.056	0.047
18. Krok Phra	0.064	0.094	0.068	*****	0.055
19. Takhli	0.059	0.089	0.069	0.059	*****
20. Soeng Sang	0.110	0.116	0.120	0.094	0.105
21. Prathai	0.115	0.113	0.122	0.099	0.107
22. Kaeng Sanam Nang	0.116	0.113	0.116	0.103	0.109
23. Sikhio	0.122	0.123	0.125	0.107	0.109
24. Dan Khun Thot	0.132	0.128	0.126	0.112	0.109

Table 16 (Continued)

All populations	Nakhon Ratchasima population				
	Soeng Sang	Prathai	Kaeng Sanam Nang	Sekhio	Dan Khun Thot
1. Lak Si	0.086	0.087	0.091	0.085	0.086
2. Rat Burana	0.091	0.091	0.098	0.099	0.111
3. Lat Krabang	0.104	0.107	0.108	0.110	0.104
4. Huai Khwang	0.123	0.126	0.116	0.113	0.120
5. Bangkok Noi	0.114	0.116	0.118	0.116	0.120
6. Bang Lamung	0.060	0.062	0.069	0.059	0.056
7. Phanat Nikhom	0.052	0.059	0.073	0.082	0.092
8. Mueang Chon Buri	0.032	0.038	0.036	0.063	0.090
9. Si Racha	0.053	0.049	0.029	0.046	0.072
10. Chaiya	0.080	0.081	0.074	0.079	0.079
11. Ban Na Doem	0.091	0.086	0.082	0.070	0.097
12. Khian Sa	0.070	0.071	0.058	0.070	0.085
13. Don Sak	0.066	0.068	0.063	0.060	0.075
14. Mueang Surat Thani	0.082	0.085	0.077	0.068	0.067
15. Mae Poen	0.080	0.090	0.086	0.096	0.112
16. Mae Wong	0.094	0.098	0.090	0.095	0.102
17. Mueang Nakhon Sawan	0.077	0.086	0.084	0.089	0.101
18. Krok Phra	0.048	0.061	0.064	0.078	0.089
19. Takhli	0.070	0.080	0.073	0.072	0.082
20. Soeng Sang	*****	0.027	0.040	0.054	0.084
21. Prathai	0.054	*****	0.034	0.048	0.085
22. Kaeng Sanam Nang	0.063	0.053	*****	0.047	0.075
23. Sikhio	0.054	0.061	0.073	*****	0.056
24. Dan Khun Thot	0.083	0.092	0.083	0.076	*****

Values above the diagonal correspond modified Rogers distance (Wright, 1978) and values below the diagonal correspond Cavalli-Sforza & Edwards (1967) chord distance

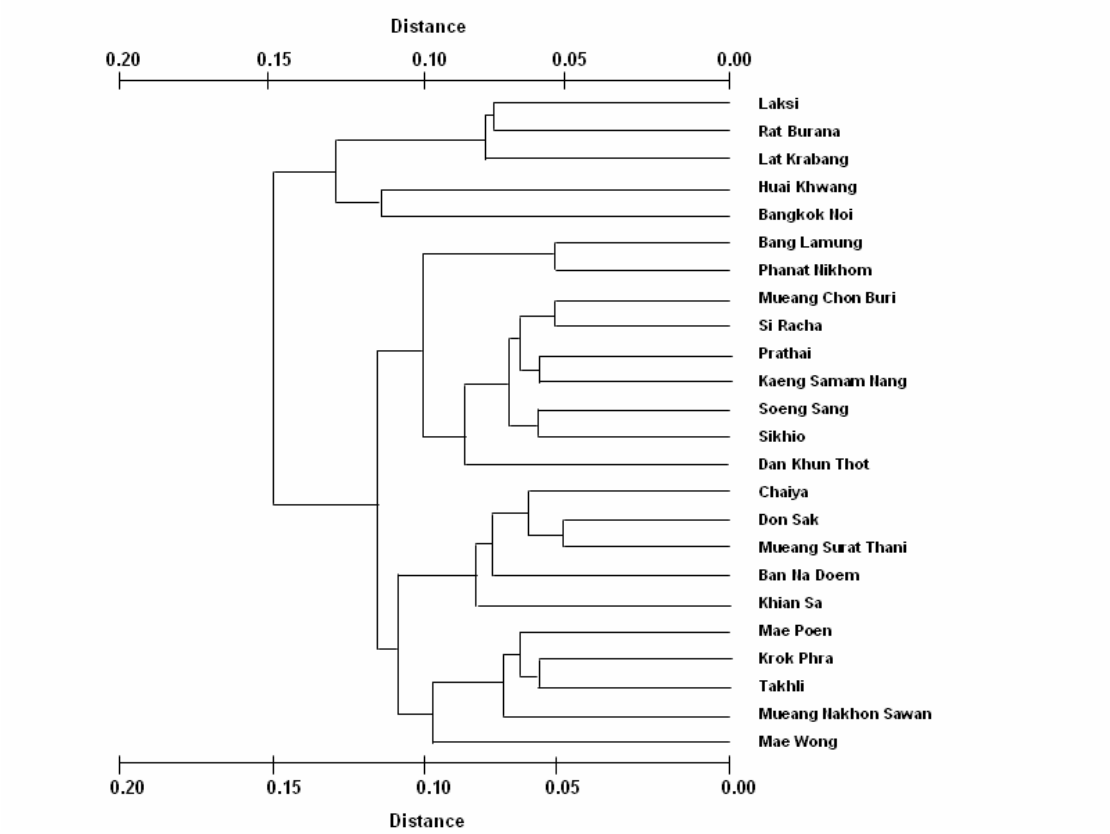

Figure 1 Unweighted pair group method averaging phenogram from Cavalli-Sforza & Edwards (1967) chord distance matrix among 24 populations of *Aedes aegypti* from 5 provinces in Thailand (cophenetic correlation = 0.928)

When among populations from each province were considered, the greatest percent polymorphism was found in Bangkok (29.0%), with the highest number of alleles per locus (2.0) and high level of heterozygosity (Ho = 0.081) (Table 17). The lowest percent polymorphism was observed from Nakhon Ratchasima (16.1%), with lowest allele per locus (1.5) and level of heterozygosity (0.055). The highest heterozygosity was found in Nakhon Sawan (0.086), whereas the lowest heterozygosity was found in Chon Buri and Nakhon Ratchasima (0.055) (Table17). All observed heterozygosities from among provinces were not significantly different from all Hardy-Weinberg expected heterozygosity ($t_{0.025} = 0.186^{ns}$, df = 4, $P > 0.05$).

In five populations in Thailand, mean value of F_{ST} calculated from all polymorphic loci showed negligible differentiation (0.050). The moderate F_{ST} values were found at *AKS-2* (0.156) and *FUM-1* (0.249). All other loci demonstrated only small F_{ST} values or negligible genetic differences (Table 18). Mean value of F_{IS} calculated from all polymorphic loci was 0.008. High positive F_{IS} value was obtained from *FUM-1* (0.790), whereas the greatest negative value was obtained from *MDH-2* (-0.250). The N_em of five populations in Thailand was 4.75 (Table 18).

Paired analyses among five populations of each province showed negligible differentiations in all combinations (Table 19). The N_em values between populations of each province in Thailand were compared and found generally high among pairwise comparison (4.96-27.53). The greatest N_em value reflecting the highest migration rate was observed between Chon Buri and Nakhon Ratchasima (27.53), whereas the smallest rate was observed between Bangkok and Nakhon Ratchasima (4.96) (Table 19). The analysis of isolation by distance indicated that there were no correlations ($P > 0.05$) between genetic and geographic distance among populations (Tables 19).

