STUDY ON GENETIC VARIATION OF FRUIT FLY PARASITOIDS (*DIACHASMIMORPHA SP.*) IN THAILAND BY ELECTROPHORETIC TECHNIQUES

DUANGTA JUNESIRIKUL

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Duangta Junesirikul
.....
Duangta Junesirikul
Candidate

Sangvorn Kitthawee
.....
Sangvorn Kitthawee, Ph.D.
Major advisor

.....
Visut Baimai, Ph.D.
Co-advisor

Sujinda Thanaphum
.....
Sujinda Thanaphum, Ph.D.
Co-advisor

Adulya Viriyavejakul
.....
Adulya Viriyavejakul, M.D., LL.B., F.R.C.P.
Dean
Faculty of Graduate Studies

.....
Vithoon Viyanant, Ph.D.
Chairman
Master of Science Program in Environmental Biology
Faculty of Science

Thesis

entitled

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was submitted to the faculty of Graduate Studies, Mahidol University for the degree of Master of Science (Environmental Biology)

on

February 26, 1997

Duangta Junesirikul

Duangta Junesirikul
Candidate

Sangvorn Kitthawee

Sangvorn Kitthawee, Ph.D.
Chairman

Visut Baimai, Ph.D.
Member

Sujinda Thanaphum

Sujinda Thanaphum, Ph.D.
Member

Adulya Viriyavejakul

Adulya Viriyavejakul, M.D., LL.B., F.R.C.P.
Dean
Faculty of Graduate Studies

P. Matangkasombut

Pornchai Matangkasombut, Ph.D., M.D.
Dean
Faculty of Science

BIOGRAPHY

NAME	Miss Duangta Junesirikul
DATE OF BIRTH	16 October B.E. 2514 (1971)
PLACE OF BIRTH	Samutsongkhram, Thailand
INSTITUTIONS ATTEND	Mahidol University, 1990-1994: Bachelor of Science (Biology)
	Mahidol University, 1994-1997: Master of Science (Environmental Biology)

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Duangta Junesirikul

ชื่อวิทยานิพนธ์ การศึกษา และ พัฒนาวิธีหาความแปรผันทางพันธุกรรมของแตนเบียน *Diachasmimorpha sp.* ในประเทศไทยโดยวิธีอิเล็คโตรโฟรีซีส

ผู้วิจัย ดวงตา จุลศิริกุล

ปริญญา วิทยาศาสตรมหาบัณฑิต (ชีววิทยาสภาวะแวดล้อม)

คณะกรรมการควบคุมวิทยานิพนธ์

สังวรณ์ กิจทวี Ph.D.

วิสุทธิ์ ใบไม้ Ph.D.

สุจินดา ธนะภูมิ Ph.D.

วันที่สำเร็จการศึกษา 26 กุมภาพันธ์ พ.ศ 2540

บทคัดย่อ

การศึกษาความแปรผันทางพันธุกรรมของแตนเบียน *Diachasmimorpha sp.* ในประเทศไทย ซึ่งได้แก่ นครปฐม สระบุรี เพชรบูรณ์ นครราชสีมา เชียงใหม่ ระนอง และชุมพร โดยใช้เทคนิค โพลิอะคริลาไมด์ เจล อิเล็คโตรโฟรีซีสในแนวราบ ของเอนไซม์ 9 ระบบ ซึ่งประกอบด้วย 13 โลไซ การวิเคราะห์ข้อมูลทำโดยการรวมข้อมูลความถี่ของจีโนไทป์ของทุกประชากร แล้ว ดำเนินการวิเคราะห์โดยใช้โปรแกรมคอมพิวเตอร์ BIOSYS-1 ซึ่งได้นำเสนอค่าที่แสดง ความแปรผันทางพันธุกรรมของแต่ละประชากรเช่น

ค่าเฉลี่ยของเฮเทโรไซโกซิตี ค่าที่แสดงความแตกต่างของแต่ละประชากร ซึ่งได้แก่ ค่าเจเนติคไอเดนติตี และ เจเนติคดีสแทนซ์ และความสัมพันธ์เชิงวิวัฒนาการที่เป็นไปได้ระหว่างประชากร ซึ่งคำนวณโดยใช้วิธี UPGMA clustering analysis

จากการวิเคราะห์ข้อมูลทั้ง 13 โลไซ พบว่า ค่าเฉลี่ยของเฮเทโรไซโกซิตีของทุกประชากรมีค่าต่ำ (0.027 - 0.094) อาจเนื่องจาก แตนเบียนชนิดนี้มีการสืบพันธุ์แบบ pathenogenesis ประกอบกับปัจจัยอื่น ๆ ที่เข้ามามีบทบาท และจากการวิเคราะห์หาความสัมพันธ์เชิงวิวัฒนาการระหว่างประชากร พบว่า แตนเบียนทั้ง 2 สปีชีส์ แยกออกจากกันที่ค่าเจเนติคไอเดนติตี = 0.43472 ในกลุ่มประชากรแตนเบียนสปีชีส์ C ประชากรแตนเบียนจาก นครปฐม กับ สระบุรี มีความสัมพันธ์ใกล้ชิดกันที่สุด คือ มีค่าเจเนติคไอเดนติตี = 0.939 และในกลุ่มประชากรแตนเบียนสปีชีส์ B ประชากร แตนเบียนจาก เชียงใหม่ กับ ชุมพร มีความสัมพันธ์ใกล้ชิดกันที่สุด คือ มีค่าเจเนติค ไอเดนติตี = 0.923 จากค่าดังกล่าว แสดงให้เห็นถึงความสัมพันธ์ที่ใกล้ชิดกันของแตนเบียน ในแต่ละกลุ่มประชากรได้เป็นอย่างดี

อย่างไรก็ตาม การศึกษาครั้งนี้ เป็นการเริ่มต้นที่จะทดลองศึกษาในด้านพันธุศาสตร์ กับแตนเบียนชนิดนี้ ผลการศึกษาที่ได้จึงจำเป็นที่จะต้องนำไปเปรียบเทียบกับการศึกษาในด้านอื่น ๆ เพื่อให้ได้ผลการศึกษาที่ชัดเจนแน่นอนต่อไป

Thesis Title Study on Genetic Variation of Fruit Fly Parasitoids (*Diachasmimorpha sp.*) in Thailand by Electrophoretic Techniques

Name Duangta Junesirikul

Degree Master of Science (Environmental Biology)

Thesis Supervisory committee

Sangvorn Kitthawee Ph.D.

Visut Baimai Ph.D.

Sujinda Thanaphum Ph.D.

Date of Graduation 26 February B.E. 2540 (1997)

Abstract

Horizontal polyacrylamide gel electrophoresis of nine enzyme systems are used to study genetic variations of *Diachasmimorpha sp.* in natural population of Thailand, which are Nakornpathom, Saraburi, Petchabun, Nakornratchasima, Chiang Mai, Ranong and Chumporn. Data analysis is performed using pooled data of genotype frequencies from all population and analyzed by computer program BIOSYS-1. Intrapopulation and interpopulation genetic variability estimates such as mean heterozygosity, genetic identity and

genetic distance estimates for genetic differentiation among groups of population and dendrograms showing possible phylogenetic relationship among groups of population are presented.

From data analysis, mean heterozygosity of all population is quite low (0.024-0.094) because of pathenogenesis and other factor effecting. From cluster analysis, the population of species C and species B diverged at the identity value of 0.43472. In population of species C, clustering level between Nakornpathom versus Saraburi is separated at the identity value of 0.939 and in population of species B, clustering level between Chiang Mai versus Chumporn is separated at the identity value of 0.923. These values show that the relationship between these groups of population are closely related.

However, this study is the first attempt to study the genetic variation of this parasitoid. It is better to confirm the result with other methods to obtain the complete result.

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Bis-acrylamide	=	N,N′-Methylene-bis-acrylamide
$MgCl_2$	=	Magnesium chloride
NAD^+	=	Nicotinamide adenine dinucleotide
$NADP^+$	=	Nicotinamide adenine dinucleotide phosphate
NBT	=	p-Nitroblue tetrazolium
PMS	=	Phenazine methosulfate
Tris	=	Tris (hydroxy methyl) aminomethane
TEMED	=	N,N,N′,N′-Tetramethylethylene diamine

Chapter I

Introduction

Insects have long been known as a kind of animal which were both useful and serious pests for fruits and vegetables. In beneficial ways they act as pollinators, producing some products such as silk, honey, lac, and capable for controlling their population. For example, parasitoids are natural enemies of some insect pests. However, they cause diseases to human and damage agricultural products, that is, mosquitoes which cause many diseases to human or fruit flies which attack and damage any kinds of fruits and vegetables.

In the past, agriculturists usually used insecticides to eliminate insect pests because of their low cost and convenience. However, besides destroying insect pests, the use of insecticides also causes many problems. First, as for ecological effect, it disturbs the balance of interaction of predators, hosts and parasitoids. Second, the remaining of insecticides is persistent in the environment and may be harmful to human both in direct and indirect ways. Third, pests will develop the resistance to insecticides. This is the one of the most

important problems because when the insects are resistant to insecticides, the agriculturist will use more amount of insecticides to get rid of them. It certainly causes more damage to the environment. Entomologists, therefore, are finding a way to control insect pests and they are now interested in **biological control,** which can be defined as the regulation by natural enemies (parasites, predators and pathogens) of another organism's population density at a lower average than would otherwise occur. (DeBach, 1974)

However, the use of the biological control method to control insect pests, entomologists should find out the basic background knowledge of both insects pest and their enemies. In Thailand, the Oriental fruit fly *Bactocera sp.* is the major pest of most economical agricultural products (Department of Agriculture Thailand, 1991). However, in nature there are many kinds of natural enemies which control them, such as egg-parasitoids, larval-parasitoids, pupal-parasitoids (Wharton and Gilstrap, 1983; Ramada and Wong, 1990; Stark, et al,1991; White and Elson-Harris,1992). *Diachasmimorpha sp.* is the braconid parasitoid (Hymenoptera : Braconidae) which attacks fruit flies and can be found in Thailand's ecosystem. Anyways, this method

is not popular because the basic background knowledge of *Diachasmimorpha sp.* is not clear (Wharton and Giltsrap, 1983). Today, advanced biochemical genetics is becoming an effective tool for taxonomists and entomologists to identify them and study their variation.

The biology of fruit flies and their problems

Family Tephritidae is one of the largest families of Diptera and it is the most economically important (White and Elson-Harris, 1992). There are approximately more than 800 species found in the oriental region (Ibrahim, 1990). They have been attacking various fruits and vegetables including flower heads, leaves, stems and roots. There are two major groups of tephritid fruit flies. One group of Tephritid fruit flies comprising around 35% of all tephritid fruit flies, attack freshy fruits. Another group constituting about 40%, attack flower heads. The remaining species comprising about 25%, attack flowers or other kinds of plant tissues (White and Elson-Harris, 1992).

Adult tephritid fruit flies are moderately large. The wings of most of its species are pictured with dark bands and other patterns (Ibrahim, 1990). The females oviposit their eggs in plant tissues. The

eggs will develop to larvae. The larvae of most species develop, live and feed on plant tissues, and the third instar larvae move to the soil, form a puparium and then emerge to adult. Emerging adults require about a week to reach sexual maturity. After mating, the new developmental cycle can be initiated (White and Elson-Harris, 1992). This fly has been proven difficult to control with residual insecticides because it has developed resistance. It poses a serious problem for control. New method, integrated control strategies have been proposed. In order to develop this method, additional information concerning tephritids biology and their parasitoids is needed.

The biology of fruit fly parasitoids

Hymenopteran parasitoids are clearly the most important group of biological control agents (LaSelle, 1993). The braconid parasitoids (Hymenoptera : Braconidae) have been reported as parasitoids of many species of tephritid fruit flies (Stark, et al, 1991; Messing, et al, 1994; Ramada, et al, 1994, 1995).

The range of body length of *Diachasmimorpha sp.* is between 3.5-5.0 mm and one of the common characteristics of the

braconid parasitoid is the long expended ovipositor of the female. The range of the ovipositor length is between 2.5-5.5 mm. The body color of the adult parasitoid is varied in yellowish brown tone but in some species their body color is black. Mating behavior occurred within a day after emergence and the first batch of eggs were laid on the first day (Bess and Haramoto, 1961). The fertilized eggs develop to females whereas unfertilized eggs develop parthenogenitically to males (Suomalainen, et al, 1987) (Fig. 1).

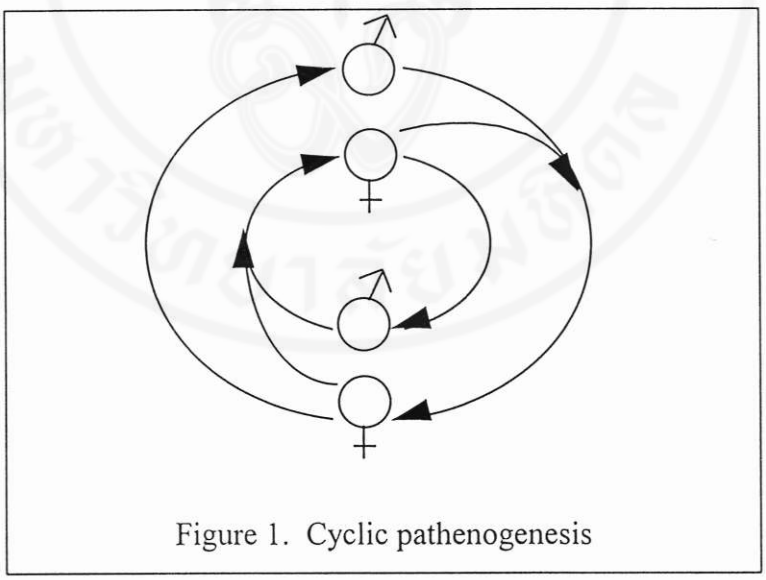


Figure 1. Cyclic pathenogenesis

Diachasmimorpha, a member of the family Braconidae, is one of the most common and widespread genus. It has been found that there are several species in this group. In Thailand, I have also found that it may be consist of several species (i.e. species B and species C,

etc.) when identify by morphology (table1). For my specimens, species B may be synonymized to *Biosteres arisanus* but species C is in fact similar to *Diachasmimorpha longicaudata* (Wharton and Gilstrap, 1983). It is distribute in Indo-Australian region (Wharton and Gilstrap, 1983). It has been found that *D. longicaudata* is in fact a conplex species group consisting of at least 7 subspecies when identify by morphology (Clausen, 1978) but Wharton and March (1978) decleared that the *longicaudatus* complex needed to be examined in much more detailed.

From surveying around Thailand, there are about 6 species which I identify it by morphology. The description of each species was identified followed Wharton and Gilstrap's key (1983) (table 1). Species C which have brown body and appically sinous ovipositor are similar to *D. longicaudata* and species B which have black body, narrowed tip ovipositor and reddish occipus may be equivalent to *Biosteres arisanus*. Other species, species A are unknown species like *Biosteres vandenboschi*, species D are unknown species and species E are unknown species like *Diachasmimorpha tryoni*. The statistical analyses of species A, species D and species E are in appendix.

The sibling species complex or isomorphic species or cryptic species as the are called, mean the species which are morphologically very similar. Such exceeding similar species pose a sinificant problem to formal taxonomists. To identify them by morphology, there was easiler in confusing within species. Modern approaches to species problems, including cytogenetics, ecological behavioral and biochemical methods, permit large scale screening of natural population for genetic attributes which have simple Mendelian properties. These methods are useful in recognition of sibling species in many groups of insect (White, 1973). In this study, electrophoresis which is the one of biochemical methods, is used to identify parasitoid species and used this result to confirm with identify by morphology.

Biochemical genetics

In 1957, Hunter and Markert developed the zymogram technique to identify enzymes in tissue extracts. This application led to the discovery of isozymes or multiple molecular forms of enzymes (Brewer, 1970). This technique was introduced to population biology by Lewontin and Hubby (1966) in *Drosophila* and by Harris (1966) in man. Studies in molecular genetics revealed that proteins are made up of one or more polypeptide which is the chain of amino acids. Different types of amino acids are coded by a set of three nucleic acids (codon) and the sequence of these codons on the DNA strand determines the sequence of amino acids in the protein coded. The substitution, addition or deletion of a base in the DNA strand will usually result in changes in the sequence of amino acids which correspond in protein synthesis. At present, advanced molecular genetics has been applied to the study of genetic variation at a level of gene action but it is time consuming and costly to be applied on a large scale. Gel electrophoresis is, therefore, commonly used. The principle of electrophoresis is to pass an electric current through an electrophoretic medium. The electric current will make it an electric field. The particle carrying electrical charges under the influence of an electric field will move. Proteins are made up of different amino

acids which contain an amino group, carboxyl group and side-chain. The side-chain of amino acids carries an electrical charge, which is determined by its composition and the pH condition of its environment. In the electrical field, proteins will move towards the opposite pole at different rates depending on their charges. Nonidentical proteins will move at different rates while identical proteins will move at the same rate resulting in the same distance under the same electrophoretic condition (Brewer, 1970; Selander, 1977; Ooi, 1990).

Among various proteins which scientists used in the study of genetics variation, enzyme is usually used in biochemical systematic studies because the mobility of certain enzymes can be detected by their specific histochemical staining. So enzyme electrophoresis has been widely used to study genetics variation in natural population and in laboratory colonies.

When the variation of specifically enzymes within and between populations or species is studied, the terms 'isozyme' and 'allozyme' need to be differentiated. Enzymes with similar function but differing in structure and coded by separate genes or loci are called isozyme while different forms of an enzyme which are products of

alternative alleles at a locus within a species are called allozyme (Ferguson, 1980). Enzymes are made up of one or more polypeptide subunits. A monomeric is enzyme made up of only one subunit while a multimeric enzyme is made up of more than one subunit. The subunit of an enzyme may be identical (homomeric as observed in homozygous genotypes) or they may be different (heteromeric as shown by heterozygous genotypes) (Ooi,1990) (Fig. 2)

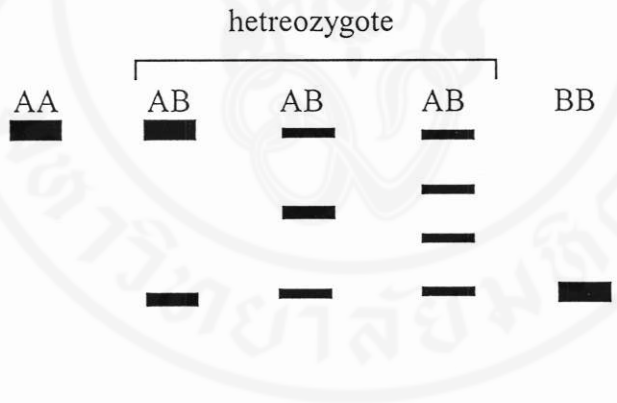


Figure 2. Example of zymogram patterns. Shown in the three center lanes are the expected zymogram patterns for a heterozygous individual when the enzyme in question is (from left to right) monomeric, dimeric or tetrameric, respectively.

