GENETIC DIFFERENTIATIONS AMONG *AEDES AEGYPTI* (LINNAEUS) POPULATIONS COLLECTED FROM TRAT PROVINCE, EAST OF THAILAND

SUPATTRA CHINAKOOL

THESIS SUBMITTED IN PARTIAL FULLFILLMENT
OF THE REQUIREMENT FOR THE DEGREE
OF MASTER OF SCIENCE (BIOTECHNOLOGY)
SCHOOL OF GRADUATE STUDIES
KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG
2003

ISBN 974-324-763-7

หัวข้อวิทยานิพนธ์	**การศึกษาความแตกต่างทางพันธุกรรมระหว่างประชากรยุงลาย Aedes aegypti (Linnaeus) จากจังหวัดตราด**
นักศึกษา	**นางสาวสุกัทรา จินากูล**
รหัสประจำตัว	39065214
ปริญญา	**วิทยาศาสตรมหาบัณฑิต**
สาขา	**เทคโนโลยีชีวภาพ**
พ.ศ.	2546
อาจารย์ผู้ควบคุมวิทยานิพนธ์	**ดร.อุ่นเรือน เพชราวัลย์**
อาจารย์ผู้ควบคุมวิทยานิพนธ์ร่วม	**ดร.เกรียงไกร เลิศทัศนีย์** **รศ.ดร.ธีรภาพ เจริญวิริยะภาพ**

บทคัดย่อ

วัตถุประสงค์ของการศึกษาครั้งนี้เป็นการเปรียบเทียบไอโซไซม์ของยุงลาย *Aedes aegypti* (Linnaeus) 6 กลุ่มประชากรจากจังหวัดตราด โดยใช้วิธี starch gel electrophoresis ในจำนวนนี้มียุงลายจากเกาะช้าง 4 กลุ่มคือ อ่าวสับปะรด, บ้านคลองสน, บ้านด่านใหม่ และบ้านสลักเพชร และอีก 2 กลุ่มจากท่าธรรมชาติและผืนแผ่นดินใหญ่อำเภอแหลมงอบ พบว่าไอโซไซม์ 5 โลไซจากทั้งหมด 13 โลไซ (loci) เป็นโพลีมอร์ฟิค (polymorphic loci) ค่าเฉลี่ยอัลลีลต่อโลคัสอยู่ในช่วง 1.4 (±0.1) ถึง 1.7 (±0.2) ค่าเฉลี่ย heterozygosities ทั้งค่าสังเกตและค่าคาดหวังอยู่ในช่วง 0.036 (±0.015) ถึง 0.171 (±0.077) และ 0.040 (±0.018) ถึง 0.149 (±0.060) ตามลำดับ สัดส่วนค่าโพลีมอร์ฟิคอยู่ในช่วง 15.4% ถึง 38.5 % ค่าระยะทางทางพันธุกรรม (Nei's genetic distance) ระหว่างประชากรทั้งหมดอยู่ในช่วง 0.001 ถึง 0.117 และค่าสถิติแบบ Wright แสดงให้เห็นว่ามีความแปรผันทางพันธุกรรมระหว่างกลุ่มประชากรมากกว่าภายในประชากรทั้งหมดรวมกัน ดังนั้นยุงลาย *Ae. aegypti* จากเกาะช้างและผืนแผ่นดินใหญ่ชายฝั่งอำเภอแหลมงอบจังหวัดตราด มีความแตกต่างทางพันธุกรรมสูงเนื่องจากมีการเคลื่อนย้ายยืนระหว่างทั้งสองพื้นที่ต่ำ

Thesis Title	Genetic differentiations among *Aedes aegypti* (Linnaeus) populations collected from Trat Province, East of Thailand
Student	Miss Supattra Chinakool
Student ID.	39065214
Degree	Master of Science
Programme	Biotechnology
Year	2003
Thesis Advisor	Dr. Ounruan Petcharawan
Thesis Co-advisor	Dr. Kriangkrai Lerdthusnee
	Assoc. Prof. Dr. Theeraphap Chareonviriyaphap

ABSTRACT

Isozymes of 6 wild-caught populations of *Aedes aegypti* (Linnaeus) were compared using starch gel electrophoresis. Four populations were collected from Ao Sapparod, Klong Son Village, Dan Mai Village and Salak Phet Village in Chang Island, the others 2 populations were collected from Tha Thammachat and mainland of Laem Ngob District, Trat Province. Five of 13 isozyme loci were polymorphic. The mean number alleles per locus ranged from 1.4 (±0.1) to 1.7 (±0.2). Both observed and expected mean heterozygosities ranged from 0.036 (±0.015) to 0.171 (±0.077) and 0.040 (±0.018) to 0.149 (±0.060), respectively. Nei's genetic distance among populations ranged from 0.001 to 0.117. Wright's *F* statistics revealed greater variation among populations than within populations. The genetic differentiation was observed between *Ae. aegypti* populations in Chang island and on the coastal areas of mainland in Laem Ngob District, Trat Province, as a low level of gene flow among these populations.

ACKNOWLEDGMENTS

I wish to express my deepest gratitude and sincere appreciation to Associate Professor Dr. Theeraphap Chareonviriyaphap for his guidance, suggestion throughout my laboratory processes and data analysis and Dr. Kriangkrai Lerdthusnee for his support this thesis.

I also wish to express my true appreciation to Dr. Ounruan Petcharawan for her valuable suggestion.

I would like to express my grateful thanks to my father, my mother, my brother and my sisters who give their love, understanding and encouragement for my graduate study.

Finally, I would like to thank all of my friends for their encouragement throughout my study.

Supattra Chinakool

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Cont.)

LIST OF TABLES (Cont.)

LIST OF TABLES (Cont.)

LIST OF FIGURES

Figure **Page**

CHAPTER 1

INTRODUCTION

1.1 Background

Aedes aegypti (Linnaeus) is a principal vector of dengue fever in some areas including Southeast Asia, the Western Pacific, Latin America, the Caribbean and Africa (Harrington and Stork 1995). Dengue fever, a human viral infection is normally an acute and non-fatal disease has become a global health problem. However, dengue hemorrhagic fever, a severe type of this disease which is associated with multiple infections has become one of the leading causes of illness and death in Thailand as well as other Asian countries (Kantachuvessiri 2002). In Thailand, dengue fever occurs throughout the country after the first outbreak of this disease in 1958. The outbreak of dengue fever in Thailand has occurred every year or every 2-3 years. Increasing in transportation has contributed to the dispersal of infected vector and veramic human (Faillox *et al.* 1995).

There have been extensively studied on genetic structure of *Ae. aegypti*, since the great variety of this mosquito in morphology, physiology, behavior and geographic with its importance in medicine. Based on *Ae. aegypti* isozyme polymorphism, the knowledge of genetic structure is useful for identification of *Ae. aegypti* populations and for analysis gene flow among *Ae. aegypti* in different geographical zones. Two subspecies of *Ae. aegypti* have been recognized in East Africa (Mattingly 1957) and the other subspecies has been recognized in South of Sahara, East Africa(Trips and Hausermann 1975). In Thailand, the presence of a single species based on genetic structure of *Ae. aegypti* populations between island and mainland of Surat-Thanee Province, South of Thailand has been reported by Chareonviriyaphap and Lerdthusnee (2002).

Chang (elephant) island, one of marine national park groups (Kut, Mak, Rat, Maichi, etc.) with an area of about 429 square kilometers. It is located on the north of the Gulf of Thailand, about 315 and 9 kilometers from East Bangkok and Laem Ngob district, respectively. Chang island has an population of about 6,000 distributed along the coastal plain around the island, most of them are fishing. The dominant vegetation on Chang island is coconut palm. The climate of Chang island is affected by the monsoons. Rainfall occurs throughout the year usually May to October, most of the time due to the southwest monsoon. The mean annual rainfall is about 5,500-6,500 millimeters and the mean annual temperature is 27.7°C. Many environmental

changes have created situations that have favored condition for increasing *Ae. aegypti* in Chang island. However, little is known about the movement of *Ae. aegypti* populations, especially among island and mainland.

Starch gel electrophoresis technique was available to investigate whether there are any significant differences in genetic structure of *Ae. aegypti* populations in the mainland and island of Trat Province, East of Thailand. In this study, the pattern of gene flow between island and mainland populations and among the 4 island populations were analyzed, using genetic profiles of *Ae. aegypti* from 2 geographical zones.

1.2 Objectives

1. To investigate the genetic structure of *Ae. aegypti* populations in island and mainland of Trat Province.

2. To analyze the pattern of gene flow of *Ae. aegypti* populations in island and mainland of Trat Province.

1.3 Research Plans

1. Collect and identify mosquito specimen from Chang island and from the coastal areas in Laem-Ngob District, Trat Province.

2. Investigate the isozyme of *Ae.aegypti* using starch gel electrophoresis.

3. Analyze and compare the genetic structure of *Ae. aegypti* populations from the island and mainland in Trat Province using the computer program, BIOSYS-1 (Swofford and Selanda. 1981).

4. Produce the phenogram by Unweighted Pair Group Method Averaging (UPGMA) for cluster analysis according to Nei's (1978) unbiased genetic distance.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy of *Aedes aegypti*

Aedes aegypti is a medium-sized mosquito belongs to Class Insecta, Order Diptera, Family Culicidae and Genus *Aedes*. It was first described by Linnaeus in 1762 (Christopher 1960). The origin of *Ae. aegypti* is the African forest (Wallis and Tabachnick 1990). In general, this mosquito is known by different common names such as yellow fever mosquito, black and white mosquito, day-biting mosquito, domestic mosquito (Christopher 1960) and the mosquito of seaport (Horsfall 1972).

This species have been recognized as species complex comprising at least 3 species namely *Ae. aegypti aegypti*, *Ae. aegypti formorsus* (Walker) and *Ae. aegypti queenlandensis* (Mattingly 1957 ; Trips and Hausermann 1975). The subspecies *Ae. aegypti aegypti*, a brown and domestic form distributed widely throughout the range of the species except in Africa South of Sahara, where the other subspecies *Ae. aegypti formorsus* (Walker), a black and forest form have found. The subspecies *Ae. aegypti queenlandensis*, a pale and peridomerstic form has been distributed in the same area of *Ae. aegypti formorsus* on the coast of East of Afreica.

2.2 Biology of *Aedes aegypti*

Aedes aegypti has a complete metamorphosis of 4 different forms (egg, larva, pupa and adult). A batch of eggs is laid separately on the moist surface just above the waterline of the water container. The fertilized female lay its eggs mostly in containers inside human habitats. Only a small amount of eggs are laid in containers outside human habitats (Harrington and Stork 1995). The eggs (elongated tubular, about 1 millimeter in length) are white and turn to dark within 2 hours. The eggs develop to a fully stage within 30 to 40 hours, by the first 16 to 24 hours they take up very high water to remain viable (Christopher 1960 ; Kliewer 1961). The fully developed eggs can survive in dry condition for months up to 1 year (Christopher 1960 ; Horsfall 1972) and can hatch into larva stage as soon as reflooded.

Like others mosquito larva, there are 3 different regions in the body : sclerotised head, a broad thorax and segmented abdomen. The *Ae. aegypti* larvae can hang head down from the

water surface, with breath and feed below the water surface in the same time, since they have a short siphon with interior respiratory tube at the terminal segment of its abdomen. The larvae are found in the container with clean and still water, occasionally found in dirty water. The larvae feed on the small particle in the water container (Womack 1993). The larvae quickly move to the bottom of water container when they are disturbed because of their sensitivity to the water vibration and light intensity. The lavae shed their skin 4 times and develop into pupa within 6-8 days (Christopher 1960).

The pupal stage remain an aquatic living and have a cephalothorax (the combined part of the head and the thorax) which is jointed to a segmented abdomen. They can breath through a tubular respiratory horn which is located on the posterior cephalothorax. The food does not essential for this resting stage. The pupae take 1-2 days before the adults emerge (Christopher 1960).

The adults are medium size and easily recognized by silver-white lyre-shaped pattern on the mesonotum with the white band on its abdomen and its hind tarsi. Fertilized female lay eggs after feeding blood. Both sexes have similar feeding period, the female is feed only on human. There are 2 biting peaks ; before sunset and after sunrise at the area associated with human habitats. Blood meal are essential for the maturation of eggs. Each fertilized female lay eggs 5 to 6 time, maximum to 116 eggs in their life cycle (Brigel 1990).

2.3 Distribution of *Aedes aegypti*

The origin of *Ae. aegypti* is the African forest. The African slave tradition in about 1500s had begun the spread of this mosquitoes into others areas, subsequently the transportation by sea is the importance factor to introduce this mosquitoes to others areas of the world (Service 1997). At present, *Ae. aegypti* is widely distributed throughout the temperate region, which roughly correlated with range extending from 40°S to 40°N latitude (Womack 1993). The distribution of this mosquito is dominated by the limitation of life factor such as; temperature, rainfall, season, habitat and dispersal (Scanlon. 1967 ; Fontenille and Rodhain 1989 ; Kemp and Jupp 1991). The adults mosquitoes can not resist to cold climate and a temperature of 6°C for 24 hours, or 42 °C for 5 minutes. The temperature range from -8 to 46 °C and 20 to 46 °C are suitable for the larvae and the eggs, respectively (Slosek 1986). Low populations of *Ae. aegypti* are obvious in dry season and become higher in rainy season (Schultz 1993). The different containers are the

importance habitats of *Ae. aegypti* larvae such as discarded automobile tires, garden vessels, rubbish, rock hole, coconut shells, water jars, drum, cement cistern (Trpis 1972 ; Jumali *et al.* 1979 ; Chamber *et al.* 1986 ; Tidwell *et al.* 1990 ; Kittiyapong and Strickman 1993). In flight ability, *Ae. aegypti* can not fly for a long distance from human habitats (Mattingly. 1969). Sheppard *et al.* (1969) reported that the average distance flight of this mosquito in Bangkok was 37 meters. In Thailand, this mosquito is distributed in throughout the country, but the abundant period commonly occurs during the rainy season.

2.4 Isozyme electrophoresis

Advantage in biochemistry and molecular biology has allowed the differentiation of related animal and plant species. Isozymes electrophoresis is extensive used for studies the relationship at population and species level. It is involed the protein separation on supporting media using electrical current, and the enzyme staining reaction. The migration rates of protein through the media depend upon the net charge, size and shape of the protein. Each band appears on the gel corresponds to a different protein, which is controlled by one, two or several genes. A single or multilocus banding pattern is easily interpreted following Mendelian law. In addition, it provide heterozygote banding pattern from which estimates of deviation from Hardy-Weinberg

2.5 Genetic structure of *Aedes aegypti*

Aedes aegypti is widely distributed throughout the tropical and subtropical zone. For many years there were some studies that indicated the genetic differentiation among different *Ae. aegypti* populations. The in formations of isozyme electrophresis were available to identify *Ae. aegypti* from worldwide into 8 geographical groups including feral form *Ae.aegypti formorsus* in East and West Africa and domestic form *Ae. aegypti aegypti* in Southeast Asia-Pacific, the Caribbean, Southeast U.S., Southwest U.S.-Mexico, Central-South America and East Africa (Tabachnik and Powell 1979 ; Powell and Tabachnik 1980 ; Tabachnik 1991).

The study of genetic structure of domestic and feral *Ae. aegypti* in East Africa were done by Scott and McClelland (1975) and Tabachnick *et al.* (1979). Based on the 15 polymorphic loci, in some loci showed genetic differentiations between the 2 forms of *Ae. aegypti* ; however, there no different in mean heterozygosity ($P<0.05$). In addition, a low level of gene flow was observed between the 2 forms of *Ae. aegypti*.. From these results and the distinct behavioral

feature, they divided these 2 forms into subspecies (Tabachnick *et al.* 1979). Contrast to the studies on genetic structure of domestic and feral *Ae. aegypti* Caribbean island of Angulla (Wallis and Tabachnik. 1990).