Table 17 Genetic variability at 31 loci of pooled populations of *Aedes aegypti* from 5 provinces in Thailand

Population	Average alleles per locus	% polymorphic loci [1]	Mean heterozygosity	
			H_{obs}	H_{exp} [2]
Bangkok	2.0 ± 0.3	29.0	0.081 ± 0.026	0.098 ± 0.031
Chon Buri	1.5 ± 0.2	19.4	0.055 ± 0.022	0.056 ± 0.023
Surat Thani	1.7 ± 0.2	22.6	0.082 ± 0.028	0.078 ± 0.027
Nakhon Sawan	1.6 ± 0.2	22.6	0.086 ± 0.030	0.078 ± 0.027
Nakhon Ratchasima	1.5 ± 0.2	16.1	0.055 ± 0.022	0.053 ± 0.022
			$t_{0.025} = 0.186^{ns}$	

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

[2] Unbiased estimate and standard error (Nei, 1978)

Table 18 *F*-statistic analysis of polymorphic loci in 5 populations of *Aedes aegypti* in Thailand

Locus	F_{IS} [1]	F_{ST} [2]
ACO-1	-0.003	0.002
ACO-2	-0.123	0.070
AKS-2	-0.237	0.156
AKS-3	-0.015	0.011
ARK-2	-0.037	0.026
ARK-3	0.004	0.018
ATA-1	-0.069	0.026
ATA-2	-0.239	0.090
FUM-1	0.790	0.249
GPD-1	-0.008	0.004
HAD-1	-0.043	0.026
HK-2	-0.003	0.003
IDH-1	0.202	0.024
MDH-1	0.055	0.027
MDH-2	-0.250	0.052
ME-1	-0.099	0.043
PGM-1	0.082	0.016
PK-2	-0.008	0.005
PK-3	-0.003	0.002
Mean	0.008	0.050
		$N_e m = 4.75$

[1] F_{IS} = Inbreeding coefficient

[2] $F_{ST} > 0.25$ Great genetic differentiation among the subpopulations

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

Table 19 Pairwise *F*-statistics at all loci among 5 populations of *Aedes aegypti* from Thailand

Populations compared	F_{ST} [1]	Effective migration rate ($N_e m$)	Distance (Km)
Bangkok : Chon Buri	0.042	5.70	81
Bangkok : Surat Thani	0.037	6.51	644
Bangkok : Nakhon Sawan	0.043	5.56	240
Bangkok : Nakhon Ratchasima	0.048	4.96	259
Chon Buri : Surat Thani	0.029	8.37	725
Chon Buri : Nakhon Sawan	0.023	10.62	321
Chon Buri : Nakhon Ratchasima	0.009	27.53	280
Surat Thani : Nakhon Sawan	0.017	14.46	884
Surat Thani : Nakhon Ratchasima	0.025	9.75	903
Nakhon Sawan : Nakhon Ratchasima	0.031	7.81	327
Coefficient of determination of isolation by distance between populations, $r^2 = 0.17^{ns}$			

[1] $F_{ST} > 0.25$ Great differentiation

$0.25 > F_{ST} > 0.15$ Moderately great differentiation

$0.15 > F_{ST} > 0.05$ Small differentiation

$F_{ST} \leq 0.05$ Negligible differentiation

When the genetic distances were calculated among populations from each province, the modified Rogers distances (Wright, 1978) of any two varied from 0.031-0.089 (Table 20). The modified Rogers distances were clustered by the unweighted pair group method (UPGMA) to produce the phenogram as shown in Figure 2. The phenogram showed similar branching patterns as in Figure 1. The Chon Buri population was closely related to the Nakhon Ratchasima population, whereas the Surat Thani population was distantly related to the Nakhon Sawan population. The Chon Buri and Nakhon Ratchasima populations as well as the Surat Thani and Nakhon Sawan populations were more distantly related, whereas the Bangkok population was markedly different from all other populations (Figure 2).

Table 20 Matrix of genetic distance between 5 *Aedes aegypti* populations from 5 provinces in Thailand

Population	Population				
	Bangkok	Chon Buri	Surat Thani	Nakhon Sawan	Nakhon Ratchasima
1. Bangkok	*****	0.082	0.082	0.089	0.087
2. Chon Buri	0.131	*****	0.063	0.056	0.031
3. Surat Thani	0.120	0.095	*****	0.051	0.058
4. Nakhon Sawan	0.125	0.085	0.090	*****	0.064
5. Nakhon Ratchasima	0.136	0.059	0.085	0.098	*****

Values above the diagonal correspond modified Rogers distance (Wright, 1978) and values below the diagonal correspond Cavalli-Sforza & Edwards (1967) chord distance

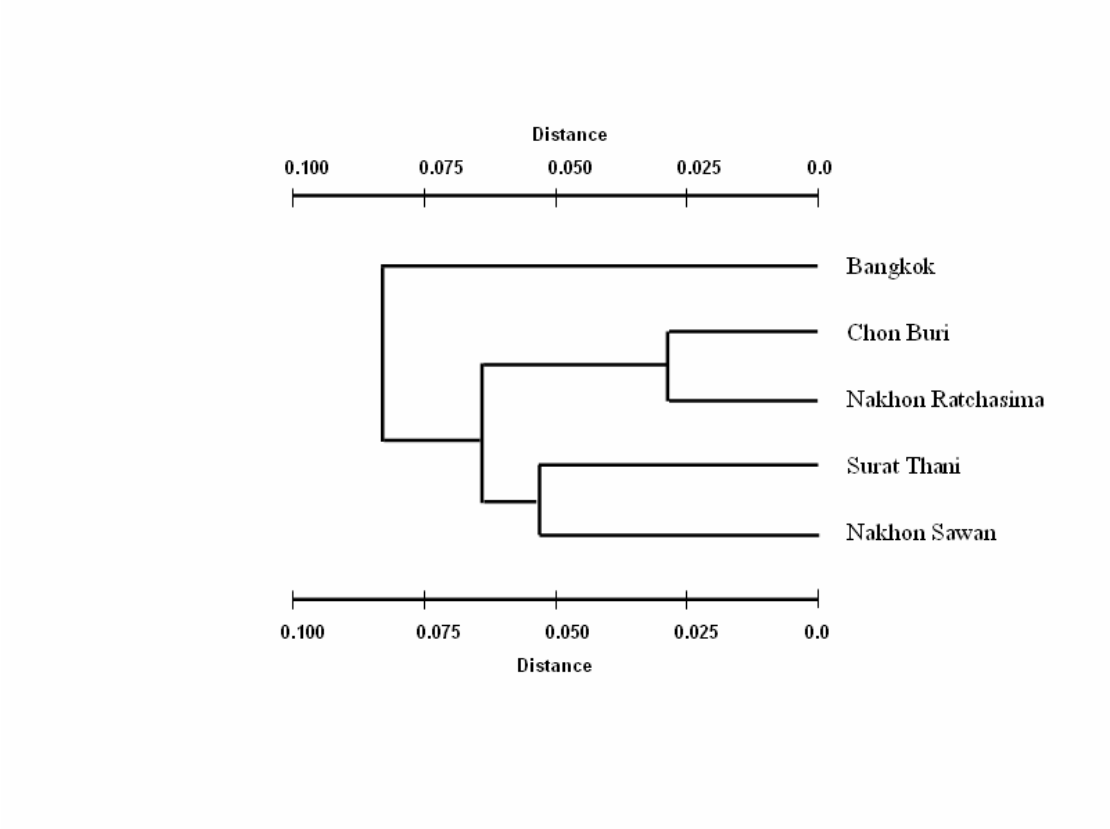

Figure 2 Unweighted pair group method averaging phenogram from modified Rogers distance (Wright, 1978) matrix among pooled populations of *Aedes aegypti* from 5 provinces in Thailand (cophenetic correlation = 0.986)

DISCUSSION

Aedes aegypti, one of the most important vectors of dengue virus, is present throughout Thailand. Progressive urbanization and other human activities have decreased natural *Aedes* larval habitats in most area of Thailand in favor to artificial ones, which have greatly assisted the expansion of *Ae. aegypti* and displacement of native species (Pant *et al*., 1973). The use of insecticides for *Ae. aegypti* vector control has had a major impact on the genetic structure and gene flow of exposed mosquito populations in many places like French Polynesia and Thailand (Wallis *et al.*, 1984; Failloux *et al*., 1995; Lerdthusnee and Chareonviriyaphap, 1999; Paupy *et al*., 2000).

This study was designed to examine the patterns of gene flow in *Ae. aegypti* at an extensive geographic dengue outbreak area of Thailand by performing the isozyme analysis of gene flow within and among the five leading dengue outbreak provinces. Gene flow and effective migration rates among five populations within 81-903 kilometers were obtained from comparative gene frequencies. In brief, the design of this study facilitated the analysis of gene flow in two different levels, among populations within the provinces and among the provinces.

Five important results were demonstrated from this study. The first finding was that *Ae. aegypti* in Thailand had extensive gene flow among populations of 2.5 reproductive migrants per generation. The second finding related to higher rate of gene flow could be observed between populations within the province. The third finding was the low genetic differentiation occurred among *Ae. aegypti* populations in Thailand. The fourth finding was no isolation between *Ae. aegypti* populations by distance. The fifth finding was that all the 24 *Ae. aegypti* populations studied were considered to be conspecific populations with some minor genetic variation. The understanding of distribution movement or dispersal of mosquito populations could facilitate the control program.