Acrylamide gel electrophoresis was introduced in 1959 by Raymond and Weintraub, Ornstein and David. Acrylamide have the advantages over the other supporting media. The pore side of gel can

change by adjusting the concentration of acrylamide. This property allows gels to construct with a varying sieving effect and they are not as fragile as starch gel. The property also makes them easier to handle. As the very important advantage, gels are clear, making isozyme bands easier to interpret. Besides the choice of supporting media, there are many factors that should be considered to obtain the sharp and well visualized enzyme band. That is, the pH of buffer solution, voltage of the electrical current and the staining techniques (Ferguson, 1980; Brewer ,1970). Enzyme electrophoresis have provided an excellent alternative method for the characterization of species diversity and sibling species. By appropriate subjection to electrophoresis and staining, different enzymes may facilitate separation of closely related species or even enzymatically distinct population and genotypes within species.

These diagnostic methods were widely used to estimate genetic diversity at different taxonomic levels of organisms. In 1977, Harrison studied enzyme variation in sibling species of crickets. Allozymic variation in *Daphnia obtusia* form the midwestern United States and Southern Canada was determined by Spitze in 1993 and the

genetic differentiation of filarial worms *Onchocerca volvurus* (Nematoda, Filarioidea) in Africa was studied by Cianchi et al. in 1985.

For insects in order Hymenoptera, there are a lot of genetic studies based on enzyme electrophoresis. Sanetra et al. studied enzyme polymorphism in ant genus *Tetramorium* Mayr and its social parasites (Hymenoptera : Formicidae) in 1994. A comparison of genetic variation in two sibling species pairs of haplodiploid insects was determined by Rosenmerer and Packer in 1993. In Thailand, enzyme electrophoresis was utilized to understand the genetic structure of many *Anopheles sp.* by Green et al. in 1990 and 1992. However, there are no reports about the use of enzyme electrophoresis in the genetic variation of *Diachasmimorpha sp.* The work report here is a biochemical study on genetic variation using enzyme electrophoretic techniques of *Diachasmimorpha sp.* in Thailand. Nine enzyme systems were employed to study the genetic variability among wild population of *Diachasmimorpha sp.* Genetic variability and statistical analysis of each enzyme locus, including possible phylogenetic relationships of species members within the *Diachasmimorpha sp.* exploited in this study, are presented.

Table 1. Collection localities, host plants of fruit fly, fruit flies and their parasitoids collected in Thailand.

Code	Location	Host plant	Host fly	Parasitoid	Remark	Description
CM(V)19	Chiang mai	*Randia eucodon*	*Bactocera pyrifoliae*	species B	20/11/95	**species A ;**
CP(I)7	Chumphon	*Musa acuminata*	*Bactocera dorsalis*	species B	18/01/96	-black body
KY1	Khao yai	*Polyalthia viridis*	*Bactocera sp.* like	species B	09/05/96 Dong Ka Ting	-ovipositor not strongly narrowed at tip
			dorsalis	species C		-occipus black
KY4	Khao yai	*Pentace burmanica*	*Bactocera sp.* like	species B		
			dorsalis	species C		**species B ;**
NP	Nakhornpathom	*Psidium guajava*	*Bactocera correcta*	species C		-black body
				species B		-ovipositor narrowed at tip
PE(U)28	Petchabun	*Spondias pinnata*	*Bactocera sp.*	species A	20/12/95 T. Nong Mae Na	-occipus reddish
PE(U)30	Petchabun	*Spondias bipinnata*	*Bactocera sp.*	species A	21/12/95 T. Nong Mae Na	
				Opius incisi		**species C ;**
PE(U)32	Petchabun	*Musa acuminata*	*Bactocera sp.*	species B	21/12/95 T. Khao Koa	-brown body
						-ovipositor appically sinous

Code	Location	Host plant	Host fly	Parasitoid	Remark	Description
RN63	Ranong	*Cleidon javanium*	*Bactocera carambolae* *Bactocera dorsalis*	species D	28/06/96 T. Hat Som Pean	**species D ;** -orange body and small size
RN142	Ranong	*Strychnos sp.*	*Bactocera(z)nongensis*	species E	23/01/96 T. Hat Som Pean	-ovipositor short and straight at tip
RN232	Ranong	*Gacinia costata*	*Bactocera aptata*	species C	14/05/96 T. Hat Som Pean	**species E ;** -brown body
RN376	Ranong	*Cleistocalyx operculatus*	*Bactocera sp.*	species D	23/06/96 T. Hat Som Pean	-ovipositor appically sinous -hind femora predominantly black
RN388	Ranong	*Eugenia formosana*	*Bactocera sp.*	species D	21/07/96 T. Bang Non	*Opius incisi* ;
RN413	Ranong	*Randia eucodon*	*Bactocera sp.*	species A species C	27/07/96 T. Bang Non	-stigma bicolour
SR	Saraburi	*Artabotrys siamensis*	*Bactocera dorsalis* Na-bak	species B species C	14/05/96	

Figure 3. Map of Thailand showing field collections at seven localities of species B and C of *Diachasmimorpha* utilized for enzyme electrophoresis study.

Chapter II

Objectives

The objectives of this study are as follow:

1. To develop the method of enzyme electrophoresis to be used as genetic markers for identify species of fruit fly parasitoids (*Diachasmimorpha sp.*) in Thailand.

2. To estimate the genetic variation of *Diachasmimorpha sp.* and establish possible phylogenetic relationships in different population.

3. To compare the relationship between parasitoids and their hosts by electrophoretic technique.

4. To use this study as the basic background for other braconid parasitoids.

Chapter III

Materials and Methods

3.1 Fruit fly and Parasitoid collection

Fruit flies and parasitoids are obtained by collecting fruits infested with the fruit fly larvae in the field. Then put them into plastic box with sawdust, allow them to pupate and emerge either as adult fruit flies or as adult parasitoids. For propagation in laboratory, fruit fly larvae are exposed to adult parasitoids for parasitization. After that larvae are allow to pupate and emerge. The larvae which are parasitized by parasitoids, they will emerged to be parasitoid unless they will emerged to be fruit flies.

3.2 Electrophoresis

Horizontal polyacrylamide gel electrophoresis followed procedure described by Green et al., 1990 and by Harris and Hopkinson, 1976 with some modifications. The glass plates 20 cm long, 19 cm wide fixing with glass side pieces 19 cm long, 1 cm wide and 0.2 cm thick on both sides for provide the thickness of the gel is using as mold for preparing the gel. The mold for gel polymerization is prepared by

arranging 4 of the mold glass into a stack and covering them with a glass plate of the same size. Then inserting them into a plastic bag and sandwiched between two glass plates, seal with masking tape to prevent any gel solution leakage except the top side of the mold which use for pouring the gel solution.

Gel and Buffer systems

Two kinds of buffer systems are used in this enzyme assay study.

1. TC (Tris-Citrate) pH 7.1

Trisma base	4.090 g.
Citric acid	2.310 g.
Distilled water	1000.000 ml.

2. TC (Tris-Citrate) pH 7.1 dilute

Trisma base	2.045 g.
Citric acid	1.155 g.
Distilled water	1000.000 ml.

The concentration of gel for every buffer system is 5.8 % and the ratio of acrylamide/bis acrylamide is 3 %. The components of the gel are as follow:

TC buffer	280.000 ml.	
Acrylamide	16.240 g.	
Bis-acrylamide	0.487 g.	
Sucrose	7.000 g.	(2.5 % of final volume)
Kodak photo flo®	1.400 ml.	(0.5 % of final volume)
TEMED	0.210 ml.	
Ammonium persulfate	0.210 g.	

Dissolve acrylamide, bis acrylamide, sucrose and photo flo in TC buffer together by stirring, then add TEMED and ammonium persulfate rapidly. The gel solution is then pour into the mold, overlayed with distilled water. The gel becomes polymerized at room temperature for next 30 minutes, and then refrigerated overnight.

Gel and Sample preparation

The mixture of grinding solution is 1% tritonX-100 and 0.1% bromphenol blue in distilled water, place the solution in an ice

bath. Each sample is placed on a glass slide and homogenized with a ground glass rod in 15 µl. of grinding solution. The homogenate is absorbed onto a small pieces of Whatman filter paper No. 1, size 0.15 cm wide x 0.45 cm long.

To use, the polymerized gel is removed from the glass mold and covered with plastic sheet, then cut to made slots for sample application. The slots are about 0.5 cm long and 0.15 cm apart. The gel is cut at predetermined line into two tiers. So that one gel plate can be used for staining two enzyme systems. Place the gel plates on the cooling brass plates, then inserted the filter papers which absorbed the homogenized sample into the cut slots of the gel. Twenty-five samples and one dye marker were applied into each gel. After that, the gel is removed from the glass mold and sandwiched between two plastic sheets, then fill the buffer tanks with the appropriate buffer. Connect the gel to electrode buffer compartment by wicks made of paper towels on both ends of the gel. Place the gel between the lower and upper cooling brass plate and connect the electric cords of the power supply to the electrode tanks. The electrophoresis runs from cathode to anode at constant voltage, 450 volts DC. The electrical power was applied until

bromphenol blue markers reach 5 cm. from the origin. Then switched off the power supply and the gels are ready for histochemical staining.

Histochemical staining

Activity of each enzyme is assayed using a histochemical staining method which is specific to each enzyme system. The staining recipes solutions and their chemical reactions are as follow:

Staining recipes

PGD (Phosphogluconate dehydrogenase)

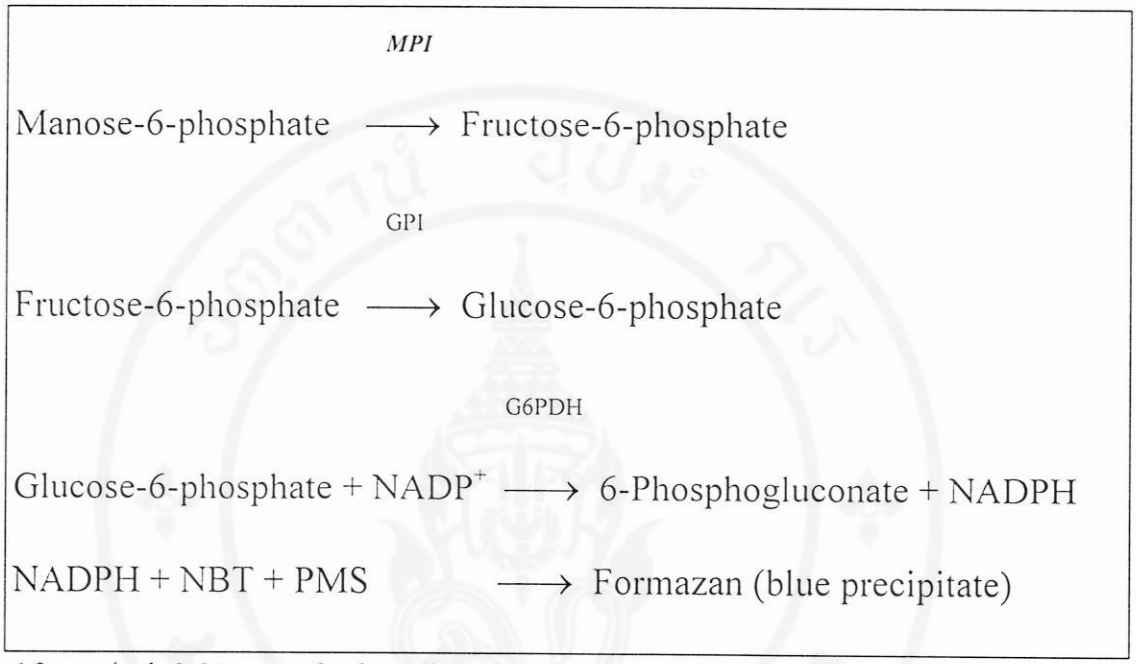
Other names: 6-Phosphogluconic dehydrogenase; 6-Glucosephosphate dehydrogenase.

PGD
6-Phosphogluconate + $NADP^+$ $\longrightarrow$ Ribulose-5-Phosphate + NADPH
NADPH + NBT + PMS $\longrightarrow$ Formazan (blue precipitate)

1 mg/ml. Sodium-6-phosphate gluconic acid	3.00 ml.
0.2M Tris-HCl pH 8.0	20.00 ml.
10 mg/ml. $MgCl_2$	2.80 ml.
10 mg/ml. $NADP^+$	1.40 ml.
10 mg/ml. NBT	0.70 ml.
Incubate at least 1-2 hour	
Add PMS	4.00 mg.

MPI (Manosephosphate isomerase)

Other name: Manose-6-phosphate isomerase.



10 mg/ml. Manose-6-phosphate	2.10 ml.
0.2M Tris-HCl pH 8.0	20.00 ml.
10 mg/ml. $MgCl_2$	2.80 ml.
Glucose-6-phosphate dehydrogenase	50.00 µl.
Phosphoglucose isomerase	15.00 µl.
10 mg/ml. $NADP^+$	1.40 ml.
10 mg/ml. NBT	0.70 ml.
Incubate at least 1-2 hour	
Add PMS	4.00 mg.

G6PDH (Glucose-6-phosphate dehydrogenase)

Other name: D-Glucose-6-phosphate:$NADP^+$-1-oxidoreductase

G6PDH
D-Glucose-6-phosphate+ $NADP^+$ ⟶ 6-Phosphogluconate+NADPH
NADPH + NBT + PMS ⟶ Formazan (blue precipitate)

Sodium-α-D-Glucose-6-phosphate	50.00 mg.
1.0M Tris-HCl pH 8.0	5.00 ml.
0.2 M $MgCl_2$	5.00 ml.
10 mg/ml. $NADP^+$	1.00 ml.
10mg/ml NBT	2.00 ml.
Add Dist. H_2O	28.00 ml.
Incubate at least 1 hour.	
Add PMS	5.00 mg.

PGM (Phosphoglucomutase)

Other name: Glucose phosphomutase.

$$\text{Glucose-1-phosphate} \xrightarrow{PGM} \text{Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + NADP^{+} \xrightarrow{G6PDH} \text{6-Phosphogluconate} + NDAPH$$

$$NADPH + NBT + PMS \longrightarrow \text{Formazan (blue precipitate)}$$

Sodium-α-D-Glucose-1-phosphate	0.20 g.
1.0M Tris-HCl pH 8.0	5.00 ml.
0.1 M $MgCl_2$	10.00 ml.
Glucose-6-phosphate dehydrogenase	100.00 µl.
10 mg/ml. $NADP^{+}$	1.00 ml.
10 mg/ml NBT	2.00 ml.
Incubate at least 1 hour.	
Add PMS	4.00 mg.

GPI (Glucosephosphate isomerase)

Other names : Phosphohexose isomerase; Phosphohexomutase; Hexose phosphate isomerase; Oxoisomerase; Glucose-6-phosphate isomerase; Phosphosaccharomutase; Phosphoglucosisomerase; D - glucose - 6 - ketol isolmerase.

GPI
D-Fructose-6-phosphate $\longrightarrow$ D-Glucose-6-phosphate
G6PDH
D-Glucose-6-phosphate + $NADP^+$ $\longrightarrow$ 6-Phosphogluconate + NADPH
NADPH + NBT + PMS $\longrightarrow$ Formazan (blue precipitate)

10 mg/ml. Fructose-6-phosphate	1.00 ml.
1.0M Tris-HCl pH 8.0	2.50 ml.
0.1 M $MgCl_2$	2.50 ml.
Glucose-6-phosphate dehydrogenase	50.00 µl.
10 mg/ml. $NADP^+$	0.50 ml.
10 mg/ml. NBT	0.75 ml
Add dist. H_2O to	20.00 ml.
Incubate at least 1 hour.	
Add PMS	5.00 mg.

IDH (Isocitrate dehydrogenase)

	IDH	
Isocitric acid + $NADP^+$	⟶	2-Oxoglutarate + NADPH
NADPH + NBT + PMS	⟶	Formazan (blue precipitate)

0.06M Sodium isocitrate	0.50	ml.
1M Tris-HCl pH 8.5	2.50	ml.
0.1 M $MgCl_2$	2.50	ml.
10 mg/ml. $NADP^+$	0.75	ml.
10 mg/ml. NBT	0.75	ml.
Add dist. H_2O to	40.00	ml.
Incubate at least 1 hour.		
Add PMS	4.00	mg.

MDH (Malate dehydrogenase)

MDH
D,L-Malic acid + NAD^+ ⟶ Oxaloacetate +NADH
NADH + NBT + PMS ⟶ Formazan (blue precipitate)

1.0M Sodium malate	0.50	ml.
1.0M Tris-HCl pH 8.5	5.00	ml.
10 mg/ml. NAD^+	0.75	ml.
10 mg/ml. NBT	1.25	ml.
Add dist. H_2O to	50.00	ml.
Incubate at least 1 hour.		
Add PMS	4.00	mg.

ME (Malic enzyme)

Other name: Malate dehydrogenase-$NADP^-$ dependant.

ME
D,L-Malic acid + $NADP^+$ ⟶ Pyruvate + NADPH
NADPH + NBT + PMS ⟶ Formazan (blue precipitate)

1.0M Malic acid pH 7.0	0.20	ml.
1.0M Tris-HCl pH 8.5	2.50	ml.
0.1 M $MgCl_2$	1.25	ml.
10 mg/ml. $NADP^+$	1.00	ml.
10 mg/ml. NBT	1.00	ml.
Add dist. H_2O to	50.00	ml.
Incubate at least 1 hour.		
Add PMS	5.00	mg.

G3PDH (Glycerol-3-phosphate dehydrogenase)

Other names: Glycerophosphate dehydrogenase; NAD^+-α-glycerophosphate dehydrogenase.

G3PDH
D,L-α-Glycerophosphate+NAD^+ → Dihydroxyacetonephosphate+NADH
NADH + NBT + PMS → Formazan (blue precipitate)

D,L-α-Glycerophosphate	1.30 g.
0.2M Tris-HCl pH 8.0	50.00 ml.
0.1 M $MgCl_2$	1.00 ml.
Adjust pH to 8.0 by 5N HCl	
10 mg/ml. NAD^+	1.00 ml.
10 mg/ml. NBT	0.50 ml.
Incubate at least 1 hour.	
Add PMS	5.00 mg.

For histochemical staining, cut the gels at line where samples were applied. Placed each cut gel into a plastic box lined with plastic wrap and containing the appropriate staining solution. Put this plastic box in incubator at 37°C at least one hour, then added PMS and reincubated until the electromophs fully developed. Rinse the gels with tap water and fixed in fixing solution (glacial acetic acid 10%, 95% ethyl alcohol 6.4% and distilled water 83.6%) for at least 15 minutes. The fixative stop the staining activity, clear the background and preserve the electromophs. Store the gels in tap water overnight, then dry the gel and keep for use in analyzed electrophoretic data.