Genetic variability of *Ae. aegypti* in 18 collections of Caribbean islands were analyzed using starch gel electrophoresis (Wallis *et al* 1984). Similarity of genetic structure throughout the studied areas were found. Although there were some relationship between geographic and genetic distance, some sample collections did not correlate to any geographic pattern. These resulted from a high level of gene flow among island and with mainland of America. Contrast to the study of the relationship of *Ae. aegypti* and the epidemic of dengue fever in French Polynesia islands (Failloux *et al.* 1995). They analyzed the genetic structure of *Ae. aegypti* in attempt to analyze the pattern of gene flow between the islands. A low level of gene flow among islands were found. Thus the epidemic of dengue fever in French Polynesia islands could be resulted from the movement of infected human from place to place.

In Argentina, the genetic structure of *Ae. aegypti* were studied for the attemption to determine the genetic distance among different provinces which are abundant with this mosquito. There no relationship between geographic and genetic distance, as the recent introduction of individuals into the studied areas. The low level of genetic differentiations were observed among 3 different locations (Sousa *et al.* 2000). They noticed that the distribution of *Ae. aegypti* in Argentina depend upon the commercial trade from others countries.

The genetic structure of domestic *Ae. aegypti* in East African villages less than 2 kilometers apart was analyzed on 4 loci : *Est-6, Idh-1, Mdh-1* and *Pgm*-1 (Tabachnik and Powell 1978). They found no genetic subdivision within a village with low gene flow between villages. They also found genetic different between villages, this due to natural selection or random genetic drift. In the other hand, Harrington *et al.* (1984) examined genetic structure of urban *Ae. aegypti* in USA. Samples were collected from tire dumps, which are the common larval habitats in this country. Based on 10 enzyme loci : *Hk, Mdh, Pgm, Pgi, Ald, Ae, Fum, α-Gpd, Me* and *6-Pgd*, they found the low genetic differentiation among different populations in study areas. However, the genetic variation of *Ae. aegypti*the was also obtained examination the only one enzyme system : the phosphoglucomutase (*Pgm*) (Bullini and Coluzzi. 1972), Esterase (*Est*) (Briegel and Freyvoge 1973) and phosphate system (Igbokwe 1982).

In Thailand, Chareonviriyaphap and Lerdthusnee (2002) studied the genetic structure *Ae. aegypti* populations in Samui island and mainland of Surat-Thanee province. They found the low

level of genetic variability in these populations. Low level of genetic exchange between island and mainland populations were observed, these results due to a low level of gene flow between island and mainland populations. In addition, Lerdthusnee and Chareonviriyaphap (1999) observed the genetic differentiation among the *Ae. aegypti* populations which were collected before and after the mosquitoes control program in Tak Province. Similarity to the study of the genetic structure of *Ae. aegypti* from different subdistrict in Chaiengmai Province (Mousson *et al.* 2002). The genetic differentiation among these locations were found, this evidence was the effect of insecticide control in the study sites. Komalamisra *et al.* (2001) examined genetic structure of *Ae.aegypti* from different provinces. They reported no genetic differentiations among these mosquito populations ; although the morphological study showed the different form of these mosquito.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

1. Accessories for electrophoresis : U-mold containing solidified starch gel covered with saran wrap, electrode buffer chamber and accessories for gel preparation : cutting plate, gel slicer, L-shaped slot guide and spatula. (Figure 3.2)

2. Aspirator pump model B-169 (BUCHI Labortechnik AG, Switzerland)

3. Balance model PB 153 (METTLER TOLEDO, Switzerland)

4. Bunsen burner

5. Cellulose polyacetate (Gelman Sciences Inn., USA)

6. Deep-freezer (-80°C) model U 420 (Heto-Holten A/S)

7. Magnetic stirrer (MICROSTIRER, VELP SCIENTIFICA, Italy)

8. Oven model BE 500 (MEMMERT GmbH + Co.KG, Germany)

9. Perspex grinding block

10. pH meter model pH 325A (WTW, Germany)

11. Power supply model EC 105 (E-C Apparatus Corporation)

12. Staining box with lid

13. Vortex mixer (VIBROMIX, Italy)

3.2 Mosquitoes Collection

Both larval and pupa mosquitoes were collected from 2 geographical zones, island and mainland of Trat Province, East of Thailand (Figure 3.1). Four populations of Chang island namely Ao Sapparod, Dan Mai and Salak Petch and the others 2 populations were collected from Tha Thammachat and mainland of Laem Ngob District, Trat Province. All populations were located about 2-25 km apart ; the distance between Klong Son and Ao Sapparod, Ao Sapparod and Dan Mai, Dan Mai and Salak Petch, Tha Thammachat and Ao Sapparod, Laem Ngob And Dan Mai and Tha Thammachat and Laem Ngob are about 2, 10, 14, 5, 9 and 17 km, respectively. All mosquitoes were reared to adult stage and then stored at -80°C for isozyme analysis. The species of all mosquitoes were recorded and identified.

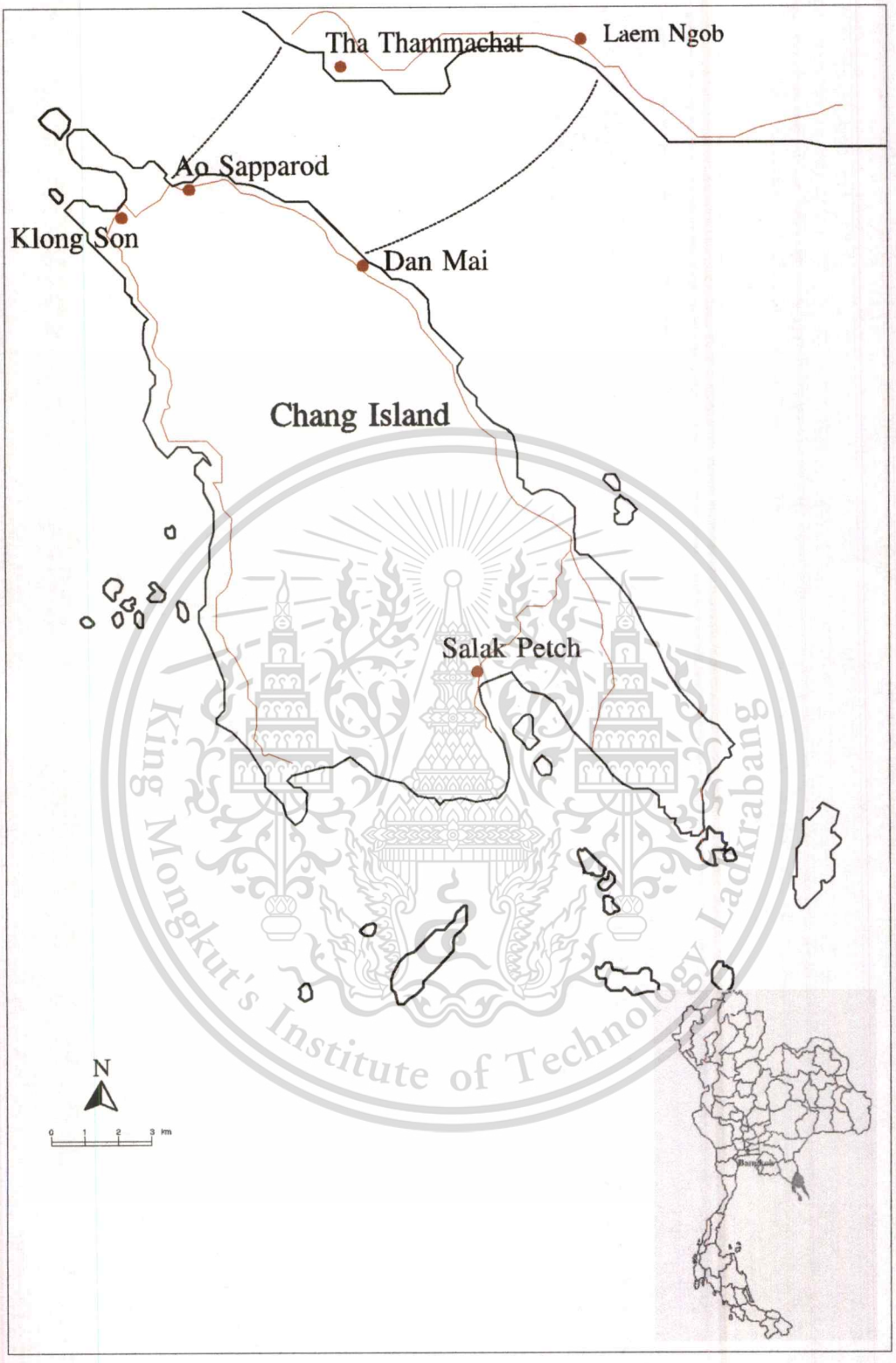

Figure 3.1 Collection sites of Aedes aegypti populations from Ao Sapparod, Klong Son, Dan Mai and Salak Petch in Chang Island, Tha Thammachat and mainland of Laem Ngob District, Trat Province.

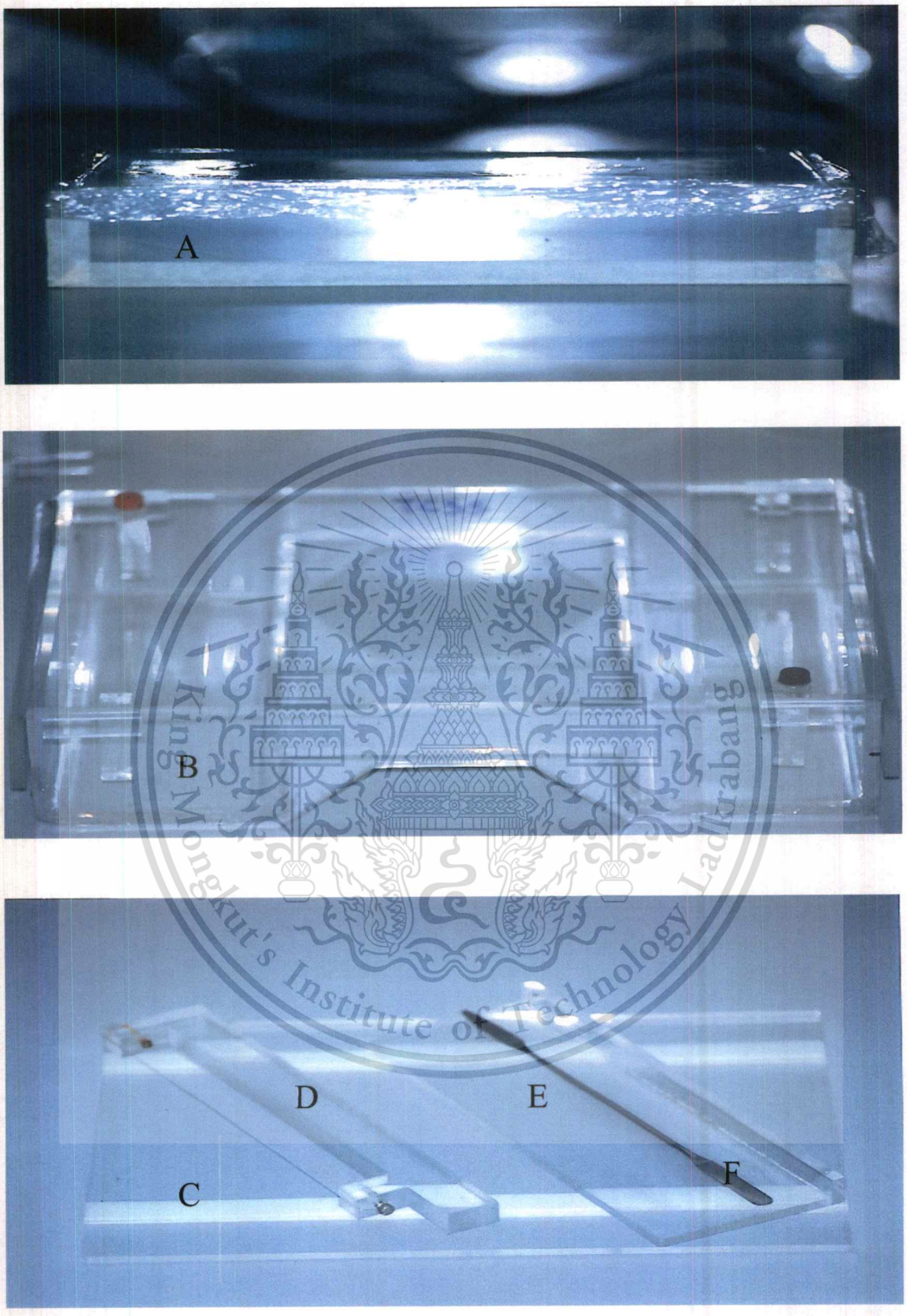

Figure 3.2 Accessories for electrophoresis : U-mold containing solidified starch gel covered with saran wrap(A), electrode buffer chamber (B) and accessories for gel preparation : cutting plate (C), gel slicer (D), L-shaped slot guide (E) and spatula (F).

3.3 Sample preparation

Aedes aegypti mosquitoes were placed in a Perspex grinding block. Twenty-five microliters of lysis buffer (0.605 g Trisma, 0.02 g EDTA and 0.005 g NADP in 50 ml dH_2O, adjust pH to 7.0 with HCl plus 1 drop mercaptoethanal) was added to each well. Individual mosquitoes was then ground with a glass rod until the mosquito was homogenous. The homogenates were absorbed onto 4 × 11 millimeters of cellulose polyacetate paper. The saturated paper were inserted in the sample slot of the prepared starch gel.

3.4 Starch gel preparation

Horizontal starch gel was prepared by using a concentration of 11.1 and 4.4 % (w/v) of hydrolyzed potato starch and sucrose, respectively in the suitably-chosen buffer (Appendix 2) namely Lithium hydroxide (LiOH) (Pasteur *et al.* 1988), Morpholine (Morph) (Clayton and Tretiak. 1972), Tris-citrate buffer system (TCss) (Shaw and Prasad 1970) and Tris-malate-ethylenediamiestetracetic acid (TMED) (Pasteur *et al.* 1988). The 450 milliliters suspension in a 1 liter Erlenmeyer flask was then heat over Bunsen burner with constantly swirling. When the suspension become viscous, heating and swirling must be continued for 60 seconds (until the suspension was converted into a transparent solution). The solution was then degassed under vacuum about 20 seconds. The solution was poured into the U-mold with masking tape shut along the bottom of the side walls. The gel was allowed to set for 1 hour at room temperature. After that the gel was covered with Saran wrap to prevent the hydration, and it was placed at room temperature overnight. Before use, the gel was cooled in a refrigerator for a half hour and thereafter it was cut vertically as the sample slot with a scalpel, about 4.5 cm from one side using a L- shaped slot guide.

3.5 Loading the samples

The pieces of the cellulose polyacetate paper previously saturated with the sample solution were inserted into a vertical cutting in the gel. After loading the samples, the gel surface was covered with Saran wrap and was placed over the gel with a crushed ice box.

3.6 Running gel electrophoresis

Isozyme electrophoresis were performed on horizontal starch gel. The process of electrophoresis were conducted as described by Lerdthusnee and Chareonviriyaphap (1999). A total of 20 enzyme systems were performed, as followed : Aconitase (*Acon*, E.C.4.2.1.3), Adenylate kinase (*Ak*, E.C.2.7.4.3), Aldehyde oxidase (*Ao*, E.C. 1.2.3.1), Arginase kinase (*Argk*, E.C.2.7.3.3), Esterase (*Est*, E.C. 3.1.1.1), Glycerol dehydrogenase (*Gcd*, E.C. 1.1.1.72), Glucose-6-phosphate dehydrogenase, (*G6pdh*, E.C.1.1.1.49), Glutamate oxaloacetate transminase (*Got*, E.C. 2.6.1.1), α-glycerophosphate dehydrogenase (*α-Gpdh*, E.C.1.1.1.8), β-hydroxyacid dehydrogenase (*Had*, E.C.1.1.1.30), Hexokinase (*Hk*, E.C. 2.7.1.1), Isocitric dehydrogenase (*Idh*, E.C.1.1.1.42), Malate dehydrogenase (*Mdh*, E.C.1.1.1.37), Malic enzyme (Me, E.C. 1.1.1.40), Mannose-6-phosphate isomerase (*Mpi*, E.C.5.3.1.8), 6-Phosphogluconate dehydrogenase (*6Pgd*, E.C. 1.1.1.43), Phosphoglucose isomerase (*Pgi*, E.C.5.3.1.9), Phosphoglucomutase (*Pgm*, E.C. 5.4.2.2) and Xanthine dehydroganase (E.C. 1.2.1.37). Four buffer systems : Morph, LiOH, TCss and TMED were used in this study. Electrophoresis were performed at the constant voltage of 16 V/cm, for 4 hours in LiOH, Morph and TCss and 6 V/cm, for 6 hours in TMED buffer system.