In this study; the mean number of alleles per locus for all 31 loci of all 24 populations studied were between 1.2 and 1.7 which were lower than the 2.1 and 2.5 for all 20 loci of *Ae. aegypti* along the coastal area of Gulf of Thailand; covering 6 following provinces, Trat, Samut Prakan, Bangkok, Chumphon, Surat Thani and Songkhla (Netthanomsak, 2004). The Bangkok population showed the greatest genetic polymorphism. The Huai Khwang (central Bangkok) population demonstrated the greatest percentage of polymorphism (35.5%) compared with other locations in Thailand. However, the percent polymorphism from all collections were lower (12.9-35.5%) than those reported elsewhere, 37.5-50.0% by Dinardo-Miranda and Contel (1996), 44.4-55.6% by Costa Fraga *et al*. (2003) in Brazil, 59% by Tabachnick and Powell (1976) as well as 40-50% by Netthanomsak (2004). In 2000, Sousa *et al*. (2000) found wide ranges of polymorphic loci (27.3-63.6%) in *Ae. aegypti* populations from Argentina. However, the percent polymorphic loci of all populations in Thailand were similar to those reported from southern Thailand (24.2-36.4%) (Chareonviriyaphap and Lerdthusnee, 2002). Instead, *Ae. aegypti* populations from Houston (USA) had rather higher percent polymorphic loci of 30 to 40% (Harrington *et al*., 1984). Greater level of polymorphism found in Bangkok populations, especially from Huai Khwang and Bangkok Noi districts, could be the result of multiple introductions of different subpopulation samples of the gene pool of the species. The Chon Buri populations, especially those of Phanat Nikhom and Mueang Chon Buri as well as the Nakhon Ratchasima populations, especially those of Prathai and Sikhio had relatively lower polymorphic loci (12.9%). Furthermore, both Chon Buri and Nakhon Ratchasima populations also had the lowest mean heterozygosity (H_{exp} = 0.055) from pooled populations as well as the lowest F_{ST} (0.009) from pairwise *F*-statistics between provinces, however, they had the highest effective migration rate (27.53) between them. These two populations showed evidence indicating the highest gene flow of *Ae. aegypti* in Thailand.

The highest mean heterozygosity values of Huai Kwang population indicated that this population was the most variable among all 24 populations. It was also found that the average of H_{exp} of populations in Bangkok was the highest (0.098), whereas those from others four provinces were lower, ranging from 0.053 to 0.078. The Bangkok populations demonstrated greatest genetic polymorphism and contained many unique alleles (*ATA-1* allele141; *FUM-1* allele 27; *HK-2* allele 87; *IDH-1* allele 73, 92 and 144; *MDH-1* allele 142 and *PGM-1* allele 185). Indeed,

the *Ae. aegypti* populations in Bangkok could be considered essentially panmictic based on the results reported here. The Bangkok area is a large, heavily populated residential and commercial area. There are numerous physical means of transport from public and private sectors in the metropolitan area. Land and boat traffic are believed to have played a significant role in the distribution and expansion of *Ae. aegypti* in Thailand (Pant *et al.*, 1973). In addition, the active movement of mosquitoes in such metropolitan area may play a significant role in population expansion. Opportunities for introduction and re-introduction of this species in and around busy urban areas have continued to increase with expansion of transport networks. The relative ease of *Ae. aegypti* dispersal is a product of its bionomics, especially in the readily transportable eggs and larval stages (Southwood *et al.*, 1972)

Results indicated that vector control programs in Bangkok have had little direct impact on the genetic structure of these mosquito populations as indicated by a uniformity of genetic variation and the absence of any indication of small population size with resulting genetic drift effects. The rapid re-establishment by migrant mosquitoes from nearby areas or rapid adaptation to new areas likely plays a significant role in maintaining the large panmictic population. These results are in agreement with the genetic differentiation seen among *Ae. aegypti* populations in the United States, French Polynesia and Colombia (Tabachnick, 1982; Failloux *et al.*, 1995; Ocampo and Wesson, 2004)

Expected mean heterozygosity from all populations in Thailand ranged from 0.037 to 0.123 which was lower than that observed from *Ae. aegypti* along the coastal area of Gulf of Thailand (0.224-0.248) (Netthanomsak, 2004) as well as that from Argentina (0.090-0.161) (Sousa *et al*., 2000). The average expected heterozygosity (H_{exp}) from all populations in this study (H_{exp} = 0.071) was comparatively lower than earlier reports. Tabachnick and Powell (1978) reported a mean H_{exp} of 0.152 among 23 loci from worldwide populations of *Ae. aegypti* collections as well as Tabachnick *et al*. (1979) who reported the H_{exp} of 0.141 for domestic and 0.163 for East African wild populations. Also, Tabachnick (1982) detected H_{exp} = 0.118 in the Caribbean, however, Harrington *et al.* (1984) detected lower H_{exp} (0.097) in Houston populations. Mean H_{exp} of 0.163 was detected from 11 allozyme loci in Puerto Rico (Wallis *et al.*, 1984) and 0.156 among 18 loci in Manaus, Brazil (Costa Fraga *et al.*, 2003) as well as 0.192 from Brasilia

(Brazil), whereas in São Paulo populations it varied from 0.48 to 0.53 (Dinardo-Miranda and Contel, 1996). In southern Thailand, the H_{exp} of 0.121 was detected by Chareonviriyaphap and Lerdthusnee (2002). The average expected heterozygosity (H_{exp}) from all populations in this study (H_{exp} = 0.071) was much lower than those among 57 RAPD loci from Puerto Rico (H_{exp} = 0.354) (Apostol *et al.*, 1994) and 60 RAPD loci from northern coast of Mexico (H_{exp} = 0.339) (Gorrochotegui-Escalante, 2000). Average heterozygosity of *Ae. aegypti* populations from this study, however, was approximately similar to those from Asian populations reported by Tabachnick (1991) (H_{exp} = 0.090). Low genetic variability in *Ae. aegypti* populations in Thailand could possibly cause from a vigor vector control during the collection period and this may result in loss of genetic variation. Consequently, the decrease of the genetic variability from the result could be lead to the low numbers of allelic polymorphic loci. In addition, a small sample size and genetic drift from vigorous vector control could also be accounted for low heterozygosity.

The mean Wright's coefficient F_{ST} value, used to measure the amount of genetic differentiation among subpopulations, demonstrated small differentiation (0.091) among all 24 subpopulations in Thailand which was slightly higher than the F_{ST} value (0.065) obtained from Argentina, indicating low levels of genetic differentiation among populations from different localties. Nevertheless, Dinardo-Miranda and Contel (1996) found lower F_{ST} values (0.018) in São Paulo populations. As observed in Thailand, genetic differentiation based on F_{ST} estimates tended to be higher (0.091) when all populations over Thailand were compared (larger area), although smaller F_{ST} estimates were observed when the populations within province were compared (0.026-0.053) (smaller area), this may attribute to a greater heterogeneity of habitats. The findings of small to negligible F_{ST} values for all populations in Thailand indicated the absence of a species complex or the speciation process of *Ae. aegypti* in Thailand. Higher F_{ST} value (0.150) was reported from populations of *Ae. aegypti* from islands of the French Polynesia (Failloux *et al*., 1995) as well as from along the coastal area of Gulf of Thailand (F_{ST} = 0.108) (Netthanomsak, 2004). The F_{ST} values of Bangkok, Chon Buri and Nakhon Ratchasima (F_{ST} = 0.052, 0.053 and 0.051, respectively) were similar to the result of Mousson *et al*. (2002) who studied *Ae. aegypti* populations in Chiang Mai, Thailand (F_{ST} = 0.053), however, the F_{ST} values of Surat Thani and Nakhon Sawan populations were lower (F_{ST} = 0.026 and 0.046, respectively). The F_{IS} values of Bangkok and Nakhon Sawan populations (0.115 and -0.156, respectively)

showed some degree of non random mating which Bangkok tended to be rather heterozygote deficiency although Nakhon Sawan to be rather heterozygote excess. However, the F_{ST} value of Bangkok populations showed small differentiation (0.052), whereas Nakhon Sawan populations showed only negligible differentiation (0.046). The positive F_{IS} value of Bangkok was contrast to those of the other provinces which showed negative F_{IS} values. This could possibly be from a vigor vector control in Bangkok during the collection period. It was concluded that all 24 populations remained the same species with some minor variations and local effects of vector control which increased F_{ST} value by bottleneck effect would quickly be broken down by gene flow.