Analysis of electrophoretic data

Identification of electromorphs is possible by use of reference standards for each gel. For each locus, the most common allele in all population is taken as the reference allele (100), and the relative mobility of other allele is calculated from the ratio of distance migrated from the origin in the gel to that migrated by the reference allele (100). Genotypic frequencies of each enzyme locus in different population are used as input data for further analysis using a computer program BIOSYS-1 version 1.7 which is the FORTRAN IV computer program for

the analysis of electrophoretically detectable allelic variation. The analyses of primary genetic variability, such as, allelic frequencies, mean number of alleles per locus, percentage of loci polymorphic, heterozygosity and Chi-square test for deviation from Hardy-Weinberg are performed. Similarity and distance coefficients are calculated. Nei's (1978) unbiased genetic identity and genetic distance are used for clustering analysis by UPGMA. The dendrograms from the mentioned methods are also presented.

Table 2. Enzyme names, enzyme commission numbers, their abbreviations and buffer systems used for their analyses.

Enzyme Name	Enzyme Commission Number	Abbreviation	Buffer System and pH
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	TC pH 7.1
Malate dehydrogenase	1.1.1.37	MDH	TC pH 7.1
Malic enzyme	1.1.1.40	ME	TC pH 7.1
Isocitrate dehydrogenase	1.1.1.42	IDH	TC pH 7.1
Phosphogluconate dehydrogenase	1.1.1.44	PGD	TC pH 7.1 (dilute)
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH	TC pH 7.1 (dilute)
Phosphoglucomutase	2.7.5.1	PGM	TC pH 7.1
Manosephosphate isomerase	5.3.1.8	MPI	TC pH 7.1
Glucosephosphate isomerase	5.3.1.9	GPI	TC pH 7.1

Chapter IV

Results

Because there are no reports about the use of enzyme electrophoretic techniques in understanding the genetic variation of *Diachasmimorpha sp.* , there are no staining recipe specific for this parasitoid. In this study, with some modification from the recipe of Harris and Hopkinson (1976) and other recipes. There is an attempt to use them with insects in family Braconidae, two kinds of braconid parasitoid, *Diachasmimorpha sp.* (the parasitoid of fruit fly) and *Chelonus sp.* (the parasitoid of fruit moth). In this report, *Chelonus sp.* is used for testing electrophoretic techniques. The photographs shows that this recipe fully work in *Diachasmimorpha sp.* and partially work in *Chelonus sp.* and also shows that this technique can differentiate insects in the same family, same genus. Furthermore, this technique can be used to identify the sibling species complex of insects. In this electrophoretic study, thirteen loci with independent segregation are found in nine enzyme systems. Of these, only *Me* locus has only identical electrophoretic bandmorphs in all individuals of all population (figure 20). The remaining twelve loci, *G3pdh-1, G3pdh-2, G3pdh-3, Gpi,*

G6pdh, Idh, Mdh-1, Mdh-2, Mpi, Pgd, Pgm-1 and *Pgm-2* have polymorphic bandmorphs.

4.1 The electrophoretic patterns

Glycerol-3-phosphate dehydrogenase (G3PDH)

There are three electrophoretic loci of glycerol-3-phosphate dehydrogenase isozyme; designated as *G3pdh-1, G3pdh-2* and *G3pdh-3* (figure 4-5). It is found that *G3pdh-1* and *G3pdh-3* code for dimeric enzyme and *G3pdh-2* code for monomeric enzyme, *G3pdh-1* and *G3pdh-2* consist of three alleles while *G3pdh-3* consists of two alleles

G3pdh-1 is shown as a monomorphic locus with relative mobility 100 ($G3pdh\text{-}1^{A}$) in Nakornpathom species C. In other population there are alleles with mobility 107 ($G3pdh\text{-}1^{C}$) and 93 ($G3pdh\text{-}1^{B}$) and common allele.

G3pdh-2 is shown as a monomorphic locus with relative mobility 100 ($G3pdh\text{-}2^{A}$) in most population except Chiangmai species B and Petchabun species B which have alleles with mobility 105($G3pdh\text{-}2^{C}$) and 95 ($G3pdh\text{-}2^{B}$) and common allele.

G3pdh-3 is found to be a monomorphic locus with relative mobility 100 ($G3pdh\text{-}3^{A}$) in almost population except Saraburi species C,

and Chiangmai species B which have both alleles, common allele and allele with mobility 115 (*G3pdh-3*^{B}).

Glucosephosphate isomerase (GPI)

There is only one locus that has been found in all population studied (figure 6-7). There are three alleles in this locus, alleles *Gpi*^{A} (relative mobility 100), *Gpi*^{B} (relative mobility 80) and *Gpi*^{C} (relative mobility 60) and this locus code for dimeric enzyme.

Glucose-6-phosphate dehydrogenase (G6PDH)

There are four alleles of G6PDH isozyme that has been observed in one locus of G6PDH (figure 8-9), designated as alleles *G6pdh*^{A} (relative mobility 100), *G6pdh*^{B} (relative mobility 113), *G6pdh*^{C} (relative mobility 126) and *G6pdh*^{D} (relative mobility 139). In Chiangmai species B and Chumporn species B, *G6pdh* is fixed at allele *G6pdh*^{B} in all individuals.

Isocitrate dehydrogenase (IDH)

This isozyme shows only one locus which codes for monomeric enzyme (figure 10-11). This locus consists of seven alleles,

with the relative mobility 100 is allele Idh^A . Alleles Idh^B , Idh^C , Idh^D , Idh^E , Idh^F and Idh^G have the mobility 87, 74, 61, 48, 35 and 113, respectively. In Ranong species C, Nakornratchasima species C and Nakornratchasima species B, Idh is fixed at allele Idh^G and in Chumporn species B, it is fixed at allele Idh^F.

Malate dehydrogenase (MDH)

There are two electrophoretic loci of MDH isozyme. *Mdh-1* migrates anodally whereas *Mdh-2* migrates cathodally (figure 12-13). Each locus comprise three alleles and code for dimeric enzyme.

Mdh-1 consists of three alleles, designated as $Mdh\text{-}1^A$ (relative mobility 100), $Mdh\text{-}1^B$ (relative mobility 75) and $Mdh\text{-}1^C$ (relative mobility 125). In Nakornpathom species C and Saraburi species C, *Mdh-1* locus is shown as monomorphic locus with relative mobility 100 ($Mdh\text{-}1^A$) and with relative mobility 125 ($Mdh\text{-}1^C$) in Chumporn species B.

Mdh-2 consists of three alleles, designated as $Mdh\text{-}2^A$ (relative mobility 100), $Mdh\text{-}1^B$ (relative mobility 38) and $Mdh\text{-}1^C$ (relative mobility 123) and this locus exhibits itself as a monomorphic locus with mobility 123 in all population of species B in all individuals.

Manosephosphate isomerase (MPI)

There is only one locus that has been observed in MPI isozyme and this locus codes for monomeric enzyme (figure 14-15). There are five alleles in this locus namely, Mpi^{A} with relative mobility 100, Mpi^{B} with relative mobility 107, Mpi^{C} with relative mobility 114, Mpi^{D} with relative mobility 93 and Mpi^{E} with relative mobility 86.

Phosphogluconate dehydrogenase (PGD)

There is only one dimeric locus in PGD isozyme (figure 16-17). This locus consists of four alleles, alleles Pdg^{A} (relative mobility 100), Pdg^{B} (relative mobility 130), Pdg^{C} (relative mobility 160) and Pdg^{D} (relative mobility 70). In Chumporn *Pgd* is fixed at allele with relative mobility 100 (Pdg^{A}) in all individuals.

Phosphoglucomutase (PGM)

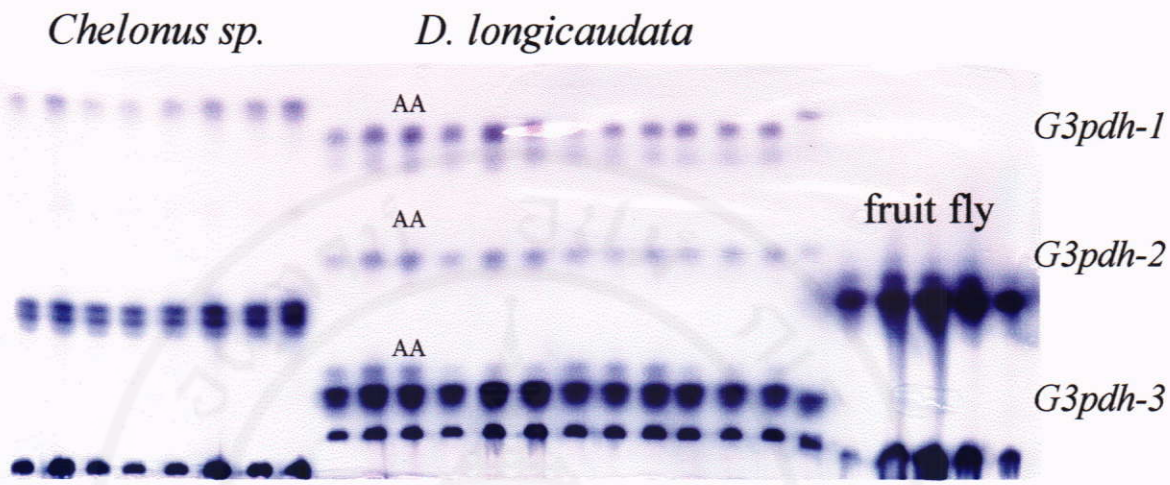
There are two monomeric loci for PGM isozyme (figure 18-19). At *Pgm-1* locus, there are five alleles while *Pgm-2* locus comprises three alleles.

Pgm-1 consists of alleles $Pgm\text{-}1^{A}$ with relative mobility 100, $Pgm\text{-}1^{B}$ with relative mobility 109, $Pgm\text{-}1^{C}$ with relative mobility 91, $Pgm\text{-}1^{D}$ with relative mobility 82 and $Pgm\text{-}1^{E}$ with relative mobility 73.

Pgm-2 consists of alleles $Pgm\text{-}2^{A}$ with relative mobility 100, $Pgm\text{-}2^{B}$ with relative mobility 87 and $Pgm\text{-}2^{C}$ with relative mobility 74.

Figure 4. Photograph of gel showing the example of electromorph patterns of the isozyme Glycerol-3-phosphate dehydrogenase (G3PDH) of species C (*D. longicaudata*) from Nakornpathom compared with moth parasitoid (*Chelonus sp.*) and fruit fly (*B. correcta*).

Figure 5. Diagram of electromorphs showing allelic and genotypic patterns of the isozyme Glycerol-3-phosphate dehydrogenase (G3PDH) of species B and C of *Diachasmimorpha* from natural populations in Thailand.



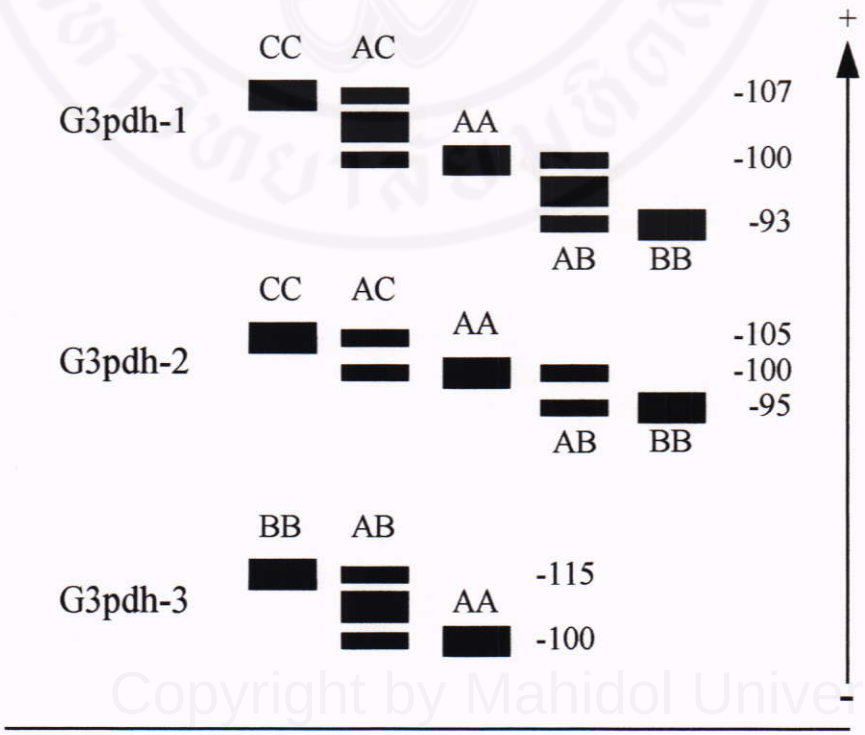
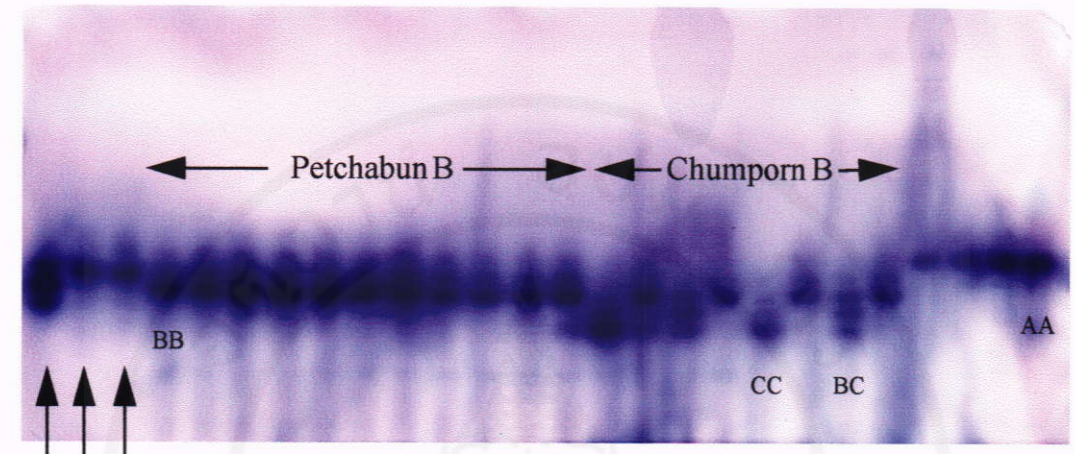


Figure 6. Photograph of gel showing the example of electromorph patterns of the isozyme Glucosephosphate isomerase (GPI) of two populations of species B compared with moth parasitoid (*Chelonus sp.*).

Figure 7. Diagram of electromorphs showing allelic and genotypic patterns of the isozyme Glucosephosphate isomerase (GPI) of *Diachasmimorpha sp*. from natural populations in Thailand.



Chelonus sp.

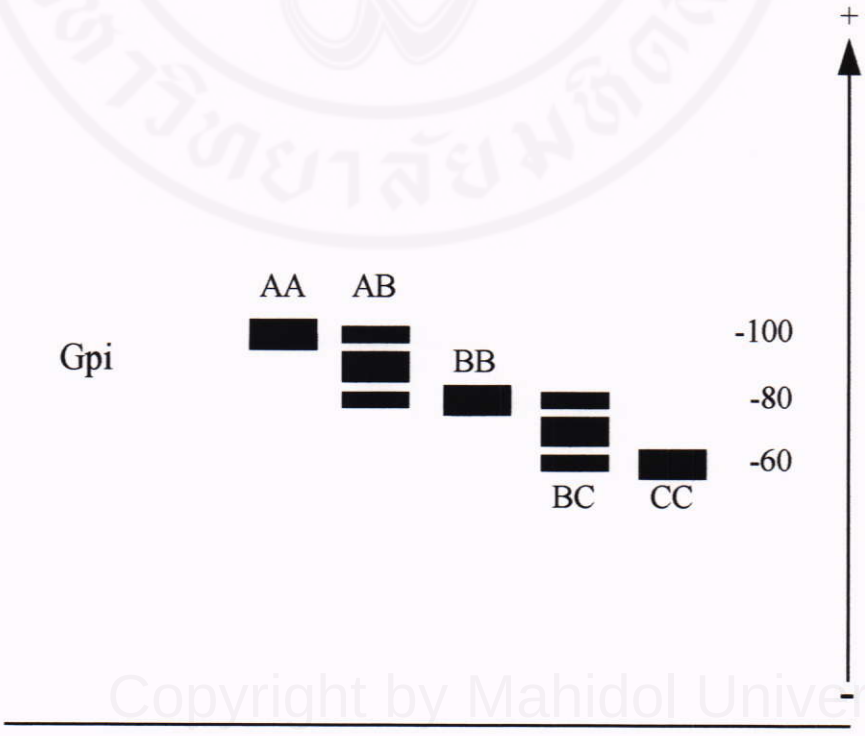
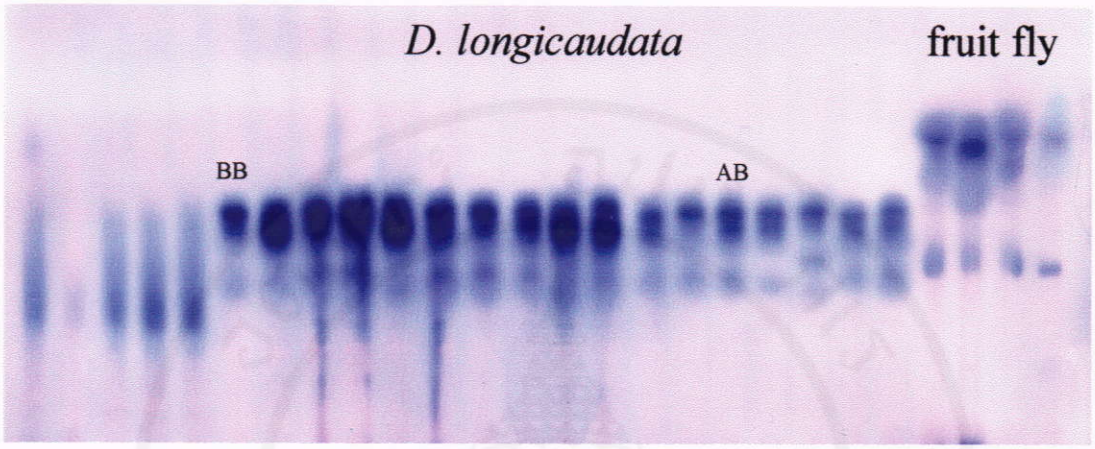


Figure 8. Photograph of gel showing the example of electromorph patterns of the isozyme Glucose-6-phosphate dehydrogenase (G6PDH) of species C (*D. longicaudata*) from Nakornpathom compared with fruit fly (*B.correcta*).

Figure 9. Diagram of electromorphs showing allelic and genotypic patterns of the isozyme Glucose-6-phosphate dehydrogenase (G6PDH) of *Diachasmimorpha sp*. from natural populations in Thailand.