3.7 Slicing the gels after electrophoresis

Starch gel was sliced longitudinally into several thin slices because stains will not penetrate the whole slab gel. After electrophoresis was completed, the paper pieces was removed. Cutting the unnecessary edged of the gel and marking the angle at the right of the gel for the termination. The gel was transferred to a slicing plate and sliced with a thin steel wire. Starch gel must be cut at least once before staining..

3.8 Enzyme activity staining

All staining reagents were mixed in 50 ml suitable buffer ; DH buffer, Phosphate Na/Na_2, Phosphate Na_2/K and Tri-HCl (Appendix 2). There are staining solutions that contain a specific substrate for enzyme to be assays (Appendix 2). When staining was sufficient for interpretation, the enzyme reaction were stopped with fixative solution (Methanol : dH_2O : Acetic acid in ratio 10:10:1).

3.9 Data analysis

Analysis of alleles frequencies, heterozygosity, Hardy-Weinberg equilibrium and genetic distance were calculated using BIOSYS-1 (Swofford and Selanda 1981). Each population was analyzed by the computation of allele frequencies, heterozygosity per locus, genetic variability and a test for conformance to Hardy-Weinberg equilibrium at each loci by a chi-square test. Differentiation among populations were determined by F-statistic. The phenogram were produced by UPGMA for cluster analysis according to Nei's (1978) unbiased genetic distance.

CHAPTER 4

RESULTS

4.1 Enzyme systems

Of the 20 enzyme systems were detected (Table 4.2), 13 (*Acon, Aks, Argk, Est, G6pdh, Gpdh, Had, Idh, Mdh, Mp,I, 6Pgd, Pgi* and *Pgm*) were used in this study. These 13 enzyme systems presented consistent bands (Figure 4.1). Four enzyme systems (*Ao, Hk, Me* and *Xdh*) failed to stain, the others two (*Got* and *Gcd*) showed inconsistent bands.

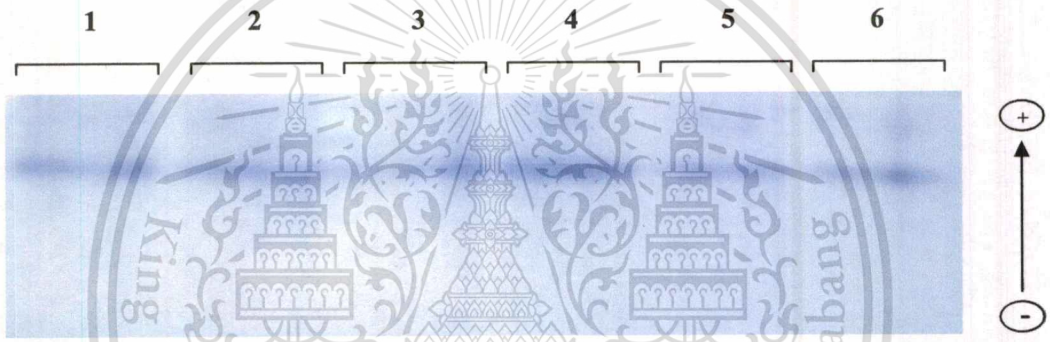

Figure 4.1 Electrophoresis pattern of Arginase kinase (*Argk*) from *Aedes aegypti* populations collected from 6 different locations. Each lane consists of 5 bands of individuals : Lane 1 from Ao Sapparod, Lane 2 from Klong Son, Lane 3 from Dan Mai, Lane 4 from Salak Petch, Lane 5 from Tha Thammachat and Lane 6 from Laem Ngob.

4.2 Population genetic structure

All enzyme systems used for this study had 1 locus (Table 4.1). Five of the 13 loci : *Acon-1, Est-1, Idh-1, Mdh-1* and *Pgm-1* were polymorphic (by 99% criterion) in all populations, whereas *Aks-1, Argk-1, G6p-1, Gpd-1, Mpi-1, 6Pgd-1* and *Pgi-1* were monomorphic loci in all populations. The genetic frequencies of all polymorphic loci for each population were showed in Table 4.2. The most polymorphic loci were *Idh-1, Mdh-1* and *Pgm-1* with 2 alleles, followed by *Est-1* and *Had-1* with 2 alleles. An unique allele, Had_{63} was found only in Tha Thammachat population. There was a fixed allelic difference between *Ae. aegypti* from Laem Ngob and the remaining populations. Measures of genetic variability at 13 loci of *Ae. aegypti* in all populations were summarized in Table 4.3. The percentage of polymorphic loci in all populations ranged

from 15.4 % to 38.5 % : the mean number of alleles per ranged from 1.4 (±0.1) to 1.7 (±0.2) and the mean expected heterozygosity ranged from 0.040 (±0.018) to 0.119 (±0.060). However, there was no difference in the mean expected heterozygosity between island and mainland populations since the broadly overlapping standard errors. In addition, the percentage of polymorphic loci for all populations ranged from 38.5 % to 46.2 % when considering at 99% criterion (Table 4.4).

Genetic frequencies deviated from the Hardy-Weinberg Equilibrium ($P<0.05$) among the 6 populations at 4 of all loci (Table 4.5). *Est-1* and *Had-1* deviated in Tha Thammachat population. *Idh-1* was not in the Hardy-Weinberg Equilibrium in Dan Mai population. *Pgm-1* did not conform to the Hardy-Weinberg Equilibrium in Ao Sapparod and Klong Son populations. All of the deviations due to heterozygote deficiencies (Fixation index ; $F > 0$)(Table 4.6), except at *Had-1* in Tha Thammachat population which was excessive of heterozygote. However, these deviations were not greater than the 5 % expected by chance ($P<0.05$). Considering the 2 different geographical areas ; island and mainland populations were not in the Hardy-Weinberg Equilibrium at 2.6 % and 1.3 % expected by chance, respectively. Only *Acon-1* showed genetic frequency equal to the Hardy-Weinberg Expectation ($x^2 = 0$) in Klong Son, Salak Petch and Tha Thammachat populations (Table 4.6).

The genetic variation among any populations of *Ae.aegypti* were compared as follow :

4.2.1 Ao Sapparod and Klong Son. Similarity of genetic frequencies between these 2 populations were found, except at *Idh-1, Mdh-1* and *Pgm-1*. Idh_{109} and Pgm_{127} alleles were found only in Klong Son population. *Mdh-1* showed highly differentiation between the 2 populations : Mdh_{100} was the most common allele in Ao Sapparod populations, Mdh_{90} Mdh_{100} occurred with equal frequency in Klong Son populations and Mdh_{112} allele was found only in Ao Sapparod population. Heterozygosity of *Acon-1* were relatively higher in Ao Sapparod than in Kong Son populations, whereas those of *Idh-1* and *Mdh-1* were relatively lower in Ao Sapparod than in Kong Son populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Pgm-1*.

4.2.2 Ao Sapparod and Dan Mai. Similarity of genetic frequencies between these 2 populations were found, except at *Idh-1, Mdh-1* and *Pgm-1*. Mdh_{112} allele was found only in Ao Sapparod population, whereas Idh_{109} allele was found only in Dan Mai populations. Pgm_{127} allele was found relatively higher in Ao Sapparod than in Dan Mai populations. Heterozygosity of *Idh*-1 and *Pgm-1* were relatively lower in Ao Sapparod than in Dan Mai populations, whereas those of *Mdh-1* locus was relatively higher in Ao Sapparod than in Dan Mai populations. Contingency chi-

square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Pgm-1* and *Idh*-1.

Table 4.1 Electrophoretic enzyme systems studied on *Aedes aegypti* adults.

Enzyme system	E.C.Number[a]	Symbol	No.Loci[b]	Buffer[c]
Aconitase	4.2.1.3	*Acon*	1	TMED
Adenylate kinase	2.7.4.3	*Aks*	1	TCss
Arginase kinase	2.7.3.3	*Argk*	1	TCss
Aldehyde oxidase	1.2.3.1	*Ao*	-	LiOH
Esterase	3.1.1.1	*Est*	1	Morph
Glycerol dehydrogenase	1.1.1.72	*Gcd*	-	TMED
Glutamate oxaloacetate transminase	2.6.1.1	*Got*	-	Morph
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6pdh*	1	TMED
α-glycerophosphate dehydrogenase	1.1.1.8	*Gpdh*	1	TCss
β-hydroxyacid dehydrogenase	1.1.1.30	*Had*	1	TMED
Hexokinase	2.7.1.1	*Hk*	-	TMED
Isocitric dehydrogenase	1.1.1.42	*Idh*	1	Morph
Malate dehydrogenase	1.1.1.37	*Mdh*	1	TMED
Malic enzyme	1.1.1.40	*Me*	-	LiOH
Mannose-6-phosphate isomerase	5.3.1.8	*Mpi*	1	TMED
6-Phosphogluconate dehydrogenase	1.1.1.43	*6Pgd*	1	TMED
Phosphoglucose isomerase	5.3.1.9	*Pgi*	1	TCss
Phosphoglucomutase	5.4.2.2	*Pgm*	1	Morph
Xanthine dehydroganase	1.2.1.37	*Xdh*	-	LiOH

[a] Enzyme commission number

[b] Number of scorable bands per phenotype

[c] Refer to electrophoretic buffer (See materials and Methods)

4.2.3 Ao Sapparod and Salak Petch. Distinctness of genetic frequencies between these 2 populations was found at *Idh-1* : Idh_{109} allele were found only in Salak Petch population, whereas Idh_{119} allele were found only in Ao Sapparod population. The remaining loci were similar in

genetic frequencies. Most of the heterozygotes were relatively higher in Ao Sapparod than in Salak Petch populations, except at *Pgm-1* locus which showed similarity frequency. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Pgm-1*.

4.2.4 Klong Son and Dan Mai. Distinctness of genetic frequencies between these 2 populations were found at *Acon -1, Idh-1, Pgm-1* and *Mdh-1*. $Acon_{94}$ and Idh_{109} alleles were found relatively lower in Klong Son than in Dan mai populations, whereas Pgm_{127} allele was found only in Klong Son populations. *Mdh-1* showed highly differentiation between the 2 populations : Mdh_{100} was the most common allele in Dan Mai populations, whereas Mdh_{90} and Mdh_{100} alleles were found in Klong Son populations with equal frequency. Heterozygosity of *Acon-1, Idh-1* and *Pgm-1* were relatively lower in Klong Son than in Dan mai populations, whereas those of *Mdh-1* were clearly high in Klong Son population. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Pgm-1* and *Idh*-1.

4.2.5 Klong Son and Salak Petch. Distinctness of alleles frequencies between these 2 populations were found at *Idh-1, Pgm-1* and *Mdh-1*. Idh_{119} and Pgm_{127} alleles were found only in Klong Son population. *Mdh-1* showed highly differentiation between the 2 populations : Mdh_{100} was the most common allele in Salak Petch population, whereas Mdh_{90} and Mdh_{100} alleles occurred with equal frequency in Klong Son population and Mdh_{112} allele was found only in Salak Petch population. Heterozygosity of *Idh-1* and *Mdh-1* were relatively higher in Klong Son than in Salak Petch populations. The remaining heterozygotes did not differ in genetic frequencies. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Pgm-1*.

4.2.6 Dan mai and Salak Petch. Distinctness of genetic frequencies between these 2 populations were found at *Acon-1, Idh-1* and *Mdh-1*. $Acon_{94}$ allele occurred higher in Dan Mai than in Salak Petch populations and $Acon_{100}$ allele occurred higher in Salak Petch than in Dan Mai populations. Idh_{119} allele was found only in Dan Mai populations and Mdh_{112} allele was found only in Salak Petch population. In addition, Idh_{109} allele was relatively higher in Dan Mai than in Salak Petch populations. Heterozygosity of *Acon-1*, *Idh-1* and *Mdh-1* were relatively higher in Dan Mai than in Salak Petch populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Idh*-1.

4.2.7 Tha Thammachat and Laem Ngob. There was a fixed allelic difference between these

Table 4.2 Allele frequencies at 13 loci in 6 populations of *Aedes aegypti.*

	Population					
Locus	AS	KS	DM	SP	TT	LM
Acon-1						
(N[a])	40	31	21	26	30	30
94	.063	.016	.119	.038	.017	.083
100	.938	.984	.881	.962	.983	.917
(H[b])	.119	.032	.215	.075	033	.155
Ak-1						
(N)	34	24	17	24	31	23
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	.000	.000	.000	.000	.000	.000
Argk-1						
(N)	44	31	21	29	30	33
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	.000	.000	.000	.000	.000	.000
Est-1						
(N)	33	38	15	33	43	32
100	.909	.895	.933	.970	.930	.938
103	.091	.105	.067	.030	.070	.063
(H)	.168	.191	.129	.060	.131	.119
G6pdh-1						
(N)	22	24	15	23	20	25
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	.000	.000	.000	.000	.000	.000
Gpdh-1						
(N)	49	25	17	44	56	45
85	.000	.000	.000	.000	.000	1.000
100	1.000	1.000	1.000	1.000	1.000	.000
(H)	.000	.000	.000	.000	.000	.000
Had-1						
(N)	18	30	16	29	25	26

Table 4.2 (Cont.)

	Population					
Locus	AS	KS	DM	SP	TT	LM
63	.000	.000	.000	.000	.400	.000
100	1.000	1.000	1.000	1.000	.600	1.000
(H)	.000	.000	.000	.000	.490	.000
Idh-1						
(N)	29	41	15	34	27	35
100	.879	.829	.733	.971	.833	.914
109	.000	.085	.200	.029	.093	.000
119	.121	.085	.067	.000	.074	.086
(H)	.216	.301	.432	.058	.297	.159
Mdh-1						
(N)	8	16	13	9	15	11
90	.125	.500	.154	.056	.567	.227
100	.750	.500	.846	.944	.233	.591
112	.125	.000	.000	.077	.200	.182
(H)	.433	.516	.271	.111	.605	.593
Mpi-1						
(N)	22	20	14	27	20	27
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	.000	.000	.000	.000	.000	.000
6Pgd-1						
(N)	44	23	30	25	50	23
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	.000	.000	.000	.000	.000	.000
Pgi-1						
(N)	59	28	26	33	49	25
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	.000	.000	.000	.000	.000	.000

Table 4.2 (Cont.)

Locus	Population					
	AS	KS	DM	SP	TT	LM
Pgm-1						
(N)	35	28	13	25	27	35
77	.100	.018	.192	.120	.093	.125
100	.900	.893	.808	.880	.778	.875
127	.000	.089	.000	.000	.130	.000
(H)	.183	.198	.323	.216	.377	.222

[a] Number of specimens analyzed for a given *Aedes aegypti* population

[b] Mean heterozygosity per locus (unbiased estimated)

2 populations at *Gpdh-1*. Genetic frequencies were differed at *Had-1*, *Idh-1*,*Mdh-1* and *Pgm-1*. Heterozygosity of *Had-1* was found only in Tha Thammachat population, whereas homogeneity of this locus was found in Laem Ngob population. Idh_{109} and Pgm_{127} alleles were found only in Tha Thammachat population. Especially, *Mdh-1* showed differentiation between the 2 populations : Mdh_{90} was the common allele in Tha Thammachat population while Mdh_{100} allele was the common allele in Laem Ngob population. Heterozygosity of *Acon-1* was lower in ThaThammachat than in Laem Ngob populations, whereas those of *Idh-1* and *Pgm-1* were higher in ThaThammachat than in Laem Ngob populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Est-1* and *Had-1*.