According to Eanes and Koehn (1978), population genetic structure is a consequence from the coupling patterns and the gene flow magnitude between populations and this is expressed by the Hardy-Weinberg equilibrium deviation and by the amount of differentiation or allelic frequency between the populations. They further considered that the high gene flow rates among sub-populations and the tendency for intrapopulational random coupling may lead to a genetic structure decrease. Given this information, it is possible to admit that the F_{ST} values detected in this study may indicate the onset of a gene flow increment process as well as the random couplings occurrence since the F_{IS} value was relatively low. The low level of genetic differentiation found among all *Ae. aegypti* populations studied revealed that the *Ae. aegypti* populations in Thailand is poorly structured. The observed low genetic differentiation may reflect important less differences of vector competence, parasite susceptibility or insecticide resistance.

Gene flow estimated by the N_em estimate appeared lower among all 24 populations (2.50 reproductive migrants per generation). When populations within province were considered, the N_em estimates were 4.47 to 9.37, indicating more extensive gene flow and rapid dispersion within them, although the flight range of *Ae. aegypti* is less than 1,000 m (Hauserman *et al*., 1971; Edman *et al*., 1998). The N_em values for Bangkok, Chon Buri and Nakhon Ratchasima (4.54, 4.47 and 4.65, respectively) were similar to the report of Chareonviriyaphap and Lerdthusnee, (2002) which indicated that the populations from southern Thailand had $N_em = 4.3$ and they also reported that the N_em value was higher ($N_em = 6.3$) within island populations. Instead, the N_em values of Surat Thani population representing southern Thailand from this study showed higher

value (9.37). Populations of this species spaning in each province in Thailand also seem to be genetically very homogenous (N_em ranging from 4.47 to 9.37 individuals per generation). From paired analyses, it was interestingly found that the highest N_em between populations was observed between Chon Buri vs. Nakhon Ratchasima (27.53) which was rather higher than that of Bangkok vs. Chumphon (22.48) (Netthanomsak, 2004). This may indicate rapid and regular dispersal and intermixing between these populations. Furthermore, the evidences of high rate of gene flow occurred among populations within each province can be seen between Laksi vs. Rat Burana (17.16) and Rat Burana vs. Lat Krabang (17.16) in Bangkok, between Mueang Chon Buri vs. Si Racha (16.42) in Chon Buri, between Don Sak vs. Mueang Surat Thani (35.46) in Surat Thani, between Mae Poen vs. Takhli (22.48) in Nakhon Sawan, as well as between Soeng Sang vs. Prathai (35.46) in Nakhon Ratchasima. As a result, the *Ae. aegypti* populations in these areas seemed to be the most vulnerable for the adaptabilities to insecticide resistance as well as to habour and transmit dengue viruses. The high effective migration rate occurred from this study clearly indicated evidence of high gene flow in *Ae. aegypti* in Thailand. Highly commercial and business transportation between Nakhon Ratchasima and the eastern seaboard in Chon Buri as well as high transportation for traveling between Mueang Surat Thani and Don Sak habour to Koh Samui could be the cause of high gene flow rate between them. The ability of *Ae. aegypti* to migrate with humans in cars, boats and aircrafts is probably sufficient to disrupt some of the geographic patterns that may have been present immediately after the colonization (Wallis *et al*., 1984). However, Ayres *et al*. (2003) discussed that it must be cautioned that these rates of gene flow were inferred on the basis of differentiation rates that assumed the island-model population structure and migration-drift equilibrium, a situation that is probably unrealistic for many mosquito populations (Donelly *et al*., 1999; Fonseca *et al*., 2001). However, it has proven to be fairly robust to violations of those assumptions, covarying positively with direct estimates of migration (Neigel, 1997).

The results indicated that the populations within Bangkok, Chon Buri, Surat Thani, Nakhon Sawan and Nakhon Ratchasima were panmictic (F_{ST} = 0.052, 0.053, 0.026, 0.046 and 0.051, respectively and N_em = 4.54, 4.47, 9.37, 5.18 and 4.65, respectively), with heterogeneticity likely influenced by frequent intermixing between vector populations in urban environments with high human population densities as the evidence from the study of Tien *et al*. (1999), Paupy *et al*.

(2000) and Vazeille-Falcoz *et al*. (2001). With high levels of commerce and communication in and around the area of all provinces, frequent transfer opportunities for eggs and larvae in artificial containers in and around the area seemed to be the most likely mode of transfer.

The analysis showed no significant differences of isolation by distance between all populations in Thailand ($P > 0.05$). This result was in agreement to the result of Mousson *et* al. (2002) who studied the relationship between genetic exchanges and geographical distances of *Ae. aegypti* populations in Chiang Mai, Thailand. Likewise, this result was also in agreement to the analysis of *Ae. aegypti* populations in Thailand using NADH dehydrogenase subunit 4 mitochondrial DNA gene (Bosio *et al*., 2005). This result was also consistent with recent range expansion of *Ae. aegypti* in Thailand because under Wright's island model sufficient time has not passed for populations to approach equilibrium where isolation by distance could be detected (Slatkin, 1993). Bosio *et al*. (2005) had some explanations for this observation. First, passive movement could transport mosquitoes for long distances, increasing genetic similarity between geographically distant populations. Second, genetic drift, could cause populations to become more similar or more distinct regardless of their geographic relationship. Third, vector control efforts could impose severe genetic bottlenecks on local populations, profoundly affecting their genetic similarity with other populations.

The genetic distances obtained from Cavalli-Sforza & Edwards (1967) chord distance and modified Rogers distance (Wright, 1978) among 24 populations varied from 0.048-0.177 and 0.027-0.113, respectively. These values were relatively low, indicating low genetic differentiation among all populations. This finding confirmed a low genetic differentiation of *Ae. aegypti* populations in Thailand as well as supported the observation of no isolation by distance.

Phenograms produced by Cavalli-Sforza & Edwards (1967) chord distance and modified Rogers distance (Wright, 1978) showed minor different branching patterns. The matrix of genetic distances based on Cavalli-Sforza & Edwards (1967) chord distance between 24 *Ae. aegypti* populations in Thailand showed that the Huai Khwang vs. Phanat Nikhom populations produced the largest genetic difference (0.177), however, the least genetic differences were seen between Mueang Surat Thani vs. Don Sak populations (0.048) as well as between Si Racha vs. Mueang

Chon Buri populations (0.049). In Bangkok, Huai Khwang vs. Rat Burana populations produced the largest genetic difference (0.146), but the least genetic difference was seen between Rat Burana vs. Lak Si populations (0.072). The Huai Khwang population produced the largest genetic difference because this area is considered to be the most highly urbanized area where *Ae. aegypti* was highly structured, a finding that correlated well with increased human population densities (Paupy *et al*., 2000). It was suspected that crowded environments may have contributed to the minor genetic differences seen between populations. In Chon Buri, Si Racha vs. Phanat Nikhom produced the largest genetic difference (0.100), however, the least genetic difference was seen between Si Racha vs. Mueang Chon Buri populations (0.049). In Surat Thani, Chaiya vs. Ban Na Doem produced the largest genetic difference (0.086), but the least genetic difference was seen between Mueang Surat Thani vs. Don Sak populations (0.048). In Nakhon Sawan, Mueang Nakhon Sawan vs. Mae Wong produced the largest genetic difference (0.121), on the contrary, the least genetic differences were seen between Mae Poen vs. Takhli as well as Krok Phra vs. Takhli populations (0.059). In Nakhon Ratchasima, Dan Khun Thot vs. Prathai produced the largest genetic difference (0.092), on the other hand, the least genetic difference was seen between Kaeang Sanam Nang vs. Prathai populations (0.053).

From phenogram (Figures 1 and 2), *Ae. aegypti* in Thailand could be grouped into 3 genetically related clusters, one closed cluster from Chon Buri and Nakhon Ratchasima. Second group was seen in Surat Thani and Nakhon Sawan. The last group was Bangkok which was markedly different from all others. Genetic differentiations observed between Bangkok and the other provinces were attributed to the differences in *FUM-1* and *HK-2* loci. Bangkok populations contained many unique alleles (*ATA-1* allele 141; *FUM-1* allele 27; *HK-2* allele 87; *IDH-1* allele 73, 92 and 144; *MDH-1* allele 142 and *PGM-1* allele 185), which were not found in other populations in Thailand. Noticeably, the Chon Buri populations had two separate groups. The Mueang Chon Buri and Si Racha populations formed a tight cluster being closely related with Nakhon Ratchasima populations more than those with Bang Lamung and Phanat Nikhom populations. Therefore, the differences in the *Ae. aegypti* larval habitats may translate into the intraspecific differences.