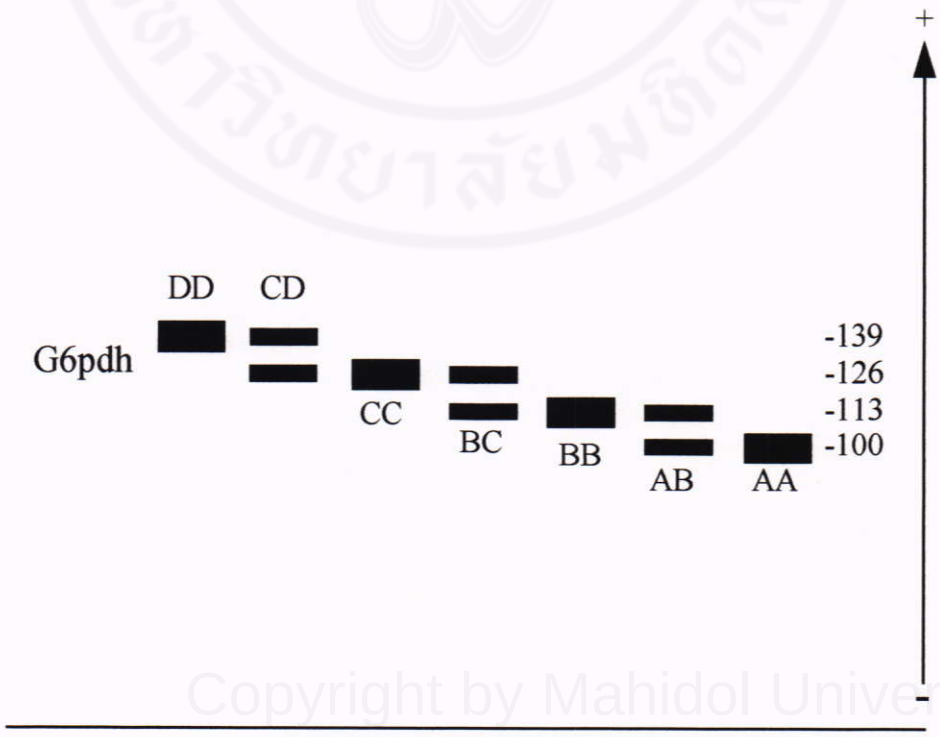
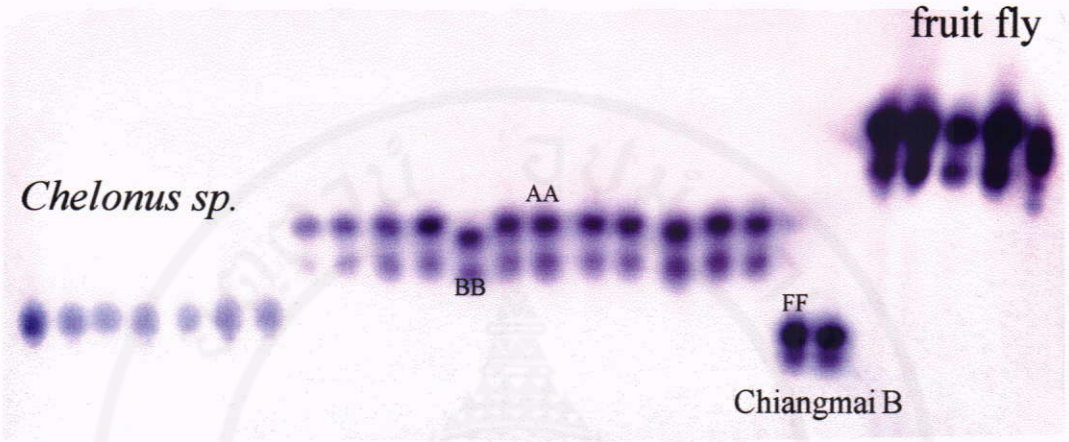


Figure 10. Photograph of gel showing the example of electromorph patterns of the isozyme Isocitrate dehydrogenase (IDH) of the species C from Nakornpathom compared with species B from Chiangmai, moth parasitoid (*Chelonus sp.*) and fruit fly (*B. correcta*).

Figure 11. Diagram of electromorphs showing allelic and genotypic patterns of the isozyme Isocitrate dehydrogenase (IDH) of the *Diachasmimorpha sp*. from natural populations in Thailand.



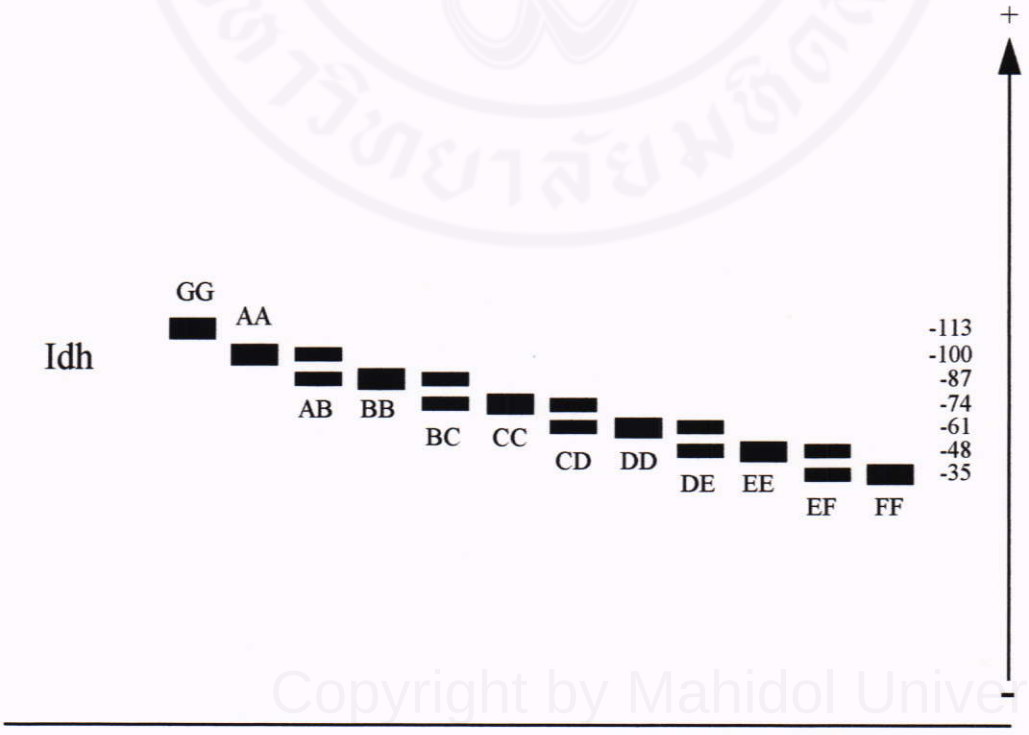
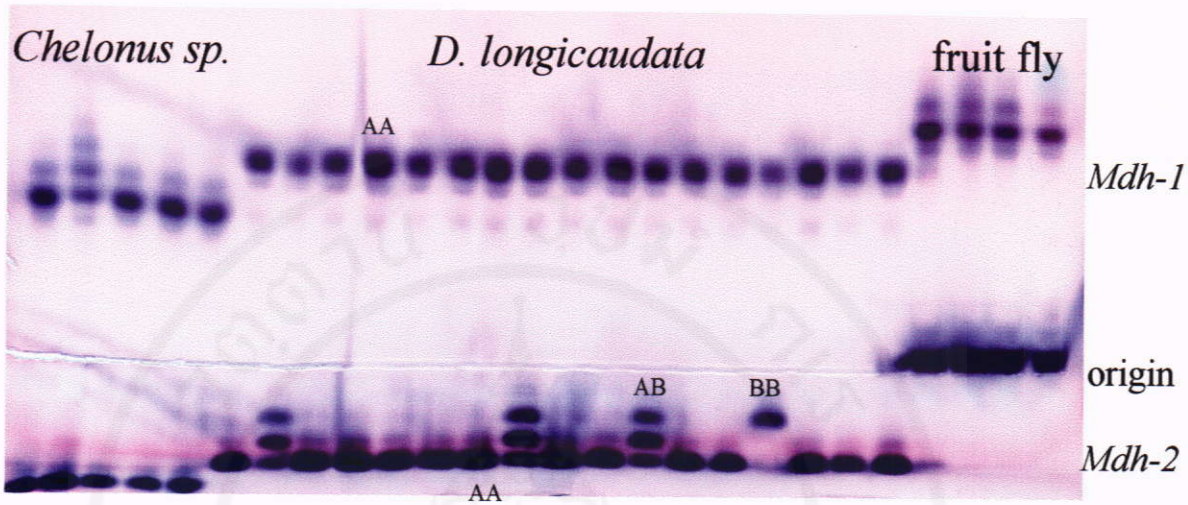


Figure 12. Photograph of gel showing the example of electromorph patterns of the isozyme Malate dehydrogenase (MDH) of the species C (*D. longicaudata*) from Nakornpathom compared with moth parasitoid (*Chelonus sp.*) and fruit fly (*B.correcta*).

Figure 13. Diagram of electromorphs showing allelic and genotypic patterns of the isozyme Malate dehydrogenase (MDH) of the *Diachasmimorpha sp.* from natural populations in Thailand.



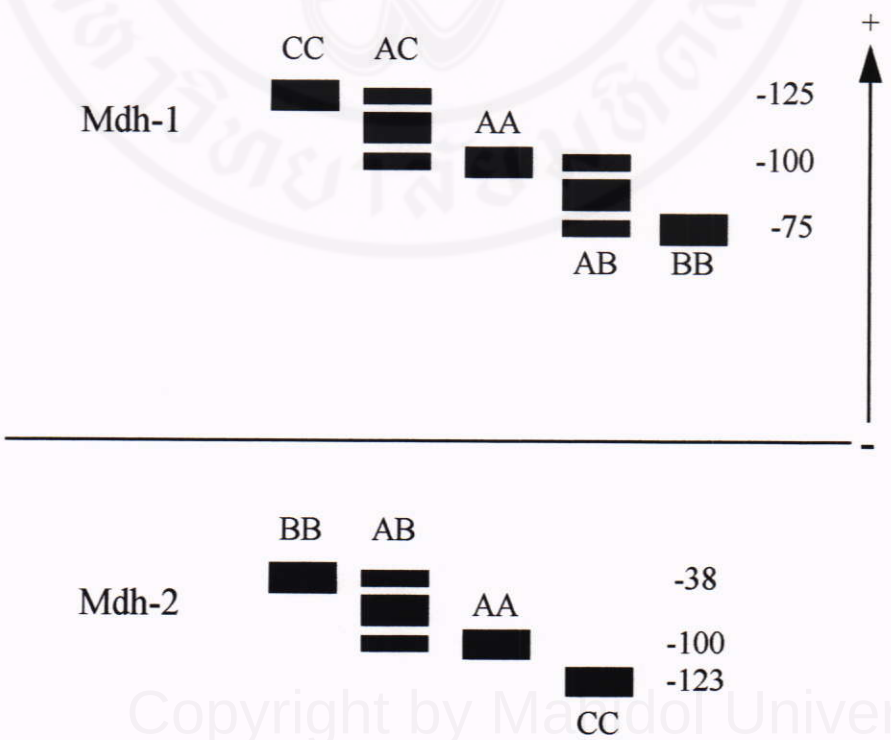
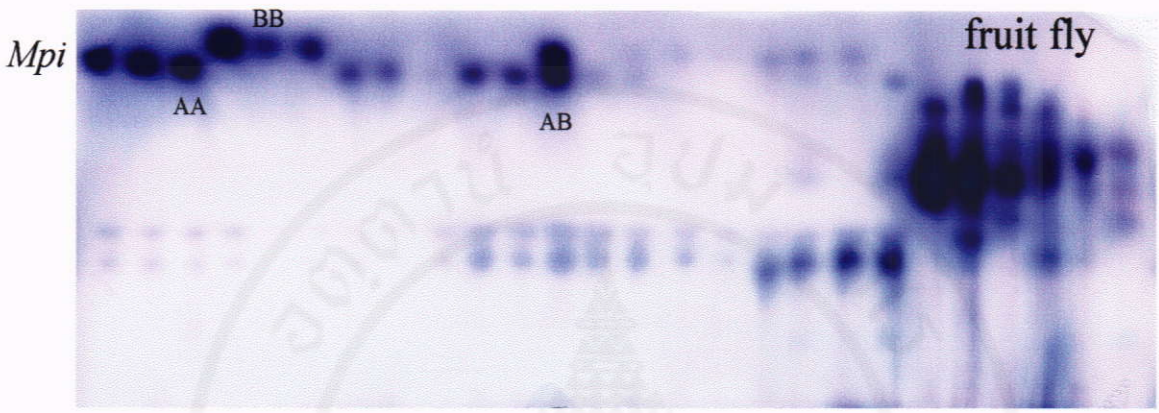


Figure 14. Photograph of gel showing the example of electromorph patterns of the isozyme Manosephosphate isomerase (MPI) of the species C from Nakornpathom compared with fruit fly (*B.correcta*).

Figure 15. Diagram of electromorphs showing allelic and genotypic patterns of the isozyme Manosephosphate isomerase (MPI) of the *Diachasmimorpha sp.* from natural populations in Thailand.



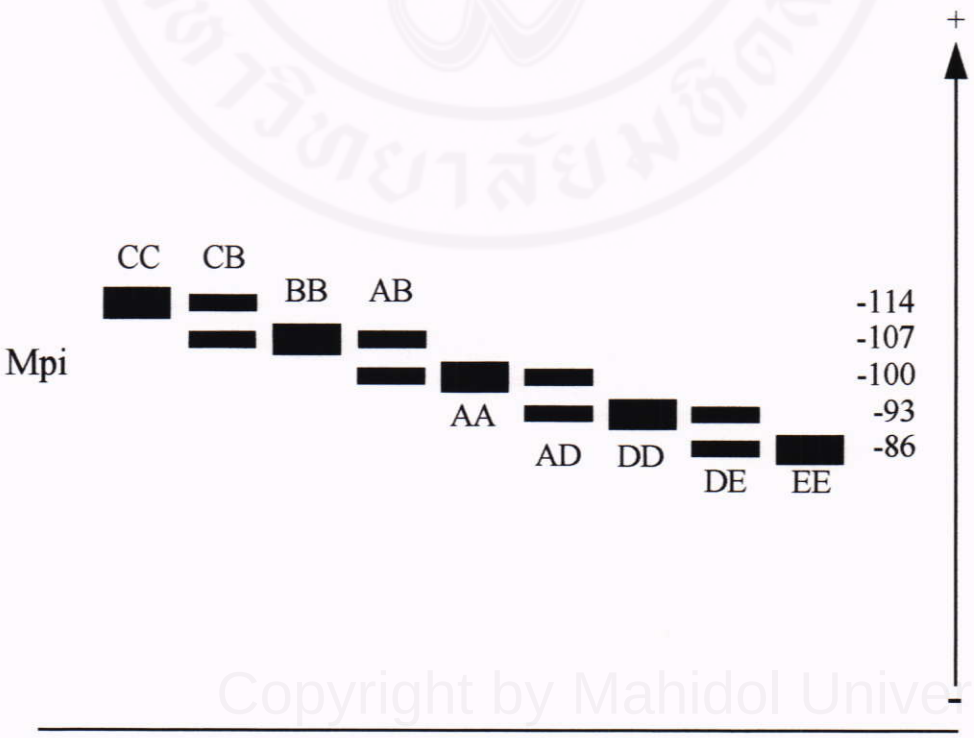
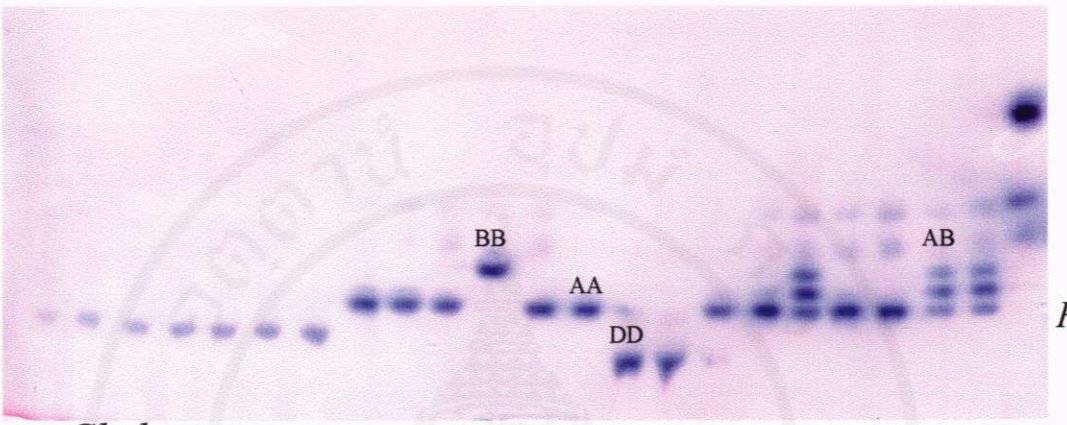


Figure 16. Photograph of gel showing the example of electromorph patterns of the isozyme Phosphogluconate dehydrogenase (PGD) of species C from Nakornpathom compared with species B from Petchabun and moth parasitoid (*Chelonus sp.*).

Figure 17. Diagram of electromorphs showing allelic and genotypic patterns of the isozyme Phosphogluconate dehydrogenase (PGD) of the *Diachasmimorpha sp.* from 42natural populations in Thailand.