4.2.8 Tha Thammachat and Ao Sapparod. Differences of genetic frequencies between these 2 populations were found at *Had-1*, *Idh-1*,*Mdh-1* and *Pgm-1*. Had_{63} , Idh_{109} and Pgm_{127} alleles were found only in Tha Thammachat populations. *Mdh-1* showed differentiation between the 2 populations : Mdh_{90} was the common allele in Tha Thammachat populations while Mdh_{100} allele was the common allele in Ao Sapparod populations. Heterozygosity of *Mdh-1* and *Pgm-1* were higher in ThaThammachat than in Ao Sapparod populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Est-1*, *Had-1* and *Pgm-1*.

4.2.9 Tha Thammaachat and Klong Son. Differences of genetic frequencies between the 2

populations were found at *Had-1*, *Mdh-1* and *Pgm-1* loci. Had_{63} allele was found only in Tha Thammachat, whereas Mdh_{100} allele was lower in Tha Thammaachat than in Klong Son populations. *Mdh-1* showed differentiation between the 2 populations : Mdh_{90} was the common allele in Tha Thammachat populations, Mdh_{90} and Mdh_{100} alleles occurred with equal frequency in Klong Son populations and Mdh_{112} allele was found only in Tha Thammachat populations. Heterozygosity of *Pgm-1* were higher in Tha Thammachat than in Klong Son populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Est-1*, *Had-1* and *Pgm-1*.

4.2.10 Tha Thammaachat and Dan Mai. Differences of genetic frequencies between the 2 populations were found at *Acon-1*, *Had-1*, *Idh-1*, *Mdh-1* and *Pgm-1*. $Acon_{94}$ and Idh_{109} alleles were found relatively lower in Tha Thammaachat than in Dan Mai populations. Had_{63} and Pgm_{127} alleles were found only in Tha Thammachat populations. There were a large differences in *Mdh-1*: Mdh_{90} was the common allele in Tha Thammachat populations while Mdh_{100} was the common allele in Dan Mai populations and Mdh_{112} allele was found only in Tha Thammachat population. Heterozygosity of *Acon-1* and *Idh-1* were relatively lower in Tha Thammachat than in Dan Mai populations, whereas *Mdh-1* was relatively in higher Tha Thammachat than in Dan Mai populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Est-1*, *Had-1* and *Idh-1*.

4.2.11 Tha Thammaachat and Salak Petch. Differences of genetic frequencies between the 2 populations were found at *Had-1*, *Idh-1*, *Mdh-1* and *Pgm-1*. Had_{63}, Idh_{119} and Pgm_{127} alleles were found only in Tha Thammachat populations. Mdh_{100} was the most common allele in Salak Petch populations, whereas Mdh_{90} was the most common allele in Tha Thammaachat population. Exception at *Had-1* locus, heterozygosity of *Idh-1*, *Mdh-1* and *Pgm-1* were found relatively higher in Tha Thammaachat than in Salak Petch populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations at *Est-1* and *Had-1*.

4.2.12 Laem Ngob and Ao Sapparod. There was a fixed allelic difference between these 2 populations at *Gpdh-1*. No differences of alleles and heterozygosity frequencies between the 2 populations, except at *Mdh-1*. Mdh_{90} allele and heterozygosity of *Mdh-1* were higher in Laem Ngob than in Ao Sapparod populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations *Pgm-1*.

4.2.13 Laem Ngob and Klong Son. A fixed allelic difference between the 2 populations

was marked at *Gpdh-1*. Difference of genetic frequencies between the 2 populations were found at *Acon-1, Idh-1, Mdh-1* and *Pgm-1*. $Acon_{94}$ allele was less higher in Laem Ngob than in Klong Son populations, whereas and Idh_{100} Mdh_{90} alleles were relatively lower in Laem Ngob than in Klong Son populations. Mdh_{100} and Mdh_{112} alleles were the most common allele and occurred only in Laem Ngob populations, respectively. Mdh_{90} and Mdh_{100} alleles occurred with equal frequency in Klong Son populations. Heterozygosity of *Acon-1* was relatively higher in Laem Ngob than in Klong Son populations, whereas *Idh-1* was relatively lower in Laem Ngob than in Klong Son populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations *Pgm-1*.

4.2.14 Laem Ngob and Dan Mai. A fixed allelic difference between the 2 populations was marked at *Gpdh-1*. Difference of genetic frequencies between the 2 populations were found at *Idh-1, Mdh-1* and *Pgm-1*. Idh_{109} allele was found only in Dan Mai population, whereas Mdh_{112} allele was found only in Laem Ngob population. Heterozygosity of *Idh-1* was relatively lower in Laem Ngob than in Dan Mai populations and *Mdh-1* was relatively higher in Laem Ngob than in Dan Mai populations. Contingency chi-square tests showed deviations from Hardy-Weinberg Equilibrium between the 2 populations *Idh-1*.

4.2.15 Laem Ngob and Salak Petch. A fixed allelic difference between the 2 populations was marked at *Gpdh-1*. Difference of genetic frequencies were found at *Idh-1* and *Mdh-1*. Idh_{109} and Idh_{119} alleles were found only in Salak Petch and Laem Ngob populations, respectively. Mdh_{100} allele was relatively lower in Laem Ngob than in Salak Petch populations. Heterozygosity of *Idh-1* and *Mdh-1* were relatively higher in Laem Ngob than in Salak Petch populations. Contingency chi-square tests showed no deviations from Hardy-Weinberg Equilibrium between the 2 populations.

Table 4.3 Measures of genetic variability at 13 loci in 6 populations of *Aedes aegypti.*

Population	Mean sample size per locus	Mean no. alleles per locus	% of loci polymorphic[a]	Mean heterozygosity: Direct count	Mean heterozygosity: Hardy- Weinberg expected[b]
Ao Sapparod	33.6 (3.9)	1.5 (.2)	38.5	.077 (.039)	.086 (.037)
Klong Son	27.6 (1.9)	1.5 (.2)	30.8	.083 (.038)	.095 (.045)
Dan Mai	17.9 (1.4)	1.5 (.2)	38.5	.061 (.023)	.105 (.043)
Salak Petch	27.8 (2.2)	1.4 (.1)	15.4	.036 (.015)	.040 (.018)
Tha Thammachat	32.5 (3.6)	1.7 (.2)	38.5	.171 (.077)	.149 (.060)
Laem Ngob	28.5 (2.3)	1.5 (.2)	38.5	.109 (.062)	.096 (.047)

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed .95

[b] Unbiased estimate (Nei, 1978)

Value in parentheses are standard errors

Table 4.4 Measures of genetic variability at 13 loci in 6 populations of *Aedes aegypti.*

Population	Mean sample size per locus	Mean no. alleles per locus	% of loci polymorphic[a]	Mean heterozygosity: Direct count	Mean heterozygosity: Hardy- Weinberg expected[b]
Ao Sapparod	33.6 (3.9)	1.5 (.2)	38.5	.077 (.039)	.086 (.037)
Klong Son	27.6 (1.9)	1.5 (.2)	38.5	.083 (.038)	.095 (.045)
Dan Mai	17.9 (1.4)	1.5 (.2)	38.5	.061 (.023)	.105 (.043)
Salak Petch	27.8 (2.2)	1.4 (.1)	38.5	.036 (.015)	.040 (.018)
Tha Thammachat	32.5 (3.6)	1.7 (.2)	46.2	.171 (.077)	.149 (.060)
Laem Ngob	28.5 (2.3)	1.5 (.2)	38.5	.109 (.062)	.096 (.047)

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed .99

[b] Unbiased estimate (Nei, 1978)

Value in parentheses are standard errors

Table 4.5 Chi-square tests for deviation from Hardy-Weinberg equilibrium among the 6 populations of *Aedes aegypti*.

Population	Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
Ao Sapparod	*Acon-1*	94/94	0	.127			
		94/100	5	4.747			
		100/100	35	35.127			
					.141	1	.708
	Est-1	100/100	28	27.231			
		100/103	4	5.538			
		103/103	1	.231			
					3.013	1	.083
	Idh-1	100/100	23	22.368			
		100/109	5	6.263			
		109/109	1	.368			
					1.355	1	.244
	Mdh-1	90/90	0	.067			
		90/100	2	1.600			
		90/112	0	.267			
		100/100	4	4.400			
		100/112	2	1.600			
		112/112	0	.067			
					.636	3	.888
	Pgm-1	77/77	2	.304			
		77/100	3	6.391			
		100/100	30	28.304			
					11.348	1	.001
Klong Son	*Acon-1*	94/94	0	.000			
		94/100	1	1.000			
		100/100	30	30.000			
					.000	1	1.000
	Est-1	100/100	31	30.373			

Table 4.5 (Cont.)

Population	Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
		100/103	6	7.253			
		103/103	1	.373			
					1.281	1	.258
	Idh-1	100/100	27	28.123			
		100/109	7	5.877			
		100/119	7	5.877			
		109/109	0	.259			
		109/119	0	.605			
		119/119	0	.259			
					1.598	3	.660
	Mdh-1	90/90	5	3.871			
		90/100	6	8.258			
		100/100	5	3.871			
					1.276	1	.259
	Pgm-1	77/77	0	.000			
		77/100	0	.909			
		100/127	1	.091			
		100/100	23	22.273			
		100/127	4	4.545			
		127/127	0	.182			
					10.271	3	.016
Dan Mai	*Acon-1*	94/94	1	.244			
		94/100	3	4.512			
		100/100	17	16.244			
					2.886	1	.089
	Est-1	100/100	13	13.034			
		100/103	2	1.931			
		103/103	0	.034.			
					.037	1	.847

Table 4.5 (Cont.)

Population	Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
	Idh-1	100/100	10	7.966			
		100/109	0	4.552			
		100/119	2	1.517			
		109/109	3	.517			
		109/119	0	.414			
		119/119	0	.034			
					17.590	3	.001
	Mdh-1	90/90	1	.240			
		90/100	2	3.527			
		100/100	10	9.240			
					3.126	1	.077
	Pgm-1	77/77	1	.444			
		77/100	3	4.200			
		100/100	9	8.400			
					1.286	1	.257
Salak Petch	*Acon-1*	94/94	0	.020			
		94/100	2	1.961			
		100/100	24	24.020			
					.020	1	.886
	Est-1	100/100	31	31.015			
		100/103	2	1.969			
		103/103	0	.015			
					.016	1	.900
	Idh-1	100/100	32	32.017			
		100/109	2	1.970			
		109/109	1	.015			
					.015	1	.901
	Mdh-1	90/90	0	.000			
		90/100	1	1.000			

Table 4.5 (Cont.)

Population	Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
		100/100	8	8.000			
					.000	1	1.000
	Pgm-1	77/77	1	.306			
		77/100	4	5.388			
		100/100	20	19.306			
					1.955	1	.162
Tha Thammachat	*Acon-1*	94/94	0	0. 000			
		94/100	1	1.000			
		100/100	29	29.000			
					.000	1	1.000
	Est-1	100/100	38	37.176			
		100/103	4	5.647			
		103/103	1	.176			
					4 .342	1	.037
	Had-1	63/100	0	3.878			
		63/100	20	12.245			
		100/100	5	8.878			
					10.483	1	.001
	Idh-1	100/100	19	18.679			
		100/109	3	4.245			
		100/119	4	3.396			
		109/109	1	.189			
		109/119	0	.377			
		119/119	0	.113			
					4.457	3	.216
	Mdh-1	90/90	4	4.690			
		90/100	4	4.103			
		90/112	5	3.517			
		100/100	1	.724			

Table 4.5 (Cont.)

Population	Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
		100/112	1	.448			
		112/112	0	.517			
					1.490	3	.685
	Pgm-1	77/77	1	.189			
		77/100	4	3.962			
		77/127	0	.660			
		100/100	16	16.245			
		100/127	7	5.547			
		127/127	0	.396			
					5.163	3	.160
Laem Ngob	*Acon-1*	94/94	0	.169			
		94/100	5	4.661			
		100/100	25	25.169			
					.195	1	.659
	Est-1	100/100	28	28.095			
		100/103	4	3.810			
		103/103	0	.095			
					.105	1	.746
	Idh-1	100/100	30	29.217			
		100/109	4	5.565			
		109/109	1	.217			
					3.279	1	.070
	Mdh-1	90/90	0	.476			
		90/100	5	3.095			
		90/112	0	.952			
		100/100	2	3.714			
		100/112	4	2.476			
		112/112	0	.286			
					4.615	3	.202

Table 4.5 (Cont.)

Population	Locus	Genotype	Observed frequency	Expected frequency	Chi-square	DF	P
	Pgm-1	77/77	1	.507			
		77/100	7	7.986			
		100/100	28	27.507			
					.610	1	.435

Table 4.6 Coefficients for heterozygote deficiency or excess at all 6 populations of *Aedes aegypti*.

Population	Locus	Observed heterozygotes	Expected heterozygotes	Fixation index (F)	D
Ao Sapparod	*Acon-1*	5	4.747	-.067	.053
	Est-1	4	5.538	.267	-.278
	Idh-1	5	6.263	.188	-.202
	Mdh-1	4	3.467	-.231	.154
	Pgm-1	3	6.391	.524	-.531
Klong Son	*Acon-1*	1	1.000	-.016	.000
	Est-1	6	7.253	162	-.173
	Idh-1	14	12.358	-.147	.133
	Mdh-1	6	8.258	.250	-.273
	Pgm-1	5	5.545	.082	-.098
Dan mai	*Acon-1*	3	4.512	.319	-.335
	Est-1	2	1.931	-.071	.036
	Idh-1	2	6.483	.681	-.691
	Mdh-1	2	3.520	.409	-.432
	Pgm-1	3	4.200	.257	-.286
Salak Petch	*Acon-1*	2	1.961	-.040	.020
	Est-1	2	1.969	-.031	.016
	Idh-1	2	1.970	-.030	.015
	Mdh-1	1	1.000	-.059	.000

Table 4.6 (Cont.)

Population	Locus	Observed heterozygotes	Expected heterozygotes	Fixation index (F)	D
	Pgm-1	4	5.388	.242	-.258
Tha Thammachat	*Acon-1*	1	1.000	-.017	.000
	Est-1	4	5.647	.283	-.292
	Had-1	20	12.245	-.667	.633
	Idh-1	7	8.019	.111	-.127
	Mdh-1	10	9.069	-.141	.103
	Pgm-1	10	10.170	-.002	-.017
Laem Ngob	*Acon-1*	5	4.661	-.091	.073
	Est-1	4	3.810	-.067	.050
	Idh-1	4	5.565	.271	-.281
	Mdh-1	9	6.524	-.445	.380
	Pgm-1	7	7.986	.111	-.123

4.3 Gene flow

Wright's *F*-statistic (Wright, 1978) (Table 4.7) : the fixation indices of individuals relative to the total subpopulation (*FIS*), the mean fixation indices of individuals relative to the total population (*FIT*) and the measure of genetic differentiation among subpopulation (*FST*) in all three geographical areas were greater than zero ($P<0.05$), except at *FIS* value in mainland population (Table 4.7). Positive *FIS* indicate excess of homozygotes and the negative *FIS* display excess of heterozygotes in the population (Wright, 1951). Excess of homozygotes were observed within combined (*FIS* = 0.032) and island (*FIS* = 0.174) populations, whereas excess of heterozygotes was observed within mainland populations (*FIS* = -0.175). *FIT* was low in both island (*FIT*= 0.250) and mainland (*FIT*= 0.174) populations. Negative *FIT* value was observed at *Had-1*(*FIT* = -.071) when considering in combined populations. Genetic differentiation was observed among all populations (*FST* = 0.270) ; considering the separated areas : *FST* estimates indicating genetic differentiations between mainland populations (*FST* = 0.077) was lower than those among island populations (*FST* = 0.297).