Dispersal of female *Ae. aegypti* is influenced by location and availability of oviposition sites (Reiter *et al*., 1995; Apostol *et al*., 1996), with dispersal rates and distance considered inversely correlated with the abundance of oviposition sites (Edman *et al*., 1998). In southern Vietnam, Huber *et al*. (2003) concluded that *Ae. aegypti* in densely populated localities favored the persistence of genetically isolated populations. In Mexico, *Ae. aegypti* was genetically isolated as a function of geographical distance (Garcia-Franco *et al*., 2002). In Colombia, spatial and temporal genetic variation between populations was observed, although there was also evidence of considerable gene flow between these urban populations (Ocampo and Wesson, 2004).

Mosquito population dispersal via human commerce is an important part of natural gene flow and influences the genetic structure of mosquito populations. This information is epidemiologically important in understanding vector biology and potential dengue transmission. The understanding of the genetic structure and gene flow of different vector populations and rate of change over time may help identify areas at greater risk for dengue outbreak. Moreover, the temporal and spatial similarities or differences with respect to expression of enzymes that confer resistance to insecticides or vector capacity for pathogens may be influenced by the patterns of gene flow between vector populations. For example, a correlation between genetic distances and variation in vector competence phenotypes in *Ae. aegypti* has been suggested (Bosio *et al*., 2000; Garcia-Franco *et al*., 2002; Ocampo and Wesson, 2004). Areas showing increased rates of gene flow and population dispersal could also share the same characteristics that influence dengue virus susceptibility and insecticide resistance patterns. Thus, moderate to high level of resistance to various insecticides in *Ae. aegypti* populations in Thailand has recently been detected (Chareonviriyaphap *et al.*, 1999; Somboon *et al*., 2003).

Insecticides were known to affect the genetic structure of exposed mosquito populations. In each treated site, a decrease in mosquito genetic variability was found after insecticidal sprays (Lerdthusnee and Chareonviriyaphap, 1999). Currently, vector control is the only available method for reducing the incidence of dengue fever. Mosquito populations can be limited by insecticides used against larvae and/or adults. Extended use of insecticides for dengue control may enhance the resistance to insecticides in mosquito populations (Pasteur and Raymond, 1996).

Furthermore, rebuilding from selected resistant individuals gives rise to a population genetically different from the original one. Therefore, knowledge about geographical genetic variation in *Ae. aegypti* populations regarding dengue transmission would be informative (Costa Fraga *et al*., 2003).

Bosio *et al*. (2005) concluded that, in the suburban/rural setting, where populations are more isolated, insecticide use could rapidly select for insecticide resistance if such variation exists in the population. Genetic bottlenecks of this kind and/or genetic drift could radically change the phenotype of the population for other important traits such as vector competence. Because gene flow from these populations appears limited, however, genotypes will spread more slowly to surroundings populations. In the urban setting, *Ae. aegypti* populations would be expected to recover quickly from insecticide treatments because they contain larger areas of panmixia. Insecticide resistance may develop more slowly because immigration of susceptible individuals from outside the treated area is not impeded. The role of gene flow in impeding movement of insecticide resistance alleles was explored in *Culex pipiens* in southern France (Lenormand *et al*., 1998). Under selection-migration equilibrium, population gene flow could be considered as a constraining force reducing the potential for local adaptation at a local scale (Lenormand *et al*., 1998). But on a wider geographical scale, gene flow could be responsible for the spread of resistance alleles by passive migration. However, depending on the frequency of insecticide application, insecticide resistance may spread by gene flow throughout the urban area from the treated population (Pasteur *et al*., 1995). If insecticides with residual activity are used, they could continue to impact gene flow for as long as they persist. This would affect migration of susceptible individuals into the treated area, but would not impede dispersal of resistant individuals out of the treated area (Bosio *et al*., 2005). Resistance genes are associated with fitness costs in populations; larval developmental time, fecundity, ability to blood feed, susceptibility to parasites or predators; can be modified by the presence of resistance genes (Chevillon *et al*., 1997). Furthermore, resistance in mosquito vectors is assumed to affect disease transmission and control (McCarroll *et al*., 2000). In most provinces of Thailand, high levels of resistance to several insecticides, including malathion, fenitrothion, temephos and some pyrethroids, have been detected in *Ae. aegypti* (Communicable Disease Control, 1995-2004; Chareonviriyaphap *et al*., 1999). Resistance will be the subject of further investigation to detect

the natural dispersion of genes responsible for insecticide resistance. Since the population genetic structure and gene flow of *Ae. aegypti* vary from one region to another in Thailand, understanding the local population dynamics and genetic exchanges between vector populations will provide estimates of their abilities to harbour and transmit dengue viruses as well as their insecticide resistance patterns. This knowledge can greatly assist predictive modeling and timely planning of surveillance as well as appropriate control strategies.

CONCLUSION

Enzyme patterns of 31 loci in twenty four populations of *Ae. aegypti* collected from the top five dengue epidemic provinces in Thailand were examined. Small genetic differentiation was obtained when all populations were compared (F_{ST} = 0.091). A population from Bangkok (Huai Khwang) showed the greatest percent polymorphic loci (35.5%), whereas two populations from Chon Buri (Phanat Nikhom and Mueang Chon Buri) and two populations from Nakhon Ratchasima (Prathai and Sikhio) demonstrated the lowest percent polymorphic loci (12.9%). Five populations from Bangkok contained several unique alleles.

Results revealed that level of gene flow rate between/among *Ae. aegypti* populations varied, depending upon the locations, epidemiological background and other related factors. Passive transportation may account for one of the most critical factors as previously described. Gene flow was 2.5 reproductive migrants per generation when all populations were compared. Among the provincial populations, the greatest gene flow was observed between Chon Buri and Nakhon Ratchasima (N_em = 27.53). Commercial and business transportations may help to increase the movement of *Ae. aegypti* mosquitoes between these two populations.

Moreover, the gene flow between populations of each district of Bangkok, Chon Buri, Surat Thani, Nakhon Sawan and Nakhon Ratchasima ranged from 4.30 to 17.61, 4.14 to 16.42, 6.89 to 35.46, 3.92 to 22.48 and 2.96 to 35.46, respectively. No fixed genetic differences were detected. Caution must be taken into account when vector control measures are implemented in the areas of high gene flow rate because high immigrants between these vector populations have occurred simultaneously. This study indicated that there was no isolation by distance among all *Ae. aegypti* populations.

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APPENDIX

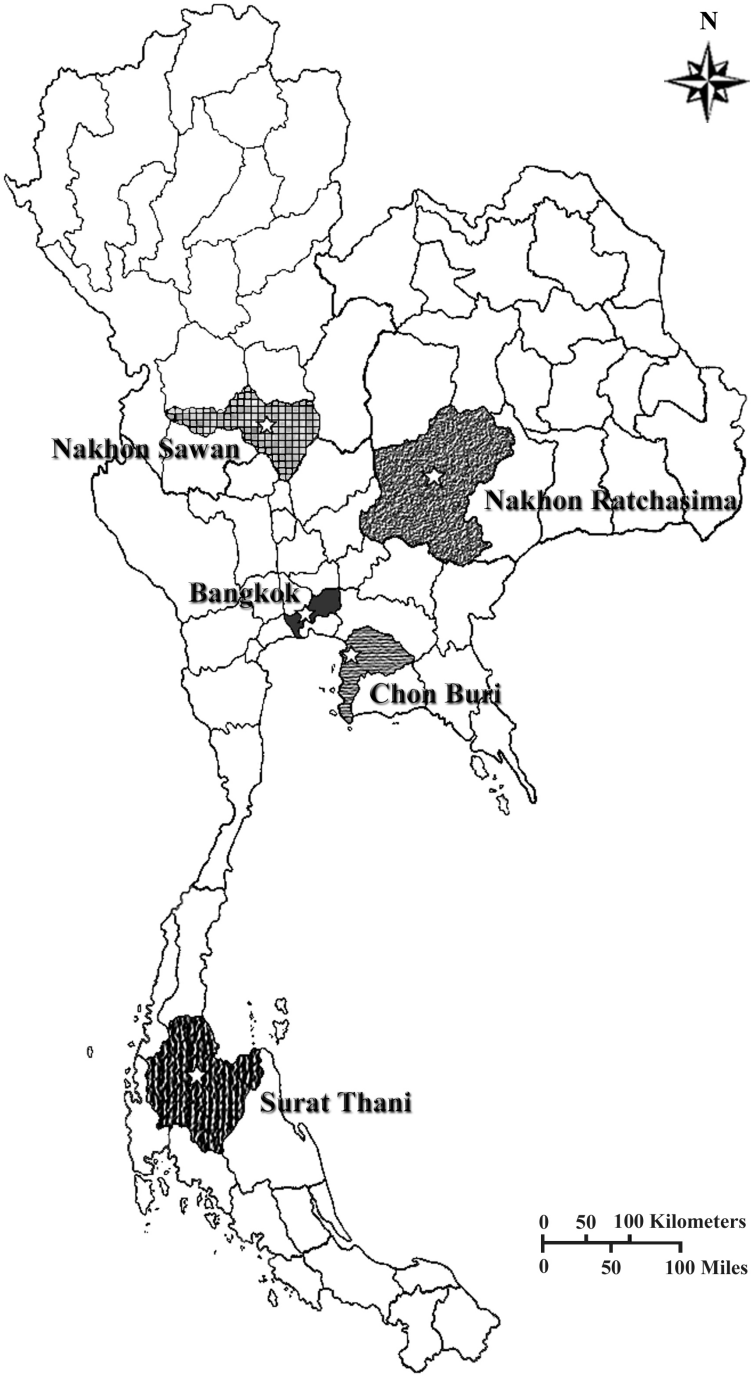
Appendix Table 1 Mortality and case rates of dengue and dengue haemorrhagic fever situation in Thailand from 2002 to 2004