Pgd

Chelonus sp. Petchabun B

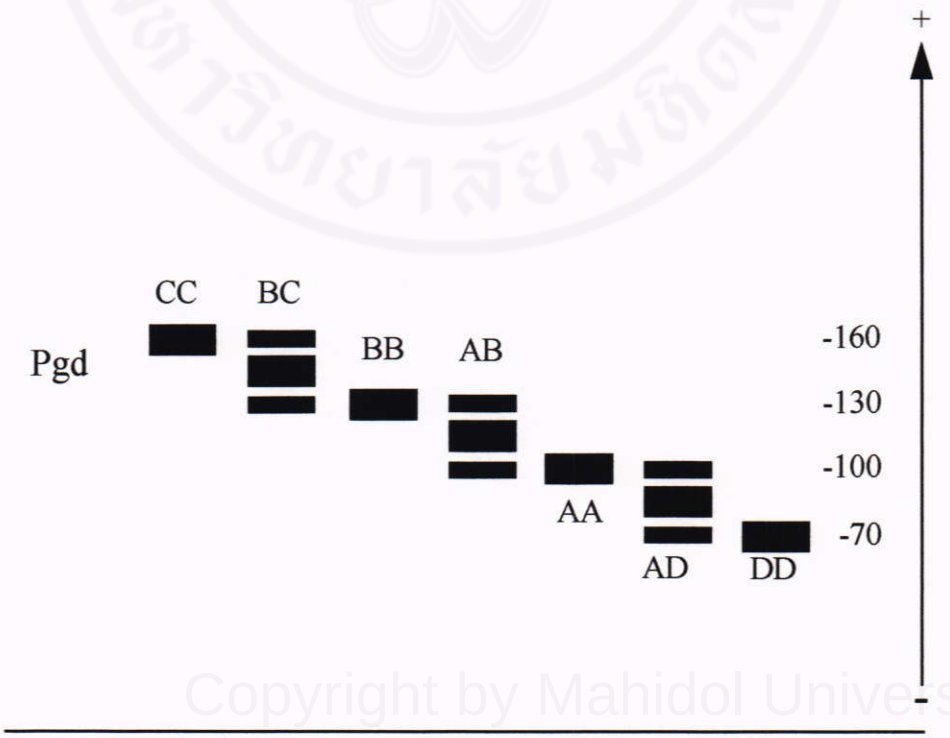
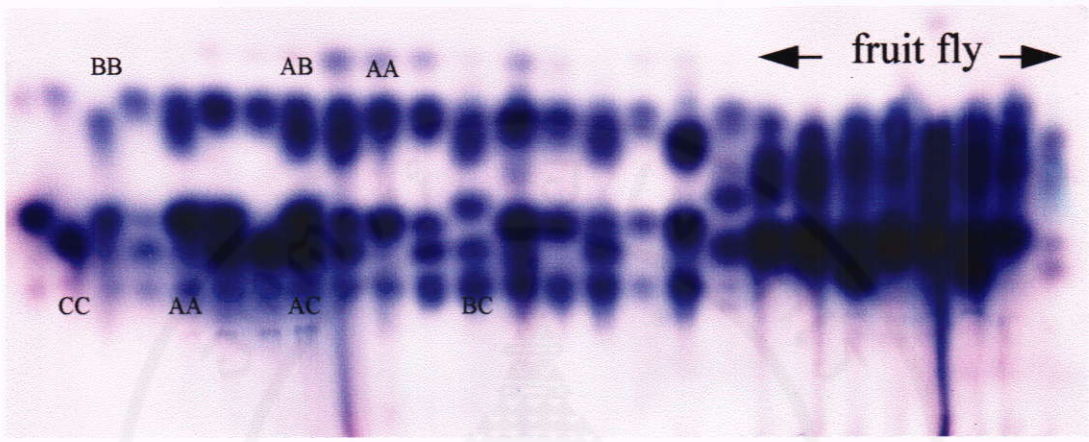


Figure 18. Photograph of gel showing the example of electromorph patterns of the isozyme Phosphoglucomutase (PGM) of the species C from Nakornpathom compared with fruit fly (*B. correcta*).

Figure 19. Diagram of electromorphs showing allelic and genotypic patterns of the isozyme Phosphoglucomutase (PGM) of the *Diachasmimorpha sp.* from natural populations in Thailand.



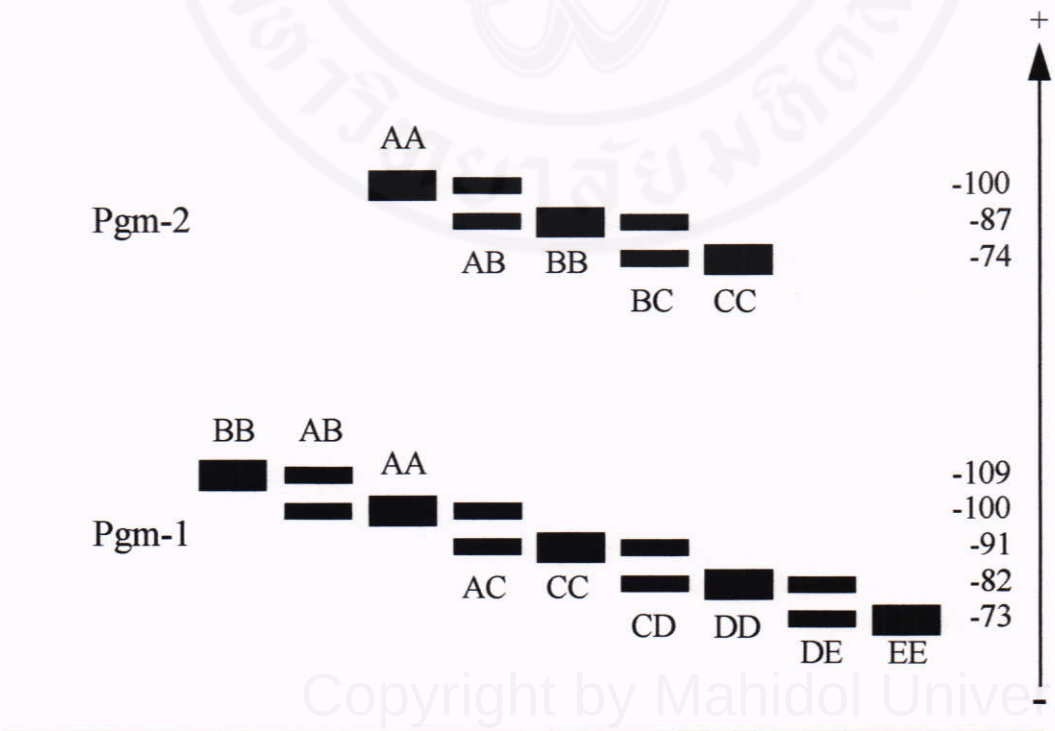
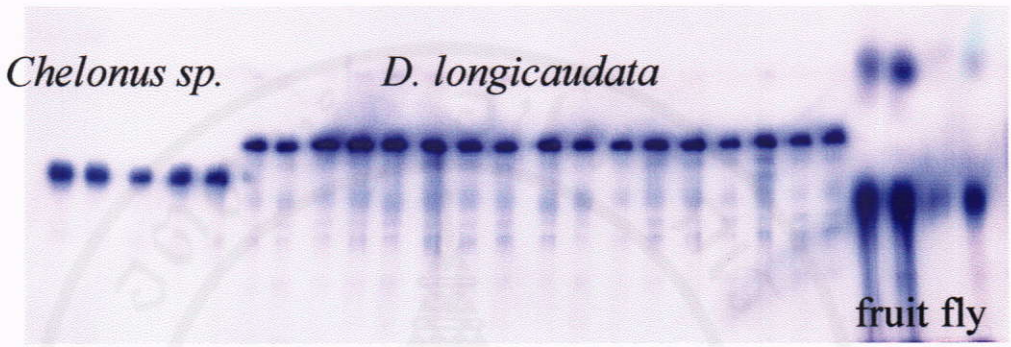
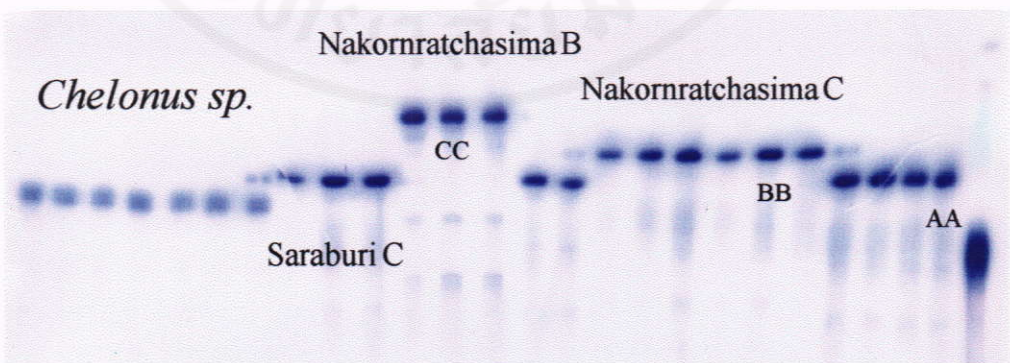


Figure 20. Above : Photograph of gel showing the example of electromorph patterns of Malic enzyme (ME) of the species C (*D. longicaudata*) from Nakornpathom compared with moth parasitoid (*Chelonus sp.*) and fruit fly (*B.correcta*).

Below : Photograph of gel showing the example of electromorph patterns of Malic enzyme (ME) of the species B from Nakornratchasima and species C from Nakornratchasima and Saraburi compared with moth parasitoid (*Chelonus sp.*).





4.2 Allelic frequencies and heterozygosity

Allelic frequencies, observed heterozygosity (Ho) and Hardy-Weinberg expected heterozygosity (He) for 12 loci, that showed variation per locus of nine groups of population in Thailand, are presented in table 3.

G3pdh-1 locus There are three alleles at the *G3pdh-1* locus. The allelic frequencies of *G3pdh-1*^{A} are high in species C with frequencies range from 0.344 to 1.000. In species B, allele *G3pdh-1*^{A} is absent, and their frequencies are 0.000. The allelic frequencies of *G3pdh-1*^{B} range from 0.000 to 0.594. The allelic frequencies of *G3pdh-1*^{C} are high in species B, their frequencies range from 0.052 to 1.000. In general, all show very low level of observed heterozygosity and expected heterozygosity.

G3pdh-2 locus There are three alleles at the *G3pdh-2* locus. The allelic frequencies of *G3pdh-2*^{A} are always high, ranging from 0.895 to 1.000. The alleles *G3pdh-2*^{B} and *G3pdh-3*^{C} are the rare alleles and present in low frequencies in Chiangmai species B and Petchabun

species B. The observed and expected heterozygosity are low in all population.

G3pdh-3 locus There are two alleles at the *G3pdh-3* locus. The allelic frequencies of $G3pdh\text{-}3^{A}$ are always high (> 0.950) and the allelic frequencies of $G3pdh\text{-}3^{B}$ are very low, ranging from 0 000 to 0.033. The expected and observed heterozygosity are very low in all population.

Gpi locus There are three alleles found in this locus. Gpi^{C} is the rarest allele and it is found only in Chumporn species B with very low frequencies. The allelic frequencies of Gpi^{A} are high in the population of species C and the allelic frequencies of Gpi^{B} are high in the population of species B. The value of expected heterozygosity ranges between 0.000 to 0.567 but the observed heterozygosity are very low in all population.

G6pdh locus There are four alleles found at the *G6pdh* locus. The allelic frequencies of $G6pdh^{A}$ are found only in Nakornpathom species C, in other population the frequencies are 0.000.

The allelic frequencies of *G6pdh*^{B} vary from 0.000 to 1.000. The allelic frequencies of *G6pdh*^{C} vary from 0.000 to 0.700. The allele *G6pdh*^{D} has been found only in Saraburi species B and the frequency is 0.633. The value of expected heterozygosity ranges from 0.000 to 0.495 and the value of observed heterozygosity ranges from 0.000 to 0.600.

Idh locus There are seven alleles found at the *Idh* locus. Alleles *Idh*^{A} and *Idh*^{B} have been found in Nakornpathom species C, and Saraburi species C. *Idh*^{E} and *Idh*^{F} have been found in Saraburi species B, Chiangmai species B, Petchabun species B and Chumporn species B, and *Idh*^{G} have been found in Ranong species C, Nakornratchasima species C and Nakornratchasima species B with the frequencies of 1.000. The observed heterozygosity in all population ranges from 0.000 to 0.333 and the expected heterozygosity is between 0.000 to 0.391.

Mdh-1 locus There are three alleles at the *Mdh-1* locus. The allelic frequencies of *Mdh-1*^{A} range from 0.125 to 1.000 and this allele is absent in Chumporn species B and Petchabun species B. The allelic frequencies of *Mdh-1*^{B} are quite low, with the value between 0.222 to 0.333. The allelic frequencies of *Mdh-1*^{C} has been found in Chiangmai

species B, Petchabun species B and Nakornratchasima species C, the frequencies range between 0.729 to 1.000. The expected heterozygosity ranges from 0.000 to 0.467 and the observed heterozygosity is quite low, ranging between 0.000 to 0.600.

Mdh-2 locus There are three alleles found at this locus. The allele $Mdh\text{-}2^{A}$ has been found in high frequencies in all population of species C, where the frequencies range from 0.944 to 1.000. The allelic frequencies of the allele $Mdh\text{-}2^{B}$ are 0.000 in most population except in Nakornpathom species C and Ranong species C but their frequencies are quite low. The allele $Mdh\text{-}2^{C}$ has been found in population of species B with the frequencies of 1.000. The observed and expected heterozygosity are very low in all population.

ME locus There are three alleles found at *Me* locus. The allele Me^{A} and Me^{B} have been found in population of species C with the frequencies of 1.000 and the allele Me^{C} has been found in population of species B with the frequencies of 1.000.

Mpi locus There are five alleles at the *Mpi* locus. The allele Mpi^A has been found in almost population except Ranong species C, Nakornratchasima species B and Saraburi species B. Allele Mpi^B has been found in almost population except Nakornratchasima species B. The allelic frequencies of Mpi^C range from 0 000 to 1.000 in Nakornratchasima species B. The expected heterozygosity in all population is quite high (> 0.45), observed heterozygosity is very low with the value ranging from 0.000 to 0.400.

Pgd locus There are four alleles found at this locus. The allelic frequencies of Pgd^A are always high (> 0.500), except in Petchabun species B and Saraburi species B. The allelic frequencies of Pgd^B vary from 0.049 to 0.214. Allele Pgd^D has been found in Chiangmai species B, Petchabun species B and Saraburi species B. The heterozygosity of all population is quite low, except in Chiangmai species B, where the values of observed and expect heterozygosity are 0.429 and 0.670, respectively.

Pgm-1 locus There are five alleles at *Pgm-1* locus. The allele $Pgm\text{-}1^C$ has been found in almost population except Petchabun

species B, with the frequencies ranging between 0.289 and 1.000. The alleles *Pgm-1*^{A} and *Pgm-1*^{B} have been found in most population with the frequencies ranging from 0.154 to 0.615 and 0.035 to 0.667, respectively, except in Nakornratchasima species C, Nakornratchasima species B and Saraburi species B. The allele *Pgm-1*^{D} has been found only in Nakornratchasima species C with the frequencies of 0.063. The expected heterozygosity in all population is quite high.

Pgm-2 locus There are three alleles found at this locus. The allelic frequencies of the allele *Pgm-2*^{A} range from 0.500 to 0.938. The allelic frequencies of the allele *Pgm-2*^{B} range from 0.063 to 1.000. The allele *Pgm-2*^{C} has been found in population from Ranong species C, Saraburi species B and Nakornratchasima species B with the frequencies of 0.056, 1.000 and 1.000, respectively. The expected heterozygosity of all population are relatively high but the observed heterozygosity is low in all population.

Table 3. Frequency of electromorph alleles, observed heterozygosity (Ho) and Hardy - Weinberg heterozygosity (He) per locus in different populations of species B and C of *Diachasmimorpha* from seven localities in Thailand (N = number of insect sampled per locus)

Locus	Population								
	NPC	RNC	NRC	SRC	NRB	SRB	CMB	CPB	PEB
(N)	271	9	16	61	5	15	19	15	20
G3pdh-1									
A	1 000	0 722	0 344	0.934	0 000	0 000	0.000	0 000	0 000
B	0 000	0 278	0.594	0.066	0 000	0 000	0 237	0 000	0 475
C	0 000	0 000	0 063	0 000	1.000	1 000	0 763	1 000	0 525
He	0 000	0 425	0 542	0 124	0 000	0 000	0 371	0 000	0 512
Ho	0 000	0 111	0 063	0.000	0 000	0.000	0.053	0 000	0 050
G3pdh-2									
A	1 000	1 000	1 000	1 000	1 000	1 000	0 895	1 000	0 975
B	0 000	0 000	0.000	0 000	0 000	0 000	0 026	0.000	0 025
C	0 000	0 000	0 000	0.000	0.000	0.000	0.079	0 000	0.000
He	0 000	0 000	0 000	0 000	0 000	0.000	0.198	0 000	0 050
Ho	0.000	0 000	0 000	0 000	0 000	0.000	0 105	0 000	0 050
G3pdh-3									
A	1 000	1 000	1 000	0 967	1 000	1 000	0.974	1.000	1.000
B	0 000	0 000	0 000	0 033	0 000	0 000	0 026	0 000	0 000
He	0 000	0 000	0.000	0 064	0.000	0.000	0.053	0.000	0 000
Ho	0 000	0 000	0 000	0 000	0.000	0 000	0 053	0 000	0 000
Gpi									
A	0 981	0 889	0 438	0.984	0 000	0 600	0 167	0.455	0 000
B	0 019	0 111	0 563	0 016	1 000	0.400	0.833	0 500	1 000
C	0 000	0 000	0 000	0.000	0 000	0 000	0 000	0 045	0 000
He	0 037	0 209	0.508	0.033	0.000	0 497	0 290	0 567	0 000
Ho	0.009	0.000	0 000	0.000	0.000	0.000	0.000	0.091	0 000
G6pgh									
A	0 927	0 000	0 000	0.000	0.000	0 000	0 000	0.000	0.000
B	0 073	0 667	0 656	0.566	0 300	0.000	1 000	1.000	0 900
C	0 000	0 333	0 344	0 434	0 700	0.367	0.000	0.000	0.100
D	0.000	0 000	0 000	0.000	0 000	0.633	0.000	0 000	0 000
He	0 135	0.471	0.466	0.495	0.467	0 480	0.000	0 000	0.185
Ho	0 069	0.222	0 188	0.148	0.600	0 067	0 000	0.000	0.100

(continued)

Locus	Population								
	NPC	RNC	NRC	SRC	NRB	SRB	CMB	CPB	PEB
Idh									
A	0 967	0 000	0 000	0 934	0 000	0 000	0 000	0 000	0 000
B	0 033	0.000	0 000	0 066	0 000	0 000	0 000	0 000	0 000
E	0.000	0.000	0 000	0 000	0 000	0 167	0 250	0 000	0 250
F	0.000	0.000	0 000	0 000	0 000	0 833	0 750	1 000	0 750
G	0.000	1.000	1 000	0 000	1 000	0 000	0 000	0 000	0 000
He	0.063	0 000	0 000	0 124	0 000	0 287	0 391	0 000	0 385
Ho	0 036	0 000	0 000	0 033	0 000	0 067	0 333	0 000	0 100
Mdh-1									
A	1 000	0 778	0 125	1 000	0 700	0.667	0 154	0 000	0 000
B	0 000	0.222	0 000	0 000	0 300	0 333	0 000	0 000	0 271
C	0 000	0 000	0 875	0 000	0 000	0 000	0 846	1 000	0 729
He	0 000	0 366	0 226	0 000	0 467	0 460	0 271	0 000	0 403
Ho	0 000	0 000	0 000	0 000	0 600	0 400	0.000	0 000	0 292
Mdh-2									
A	0 945	0 944	1 000	1 000	0 000	0 000	0 000	0 000	0 000
B	0 055	0 056	0 000	0 000	0 000	0 000	0 000	0 000	0 000
C	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000	1 000
He	0.105	0 111	0 000	0 000	0 000	0 000	0 000	0 000	0 000
Ho	0 059	0 111	0 000	0 000	0 000	0 000	0 000	0 000	0 000
Mpi									
A	0 530	0 000	0 233	0.533	0 000	0 000	0 357	0 182	0 333
B	0 470	0 222	0 167	0 418	0 000	0 067	0 643	0 455	0 250
C	0.000	0 778	0 600	0 049	1 000	0 933	0 000	0 364	0 417
He	0 499	0.366	0 577	0 543	0 000	0.129	0 495	0 658	0.712
Ho	0 265	0 222	0.067	0 180	0 000	0 000	0 143	0 182	0 333
Me									
A	1 000	0 000	0 000	1 000	0 000	0 000	0 000	0 000	0 000
B	0 000	1 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000
C	0.000	0 000	0 000	0 000	1 000	1 000	1 000	1.000	1.000
He	0 000	0.000	0 000	0 000	0 000	0.000	0 000	0 000	0.000
Ho	0 000	0.000	0 000	0 000	0 000	0.000	0 000	0 000	0 000