Considering in combined populations heterozygote deficiencies were found at *Acon-1, Est-*

1, Pgm-1 and *Idh-1* (*FIS* = 0.067, 0.118, 0.178 and 0.247, respectively). The others 2 loci showed more heterozygotes than expected in populations. *FIS* value was zero at *Gpdh-1*. Genetic differentiations among all 6 populations (*FST* = 0.270) were found at *Had-1* and *Mdh-1* : *Had-1* showed very great differentiation (*FST* > 0.25 ; *FST* = 0.357), whereas *Mdh-1* showed moderately great differentiation (0.25 >*FST*> 0.15 ; *FST* = 0.200). The remaining loci showed negligible differentiation (*FST* < 0.05). Had_{63} allele was observed only in Tha Thammachat population at a frequency of 40 % (Table 4.2). Mdh_{100} allele was observed in all island populations at high frequencies ranged from 50 to 94.4 %, whereas the mainland populations showed a lower frequencies range from 23.3 to 50 % (Table 4.2).

Table 4.7 F-statistics analysis of polymorphic in 6 populations of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]	Nm [d]
Acon-1	.067	.091	.026	9.365
Est-1	.118	.126	.008	31
Gpdh-1	0.000	1.000	1.000	0.000
Had-1	-.667	-.071	.357	.450
Idh-1	.247	.282	.047	5.069
Mdh-1	-.084	.133	.200	1.000
Pgm-1	.178	.203	.030	8.083
Mean	.032	.294	.270	0.676

[a] Fixation indices of individuals relative to the total subpopulation

[b] Fixation indices of individuals relative to the total population

[c] F-statistics

[d] Number of migrant = (1- *F(ST)*/4 *F(ST)*)

Considering in island populations : *Acon-1, Est-1, Idh-1, Mdh-1* and *Pgm-1* showed heterozygote deficiencies (*FIS*=0.129, 0.119, 0.283, 0.103 and 0.269, respectively) (Table 4.8). This suggest that random mating was occurred within population with restricted migration. Genetic differentiations among island populations were found at *Mdh-1* and *Idh-1*: *Mdh-1* showed very great differentiation (*FST* = 0.158), whereas *Idh-1* showed small differentiation (*FST* = 0.058). The remaining loci showed negligible differentiation.

Table 4.8 F-statistics analysis of polymorphic in island populations of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]	Nm [d]
Acon-1	.129	.152	.026	9.365
Est-1	.119	.129	.012	20.583
Idh-1	.283	.325	.058	4.060
Mdh-1	.103	.245	.158	1.332
Pgm-1	.269	.290	.029	8.371
Mean	.187	.250	.077	2.997

[a] Fixation indices of individuals relative to the total subpopulation

[b] Fixation indices of individuals relative to the total population

[c] F-statistics

[d] Number of migrant = (1- *F(ST)*/4 *F(ST)*)

Considering in mainland populations : *Est-1, Idh-1,* and *Pgm-1* showed heterozygote deficiencies (*FIS* = 0.117, 0.167 and 0.040, respectively) (Table 4.9), whereas *Acon-1, Had-1* and *Mdh-1* showed more heterozygotes than expected within populations (*FIS* = -0.078, -0.667 and -0.291, respectively). *FIS* value was zero at *Gpdh-1*. Genetic differentiations between the 2 populations were found at *Had-1* and *Mdh-1* : *Had-1* showed very great differentiation (*FST* = 0.250) and *Mdh-1* showed small differentiation (*FST*=0.096). The remaining loci showed negligible differentiation.

Wright's *F*-statistic were also analyzed between any populations as follow :

4.3.1 Ao Sapparod and Klong Son. Heterozygote deficiencies within populations were found at *Est-1, Mdh-1* and *Pgm-1*(+*FIS*) (Appendix Table 1), whereas homozygote deficiencies were found at *Acon-1* and *Idh-1*. However, *FIS* value was more differ longer from zero at *Acon-1* than at *Idh-1*. *Mdh-1* showed very great differentiation (*FST* = 0.108) between Ao Sapparod and Klong Son populations. The mean *FST* value was small, indicating less genetic differentiation between the 2 populations.

4.3.2 Ao Sapparod and Dan Mai populations. Heterozygote deficiencies within populations were found at all loci (+*FIS*) (Appendix Table 2). This *FIS* estimates were differ longer from zero, except at *Mdh-1*. Most of the loci showed negligible genetic differentiations. No genetic

Table 4.10 F-statistics analysis of polymorphic in 2 mainland populations of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]	Nm [d]
Acon-1	-.078	-.053	.023	10.620
Est-1	.117	.117	0.000	0.000
Gpdh-1	0.000	1.000	1.000	0.000
Had-1	-.667	-.250	.250	0.750
Idh-1	.167	.181	.017	14.456
Mdh-1	.291	-.167	.096	2.354
Pgm-1	.040	.062	.023	10.620
Mean	-.175	.174	.297	0.592

[a] Fixation indices of individuals relative to the total subpopulation

[b] Fixation indices of individuals relative to the total population

[c] F-statistics

[d] Number of migrant = (1- *F(ST)*/4 *F(ST)*)

differentiation (*FST* = 0.023) between the 2 populations were found.

4.3.3 Ao Sapparod and Salak Petch. Heterozygote deficiencies within populations were found at *Est-1, Idh-1* and *Pgm-1*(+*FIS*) (Appendix Table 3). The remaining loci : *Acon-1* and *Mdh-1* were homozygote deficiencies. *Mdh-1* showed small differentiation (*FST* = 0.054), whereas the others loci showed negligible genetic differentiations between Ao Sapparod and Klong Son populations. No genetic differentiation (*FST* = 0.028) between the 2 populations were found.

4.3.4 Klong Son and Dan Mai. Heterozygote deficiencies within populations were found at *Acon-1*, *Est-1, Idh-1, Mdh-1* and *Pgm-1* (Appendix Table 4). *Mdh-1* showed moderated great differentiation (*FST* = 0.136), whereas the others loci showed negligible genetic differentiations between Klong Son and Dan Mai. Small genetic differentiation (*FST* = 0.061) between the 2 populations were found.

4.3.5 Klong Son and Salak Petch. Heterozygote deficiencies within populations were found at *Acon-1* and *Idh-1*, whereas homozygote deficiencies within populations were found at

Est-1, Mdh-1 and *Pgm-1* (Appendix Table 5). Most of the loci showed negligible genetic differentiations, except at *Mdh-1* showed moderated genetic differentiation (*FST* = 0.246). Small genetic differentiation (*FST* = 0.117) between Klong Son and Salak Petch populations were found.

4.3.6 Dan mai and Salak Petch. Heterozygote deficiencies within populations were found at *Acon-1, Idh-1, Mdh-1* and *Pgm-1* with large values differ from zero, whereas homozygote deficiencies within populations was found at only in *Est-1*(Appendix Table 6). Exception at *Idh-1*, most of the loci showed negligible genetic differentiations. No genetic differentiations (*FST* = 0.036) between Dan mai and Salak Petch populations were found.

4.3.7 Tha Thammachat and Ao Sapparod. Heterozygote deficiencies within populations were found at 3 loci : *Est-1, Idh-1* and *Pgm-1*(+*FIS*) (Appendix Table 7), whereas homozygote deficiencies were found at *Had-1, Mdh-1* and *Acon-1.* Of the positive *FIS* values, *Had-1* value showed the largest differ from zero. Genetic differentiations between populations were found at *Had-1* and *Mdh-1* : *Had-1* showed very great differentiation (*FST* = 0.250), whereas *Mdh-1* showed moderate great differentiation (*FST* = 0.190). However, small genetic differentiations (*FST* = 0.124) between Tha Thammachat and Ao Sapparod populations were found.

4.3.8 Tha Thammaachat and Klong Son. Heterozygote deficiencies within populations were found at 3 loci : *Est-1, Mdh-1* and *Pgm-1*(+*FIS*) (Appendix Table 8), whereas homozygote deficiencies were found at *Had-1, Idh-1* and *Acon-1.* Genetic differentiations between populations were found at *Had-1* and *Mdh-1* : *Had-1* showed very great differentiation (*FST* = 0.250), whereas *Mdh-1* showed small differentiation (*FST* = 0.051). Genetic differentiation between Tha Thammaachat and Klong Son populations were small (*FST* = 0.069).

4.3.9 Tha Thammaachat and Dan Mai. Exception at *Had-1*, heterozygote deficiencies within populations were found at most of the loci (+*FIS*) (Appendix Table 9). *Had-1* and *Mdh-1* (*FST* = 0.250 and 0.258, respectively) showed very great genetic differentiations between the 2 populations. Small genetic differentiations between Tha Thammaachat and Dan Mai populations were found (*FST* = 0.132)

4.3.10 Tha Thammaachat and Salak Petch. Heterozygote deficiencies within populations were found at *Est-1, Idh-1* and *Pgm-1*(+*FIS*) (Appendix Table 10), whereas homozygote deficiencies were found at *Had-1, Mdh-1* and *Acon-1.* Both *Mdh-1* and *Had-1*showed very great genetic differentiations between populations (*FST* = 0.369 and 0.250, respectively). Moderate

genetic differentiation (*FST* = 0.199) between Tha Thammaachat and Salak Petch populations were found.

4.3.11 Laem Ngob and Ao Sapparod. Heterozygote deficiencies within populations were found at *Est-1, Idh-1* and *Pgm-1*(+*FIS*) (Appendix Table 11), whereas homozygote deficiencies were found at *Mdh-1* and *Acon-1*. *Gpdh-1* showed geographically genetic structure (*FST* = 1.000), whereas the others loci showed negligible genetic differentiations. A very great genetic differentiation (*FST* = 0.308) between Laem Ngob and Ao Sapparod populations were found.

4.3.12 Laem Ngob and Klong Son. Heterozygote deficiencies within populations were found at *Est-1* and *Pgm-1*(+*FIS*) (Appendix Table 12), whereas homozygote deficiencies were found at *Mdh-1, Acon-1* and *Idh-1*. *Gpdh-1* showed geographically genetic structure (*FST* = 1.000) and *Mdh-1* showed small genetic differentiation, whereas the others loci showed negligible genetic differentiations. A very great genetic differentiation (*FST* = 0.308) between Laem Ngob and Klong Son populations were found.

4.3.13 Laem Ngob and Dan Mai. Heterozygote deficiencies within populations were found at 3 loci : *Acon-1, Idh-1* and *Pgm-1*(+*FIS*) (Appendix Table 13), whereas homozygote deficiencies were found at *Est-1* and *Mdh-1*. *Gpdh-1* showed geographically genetic structure (*FST* = 1.000), *Idh-1* and *Mdh-1* showed small genetic differentiations, whereas the others loci showed negligible genetic differentiations. A very great genetic differentiation (*FST* = 0.302) between Laem Ngob and Dan Mai populations were found.

4.3.14 Laem Ngob and Salak Petch. Heterozygote deficiencies within populations were found at 2 loci : *Idh-1* and *Pgm-1*(+*FIS*) (Appendix Table 14), whereas homozygote deficiencies were found at *Mdh-1*,*Acon-1* and *Est-1*. *Gpdh-1* showed geographically genetic structure (*FST* = 1.000) and *Mdh-1* showed small genetic differentiation. A very great genetic differentiation (*FST* = 0.391) between Laem Ngob and Salak Petch populations were found.

Summary of *FST* pairwise comparisons were given in Table 4.11. The genetic differentiation, *FST* between any of *Ae. aegypti* from 6 populations with *FST* values ranged from 0.023 (between Ao Sapparod and Dan Mai) to 0.391 (between Laem Ngob and Salak Petch). Five of 15 comparisons (Laem Ngob – Ao Sapparod, Laem Ngob – Klong Son, Laem Ngob – Dan Mai, Laem Ngob – Salak Petch and Tha Thammachat – Laem Ngob) showed very great genetic differentiations (FST > 0.25). Number of migrants was calculated using the formula [Nm = (1-*FST*)/ 4*FST*] (Wright 1951), to indicate a level of gene flow. The number of migrants were

2.997, 0.592 and 0.676 individuals per generation in island, mainland and combined populations, respectively.

Table 4.10 F-statistics at all loci between any of 6 populations *Aedes aegypti*.

Population compared	*F(IS)*[a]	*F(IT)*[b]	*F(ST)*[c]	Nm[d]
Ao Sapparod – Klong Son	.089	.136	.052	4.558
Ao Sapparod –Dan Mai	.252	.269	.023	10.620
Ao Sapparod – Salak Petch	.073	.098	.028	8.679
Klong Son – Dan Mai	.259	.304	.061	3.848
Klong Son – Salak Petch	.096	.202	.117	1.887
Dan Mai – Salak Petch	.310	.335	.036	6.694
Tha Thammachat – Laem Ngob	-.175	.174	.297	0.591
Tha Thammachat – Ao Sapparod	-.087	.047	.124	1.766
Tha Thammachat – Klong Son	-.067	.007	.069	3.373
Tha Thammachat –Dan Mai	.061	.184	.132	1.650
Tha Thammachat – Salak Petch	-.124	.100	.199	1.006
Laem Ngob – Ao Sapparod	-.057	.269	.308	0.562
Laem Ngob – Klong Son	-.033	.286	.308	0.562
Laem Ngob – Dan Mai	.127	.390	.302	0.578
Laem Ngob – Salak Petch	-.098	.331	.391	0.389

[a] *FIS* Fixation indices of individuals relative to the total subpopulation

[b] *FIT* Fixation indices of individuals relative to the total population

[c] *FST* F-statistics

[d] Nm Number of migrant (1- *F(ST)*/4 *F(ST)*)

Table 4.11 Matrix of Nei's (1978) unbiased genetic identity above diagonal and unbiased genetic distance below diagonal

Population	Ao Sapparod	Klong Son	Dan Mai	Salak Petch	Tha Thammachat	Laem Ngob
Ao Sapparod	*****	.992	.999	.999	.968	.917
Klong Son	.008	*****	.989	.983	.983	.911
Dan Mai	.001	.011	*****	.998	.961	.910
Salak Petch	.001	.017	.002	*****	.954	.911
Tha Thammachat	.033	.017	.039	.047	*****	.889
Laem Ngob	.087	.094	.094	.093	.117	*****

4.4 Cluster analysis

Nei's (1978) genetic distance among 6 populations ranged from 0.001 to 0.117 and genetic identical ranged from 0.889 to 0.999 (Table 4.11) ; Roger's genetic distance ranged from 0.084 to 0.299 and genetic identity ranged from 0.066 to 0.317 (Table 4.12). Nei's genetic distances were used to produced a phenogram according to UPGMA method (Figure 4.2). A high level of genetic similarity of was found among the 4 island populations. The lowest level of genetic variability were found among Ao Sapparod, Dan Mai and Salak Petch populations and between Dan Mai and Salak Petch populations, where populations were differed at a maximum Nei's genetic distance of 0.001 and 0.002, respectively (Figure 4.2 and Table 4.11). The highest level of genetic variability was found between the 2 mainland populations where populations were differed from each other at a Nei's genetic distance of 0.117. These results showed that the genetic frequencies obtained from the isozyme analysis were sufficient to different between island and mainland populations. The phenogram showed that all *Ae. aegypti* populations were separated into 2 geographical groups : mainland and island. However, mainland group could be divided into 2 subgroups : since *Ae. aegypti* in Tha Thammachat population was distinct from Laem Ngob population. The pattern of the phenogram derived from Nei's genetic distance was similar to those of Roger'distance (Wright, 1978) (Figure 4.3) and Cavalli-Sforza & Edward (1967) chord and acr distance. (Figure 4.4).

Table 4.12 Matrix of modified Roger's (Wright, 1978) genetic identity above diagonal and Cavalli-Sforza & Edward (1967) chord and acr genetic distance below diagonal.