Year	Province	Population	Cases	Deaths
2002	All provinces	62,093,855	114,800	176
	- Bangkok	5,703,292	9,274	10
	- Chon Buri	1,091,375	1,728	1
	- Surat Thani	900,573	4,963	15
	- Nakhon Sawan	1,127,071	1,953	2
	- Nakhon Ratchasima	2,555,998	6,944	14
2003	All provinces	62,799,872	63,657	75
	- Bangkok	5,782,159	8,203	6
	- Chon Buri	1,129,886	1,052	2
	- Surat Thani	920,283	843	1
	- Nakhon Sawan	1,130,841	1,830	3
	- Nakhon Ratchasima	2,581,244	1,731	3
2004	All provinces	63,079,765	39,135	48
	- Bangkok	5,844,607	5,257	5
	- Chon Buri	1,157,111	218	3
	- Surat Thani	935,512	725	1
	- Nakhon Sawan	1,126,739	715	1
	- Nakhon Ratchasima	1,554,009	1,875	5

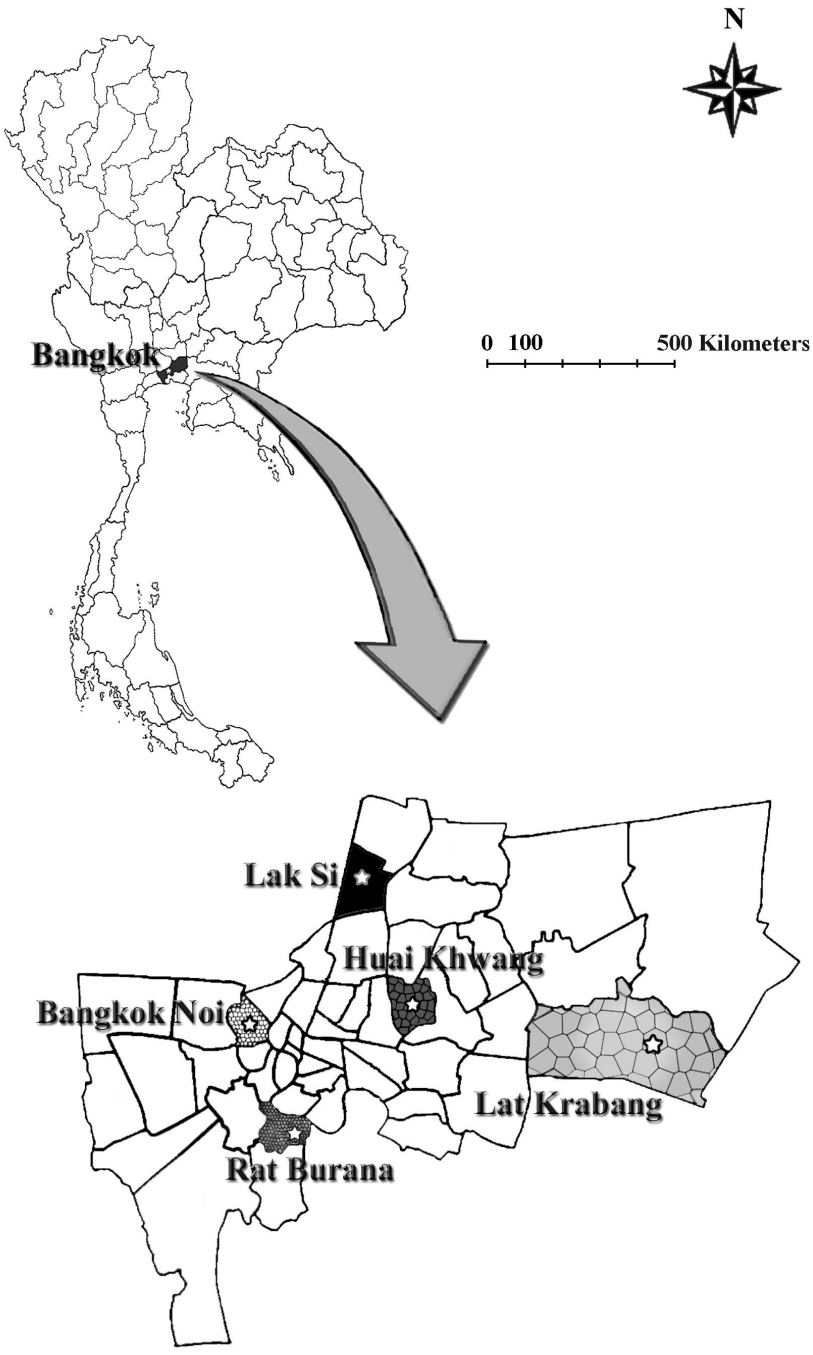
Source: Ministry of Public Health (2006)