(continued)

Locus	Population								
	NPC	RNC	NRC	SRC	NRB	SRB	CMB	CPB	PEB
Pgd									
A	0 841	0.889	0 938	0 951	1 000	0.000	0 500	1 000	0 000
B	0 159	0 111	0 063	0 049	0 000	0 000	0 214	0 000	0 000
C	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
D	0 000	0 000	0 000	0 000	0 000	1 000	0 286	0 000	1 000
He	0 267	0 209	0 121	0 094	0 000	0 000	0 670	0 000	0 000
Ho	0 244	0 222	0 000	0.033	0 000	0 000	0 429	0.000	0 000
Pgm-1									
A	0 615	0 000	0 000	0 675	0 000	0.000	0.154	0 333	0 333
B	0 093	0 389	0 000	0 035	0 000	0 000	0 308	0 167	0 667
C	0 292	0 611	0 938	0 289	1 000	1 000	0 538	0 500	0 000
D	0 000	0 000	0 063	0 000	0 000	0 000	0 000	0 000	0 000
E	0 000	0 000	0 000	0 000	0 000	0.000	0 000	0 000	0 000
He	0 529	0.503	0.121	0 463	0 000	0 000	0.615	0 638	0 455
Ho	0 254	0.111	0 000	0.123	0.000	0.000	0 000	0 333	0 000
Pgm-2									
A	0 680	0.500	0 938	0 713	0 000	0 000	0.000	0 600	0 000
B	0 320	0.444	0 063	0 287	0 000	0 000	1.000	0 400	1 000
C	0 000	0 056	0 000	0 000	1.000	1 000	0 000	0 000	0 000
He	0 436	0.582	0 121	0 413	0.000	0.000	0 000	.5.5	0 000
Ho	0 106	0 222	0 000	0 049	0.000	0 000	0 000	0 000	0.000

** Abbreviation for populations NPC=Nakornpathom species C, RNC=Ranong species C,NRC=Nakornratchasima species C, SRC=Saraburi species C, NRB= Nakornratchasima species B, CMB=Chiangmai species B, CPB=Chumphorn species B and PEB=Petchabun species B

4.3 Genetic variability analysis

Genetic variability at thirteen loci in nine gene pools of *Diachasmimorpha sp.* from seven localities in Thailand are presented in table 4. The mean number of alleles per locus ranges from 1.2 to 1.9. The percentage of loci polymorphic is high in Ranong species C, where the values are 69.2. In Chiangmai species B and Nakornratchasima species C have the same value of the percentages of loci polymorphic, it is 61.5. The percentage of loci polymorphic in Nakornpathom species C, Saraburi species C and Petchabun species B are similar, that is 46.2. The percentage of loci polymorphic in Saraburi species B and Chumporn species B are 38.5 and 30.8, respectively. The lowest polymorphism has been found in Nakornratchasima species B, where the value is 15.4. However, in general, all the population show very high polymorphism. The mean heterozygosity from Hardy-Weinberg expected (He) of all population ranges from 0.072 in Nakornratchasima species B to 0.258 in Chumporn species B.

Table 4. Genetic variability at 13 loci of different populations of species B and C of *Diachasmimorpha* in Thailand. (Standard errors in parenthesis.)

Population	Mean no of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
			Hdy Wbg expected**	Direct-count
Nakornpathom C	1 7	46 2	0.159	0 080
	(0.2)		(0 056)	(0.029)
Ranong C	1 8	69.2	0.249	0 094
	(0.2)		(0 060)	(0 028)
Nakornratchasima C	1.8	61.5	0.206	0.024
	(0.2)		(0 064)	(0 015)
Saraburi C	1 8	46.2	0 181	0.043
	(0 2)		(0.059)	(0 018)
Nakornratchasima B	1.2	15.4	0.072	0.092
	(0 1)		(0.049)	(0.062)
Saraburi B	1.4	38 5	0 143	0.041
	(0.1)		(0.058)	(0.031)
Chiangmai B	1.9	61 5	0 258	0 086
	(0 2)		(0.067)	(0.039)
Chumporn B	1 5	30 8	0 182	0 047
	(0 2)		(0.079)	(0.028)
Petchabun B	1.6	46.2	0.208	0.071
	(0.2)		(0.070)	(0.032)

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

** Unbiased estimate (Nei, 1978)

4.4 Statistical analysis

4.4.1 Chi-square test

The homogeneity of observed genotype frequencies of the data have been tested for the deviation from the expected frequencies under Hardy-Weinberg equilibrium using Chi-square goodness-of-fit at the 0.01 significance level with one degree of freedom (χ^2 value of $P \leq 0.01 = 6.64$)(Nei, 1978). When more than two allleles are presented in population, the pooling procedure is applied and pooled genotype frequencies are used. The Chi-square values of nine groups of population of *Diachasmimorpha sp* are presented in table 5. In Nakornpathom species C, the Chi-square values show that the population is not homogeneous for electromorph frequencies at loci *Gpi, G6pdh, Idh, Mdh-2, Mpi, Pgm-1* and *Pgm-2,* the values are deviated from the Hardy-Weinberg equilibrium. The population from Ranong species C is not homogeneous at loci *Gpi* and *Mdh-1* the other loci are homogeneous under Hardy - Weinberg equilibrium. The population from Nakornratchasima species C is homogeneous for electromorph frequencies at locus *G6pdh* (χ^2 value = 0.618) and loci *G3pdh-2, Idh, Mdh-2 and Me* (χ^2 value = 0.000). The population from Saraburi species

C is homogeneous under the Hardy-Weinberg equilibrium at loci *G3pdh-2, Mdh-1, Mdh-2, Me* (χ^2 value = 0.000). The population from Nakornratchasima species B is not found to deviate from Hardy-Weinberg equilibrium. The population from Saraburi species B is homogeneous for electromorph frequencies at locus *Mdh-1* (χ^2 value = 0.277) and at loci *G3pdh-1, G3pdh-2, G3pdh-3 Mdh-2, Me, Pgd, Pgm-1* and *Pgm-2* (χ^2 value = 0.000). The population from Chiangmai species B is homogeneous for electromorph frequencies under the Hardy-Weinberg equilibrium at loci *G3pdh-2, Idh, Mpi* and *Pgd* (χ^2 value = 5.139, 0.307, 4.267 and 0.340 respectively) and at loci *G6pdh, Mdh-2, Me* and *Pgm-2* (χ^2 value = 0.000). The population from Chumporn species B is homogeneous for electromorph frequencies at almost loci except *Gpi, Mpi* and *Pgm-2* and the population from Petchabun species B is homogeneous for electromorph frequencies at almost loci except *G3pdh-1, Idh* and *Pgm-1*.

4.4.2 Fixation indices (F) and Coefficient for heterozygote deficiency or excess (D)

The fixation indices (F) and Coefficient for heterozygote deficiency or excess (D) per locus in nine populations of *Diachasmimorpha sp* are presented in table 6. The value of fixation indices ranges from +1.000 to -1.000. When the fixation index value is +1.000, it means that this population is totally devoid of heterozygotes. If it equals to 0.000, this population is under the Hardy-Weinberg equilibrium and the fixation index value equals to -1.000 for the total absence of homozygotes in this population. The D values range from +1.000 (excess heterozygotes) to -1.000 (excess homozygotes). If the D value equals to 0.000, it means that both observed and expected heterozygotes have the same number.

From table 6 , the F and D values of Me locus are +1.000 and -1.000, respectively, due to the monomorphic form of this locus. These values mean that the *Me* locus is fixed for one allele. In Ranong species C at loci *Mdh-2* and *Pgd*, the F and D values are -0.059, 0.000, -0.125 and 0.000, respectively. In Nakornpathom species C at locus *Pgd*, the F and D values are 0.088 and 0.-0.090. In Chiangmai species B at locus *G3pdh-3*, the F and D values are -0.027 and 0.000, and in Petchabun species B at locus *G3pdh-3* the F and D values are -0.026 and

0.000. These values mean that these groups of population at these loci are near the Hardy-Weinberg equilibrium and they have the same number of expected and observed values of heterozygotes. In Nakornratchasima species B, the F and D values at loci *G6pdh* and *Mdh- 1* are the same, that are -0.429 and 0.286, respectively. These values can be interpret that there are excess heterozygotes in Nakornratchasima species B population. The F and D values of other groups of population at all loci are vary. However, the F and D values of all population are quite high. In general, it means that there are high frequencies of homozygote genotypes for these loci.

Table 5. Data showing the χ^2 value of different populations of species B and C of *Diachasmimorpha* in Thailand.

Locus	χ^2 value of population								
	NPC	RNC	NRC	SRC	NRB	SRB	CMB	CPB	PEB
G3pdh-1	0 000	5.908*	13 117	69 178	0 000	0.000	15.448	0 000	17 143
G3pdh-2	0 000	0 000	0 000	0.000	0 000	0.000	5.139*	0.000	0.000
G3pdh-3	0 000	0.000	0.000	80.684	0.000	0.000	0 000	0.000	0 000
Gpi	135.735	17 067	17 104	121 008	0.000	16.171	15.439	8.264	0 000
G6pdh	64 559	2 917*	6 183*	30.599	0 571*	12 057	0 000	0 000	5.474*
Idh	52 920	0 000	0.000	37.336	0 000	10.752	0 307*	0.000	11 959
Mdh-1	0 000	11 487	20.741	0.000	0.571*	0.277*	16 762	0.000	1.967*
Mdh-2	53 312	0.000	0 000	0.000	0 000	0.000	0 000	0.000	0.000
Me	0.000	0 000	0 000	0.000	0.000	0.000	0.000	0.000	0 000
Mpi	4.997	1 773*	16 171	25.446	0.000	29 037	4.267*	12.121	3.429*
Pgd	2 139*	0.067*	31 034	30 902	0 000	0.000	0.340*	0.000	0.000
Pgm-1	54.663	6 234*	31 034	30.428	0 000	0.000	14.098	6.063*	22 336
Pgm-2	125 142	6.381*	31.034	48.473	0 000	0.000	0 000	11.221	0.000

χ^2 = 0.000 mean as this locus fixed in one allele

* Under Hardy-Weinberg equilibrium (P ≥ 0 01)

** Abbreviation for populations NPC=Nakornpathom species C, RNC=Ranong species C, NRC=Nakornratchasima species C, SRC=Saraburi species C, NRB=Nakornratchasima species B, CMB=Chiangmai species B, CPB=Chumphorn species B and PEB=Petchabun species B

Table 6. Fixation indices (F) and coefficients for heterozygote deficiency or excess (D) per locus in different populations of species B and C of *Diachasmimorpha* in Thailand.

Locus	Population								
	NPC	RNC	NRC	SRC	NRB	SRB	CMB	CPB	PEB
G3pdh-1									
F	1.000	0 723	0.881	1.000	0.926	1.000	0 854	1.000	0.900
D	-1.000	-0 738	-0 885	-1.000	-0 928	-1 000	-0.858	-1 000	-0 902
G3pdh-2									
F	1 000	1 000	1.000	1.000	1 000	1.000	0.453	1.000	-0.026
D	-1 000	-1.000	-1 000	-1.000	-1.000	-1.000	-0 468	-1.000	0.000
G3pdh-3									
F	1.000	1.000	1.000	1.000	1 000	1 000	-0.027	1.000	1.000
D	-1 000	-1 000	-1 000	-1.000	-1 000	-1.000	0 000	-1.000	-1 000
Gpi									
F	0 745	1.000	1.000	1.000	1.000	1.000	1.000	0.832	0.444
D	-0 746	-1.000	-1.000	-1.000	-1 000	-1 000	-1.000	-0.840	-0.458
G6pdh									
F	0 489	0 500	0 584	0 700	-0.429	0.856	1.000	1 000	1.000
D	-0 490	-0 528	-0.597	-0.702	0.286	-0.861	-1.000	-1.000	-1 000
Idh									
F	0 426	1.000	0.903	0.732	1 000	0.760	0.111	1.000	0.733
D	-0.427	-1.000	-0.904	-0.735	-1.000	-0.768	-0.148	-1.000	-0 740
Mdh-1									
F	1.000	1.000	1.000	1.000	-0 429	0 100	1.000	1.000	0.262
D	-1.000	-1.000	-1.000	-1.000	0.286	-0.130	-1.000	-1.000	-0.277

Locus	Population								
	NPC	RNC	NRC	SRC	NRB	SRB	CMB	CPB	PEB
Mdh-2									
F	0 435	-0 059	1.000	1 000	1 000	1 000	1 000	1 000	0.262
D	-0 436	0 000	-1 000	-1.000	-1 000	-1 000	-1 000	-1.000	-0 277
Me									
F	1 000	1.000	1 000	1 000	1.000	1.000	1.000	1.000	1.000
D	-1 000	-1.000	-1 000	-1.000	-1.000	-1.000	-1.000	-1 000	-1 000
Mpi									
F	0.475	0 357	0.880	0.665	1.000	1.000	0.689	0.711	0.489
D	-0.476	-0 393	-0 884	-0 668	-1 000	-1.000	-0.711	-0.724	-0.532
Pgd									
F	0.088	-0.125	1.000	0.649	1.000	1.000	0 311	1 000	1.000
D	-0 090	0 062	-1 000	-0 652	-1 000	-1.000	-0 361	-1 000	-1 000
Pgm-1									
F	0 519	0 766	1.000	0 732	1.000	1 000	1 000	0.455	1.000
D	-0 520	-0.779	-1 000	-0.735	-1 000	-1.000	-1.000	-0.477	-1.000
Pgm-2									
F	0.757	0 596	1.000	0.880	1.000	1.000	1.000	1.000	1.000
D	-0 757	-0.618	-1 000	-0.881	-1 000	-1.000	-1 000	-1.000	-1 000

** Abbreviation for populations . NPC=Nakornpathom species C, RNC=Ranong species C, NRC=Nakornratchasima species C, SRC=Saraburi species C, NRB=Nakornratchasima species B, CMB=Chiangmai species B, CPB=Chumphorn species B and PEB=Petchabun species B

4.5 Genetic similarity and Genetic distance

Genetic similarity or identity (I) and genetic distance (D) can be used to measure the number of electrophoretically detectable substitutions per locus which have accumulated since the two groups of population are separated from the common ancestor. Similarity ranges from zero for no allele in common to one for the same alleles at identical frequencies and distance values ranged from zero to one (Nei, 1975; Ferguson, 1980). The value of Nei's standard genetic identity (I) and distance (D) and Rogers' genetic similarity and genetic distance between nine populations of *Diachasmimorpha sp.* basis on thirteen isozyme loci are presented in table 7 and table 8, respectively. The genetic identity (similarity) and genetic distance of Nei and Rogers were approximately the same value. The genetic identity of Nei among groups of population are quite high whereas Rogers' genetic identity are a little less than Nei's. The I value of Nei ranges from 0.277 to 0.939 and the values of Rogers ranges from 0.310 to 0.904. The D value of Nei ranges between 0.063 and 1.000 and the value of Rogers ranges between 0.096 and 0.690. The I value of Nei between Nakornpathom species C and Saraburi species C and between Chiangmai species B and Chumporn species B are the highest ; they are 0.923 and 0.939, respectively. In concordance

with I values, the D value between Nakornpathom species C and Saraburi species C and between Chiangmai species B and Chumporn species B are the lowest, they are 0.063 and 0.080, respectively. The I values of Nei between species C and species B range from 0.277 to 0.611 and the D values between species C and species B range from 0.492 to 1.000.

Table 7. Value of Nei 's unbiased genetic identity (I) ; below the diagonal and distance (D) ; above the diagonal between different populations of species B and C of *Diachasmimorpha* in Thailand, on the basis of 13 enzyme loci.

Population		NPC	RNC	NRC	SRC	NRB	SRB	CMB	CPB	PEB
Nakornpathom	(NPC)	*****	0 405	0 599	0 063	1 000	1.000	0 989	0 856	1.000
Ranong	(RNC)	0.667	*****	0.123	0.315	0.514	0.797	0.776	0.663	0 920
Nakornratchasima	(NRC)	0.549	0.884	*****	0.480	0 521	0.621	0.641	0 492	0 818
Saraburi	(SRC)	0.939	0.730	0.619	*****	0 936	1 000	0 868	0.723	1.000
Nakornratchasima	(NRB)	0.337	0 598	0 594	0 392	*****	0.236	0.445	0.391	0.565
Saraburi	(SRB)	0.325	0.451	0 398	0.344	0.790	*****	0.437	0.417	0 412
Chiangma	(CMB)	0.372	0.460	0.527	0.420	0.641	0 646	*****	0.080	0.085
Chumporn	(CPB)	0.425	0.515	0.611	0.485	0.677	0 659	0 923	*****	0.228
Petchabun	(PEB)	0.277	0 399	0 441	0.323	0.569	0.663	0.919	0.769	*****

Table 8. Value of Roger 's genetic similarity ; below the diagonal and genetic distance ; above the diagonal between different populations of species B and C of *Diachasmimorpha* in Thailand, on the basis of 13 enzyme loci.

Population		NPC	RNC	NRC	SRC	NRB	SRB	CMB	CPB	PEB
Nakornpathom	(NPC)	*****	0 371	0.493	0.096	0.666	0.671	0.619	0.557	0.690
Ranong	(RNC)	0 629	*****	0.207	0.321	0.442	0.551	0.557	0 490	0 599
Nakornratchasima	(NRC)	0.507	0 793	*****	0 420	0 448	0.586	0 501	0 417	0 552
Saraburi	(SRC)	0 904	0 697	0.580	*****	0 614	0 658	0 592	0.516	0.654
Nakornratchasima	(NRB)	0.334	0.558	0.552	0.386	*****	0 244	0.430	0 381	0.470
Saraburi	(SRB)	0.329	0 449	0.414	0.342	0 756	*****	0.411	0 375	0.386
Chiangma	(CMB)	0.381	0 443	0.499	0.408	0 570	0.589	*****	0 199	0.182
Chumporn	(CPB)	0.443	0 510	0 583	0.484	0.619	0.625	0 801	*****	0.299
Petchabun	(PEB)	0.310	0.401	0.448	0.346	0.530	0 614	0 818	0.701	*****

4.6 Cluster analysis

The cluster analysis of Nei's unbiased genetic identity using the unweighted pair group method with arithmetic averaging (UPGMA) among groups of population of *Diachasmimorpha sp.* is presented in table 9 and cluster analysis of Nei's unbiased genetic distance using UPGMA among groups of population is presented in table 10. Phylogenetic relationship between respective pairs of two groups of population among the nine groups of population are demonstrated by dendrograms based on the UPGMA are presented in figure 21 and 22.