Population	Ao Sapparod	Klong Son	Dan Mai	Salak Petch	Tha Thammachat	Laem Ngob
Ao Sapparod	*****	.098	.066	.059	.179	.280
Klong Son	.125	*****	.113	.132	.133	.288
Dan Mai	.108	.113	*****	.072	.194	.290
Salak Petch	.100	.132	.084	*****	.214	.291
Tha Thammachat	.178	.152	.193	.209	*****	.317
Laem Ngob	.252	.279	.276	.274	.299	*****

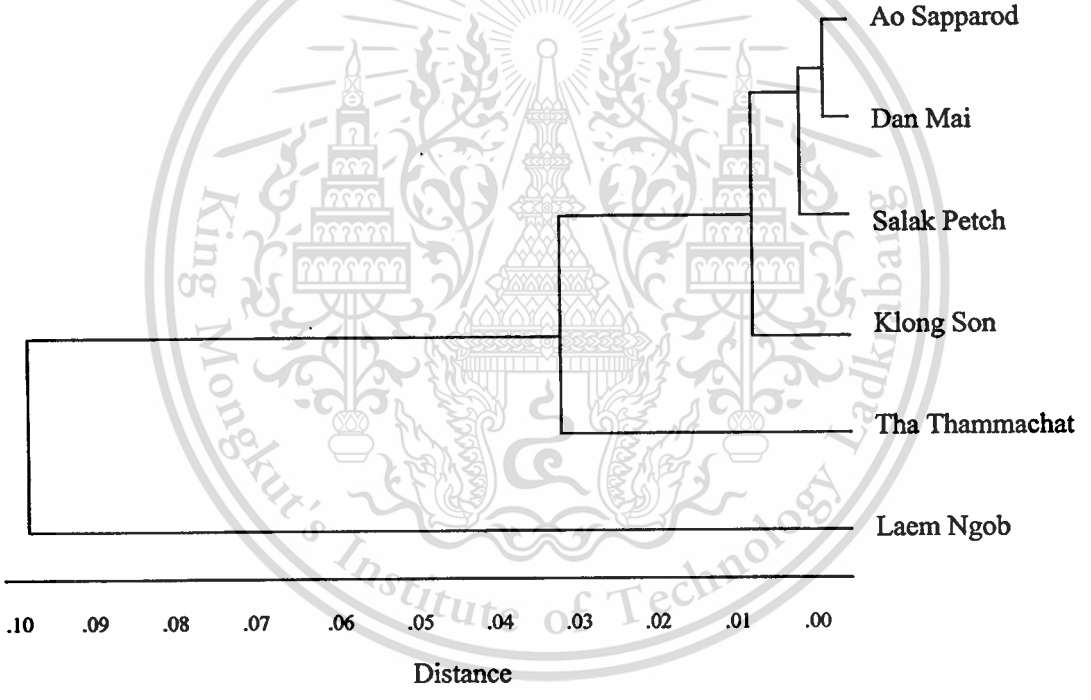

Figure 4. 2 UPGMA phenogram from Nei's (1978) unbiased genetic distance matrix for all populations of *Aedes aegypti* (Cophenetic correlation = .977)

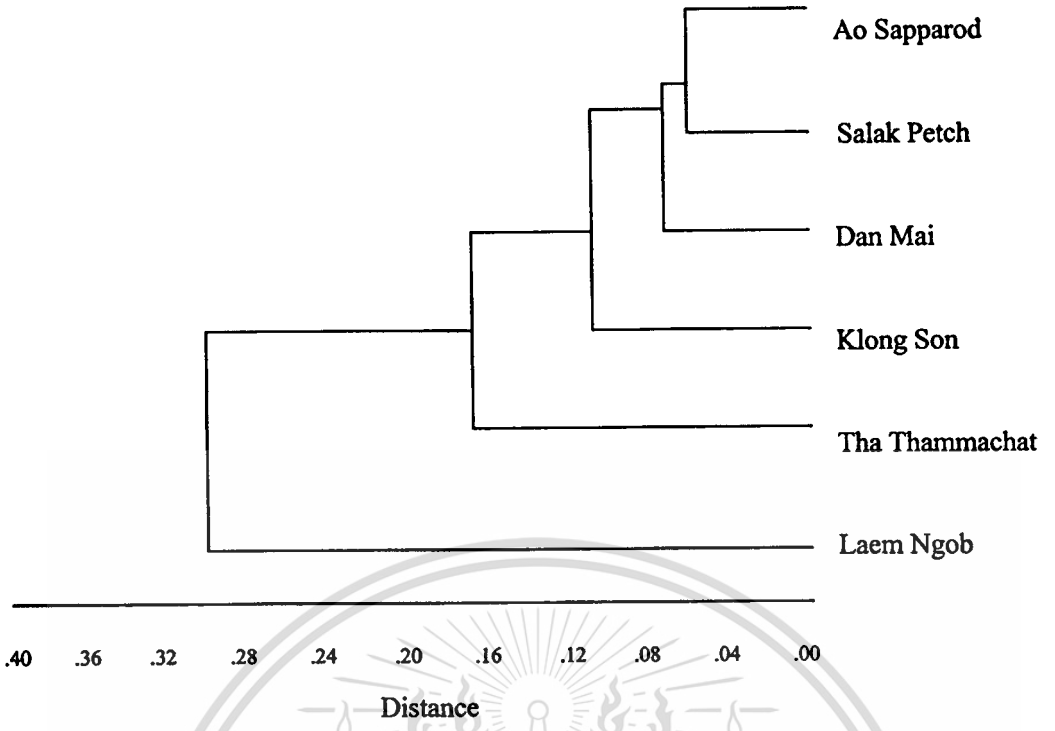

Figure 4. 3 UPGMA phenogram from modified Roger's (Wright 1978) genetic distance matrix or all populations of *Aedes aegyspti* (Cophenetic correlation = .979).

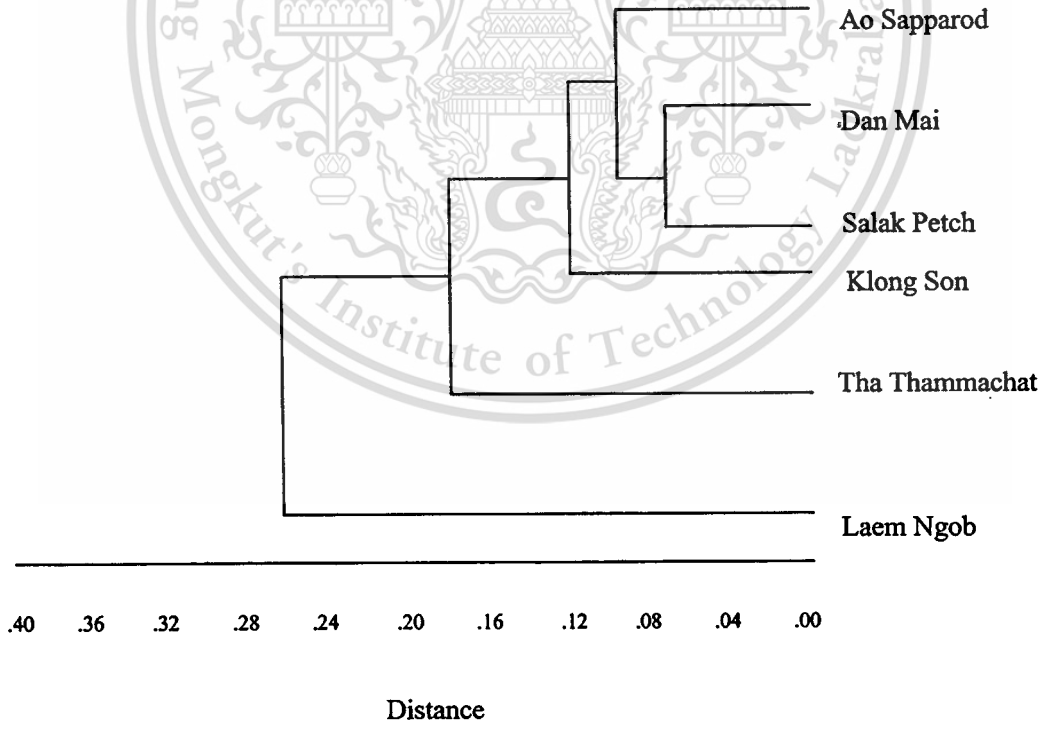

Figure 4. 4 UPGMA phenogram from Cavalli-Sforza & Edwards (1967) chord distance matrix of all populations of *Aedes aegyspti* (Cophenetic correlation = .980).

CHAPTER 5

DISCUSSION

Isozyme analysis of *Aedes aegypti* from mainland and island of Trat Province indicated a low level of genetic exchange as estimated by the fixation indices of individuals relative to the total subpopulation (*FIS* = 0.032) and population differentiation tend to be high between mainland and island. This could be result from a low level of gene flow among *Ae. aegypti* populations. A small Nm value supported a low level of gene flow among all populations. The genetic differentiation among 6 populations was also found by the large *FST* (0.270) value when all loci were consider together. Nei's genetic distance value supported the genetic differentiation among populations. There was a fixed allelic difference between the 2 mainland populations at *Gpdh-1*.

The percentage of polymorphic loci in island were varied from 15.4 % to 38.5 %, whereas in mainland populations were uniform at 38.5%. This results were differ from the previously reported in Surat-thanee Province, in which the percentage of polymorphic loci were lower in Samui island than in mainland (Chareonviriyaphap and Lerdthusnee 2002). Most of the percentage of polymorphic loci in this study were lower than the range from 37.5% to 50 % reported before for natural Brazilian *Ae. aegypti* populations (Dinardo-Miranda and Contel 1996). Exception in Salak Petch population, the percentage of polymorphic loci in this study were within the range from 27.3% to 63.6 % for Argentinean *Ae. aegypti* populations (de Sousa *et al.* 2000).

The polymorphic loci (*Est-1, Idh-1* and *Pgm-1*) deviated from Hardy-Weinberg equilibrium This finding due to heterozygosity deficiencies, likely resulted from null alleles (alleles with no function). The presence of null alleles lead to an underestimation of heterozygosity (Pasture *et al.* 1988). These deviations were not significantly greater than 5 % expected by chance, similar to the study of genetic variation in *Ae.aegypti* that related with *Dirofiralia immitis* (Nayar and Knight 2002). In addition, the particular genetic structure at *Had-1* was found only in Tha Thammachat population. This could have resulted from either recent or historical effects. Since selection can act on a single locus, whereas gene flow, genetic drift and historical effects can potentially affect all loci equally. In this study the mean expected heterozygosity ranged from 0.040 to 0.149 were similar to the range of 0.007 to 0.13 for Houston *Ae. aegypti* (Harrington *et al.* 1984). In the other hand, the higher mean expected heterozygosity of *Ae. aegypti* were

obtained from Brazil (0.48 to 0.53) (Dinardo-Miranda and Contel 1996), from Argentina (0.090 to 0.161) (de Sousa *et al.* 2000), from Kenya (0.141 to 0.163)(Tabachnik *et al.* 1979) and from Caribbean island (0.057 to 0.206) (Wallis *et al.* 1984). The mean expected heterozygosity were higher in mainland than in island populations, this could related to random mating within mainland populations. In case of island, the mean expected heterozygosity of *Ae aegypti* in the larger island populations were higher than in the smaller ones, since the more numerous introductions of the individual into the larger islands so the potential size of *Ae. aegypti* are larger and contributive of more genetic variation (Wallis *et al.* 1984). This evidence; however, did not occurred in Chang island (mean expected heterozygosity ranged from 0.040 to 0.105) when compare to Samui island (mean expected heterozygosity ranged from 0.107 to 0.121) (Chareonviriyaphap and Lerdthusnee 2002), this could be more convenience transportation (which is the importance factor for dispersal of *Ae. aegypti* from place to place) in Samui island than in Chang island.

The genetic variability in this study suggested several differences between island and mainland populations. The low genetic variability in Chang island could be subject to recent introduction of individuals. These finding are contributed the dispersal of *Ae. aegypti* into Chang island through the transportation such as passenger boats to Chang island and cars. The similarity of genetic variability in Ao Sapparod, Klong Son and Dan Mai populations could be the result of the dispersal of *Ae. aegypti* via the transportation on a wide road runs almost all the way round the island. The polymorphic loci, the mean heterozygosity and the number alleles per locus were lowest in Salak Petch population comparative to the others populations, indicating a bottleneck in establishing *Ae. aegypti* population.

Genetic differentiation was analyzed by estimating Wright's *FST*. *FST* value among island populations was lower (*FST*= 0.077) than those of combined (*FST* = 0.270) and mainland populations (*FST* = 0.297). These *FST* values indicated the genetic differentiation among all populations. *FST* (0.111) were reported for *Ae. aegypti* populations which separated less than 30 km apart (Failloux *et al.* 2002), *FST* (0.091) when populations within 60 km and *FST* (0.095) when populations over 60 km apart (Garcia-Franco *et al.* 2002). The genetic differentiation at *FST*= 0.016 ($P<10^{-6}$) were reported in Ho Chi Min City, Vietnam (Huber *et al.* 2002). *FST* values did not increasing with geographic distance in this study, whether within or between mainland and island (Table 4.10). Analysis of the allele distribution by estimating *FIS*. In this study *FIS* value was positive when considering in combined populations (0.032), but different sign from

locus to locus. *Had*-1 and *Mdh-1* were negative, whereas the remaining loci were positive. The variability of *FIS* due to null allele, natural selection or genetic drift.

Nm calculated by *FST* method, showed the different value. Nm was low when considering in combined populations (0.676) and in mainland populations (Nm = 0.592), whereas Nm in island populations was the highest value at 2.997. These Nm indicating gene flow was higher among Chang island populations than those between mainland populations or among all populations. The significant genetic structure of *Had-1* locus in Tha Thammachat population could be resulted in a low overall Nm estimate. When *Had-1* was removed from the analyses in combined populations, Nm values (0.700) were not different from those with *Had-1*. However, Nm values were lower in this study than those of *Ae. aegypti* in Madagascar : Nm = 0.9 in all population (Vazeille *et al* 2001) or in Mexico : Nm ranged from 2.5 to 19.5 (Gorrochotecgui-Escaline *et al.* 2002). Singh and Long (1992) reported that Nm value more than 2 indicating a high level of gene flow in *Drosophila* Fallen. Results from this study (*FST* and Nm estimates) indicating a low level of gene flow between *Ae. aegypti* populations from Chang island and 2 mainland of Laem Ngob District.

Nei's genetic distance among populations of *Ae. aegypti* showed vary ranged from low (0.001) to high (0.117). In contrast, Wallis and Tabachnick (1990) reported the genetic distance for *Ae. aegypti* in Caribbean islands ranged from 0.019 to 0.027, which were similar to *Ae. aegypti* in Brazil with the values ranged from 0.009 to 0.018 (Dinardo-Miranda and Contel 1996). In this study Laem Ngob populations was the most distinct of all *Ae. aeypti*, followed by Tha Thammachat population. These 2 populations were separated from the others populations by genetic distance of 0.117 and 0.047, respectively (Figure 4.2 and Table 4.11). Among populations of *Ae. aegypti* excluding Laem Ngob and Tha Thammachat populations, the maximum genetic distance was between Klong Son and the remaining populations with Nei's genetic distance of 0.012 (Figure 4.2 and Table 4.11). Even if the low distance value between Tha Thammachat population and island, the UPGMA phenogram provided the 2 geographical group : mainland and island populations. Results from Nei's genetic distance and the phenogram supported that high genetic differentiation between *Ae. aegypti* populations from Chang is and mainland of Trat Province. In addition, the informations of 15 pairwise comparisons and Nei's genetic distance indicated no relationship between geographic and genetic distance and indicated that the sea act as a barrier to gene flow for *Ae. aegypti*. Since *Ae. aegypti* have a short flight

distance (less than 500 meters) with the duration of the eggs in a dry condition. The dispersal of this mosquitoes from mainland to Chang island depend on the sea traffic.