Appendix Table 2 Populations of *Aedes aegypti* used in the study

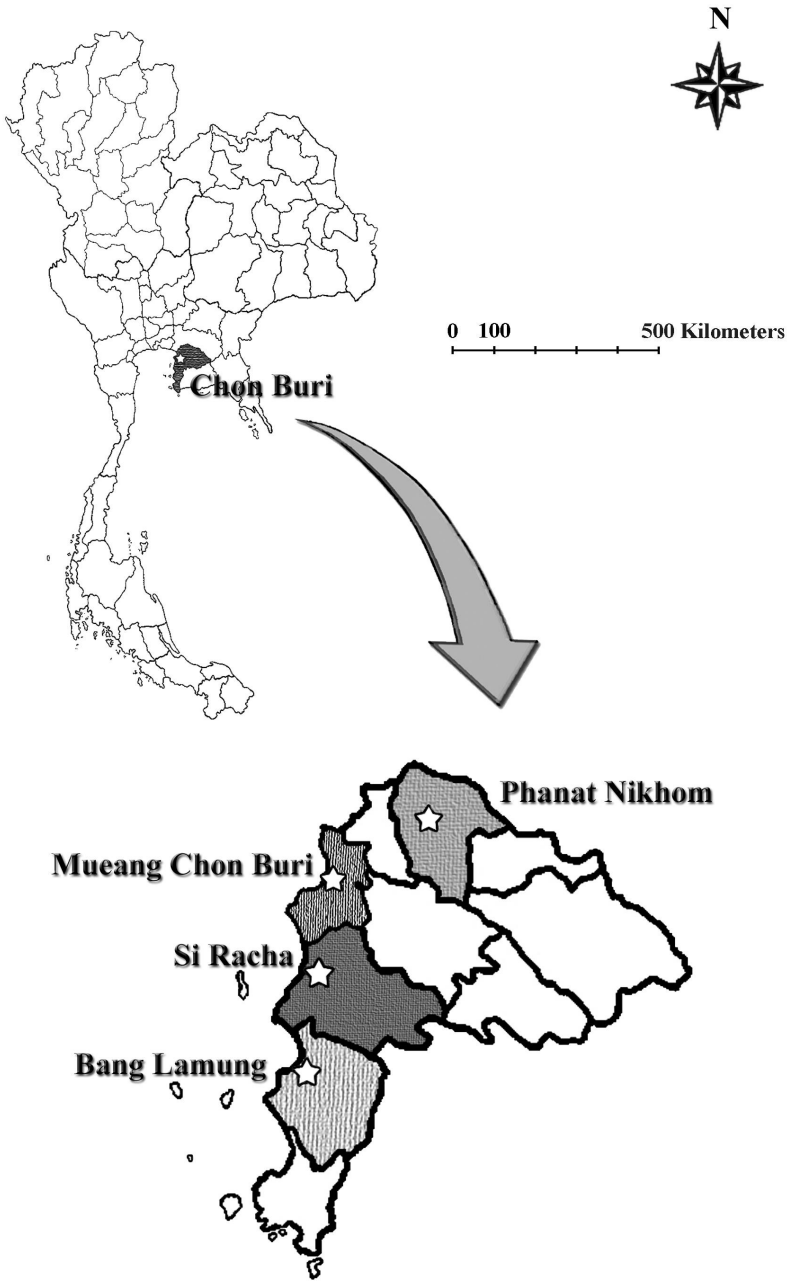
Source (Province/district)	Collection date	Type and location of habitats	Georeference coordinates
Bangkok			
-Lak Si	Apr. 2003	Water jars/Outdoor	100° 33.36 E; 13° 52.12 N
-Rat Burana	Feb. 2003	Cement tanks/Outdoor	100° 30.00 E; 13° 40.12 N
-Lat Krabang	Nov. 2003	Water jars/Indoor	100° 47.24 E; 13° 44.24 N
-Huai Khwang	Jul. 2003	Bottles refuse/Outdoor	100° 34.48 E; 13° 45.36 N
-Bangkok Noi	Feb. 2004	Water jars/Outdoor	100° 28.12 E; 13° 45.36 N
Chon Buri			
-Bang Lamung	Feb. 2004	Water jars/Indoor	100° 54.00 E; 12° 58.00 N
-Phanat Nikhom	Feb. 2004	Water jars/Indoor	101° 10.60 E; 13° 26.60 N
-Mueang Chon Buri	Mar. 2004	Water jars/Indoor	100° 58.60 E; 13° 22.00 N
-Si Racha	Mar. 2004	Water jars/Indoor	100° 55.60 E; 13° 10.00 N
Surat Thani			
-Chaiya	Aug. 2004	Water jars/Indoor	99° 13.60 E; 9° 22.60 N
-Ban Na Doem	Aug. 2004	Water jars/Indoor	99° 19.00 E; 8° 52.60 N
-Khian Sa	Aug. 2004	Water jars/Indoor	99° 13.60 E; 8° 50.60 N
-Don Sak	Aug. 2004	Water jars/Indoor	99° 40.60 E; 9° 18.00 N
-Mueang Surat Thani	Aug. 2004	Water jars/Indoor	99° 19.00 E; 9° 07.60 N
Nakhon Sawan			
-Mae Poen	Aug. 2004	Water jars/Indoor	99° 28.90 E; 15° 34.30 N
-Mae Wong	Apr. 2004	Water jars/Indoor	99° 30.05 E; 15° 46.82 N
-Mueang Nakhon Sawan	Aug. 2004	Water jars/Indoor	100° 07.00 E; 15° 40.60 N
-Krok Phra	Aug. 2004	Water jars/Indoor	100° 04.60 E; 15° 33.00 N
-Takhli	Aug. 2004	Water jars/Indoor	100° 20.60 E; 15° 15.00 N
Nakhon Ratchasima			
-Soeng Sang	Apr. 2005	Bathroom tanks/Indoor	102° 28.15 E; 14° 25.68 N
-Prathai	Apr. 2005	Bathroom tanks/Indoor	102° 39.14 E; 15° 28.90 N
-Kaeng Sanam Nang	Apr. 2005	Bathroom tanks/Indoor	102° 15.71 E; 15° 44.75 N
-Sikhio	Apr. 2005	Bathroom tanks/Indoor	101° 43.49 E; 14° 53.24 N
-Dan Khun Thot	Apr. 2005	Bathroom tanks/Indoor	101° 42.71 E; 15° 15.57 N



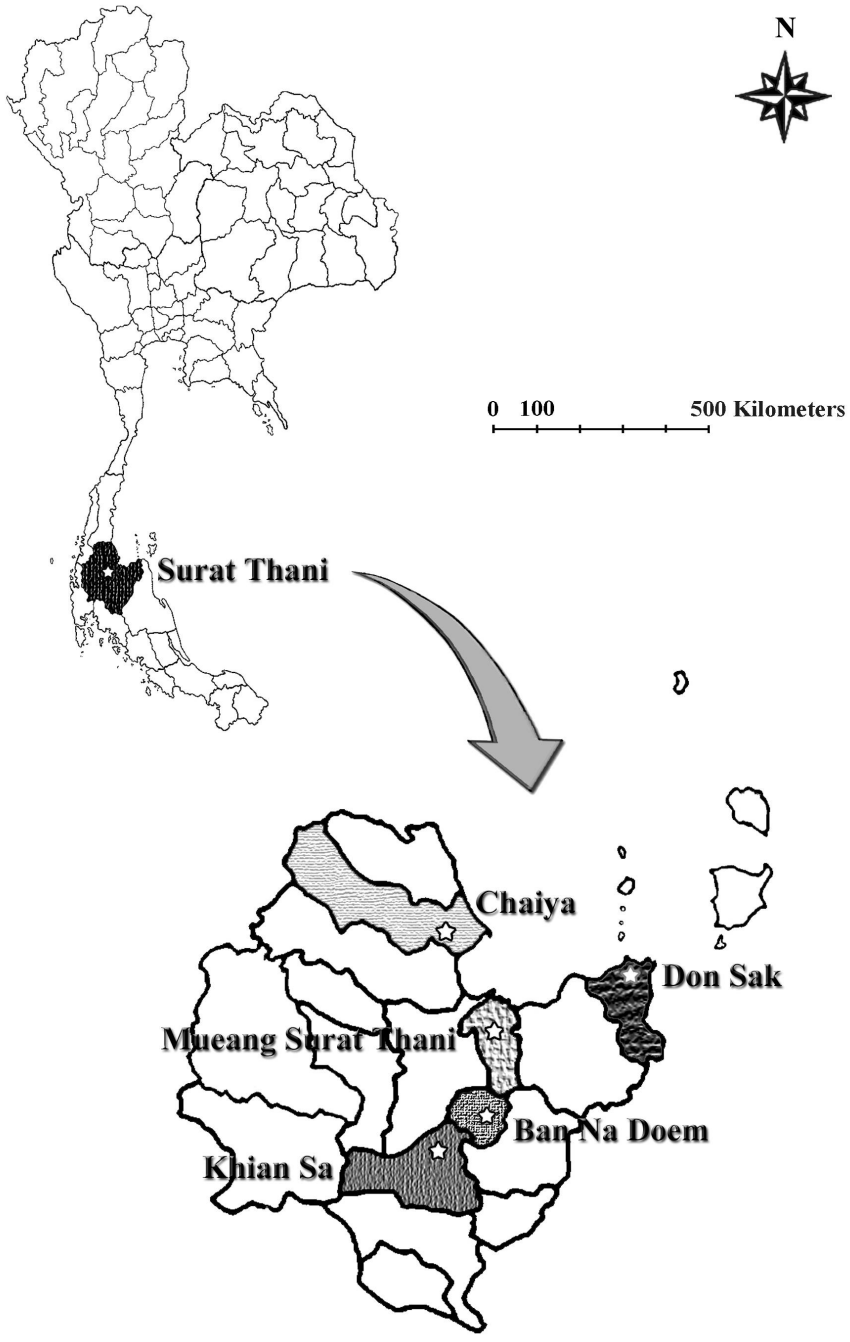
Appendix Figure 1 Map of Thailand showing the collecting sites for *Aedes aegypti* in 5 provinces