The clustering levels between pairs of population from Nakornpathom species C versus Saraburi species C, Chumporn species B versus Chiangmai species B and Ranong species C versus Nakornratchasima species C are separated at identity values 0.93864, 0.92318 and 0.88415, respectively. These values show that the relationship between these groups of population are closely related and these results were supported by the genetic distance values of clustering levels between pairs of population. As for the D values, these populations are separated at values 0.06333, 0.07994 and 0.12312, respectively. They show that these groups of population have close distance before

separation. Population of species C and species B diverged at the value of I = 0.43472, and the value of D = 0.85638.

Table 9. Cluster analysis of Nei's unbiased genetic identity between different populations of species B and C of *Diachasmimorpha* in Thailand using unweighted pair group method with arithmetic averaging (UPGMA).

Population or cluster number joined			Clustering level	Cycle
Nakornpathom C	VS	SaraburiC	0 93864	1
Ranong C	VS	Nakornratchasima C	0.88415	1
Saraburi B	VS	Nakornratchaisma B	0.78952	1
Chaingmai B	VS	Chumporn B	0.92318	1
Nakornpathom C	VS	Ranong C	0.64113	2
Chiangmai B	VS	Petchabun B	0.85727	2
Chaingmai B	VS	Nakornratchasima B	0 64228	3
Nakornpathom C	VS	Nakornratchasima B	0 43472	4

Figure 21. Dendrogram showing phylogenetic relationships between species B and C of *Diachasmimorpha* from seven localities in Thailand estimated from identity value.

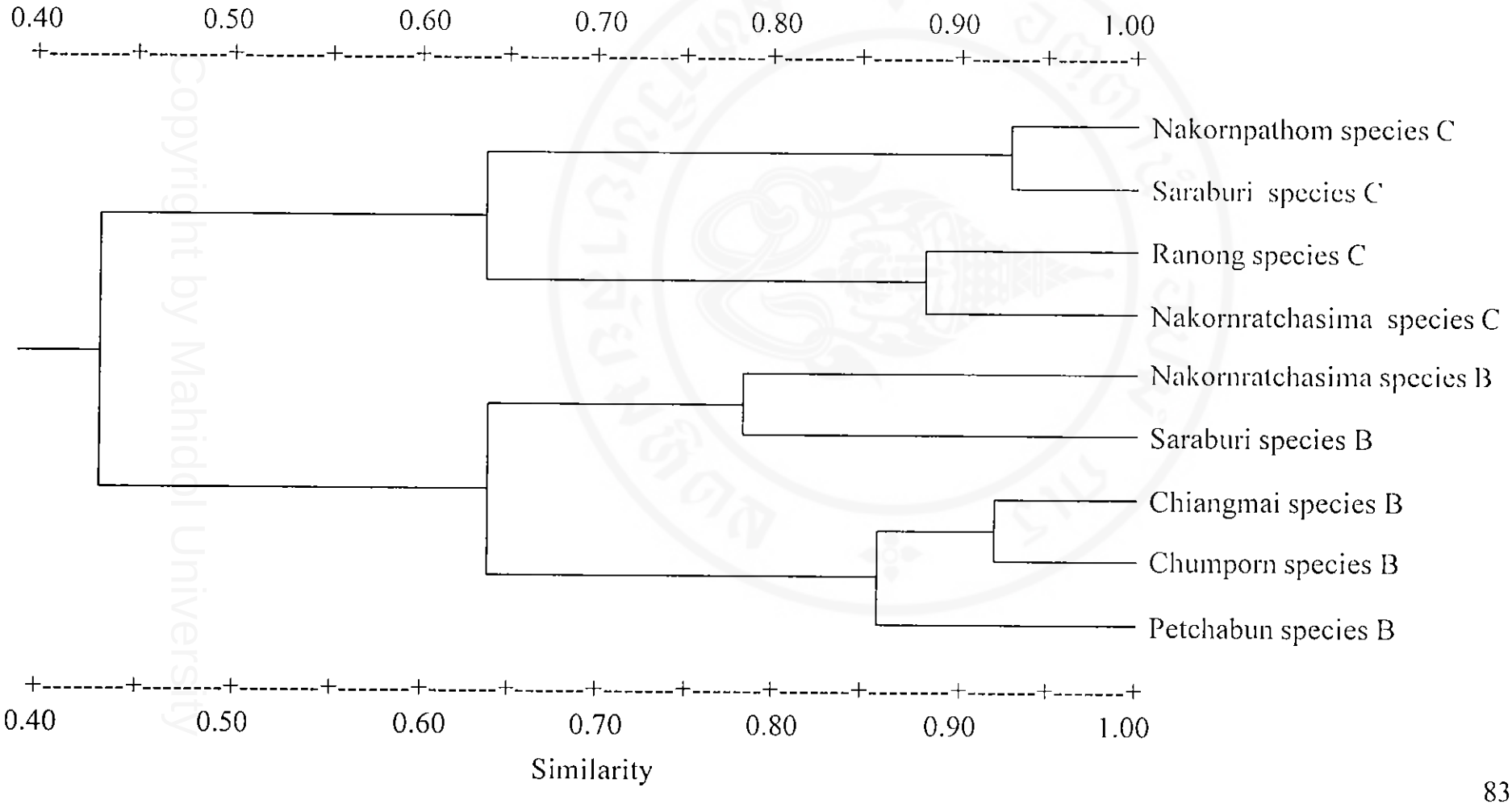
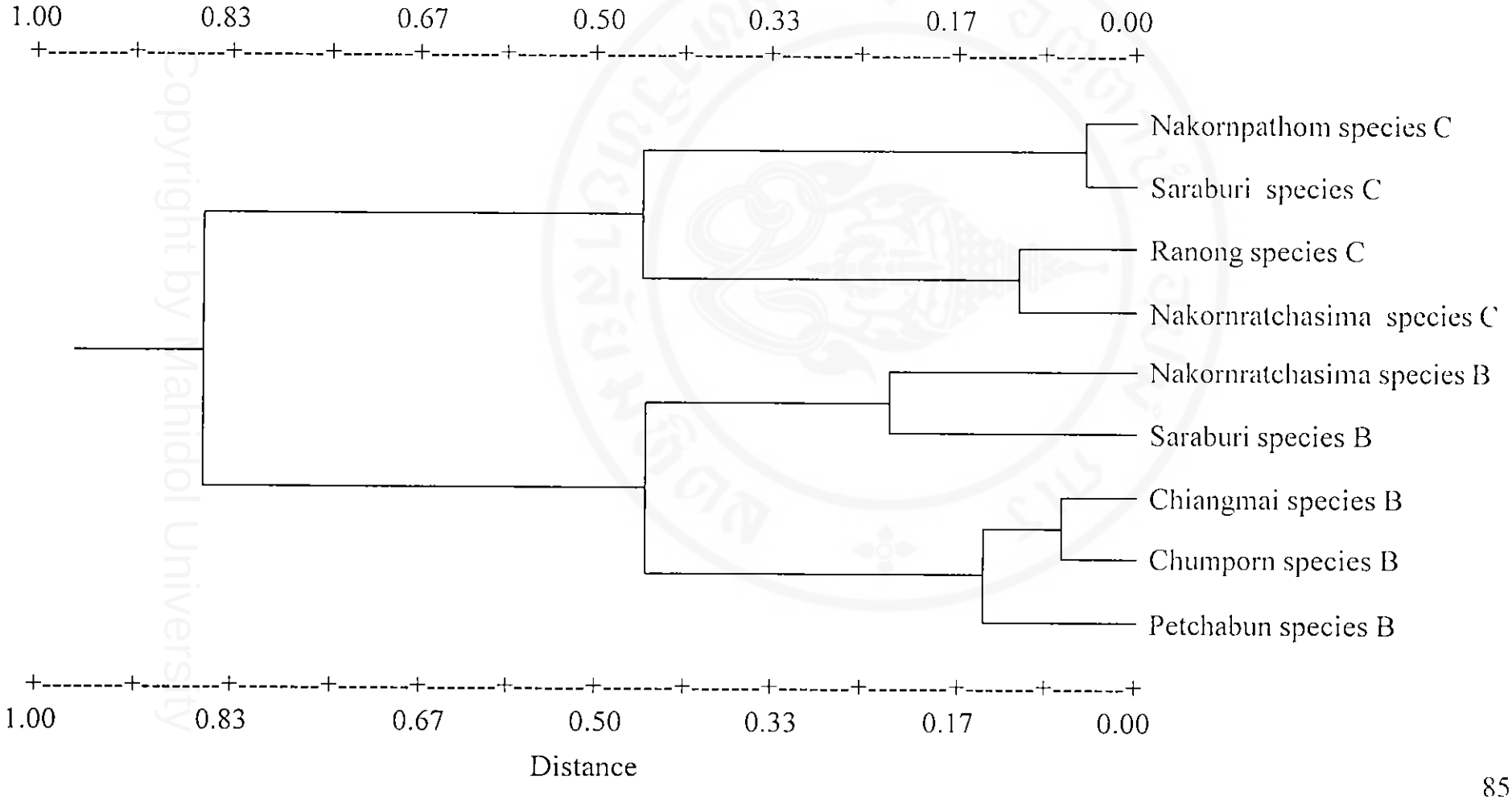


Table 10. Cluster analysis of Nei's unbiased genetic distance between different populations of species B and C of *Diachasmimorpha* in Thailand using unweighted pair group method with arithmetic averaging (UPGMA)

Population or cluster number joined		Clustering level	Cycle
Nakornpathom C	VS SaraburiC	0.06333	1
Ranong C	VS Nakornratchasima C	0.12312	1
Saraburi B	VS Nakornratchaisma B	0 23633	1
Chaingmai B	VS Chumporn B	0.07994	1
Nakornpathom C	VS Ranong C	0.4499	2
Chiangmai B	VS Petchabun B	0.15685	2
Chaingmai B	VS Nakornratchasima B	0.44429	3
Nakornpathom C	VS Nakornratchasima B	0 85638	4

Figure 22. Dendrogram showing phylogenetic relationship between species B and C of *Diachasmimorpha* from seven localities in Thailand estimated from distance value.



4.7 The relationship between host flies and their parasitoids.

The larval parasitoid, *Diachasmimorpha sp* lay their eggs into the body cavity of larvae of fruit fly and growing inside them. Then, fruit flies and their parasitoids possibly have some relationship. In this study, the electrophoresis of the larvae of parasitoid (*D longicaudata*) is run, compare with adult parasitoids and adult fruit flies to observe their relationship. Nine enzyme systems are used in this study. From these enzyme systems, three systems which are Malic enzyme (ME), Malate dehydrogenase (MDH) and Isocitrate dehydrogenase (IDH) show the relationship in electrophoretic bandmorphs. Another enzyme, Glycerol-3-phosphate dehydrogenase (G3PDH) show the unique bandmorphs. From figure 23, sample number 6 to 20 are the larvae of parasitoid. They have the different bandmorphs from adult parasitoids and their hosts. The adult parasitoids have three loci in this enzyme but their larvae have only one. The electrophoretic bandmorphs of enzyme ME, MDH and IDH show in figure 24, 25 and 26, respectively. In these enzymes, samples which are the larvae of parasitoid show the bandmorph like both adult parasitoids and adult fruit flies. From figure 25, the bandmorphs of IDH of larvae of parasitoid can identify their host species by bandmorphs which like the hosts. Samples number 25 and 26 are fruit files,

Bactocera dorsalis , and samples number 22, 23 and 24 are *Bactocera correcta.* The electrophoretic patterns of larvae of parasitoid are the same patterns as in *B correcta* It means that *B correcta* is the host of *D longicaudata*

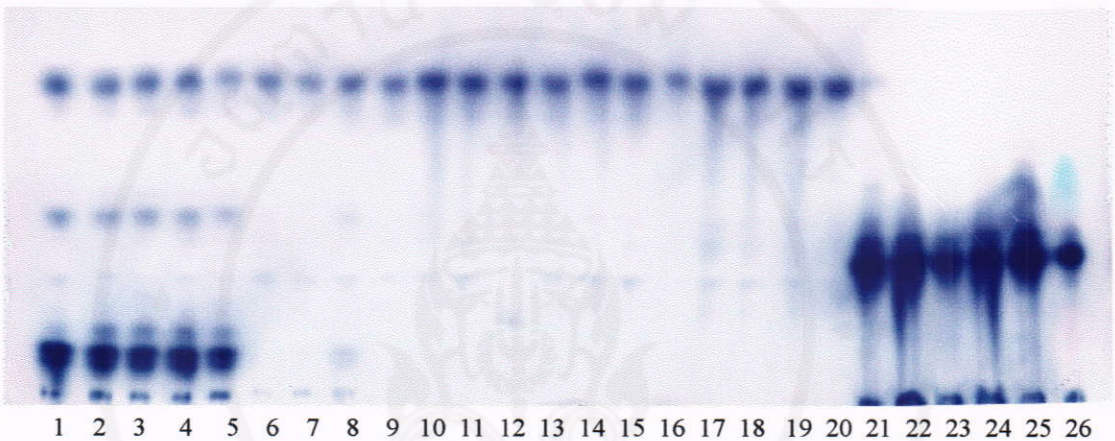


Figure 23. Photograph of gel showing electromorph patterns of the isozyme Glycerol -3 - phosphate dehydrogenase (G3PDH) of fruit flies, parasitoids and larvae of parasitoid from Nakornpathom, Thailand.
Lanes 1 to 5 are adult parasitoid.
Lanes 6 to 20 are larva of parasitoid.
Lanes 21to 26 are adult fruit fly.

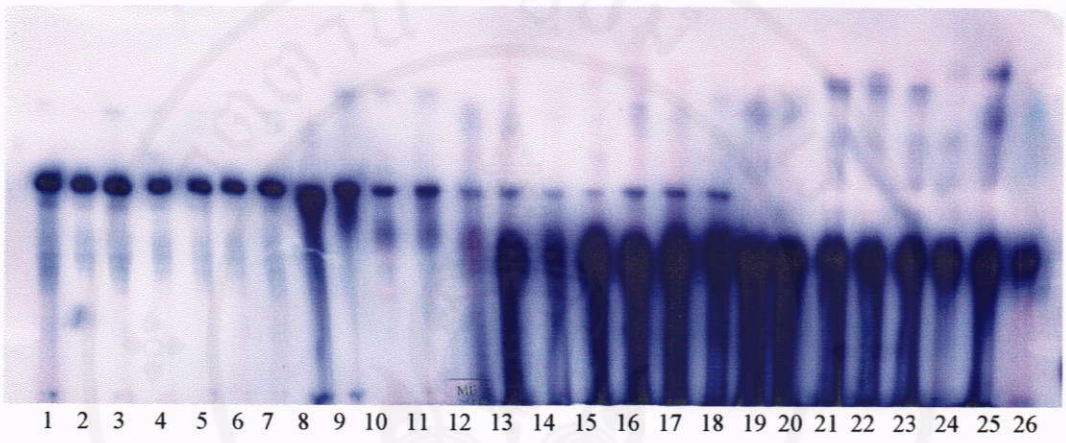


Figure 24. Photograph of gel showing electromorph patterns of the Malic enzyme (ME) of fruit flies, parasitoids and larvae of parasitoid from Nakornpathom, Thailand.
Lanes 1 to 12 are adult parasitoid.
Lanes 13 to 18 are larva of parasitoid.
Lanes 19 to 26 are adult fruit fly.

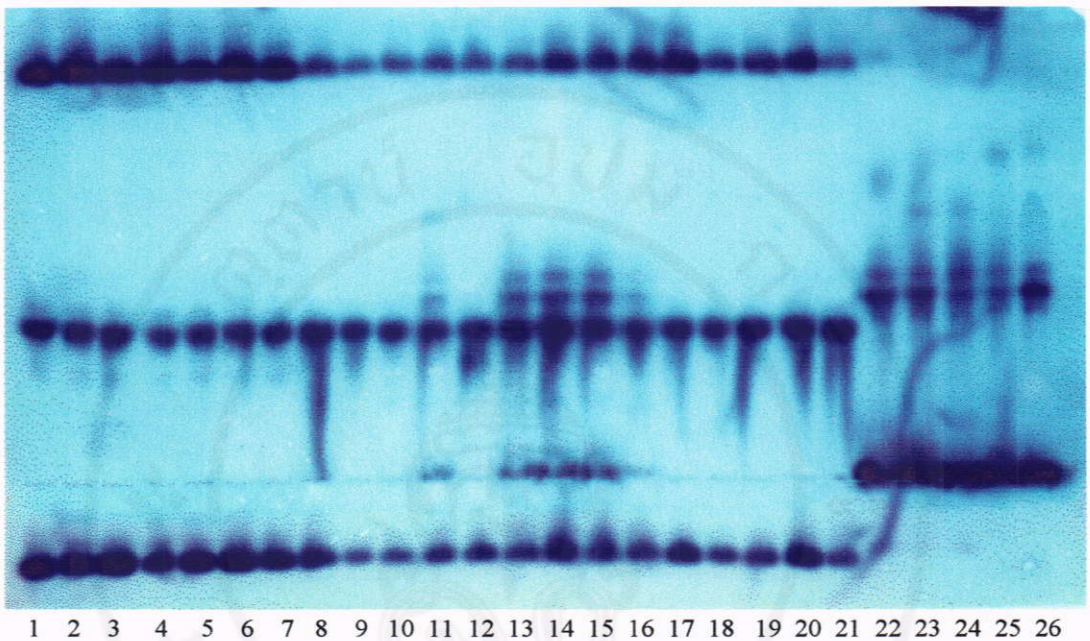


Figure 25. Photograph of gel showing electromorph patterns of the isozyme Malate dehydrogenase (MDH) of fruit flies, parasitoids and larvae of parasitoid from Nakornpathom, Thailand.
Lanes 1 to 10 and 17 to 21 are adult parasitoid.
Lanes 11 to 16 are larva of parasitoid.
Lanes 22 to 26 are adult fruit fly.

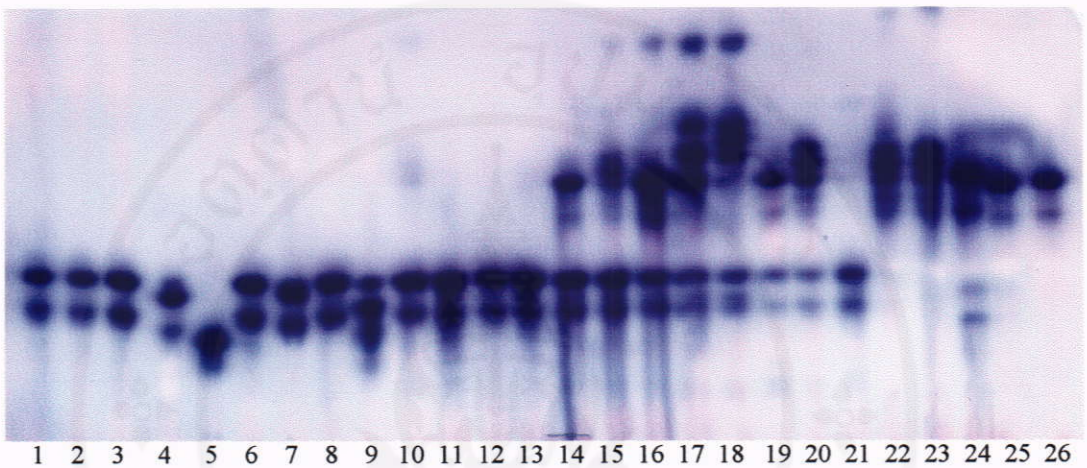


Figure 26. Photograph of gel showing electromorph patterns of the isozyme Isocitrate dehydrogenase (IDH) of fruit flies, parasitoids and larvae of parasitoid from Nakornpathom,Thailand.
Lanes 1 to 13 and 21 are adult parasitoid.
Lanes 14 to 20 are larva of parasitoid.
Lanes 22 to 26 are adult fruit fly.