CHAPTER 6

CONCLUSION

Aedes aegypti, is the most importance vector of dengue fever in Thailand as well as the other countries in Asia. Many methods were used to control this mosquito. As the great variety of *Ae. aegypti* and its importance in medicine, there have been extensively studied on genetic structure of this mosquitoes. However, little is known about genetic structure in Chang island. This study was aimed to investigate the genetic structure of *Ae. aegypti* populations and analyze the pattern of gene flow of *Ae. aegypti* populations in island and mainland of Trat Province, using starch gel electrophoresis for isozyme analysis.

Twenty enzyme systems were detected to identify the suitable enzymes. Thirteen enzymes (*Acon, Aks, Argk, Est, G6pdh, Gpdh, Had, Idh, Mdh, Mp,I, 6Pgd, Pgi* and *Pgm*) were used, these 13 enzymes showed consistent bands. Each enzyme had 1 loci, 5 of all loci (*Acon-1, Est-1, Idh-1, Mdh-1* and *Pgm-1*) were polymorphic (99% criterion) in all populations, whereas *Aks-1, Argk-1, G6p-1, Gpd-1, Mpi-1, 6Pgd-1* and *Pgi-1* were monomorphic loci in all populations. Isozyme analysis was conducted in unfed adult of *Ae. aegypti*, that reared from pupae or larvae mosquitoes collected from 4 island populations and 2 mainland populations of Trat Province, East of Thailand. Allele frequencies and variability measures, Chi-square test for deviation from Hardy-Weinberg equilibrium, Wright's *F* statistic, Nei's genetic distance and phenogram were calculated and computed by BIOSYS fortan program. There was a fixed allelic difference between the 2 mainland populations at *Gpdh-1*. The genetic variability in all populations were considered from the mean number of allele per locus, the percentage of polymorphic loci and the mean heterozygote. The mean number of allele per locus ranged from 1.4 (±0.1) to 1.7 (±0.2), the percentage of polymorphic loci ranged from 15.4% to 38.5% and the mean heterozygote (expected) ranged from 0.040 (±0.18) to 0.149 (±0 .060).

The Chi-square test for deviation from Hardy-Weinberg equilibrium found that 5 of 78 comparison (*Est-1, Had-1, Idh-1* and *Pgm-1* loci) did not conform to Hardy-Weinberg equilibrium, these deviations resulted from natural selection at *Had-1* locus and null allele at *Est-1, Idh-1* and *Pgm-1* loci. Genetic different among populations were estimated by *FST*; the mean value of *FST* in combined, island and mainland populations were 0.270, 0.077 and 0.297, respectively. These values indicated a high level of genetic differentiation between *Ae. aegypti* from island and mainland populations and indicated a lower level of genetic differentiation in

island than in mainland populations. *FIS* value indicating the genetic distribution within all population. Positive *FIS* were observed in both combined and island populations with values of 0.032 and 0.187, respectively. These values indicated the rack of heterozygotes. However, the abundant of heterozygotes were found in mainland populations with the *FIS* value of -0.175, as the high frequency of Had_{63} occurred only in Tha Thammachat population. These informations suggested that there were a low level of gene exchange between *Ae.aegypti* populations from Chang island and mainland of Trat Province. The phenogram was produced by clustering Nei's genetic matrix according to UMPGA method. Six populations of *Ae.aegypti* were clustered into 2 groups : island and mainland groups. However, the island populations could be divided into 2 subgroup.

BIBLIOGRAPHY

Briegel, H. and Freyvogel, T.A. 1973. **Non-specific esterase in several organs of *Aedes aegypti* (L.) during female adult life.** Comp. Biochem. Physio.Part B: Biochem. Mol. and Mol. Bio. 44(2) : 371-380.

Briegel, H. 1990. **Metabolic relationship between female body size, reserves and fecundity of *Aedes aegypti*.** J. Ins. Physio. 36:165-172.

Bullini, L. and Coluzzi, M. 1972. **Natural selection and genetic drift in protein polymorphism.** Nature. 239 : 160-161.

Cavilli-Sforza, L.L. and Edward, A.W.F. 1967. **Phylogenetic analysis models and estimation procedures.** Evolution. 21 : 550-570.

Chambers, D. M., Young, L. F. and Hills., Jr. H.S. 1986. **Backyard mosquito larval habitat availability and use as influced by census tract determine resident income levels.** J. Am. Mos. Cont. Assoc. 2(4) : 539-543.

Chareonviriyaphap,T. and Lerdthusnee, K. 2002. **Genetic differentiation of *Aedes aegypti* (Linn.) from mainland and island of Surat-thanee Province, East of Thailand.** J. Am. Mos. Cont. Assoc. 18(3) :173-7.

Christopher, S.R. 1960. **The yellow fever mosquitoes. Its life history, bionomics and structure.** Cambridge Univ. Press. Cambridge. 739 p.

Clayton, J. W. and Tretiak, D. N. 1972. **Amine-citrate buffer for pH control in starch gel electrophoresis.** J. Fish Res. Board Can. 29 :1169-1172.

Craig, Jr. G.B., and Vandehey, R. C. 1962. **Genetic variablilty in *Aedes aegypti* (Diptera : Culidae) I. Mutations affecting color pattern.** Ann. Entomol. Soc. Am. 55 : 47-58

de Sousa, G. B., Avilez, G. and Gradenal, C. N. 2000. **Allozymatic polymorphism in *Aedes aegypti* populations from Argentina.** J. Am. Mosq. Assoc. 16(3) : 206-209.

Dinardo-Miranda, L. L. and Contel, E. P. B. 1996. **Enzymatic variability in natural populations of *Aedes aegypti* (Diptera: Culicidae) from Brazil.** J. Med. Entomol. 33(5) :726-733.

Failloux, A.B., Darius, H. and Pasteur, N. 1995. **Genetic differentiation of *Aedes aegypti*, the vector of dengue fever in French Polynesia.** J. Am. Mosq. Cont. Assoc. 11 : 457-462.

Failloux, A.B., Fouque, F., Vazeille, M. and Rodhain, F. 2002. Short communication. **Isoenzyme differentiation of *Aedes aegypti* populations in French Guiana.** Med. Veterinary Entomol. 16(4) : 456- 460.

Fontenille, D. and Rodhain, F. 1989. **Biology and distribution of *Aedes albopictus* and *Aedes aegypti* in Madagascar.** J. Am. Mosq. Cont. Assoc. 5(2) : 219-225.

Garcia-Franco, F., De Lourdes Munoz, M., Lozano-Fuentes, S., Fernandez-Salas, I., Garcia-Rejon, J., Beaty, B.J. and Black, , W.C. 2002. **Large genetic distances among *Aedes aegypti* populations along the South Pacific coast of Mexico.** Am. J. Trop. Med. Hyg. 66(5) : 594-598.

Gillett, J.O, Corbet, P. S. and Haddow, A. J. 1961. **Observations on the oviposition cycle of *Aedes* (Stegomyia) *aegypti* (Linnaeus)VI.** Ann. Trop. Med. Parasitol. 55 : 427-43.

Gorrochotegui-Escalante, N., Gomez-Machorro, C., Lozano-Fuentes, S., Fernandez-Salas, I., De Lourdes Munoz, M., Farfan-Ale, J.A., Garcia-Rejon, J., Beaty, B.J. & Black, , W.C. 2002. **Breeding structure of *Aedes aegypti* populations in Mexico varies by region. Am.** J.Trop. Med. Hyg. 66 : 213-222.

Harrington, M. A., Hacker, C. S., Cheng, M. L. and Ferrall R. E. 1984. **Genetic variation in an urban population of *Aedes aegypti* (Diptera : Culicidae).** J. Med. Entomlol. 21(6) : 706-710.

Harrington, R. and Stork, N. E. 1995. **Insects in a changing environment.** Harcout Brace & Co., Publ., London.

Horsfall, W. R. 1972. Mosquitoes: **Their bionomic and relation to disease.** Hafner publ., New York.

Huber, K., Loan, L. L., Hoang, T. H., Ravel, S., Rodhain, F. and Failloux, A. B. 2002. **Genetic differentiation of the dengue vector, *Aedes aegypti* (Ho Chi Minh City, Vietnam) using microsatellite markers.** Mol. Ecol. 11:1629-1635.

Huber, K., Loan, L. L., Tran Huu, H., Tran Khanh, T., Rodhain, F. and Failloux, A. B. 2002.

Temporal genetic variation in *Aedes aegypti* populations in Ho Chi Minh City (Vietnam). Heredity. 89(1) : 7-14.

Igbokwe, E. C. and Downe, A. E. R. 1978. **Electrophoretic and histochemical comparison of three strains of *Aedes aegypti*.** Comp. Biochem. Physio.part B: Biochem. Mol. and Mol. Bio. 60(2) : 131-136.

Igbokwe, E.C. and Mills, M. 1982. **Electrophoretic variability in the phosphate system of the yellow-fever mosquito, *Aedes aegypti*.** Comp. Biochem. Physiol. 73(B) : 457-458.

Jumali, Sunarto, Gubler, D.J., Nalim, S. Eram,S. and Sarosso, J.S. 1979. **Epidemic dengue hemorrhagic fever in rural Indonesia.** Am. J. Trop. Med. Hyg. 28 : 717-724.

Kantachuvessiri, A. 2002. **Dengue hemorrhagic fever in Thai society.** Southeast Asian J. Trop. Med. Publ. Health. 33(1) : 56-62.

Kemp, A. and Jump, P. G. 1991. **Potential for dengue in south America : mosquito ecology with particular reference to *Aedes aegypti*.** J. Am. Mosq. Cont. Assoc. 7(4) : 574-583.

Kittayapong, P. and Strickman, D. 1993. **Distribution of container-inhabiting *Aedes* larvae (Diptera : Culicidae) at a dengue focus in Thailand.** J. Med. Entomol. 30 : 601-606.

Kliewer, J. W. 1961. **Weight and hatching of *Aedes aegypti* (Diptera : Culicidae)eggs.** Ann. Entomol. Soc. Am. 54:912-917.

Komalamisra, N., Sucharit, S., Palakul, K. and Phrummongkol, S. 2001. **Isozymes and different forms of *Aedes aegypti*, the vector of Dengue virus.** Mahidol University Ann. Res. Abstract. 28 : 276-277.

Lerdthusnee, K. and Chareonviriyapha, T. 1999. **Comparision of isozyme pattern of *Aedes aegypti* populations collected from pre- and post-*Bacillus thuringiensis israelensis* treatment sites in Thailand.** J. Am. Mosq. Cont. Assoc. 15(1) : 48-52.

Mattingly, P. F. 1957. **Genetic aspects of *Aedes aegypti* problem. II. Taxonomy and bionomics.** Ann. Trop. Med. Parasitol. 51: 392-408.

Mattingly, P. F. 1969. **The biology of mosquito-borne disease.** Allen and Unwin, London.

Mousson, L., Vazeille, M., Chawprom, S., Prajakwong, S., Rodhain, F. and Failloux, A. B. 2002. **Genetic structure of *Aedes aegypti* populations in Chiang Mai (Thailand) and relation with dengue transmission.** Trop. Med. & Int. Health. 7 (10) : 865-872.

Nayar, J. K. and Knight, J. W. 2002. **Isoenzyme variation in *Aedes aegypti* correlated with *Dirofilaria immitis* infectability.** Med. Vet. Entomol. 16(4) : 424 - 429.

Nei, M. 1978. **Estimation of average heterozygosity and genetic distance for a small number of individual.** Genetics. 89 : 583-590.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. and Britton-Davidian, J. 1988. **Practical isozyme genetic.** John Wiley & Sons, New-York.

Powell, J. R. and Tabachnick, W. J. 1980. **Genetics and the original of a vector population: *Aedes aegypti* : a case study.** Science. 208 : 1385-1387.

Scanlon, J.R. 1967. **Control of *Aedes aegypti* (Linn.) in Southeast Asia.** Symposium. 20 :108-112.

Schultz, G. W. 1993. **Seasonal abundant of dengue vectors in Manila, Republic of the Philippines.** Southeast Asian J. Trop. Med. Publ. Health. 24(2) : 369-375.

Scott, J. A. and McClelland, G. A. H. 1975. **Electrophoretic differences between sympatric ecotypes.** Nature. 256 : 405-406.

Service, M. W. 1993. **Mosquitoes (Culicidae)** pp. 120-240. In Lane, R. P. and Crosskey., R. W. (eds). Medical insects and arachnids. Chapman & Hall, London.

Service, M.W. 1997. **Mosquitoes (Dipera : Culicidae) Dispersal-The long and short of it.** J. Med. Entomol. 34(6) : 579-588.

Shaw, C. R. and Prasad, R. B. 1970. **Starch gel electrophoresis of enzyme : A complication of recipes.** Biochem. Genetics. 4 : 297-320.

Sheppard, P. M., Mcdonald, W. W., Tonn, R. J. and Grab, B. 1969. **The dynamics of an adult population of *Aedes aegypti* in relation to dengue hemorrhagic fever in Bangkok.** J. Ani. Ecol. 38 : 661-702

Singh, R.S. and Long, A.D. 1992. **Geographic variation in *Drosophila* : from molecular to morphology and back.** TREE. 7 : 340 -347.

Slosek, J. 1986. ***Aedes aegypti* mosquito in the America: a review of their interactions with the human population.** Soc. Sci. Med. 23(1) : 249-257.

Steiner, W. W. M. & Joslyn, D. J. 1979. **Electrophoretic techniques for the genetic study of mosquitoes.** Mosquito News. 39 : 35-54.

Swofford, D. L. & Selander, R. B. 1981. **BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics.** J. Heredity. 72 :281-283.

Tabachnick, W. J. and Powell, J. R. 1978. **Genetic structure of East African domestic population of *Aedes aegypti*.** Nature. 272 : 535-537.

Tabachnick, W. J. and Powell, J. R. 1979. **A world-wide survey of genetic variation in the yellow fever mosquito, *Aedes aegypti*.** Genet Res. 34 : 215-229.

Tabachnick, W. J., Munsterman, L. E. and Powell, J. R. 1979. **Genetic distinctness of sympatric forms of *Aedes aegypti* in east Africa.** Evolution. 33 : 287-295.

Tabachnick, W. J. 1991. Evolutionary genetics and arthropod-borne disease: the yellow fever mosquito. Am. Entomologist. 37 : 14-24.

Tidwell, M.A., Williams, D.C., Tidwell, T.C., Pena, C.J., Gwinn, T.A., Focks, D.A., Zaglul, L. and Mercedes, M. 1990. **Baseline data on *Aedes aegypti* populations in Santo Domingo, Dominican Republic.** J. Am. Mosq. Cont. Assoc. 6 : 514-522.

Trpis, M. 1972. **Seasonal changes in the larval populations of *Aedes aegypti* in Two biotypes in Dar es Salam,Tanzania.** Bull. World Health Org. 47 : 245-255.

Trpis, M., and Hausermann, W. 1975. **Demonstration of differentiation domesticity of *Aedes aegypti* (L.) (Diptera : Culicidae) in Africa by mark-release-recapture. Bull.** Entomol. Res. 65 : 199-208.

Vazeille, M., Mousson, L., Rakatoarivony, I., Villeret, R., Rodhain, F., Duchemin, J. B. & Failloux, A.B. 2001. **Population genetic structure and competence as a vector for dengue type 2 virus of *Aedes aegypti* and *Aedes albopictus* from Madagascar.** Am. J. Trop. Med. Hyg. **65** : 491-497.

Wallis, G. P., Tabachnick, W. J. and Powell, J. R. 1984. **Genetic heterogeneity among Caribbean populations of *Aedes aegypti*.** Am. J. Trop. Med. Hyg. 33(33) : 492-498.

Wallis, G. P. and Tabachnick, W. J. 1990. **Genetic analysis of rock hole and domestic *Aedes aegypti* on the Caribbean island of Anguilla.** J. Am. Mosq. Cont. Assoc. 6(4) : 625-630.