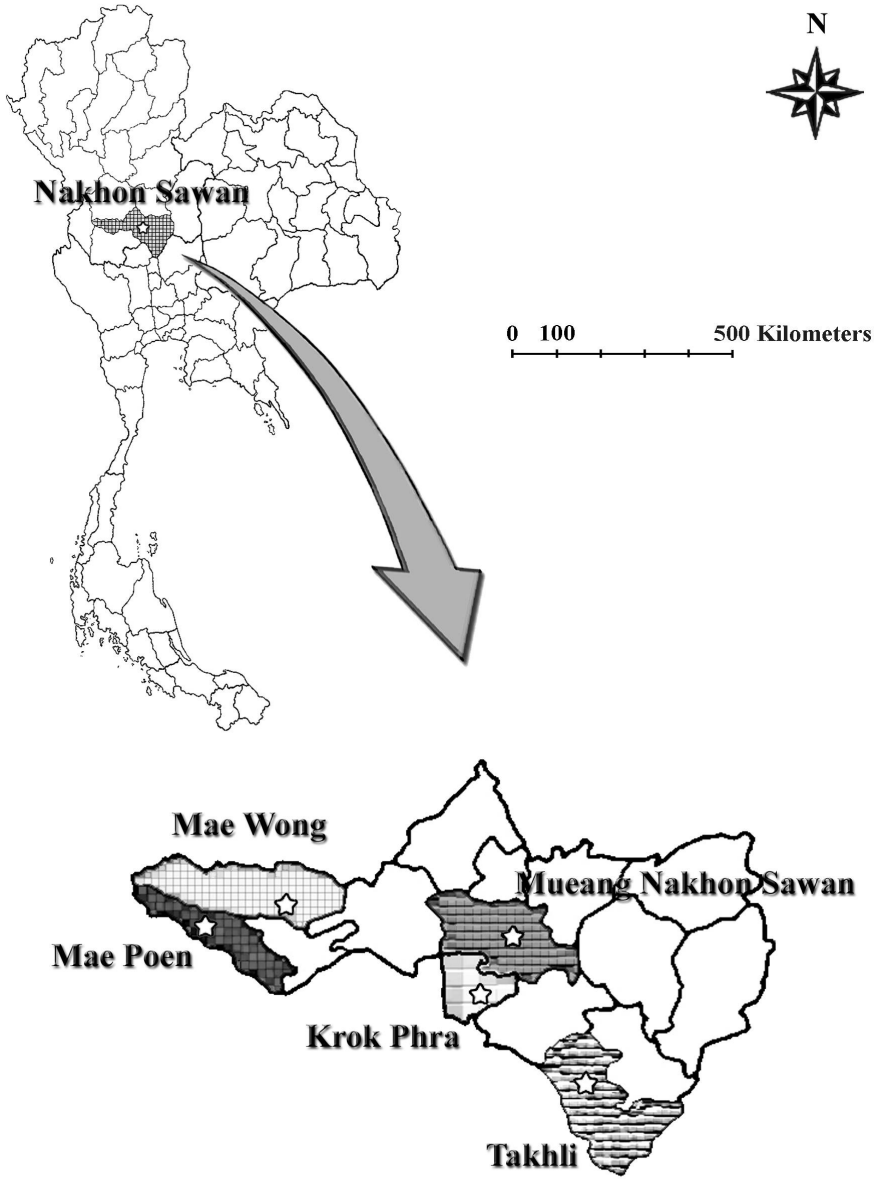
Appendix Figure 2 Map of Bangkok showing the collecting sites for *Aedes aegypti* in 5 districts



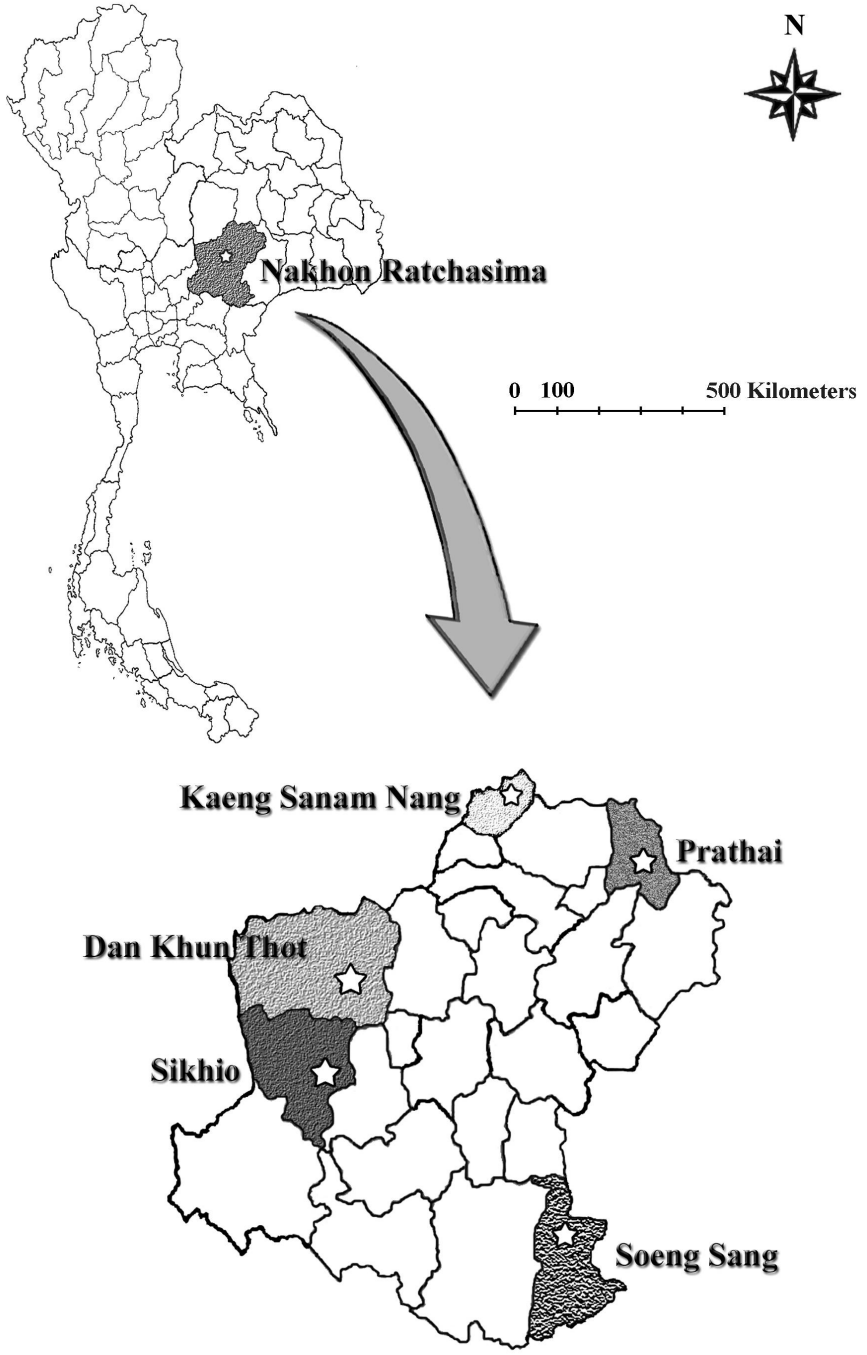
Appendix Figure 3 Map of Chon Buri province showing the collecting sites for *Aedes aegypti* in 4 districts



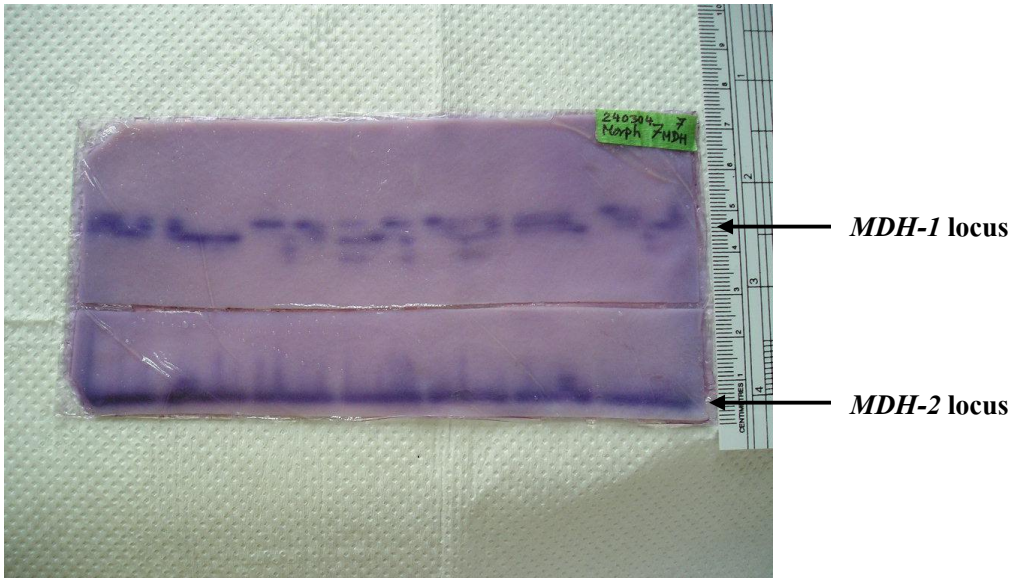
Appendix Figure 4 Map of Surat Thani province showing the collecting sites for *Aedes aegypti* in 5 districts



Appendix Figure 5 Map of Nakhon Sawan province showing the collecting sites for *Aedes aegypti* in 5 districts



Appendix Figure 6 Map of Nakhon Ratchasima province showing the collecting sites for *Aedes aegypti* in 5 districts



Appendix Figure 7 Example of electrophoretic patterns of malate dehydrogenase (MDH) from *MDH-1* and *MDH-2* loci