Chapter V

Discussion

Enzyme electrophoretic techniques have led to discover many new polymorphisms in a large variety of organism and provide new information about the nature of genetic variation and an insight into the relationships between the genotype and fitness. These techniques have been widely applied to the study of mosquitoes and *Drosophila* in many purposes (Prakash,1969; Ayala, et al, 1971; Ayala and Powell, 1972; Steiner and Joslyn, 1979; Corsaro and Munstermann, 1984). In recent years, there are reports about utilized enzyme electrophoretic techniques for extensive genetic investigations and studies of phylogenetic relationship among sibling species of Tephritid fruit flies, *Rhagoletis spp.* in North America (Berlocher, 1980 ; Berlocher, et al, 1993). In Malaysia, Ooi (1990) tried to differentiate species in *Bactocera dorsalis* complex, taxon A and taxon B, based on different mobilities of the enzyme Isocitrate dehydrogenase, Phosphogluonate dehydrogenase and aldolase but there has been no similar genetic approach in their parasitoid, *Diachasmimorpha sp.* There were only reports on the insect in order Hymenoptera. Pintereau (1993) studied

enzymatic analysis of the genus *Trichogramma* (Hymenoptera · Tricogrammitidae) in Europe. Therefore this work is the first attempt to study the genetic variation of *Diachasmimorpha sp.* by enzyme electrophoresis.

In comparison of electromorph patterns from this study to starch gel electrophoresis in human (Harris and Hopkinson, 1976), there are some differences, for example, the number of loci of all enzymes are different and the subunit structure of enzyme Glucose-6-phosphate dehydrogenase and Isocitrate dehydrogenase in *Diachasmimorpha sp.* is monomer but in human is dimer. The subunit structure of Malic enzyme in human is tetramer but in *Diachasmimorpha sp* , Malic enzyme has only single electrophoretic bandmorph. On the other hand, some similarities in electromorph patterns are found, for example, the cathodally migrating of Malate dehydrogenase locus is recorded in human as well as in *Diachasmimorpha sp*

In this study, the electrophoretic bandmorphs of each allele at each locus are not too difficult to score, except in Glycerol-3-phosphate dehydrogenase and Phosphogluconate dehydrogenase which

their bands are develop inconsistently. However, the way to improve this problem was to used the new batches of staining reagents.

Artifacts on gel can be found when there are some residue of ammonium persulfate on the gel. It is causing band splitting which give rise of subband or disappearance of bands (Brewer, 1967). Other explanations for the appearance of artifacts on gel were discussed to result from post-translational modification of proteins which could be denaturation, deamination, phosphorelation, sulphation, oxidation, reduction, addition of other molecules, aggregation or cleavage of polypeptides which causing extra band on the gel (Ferguson, 1980).

For insect which reproduce by pathenogenesis, expected heterozygosity should be used as a measure of genetic variability rather than observed becaues observed heterozygpsity is very sensitive to the mating structure of a population and the amount of inbreeding. When inbreeding is strong, or reproductive method is pathenogenesis, observed heterozygosity can be very low, while expected heterozygosity is not affect (Nei, 1975; Selander and Hudson, 1976; Lester and Selander, 1979; Gruar, 1985). The mean heterozygosity of *Diachasmimorpha sp.*

in this report ranged from 0.072 to 0 258. It is relatively high when compare with other insects. The mean heterozygosity of all insects excluding *Drosophila* was 0.107 ± 0.005, the mean heterozygosity of insects in order Diptera was 0.124 ± 0.007 and the mean heterozygosity of *Drosophila* was 0.135 ± 0.011 (Graur, 1985). Many scientists reported that hymenopteran species display levels of electrophoretic variation significantly lower than most other insects and a few of them lack variation altogether Graur (1985) reported the mean heterozygosity of insect in order Hymenoptera was 0.036 ± 0.004. The mean heterozygosity of *Opius juglandis* (Hymenoptera : Braconidae) ranged between 0.024 to 0.067 (Lester and Selander, 1979). However, the high level of mean heterozygosity may due to the small sample size (N) of some populations The size of population, may be the most important factor in determining heterozygosity (Graur, 1985).

Lester and Selander (1979) evaluated the probability of fixation of a mutant gene in a diploid and haplodoploid population for the variance effective numbers. If the populations have equal numbers of genes, the values of fixation probability are approximately equal, but if

the populations have equal numbers of individual, the fixation probability in the haplodipliod population may reach a maximum of 1.33 times that in diploid population. Fixation is a genetically situation where all members of a population are homozygous for one particular allele of a gene. Then fixation may reduce the number of heterozygote in population. Furthermore, in haplodiploid species, linkage should be increased over the level in diploids because there is no opportunity for crossing over or chromosome segregation in males. In summery, the low level of heterzygosity in hymenopterans was due to many reasons : reduced heterozygote advantage, inbreeding, eusociality, environmental stability conferred by the nest microhabitat, facilitation of exposure of deleterious genes in the haploid sex, reproduction by arrhenotokous pathenogenesis with balancing selection, small effective population size and a shorter time to fixation for new mutants than diploid population of equal size. All of these and other differences between diploid and haplodiploid populations produce the result that fewer allele are moving to fixation faster in haplodiploid populations, resulting in lower level of heterozygosity (Snyder, 1974; Metcalf, et al, 1975; Lester and Selander, 1979; Pamilo and Crozier, 1981; Graur, 1985).

In this study I use both male and female parasitoid as my sample. Many scientists comment that, in haplodiploidy system should be drop male and use only female but Crozier (1970) study the genetic polymorphism in diploid and haplodipolid populations and demonstrated that there are no significant difference in the number of polymorphism in these two genetic systems.

Based on 13 loci studied, the value of Nei's unbiased genetic identity (I) between intrapopulation ranged from 0.579 to 0.939 and the genetic distance (D) value ranged from 0.063 to 0.599. In concordance with Nei's, the value of Rogers' genetic similarity between intrapopulation range from 0.507 to 0.904 and the distance value ranged from 0.096 to 0.493. The I values of Nei and Rogers between interpopulation ranged from 0.277 to 0.611 and 0.310 to 0.583, respectively The D values of Nei and Rogers between interpopulation ranged from 0.492 to 1.000 and 0.417 to 0.690, respectively. The I values of interpopulation are smaller than those of intrapopulation and the D values of interpopulation are larger than those of intrapopulation. From the genetic similarity (or identity) estimated, the pair of population which is express highest value of Nei's genetic identity and Rogers'

genetic similarity are Nakornpathom species C and Saraburi species C, which are 0.939 and 0.904, respectively. The value of Nei's unbiased genetic identity and genetic distance were used to construct the phylogenetic tree.

The phylogenetic dendrograms produced by analysis using the Biosys-1 program based on UPGMA methods are presented in figure 21 and 22. Both dendrograms clustered the population of species C together in one branch and the population of species B together in one branch. Both dendrograms clustered Nakornpathon species C and Saraburi species C in one cluster, Ranong species C and Nakornratchasima species C in one cluster, Nakornratchasima species B and Saraburi species B in one cluster, Chiangmai species B and Chumporn species B in one cluster and Petchabun in another cluster close to Chiangmai species B and Chumporn species B cluster. These analyses indicated that there are the genetic variation within species and may be they are the species complex and need to be examined in much more detail.

In this study, the number of sample in some population was too small because they are the rare species in these locations. Nei and Roychoudhury (1974) concluded that a relatively reliable estimated of average heterozygosity and genetic distance can be obtained from a small number of indiviauals if a large number of loci are examined.

The relationship between fruit flies and their parasitoids is studied by electrophoretic techniques. Electrophoresis of fruit fly, parasitoid larva and adult parasitoid (Fig. 23, 24, 25 and 26) show the bandmorph relationship in enzyme Malate dehydrogenase, Isocitrate dehydrogenase, Malic enzyme and Glycerol-3-phosphate dehydragenase. Parasitoid larvae show band which like their host and adult parasitoid. Many papers reported that the larva of hymenopteran parasitoids can produce specialized cell which derived from the extra embryonic membrane of the egg. These cells are known as teratocytes. They are liberated into the host hamocoel and increase in size during growth of the parasitoid larva. The major function of teratocytes is supplying nutrition to the parasitoid larva. The surface of teratocytes is densely packed with microvilli then they can absorb nutrients from the host. The number of teratocytes decreased gradually and few or no teratocytes remained in the

host body cavity after the parasitoid larva egressed, it is likely that parasitoid larva ingest teratocytes. The parasitoid larva may either feed directly on the teratocyte cells or they may take up those proteins that have been secreted into the host hemolymph. Dahlman (1991) proposed that teratocytes could serve three major functions : (1) immunosupression of host defenses ; (2) secretion of enzymes and other factors influencing the host physiology ; and (3) supplying nutrition to the parasitoid. Okuda and Kadono-Okuda (1995) observed the teratocytes of braconid wasp, *Perilitus coccinellae* and analyzed the proteins which synthesized by the teratocytes. They suggest that the teratocytes of *P. coccinellae* have a role in providing nutrition to the parasitoid larva, however, the function of teratocytes still remains unclear (Salt, 1971; Vison and Iwantsch, 1980; Okuda and Kadono-Okada, 1995 ; Strand and Obrycki, 1996). From this evidence, it is possibly that there is host hemolymph contain in teratocytes and when parasitoid larva ingest teratocytes, host hemolymph will transfer to the body cavity of parasitoid larva. When the electrophoresis of these larvae was run, they may have both parasitoid and host bands as present in this study. The evidence obtained in this study may apply to identify the species of host fly because fruit fly in different species give the different electrophoretic

bandmorph. When electrophoresis was run, we can compare the bandmorph and expect the species of host of the parasitoid.

Chapter VI

Conclusion

In this study, nine enzyme systems were selected from fourteen systems for the first trial. In these nine systems, thirteen enzyme loci were detected. Every enzyme systems showed the genetic variation and interesting electrophoretic bandmorph. Only the Malic enzyme system did not show the polymorphism but it can differentiate parasitoid in this study from some different locations.

Genetic variability estimation among population of *Diachasmimorpha sp* has shown that population of *Diachasmimorpha sp* from different regions of Thailand exhibited the high variation as observed from the range of the genetic similarity (0.027 - 0.939). Phylogenetic dendrograms produced by analysis using the Biosys-1 program based on UPGMA methods showed the relationship between parasitoid from different locations. The population from Nakornpathom species C was closely related to that from Saraburi species C in the same ways as the population from Chiang mai species B related to that from Chumphorn species B. From this study, the number of samples

examined in some population was too small. It is better to repeat this experiment by increase the number of sample and confirm the result with other methods such as results from cytogenetic methods, morphology or molecular genetics because electrophoresis of soluble proteins does not detect all the biochemical polymorphism which exists in natural population.

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APPENDIX

Abbreviation for gene pools :

PEO = Petchabun *Opius incisi*

PEA = Petchabun species A

RNE = Ranong species E

RND = Ranong species D

Refer to table 1 for description of each species.

Appendix 1 Frequency of electromorph alleles, observed heterozygosity (Ho)and Hardy-Weinberg heterozygosity (He) per locus in four populations of *Diachasmimorpha sp* from two localities in Thailand. (N = number of insect sampled per locus.)

Locus	Population			
	PEO	PEA	RND	RNE
(N)	10	24	16	24
G3pdh-1				
B	1 000	0.979	1 000	1.000
C	0 000	0.021	0 000	0 000
He	0 000	0 042	0 000	0 000
Ho	0 000	0 042	0 000	0 000
G3pdh-2				
A	0 400	0.552	1.000	1 000
C	0 600	0 448	0.000	0 000
He	0 505	0 503	0 000	0 000
Ho	0.000	0.000	0 000	0.000
G3pdh-3				
A	1 000	0 980	1 000	1.000
B	0 000	0 020	0 000	0 000
He	0.000	0.040	0.000	0 000
Ho	0 000	0 040	0.000	0.000
Gpi				
A	0 900	1 000	0 000	0 000
B	0 100	0.780	0 063	0.958
C	0 000	0 120	0.938	0.042
He	0.189	0.375	0.121	0 082
Ho	0 000	0.120	0 000	0.000
G6pgh				
A	0 000	0.000	0 000	0.125
B	1 000	0 880	0.375	0 792
C	0 000	0.120	0.438	0.083
D	0 000	0 000	0.188	0.000
He	0.000	0 216	0.653	0 358
Ho	0 000	0.160	0 375	0.333

(continued)

Locus	Population			
	PEO	PEA	RND	RNE
Idh				
A	0 700	0.000	0.438	0 000
B	0.300	0.000	0.000	0 000
C	0 000	0 804	0 000	0 000
D	0 000	0 196	0 000	0 000
G	0 000	0 000	0 563	1.000
He	0 442	0 321	0 508	0 000
Ho	0 400	0 250	0 000	0 000
Mdh-1				
A	0 400	0 519	0.813	0.000
B	0 000	0 115	0.000	0.000
C	0 600	0 365	0.188	1.000
He	0 505	0 595	0.315	0 000
Ho	0 000	0 038	0.000	0.000
Mdh-2				
A	1.000	0 000	1 000	1.000
C	0 000	1.000	0.000	0 000
He	0.000	0 000	0.000	0 000
Ho	0 000	0.000	0 000	0 000
Mpi				
A	0 000	0 278	0 000	0.000
B	0 000	0.389	0.000	0.000
C	0 000	0 333	0 000	0.000
D	0 800	0 000	0 250	0.438
E	0 200	0.000	0 750	0.563
He	0 337	0.699	0 387	0.503
Ho	0.200	0 111	0.125	0 208
Me				
B	0.000	0 000	0.000	1 000
C	1 000	1.000	1.000	0.000
He	0.000	0 000	0.000	0.000
Ho	0 000	0.000	0 000	0 000

(continued)

Locus	Population			
	PEO	PEA	RND	RNE
Pgd				
A	0 000	0 469	0 625	1 000
B	0 938	0 344	0 375	0 000
C	0 063	0 000	0 000	0 000
D	0 000	0 188	0 000	0 000
He	0 125	0 647	0 484	0 000
Ho	0 125	0 188	0.000	0 000
Pgm-1				
A	0 556	0 000	0 000	0 500
B	0 000	0 000	0 000	0 500
C	0 444	0 000	0 500	0 000
D	0 000	0 591	0 406	0 000
E	0 000	0 409	0 094	0 000
He	0 523	0 495	0 595	0 511
Ho	0.000	0 182	0 313	0.083
Pgm-2				
A	0 714	0.091	0.656	0 896
B	0 286	0.909	0 344	0 104
He	0 440	0 169	0 466	0.191
Ho	0 000	0.000	0 188	0.042

Appendix 2. Genetic variability at 13 loci in four populations of *Diachasmimorpha sp*. from two localities in Thailand. (Standard errors in parenthesis.)

Population	Mean no of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
			HdyWbg expected**	Direct-count
Petchabun O	1 6	61.5	0 236	0.056
	(0 1)		(0.063)	(0.034)
Petchabun A	2 2	69.2	0 316	0.087
	(0.2)		(0.071)	(0.024)
Ranong D	1.8	61.5	0 217	0.077
	(0.2)		(0.071)	(0 037)
Ranong E	1.5	30.8	0 126	0.051
	(0 2)		(0 055)	(0.029)

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

** Unbiased estimate (Nei, 1978)

Appendix 3. Data showing the χ^2 value of four populations of *Diachasmimorpha sp* from two localities in Thailand

Locus	χ^2 value of population			
	PEO	PEA	RND	RNE
G3pdh-1	0.000	0 000	0 000	0.000
G3pdh-2	11.221	30.059	0.000	0.000
G3pdh-3	0.000	0 000	0.000	0.000
Gpi	19.059	30.181	31.034	47.002
G6pdh	0.000	1.955*	9.661*	7.534*
Idh	0.105*	1.500*	17.104	0 000
Mdh-1	11.221	53.413	18.720	0.000
Mdh-2	0.000	0.000	0.000	0 000
Me	0.000	0.000	0.000	0.000
Mpi	2.113*	15.771	8.201	8.598
Pgd	0.000	23.938	17.225	0.000
Pgm-1	10.159	9.251	10.767*	17 537
Pgm-2	8.889	28 718	6 183*	17.972

χ^2 = 0.000 mean as this locus fixed in one allele

* Under Hardy-Weinberg equilibrium ($P \geq 0\ 01$)

Appendix 4. Fixation indices (F) and coefficients for heterozygote deficiency or excess (D) per locus in four populations of Diachasmimorpha sp. from two localities in Thailand

Locus	Population			
	PEO	PEA	RND	RNE
G3pdh-1				
F	1 000	-0.021	1.000	1 000
D	-1.000	0.000	-1.000	-1.000
G3pdh-2				
F	1.000	1.000	1.000	1.000
D	-1 000	-1.000	-1 000	-1 000
G3pdh-3				
F	1 000	-0 020	1 000	1.000
D	-1 000	0.000	-1.000	-1 000
Gpi				
F	1.000	0.673	1 000	1 000
D	-1 000	-0.680	-1.000	-1.000
G6pdh				
F	1.000	0.242	0.407	0.050
D	-1.000	-0.258	-0.426	-0.069
Idh				
F	0.048	0 208	1.000	1.000
D	-0 095	-0.222	-1.000	-1 000
Mdh-1				
F	1.000	0.934	1.000	1.000
D	-1.000	-0.935	-1.000	-1.000

Locus	Population			
	PEO	PEA	RND	RNE
Mdh-2				
F	1.000	0 934	1.000	1 000
D	-1 000	-0.935	-1 000	-1.000
Me				
F	1.000	1 000	1 000	1.000
D	-1.000	-1.000	-1.000	-1.000
Mpi				
F	0 375	0.832	0.667	0.577
D	-0 406	-0 841	-0 667	-0 586
Pgd				
F	-0.067	0.701	1.000	1 000
D	0 000	-0 710	-1.000	-1.000
Pgm-1				
F	1.000	0.624	0 458	0 833
D	-1 000	-0 632	-0.475	-0.837
Pgm-2				
F	1.000	1.000	0.584	0 777
D	-1 000	-1.000	-0.597	-0.781