Womack, M. 1993. **The yellow fever mosquito, *Aedes aegypti*.** Wing Beats. 5(4):4.

Wright, S. 1951. **The genetic structure of populations.** Annu.Eugenet. 15 : 323-354.

Wright, S. 1978. **Evolution and genetic of populations. Vol.4, Variability within and among natural populations.** University of Chicago Press. Chicago.

APPENDIX

Appendix 1

Chemical substrates

Most of the chemical substrates are available from Sigma.

Product	Reference
cis-aconitic acid	A 3412
adenosine 5′ diphosphate	A 2754
L-Cystine sulfinic acid	C 4418
fast blue BB salt	F 3378
fructose-6-phosphate	F 3627
disodium glucose-1- phosphate (G1PDH)	G 7000
glycerol	
DL-α-Glycerophosphate	G 6014
α-D-Glucose	G 7528
α-gluconic dehydrogenate	G 5636
glucose-6-phosphate dehydrogenase	G 8878
D-Gluconic acid	G 9005
hexokinase type III, yeast	H 5000
DL-Isocitric acid	I 6768
isocitric dehydrogenase	I 2516
α-Ketoglutaric acid	K 1750
DL-Malic acid	M 0875
magnesium chloride anhydrous	M 8266
mannose-6-phosphate	
thiazolyl blue (MTT)	M 2128
α-Naphthyl acetate	N 8505
β-Naphthyl acetate	N 6877
α-Naphthyl propionate	N 0376
β-Naphthyl propionate	N 0501
β-Nicotinamide adenine dinucleotide (βNAD)	N 7004

Product	Reference
nicotinamide adenine dinucleotide phosphate (NADP)	N 3886
nitro blue tetrazolium, grade III	N 6878
phenazine metho sSulphate (PMS)	P 9605
6-Phosphogluconic acid	P 7877
phospho-L-arginine	P 5139
phosphoglucose isomerase	P 9956
sodium chloride (NaCl)	S 7653
sodium hydroxide (NaOH)	S 5881
acetic acid Glacial (Analytical reagent)	A 8401 (LAB-SCAN)
citric acid (monohydrate)	C 7129
hydrolyzed potato starch	S 40501 Lot 048H0286
trizma base	T 6791
methanol (anhydrous)	UN 1230 (LabGuard)
hydrochloride (HCl)	
sucrose	S 9378
N-(3-Aminopropyl)-morpholine	A 9028
potassium phosphate	P 5379
sodium phosphate monohydrate ($NaH_2PO.H_2O$)	S 9638
sodium phosphate anhydrate (Na_2HPO)	S 9763
bromphenol blue	18030 C00967 (Fluka)
ethylenodiamine tetraacetic acid (EDTA)	ED 233
acetone	UN 1090 (BDH)

Appendix 2

Reagent preparation

2.1 Electrophoresis buffer formulations

2.1.1 Morpholine Buffer

Electrode buffer pH 6.1

Citric acid	8.4 g
H_2O	900 ml
Adjust to pH 6.1 with N-(3-amino-propyl-Morpholine)	± 10 ml
H_2O, make up to 1,000 ml	

Gel buffer pH 6.1 (1:20 dilution of electrode buffer)

2.1.2 TCss 6.3 (Tris-Citrate pH 6.3)

Electrode buffer pH 6.3

Trisma (0.22M)	27.0 g
Citric acid (monohydrate) (0.094M)	15.5 g
H_2O, make up to 1,000 ml	
Adjust to pH 6.3 with Trisma (1M) or Citric acid (1M)	

Gel buffer pH 6.7

Trisma (0.08M)	1.2 g
Citric acid (monohydrate) (0.003M)	0.5 g
H_2O, make up 1,000 ml	
Adjust to pH 6.7 with Trisma (1M) or Citric acid (1M)	

2.1.3 TMED 7.4 (Tris-malate-ethylenediamiestetracetic acid, pH 7.4)

Electrode buffer pH 7.4

Trisma (0.1M)	12.3 g
Malic anhydride(0.1M)	9.8 g
EDTA (0.01M)	3.72 g
$Mg_2Cl,6H_2O$ (0.01M)	2.0 g

H_2O, make up to 1,000 ml

Adjust to pH 7.4 with NaOH in Flakes.

For further adjustments, use Trisma (1M) or malic anhydride (1M).

Gel buffer pH 7.4 (1:9 dilution of electrode buffer)

Adjust pH to 7.4 with Trisma (1M) or malic anhydride (1M)

2.1.4 LiOH (Litium hydroxide

Electrode buffer pH 8.25

Lithium hydroxide	1.50 g
Boric acid (0.194 M)	12.00 g
H_2O, make up to 1,000 ml	

Gel buffer pH 8.45 (1:9 dilution of electrode buffer)

Trisma (0.074 M)	9.00 g
Citric acid (0.009M)	1.9 g
H_2O, make up to 1,000 ml	
Adjust pH to 8.45	

2.2 Staining buffer formulations

2.2.1 DH Buffer (0.4 M), pH 8.4

Stock :

Trisma	49.4 g
ConcHCl	7.5 ml
H_2O, make up to 1,000 ml	

Before use dilute 1 part of stock plus 3 parts of H_2O

2.2.2 Phosphate C (Phosphate Na/Na_2) (0.1M), pH 6.0 (for *Est*)

NaH_2PO	12.5 g
Na_2HPO_4(anhydrous)	2.8 g
H_2O, make up to 1,000 ml	

2.2.3 Phosphate F (Phosphate Na_2/K) (0.1M), pH 6.5 (for ***Est***)

KH_2PO_4	9.2 g

Na_2HPO_4(anhydrous)	4.8 g
H_2O, make up to 1,000 ml	

2.2.4 Tris-HCl (0.5m), pH7.1 (for *Ak* and *Argk*)

Stock :

Trisma	60.55 g
H_2O, make up to 1,000 ml	
Adjust pH with Conc HCl	
Before use add 45 ml of H_2O to 5 ml of stock	

2.3 Specific staining solutions for detect enzyme activities

2.3.1 Aconitase

Buffer :

DH buffer	50 ml

Stain :

Mg_2Cl	200 mg
NADP	10 mg
Aconiticacid	75 mg
Isocitrate dehydrogenase	50 units
MTT	10 mg
PMS	1 mg

Incubate with gel in the dark until blue bands appear on the clear background.

2.3.2 Adenylate kinase

Buffer :

0.5M Tris-HCl pH 7.1	5 ml
H_2O	45 ml

Stain :

G6PDH	40 units
NADP	12.5 mg
Mg_2Cl	12.5 mg
Glucose	45 mg

ADP	10 mg
Hexokinase	80 units
MTT	5 mg
Incubate with gel for 10 minute, then add :	
PMS	1.25 mg

Incubate with gel in the dark until blue bands appear.

2.3.3 Arginase kinase

Buffer :

0.5M Tris-HCl pH 7.1	5 ml
H_2O	45 ml

Stain :

Glucose	45 mg
G6PDH	40 units
NADP	5 mg
Mg_2Cl	50 mg
ADP	10 mg
Hexokinase	80 units
Phospho-L-arginine	10 mg
MTT	10 mg
Incubate with gel for 10 minute, then add:	
PMS	1 mg

Incubate with gel in the dark until blue bands appear.

2.3.4 Esterase

Buffer:

Phosphate F	60 ml

Stain :

α-Naphthyl acetate : 2 % in acetone	1 ml
β -Naphthyl acetate : 2 % in acetone	1 ml
Incubate with gel for 2-3 minutes at room temperature, then add :	
Fast Blue BB	1 ml

Incubate with gel at room temperature the bands will appear within 30 minutes. Fix as soon as possible.

2.3.5 Glucose-6-phosphate dehydrogenase

Buffer :

DH	50 ml

Stain :

NADP	10 mg
Mg_2Cl	50 mg
Glucose-6-phosphate	100 mg
NBT	20 mg
PMS	1 mg

Incubate at 37°C at least 1 hour. Fix as soon as possible to stop the background become too colored.

2.3.6 α-Glycerophosphate dehydrogenase

Buffer :

DH Buffer	50 ml

Stain :

α- Glyceerophosphate	100 mg
NAD	12.5 mg
NBT	20 mg
PMS	1 mg

Incubate with gel at 37°C until the bands appear (45-60 minutes). Fix when the stain is satisfactory.

2.3.7 β-Hydroxyacid dehydrogenase

Buffer :

DH Buffer	50 ml

Stain :

NADP	5 mg
NAD	12.5 mg
NaCl	150 mg

Gluconic acid	200 mg
MTT	10 mg
PMS	1 mg

Incubate with gel at 37°C until the bands appear (45-60 minutes). Fix when the stain is satisfactory.

2.3.8 Isocitrate dehydrogenase

Buffer :

DH Buffer	50 ml

Stain :

Sodium isocitrate	50 mg
Mg_2Cl	50 mg
NADP	5 mg
MTT	10 mg
PMS	1 mg

Incubate with gel in the dark the clear blue bands appear after about 30 minutes.

2.3.9 Malate dehydrogenase

Buffer :

DH Buffer	50 ml

Stain :

NAD	25 mg
Malate pH 7	50 mg
MTT	10 mg
PMS	1 mg

Incubate with gel in the dark the blue bands appear after around 30-60 minutes.

2.3.10 Mannose-6-phosphate isomerase

Buffer:

DH	50 ml

Stain :

NADP	12.5 ml
Mannose-6-phosphate	60 mg

Pgi	80 units
G6PDH	40 units
Mg_2Cl	50 mg
MTT	10 mg
PMS	1 mg

Incubate with gel at 37°C (60 minutes).

2.3.11 6-Phosphogluconic dehydrogenase

Buffer :

DH Buffer	50 ml

Stain :

NADP	10 mg
Mg_2Cl	50 mg
Na_2-6-phosphogluconate	15 mg
NBT	20 mg
PMS	1 mg

Incubate with gel at 37°C until blue bands appear (60-90 minutes).

2.3.12 Phosphoglucose isomerase

Buffer :

DH Buffer	50 ml

Stain :

NADP	5 mg
G6PDH	40 units
D-Fructose-6-Phosphate	12.5 mg
MTT	5 mg

Incubate 30 minutes at 37 °C then add PMS 1 mg

2.3.13 Phosphoglucomutase

Buffer :

DH Buffer	50 ml

Stain :

Glucose-1-phosphate $Na_2 4H_2O$	50 mg

NADP	5 mg
Mg_2Cl	25 mg
Glucose-6-phosphate	40 units
MTT	5 mg
PMS	0.5 mg

Incubate with gel in the dark blue bands appear after around 30-60 minutes. Fix as soon as possible.

Appendix Table 1 F-statistics analysis of polymorphic in 2 populations (Ao Sapparod-Klong Son) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.056	-.041	.014
Est-1	.211	.211	.001
Idh-1	-.008	.003	.011
Mdh-1	.034	.138	.108
Pgm-1	.294	.308	.019
Mean	.089	.136	.052

Appendix Table 2 F-statistics analysis of polymorphic in 2 populations (Ao Sapparod-Dan Mai) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	.181	.189	.010
Est-1	.121	.123	.003
Idh-1	.515	.538	.049
Mdh-1	.019	.038	.019
Pgm-1	.355	.366	.017
Mean	.252	.269	.023

Appendix Table 3 F-statistics analysis of polymorphic in 2 populations (Ao Sapparod-Salak Petch) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.056	-.053	.003
Est-1	.189	.202	.016
Idh-1	.141	.178	.042
Mdh-1	-.195	-.131	.054
Pgm-1	.372	.373	.001
Mean	.073	.098	.028

Appendix Table 4 F-statistics analysis of polymorphic in 2 populations (Klong Son-Dan Mai) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	.275	.305	.042
Est-1	.069	.073	.005
Idh-1	.336	.347	.016
Mdh-1	.304	.399	.136
Pgm-1	.190	.225	.043
Mean	.259	.304	.061

Appendix Table 5 F-statistics analysis of polymorphic in 2 populations (Klong Son-Salak Petch) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.033	-.028	.005
Est-1	.116	.136	.022
Idh-1	-.128	-.082	.041
Mdh-1	.196	.394	.246
Pgm-1	.165	.184	.022
Mean	.096	.202	.117

Appendix Table 6 F-statistics analysis of polymorphic in 2 populations (Dan Mai-Salak Petch) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	.225	.243	.022
Est-1	-.059	-.051	.007
Idh-1	.595	.630	.086
Mdh-1	.275	.293	.026
Pgm-1	.251	.259	.010
Mean	.310	.335	.036

Appendix Table 7 F-statistics analysis of polymorphic in 2 populations (Tha Thammachat-Ao Sapparod) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.056	-.041	.014
Est-1	.274	.275	.002
Had-1	-.667	-.250	.250
Idh-1	.143	.154	.013
Mdh-1	-.178	.047	.191
Pgm-1	.170	.194	.028
Mean	-.087	.047	.124

Appendix Table 8 F-statistics analysis of polymorphic in 2 populations (Tha Thammachat-Klong Son) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.017	-.017	.000
Est-1	.211	.214	.004
Had-1	-.667	-.250	.250
Idh-1	-.019	-.019	.000
Mdh-1	.039	.088	.051
Pgm-1	.027	.044	.018
Mean	-.067	.007	.069

Appendix Table 9 F-statistics analysis of polymorphic in 2 populations (Tha Thammachat-Dan Mai) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	.274	.304	.041
Est-1	.110	.110	.000
Had-1	-.667	-.250	.250
Idh-1	.446	.455	.015
Mdh-1	.029	.279	.258
Pgm-1	.116	.134	.020
Mean	.061	.184	.132

Appendix Table 10 F-statistics analysis of polymorphic in 2 populations (Tha Thammachat-Salak Petch) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.033	-.028	.004
Est-1	.185	.192	.008
Had-1	-.667	-.250	.250
Idh-I	.088	.123	.039
Mdh-1	-.128	.288	.369
Pgm-1	.087	.108	.024
Mean	-.124	.100	.199

Appendix Table 11 F-statistics analysis of polymorphic in 2 populations (Laem Ngob-Ao Sapparod) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.080	-.079	.002
Est-1	.128	.131	.003
Gpdh-1	...	1.000	1.000
Idh-1	.223	.226	.003
Mdh-1	-.356	-.329	.020
Pgm-1	.297	.299	.002
Mean	-.057	.269	.308

Appendix Table 12 F-statistics analysis of polymorphic in 2 populations (Laem Ngob-Klong Son) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.078	-.052	.024
Est-1	.074	.080	.006
Gpdh-1	...	1.000	1.000
Idh-1	-.003	.013	.016
Mdh-1	-.119	-.062	.051
Pgm-1	.097	.118	.023
Mean	-.033	.286	.308

Appendix Table 13 F-statistics analysis of polymorphic in 2 populations (Laem Ngob-Dan Mai)of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	.146	.149	.004
Est-1	-.069	-.069	.000
Gpdh-1	...	1.000	1.000
Idh-1	.569	.595	.060
Mdh-1	-.176	-.107	.059
Pgm-1	.197	.204	.008
Mean	.127	.390	.302

Appendix Table 14 F-statistics analysis of polymorphic in 2 populations (Laem Ngob-Salak Petch) of *Aedes aegypti* at all loci.

Locus	*F(IS)* [a]	*F(IT)* [b]	*F(ST)* [c]
Acon-1	-.074	-.065	.009
Est-1	-.055	-.049	.006
Gpdh-1	...	1.000	1.000
Idh-1	.190	.211	.026
Mdh-1	-.385	-.215	.123
Pgm-1	.176	.176	.000
Mean	-.098	.331	.391

BIOGRAPGHY

Miss Supattra Chinakool was born on March 21, 1973 in Nakhon Ratchasima Province. She studied and received a secondary education in the birthplace. In 1996, she educated a Bachelor degree of Science in Agricultural from King Mongkut's Institute of Technology Ladkrabang, where she has been studying a Master of Science in Biotechnology at Faculty of Science.