POPULATION GENETICS OF FRUIT FLY *BACTROCERA CORRECTA* (BEZZI) (DIPTERA: TEPHRITIDAE) IN THAILAND

CHONTICHA KUNPROM

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Mahasarakham University
November 2014

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The examining committee has unanimously approved this thesis, submitted by Mr. Quoc Tuan Tran, as a partial fulfillment of the requirements for the Master of Fine Arts Program in Visual Arts at Mahasarakham University.

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Chonticha Kunprom

ชื่อเรื่อง พันธุศาสตร์เชิงประชากรของแมลงวันผลไม้ *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) ในประเทศไทย
ผู้วิจัย นางสาวชลธิชา ขุนพรม
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บทคัดย่อ

แมลงวันฝรั่ง (*Bactrocera correcta*) (Bezzi) เป็นศัตรูพืชที่สำคัญในสกุล *Bactrocera* สามารถเข้าทำลายพืชมากกว่า 60 ชนิด ใน 30 วงศ์ หลายชนิดเป็นพืชที่มีความสำคัญทางเศรษฐกิจ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความแปรผันทางพันธุกรรม โครงสร้างทางพันธุกรรม และประวัติศาสตร์ประชากรของแมลงวันผลไม้ *B. correcta* ในประเทศไทย โดยใช้ยีน cytochrome *c* oxidase subunit I (COI) ในไมโทรคอนเดรียลดีเอ็นเอ จากการศึกษาตัวอย่างแมลงวันผลไม้ *B. correcta* ทั้งหมด 171 ตัวอย่าง จาก 15 ประชากร พบว่า แมลงวันผลไม้ *B. correcta* มีความแปรผันทางพันธุกรรมสูง (0.0076-0.0325) ซึ่งมีสาเหตุมาจากความแตกต่างของสายวิวัฒนาการโดยแสดงให้เห็นจากการวิเคราะห์ด้วยวิธี median joining (MJ) จากการวิเคราะห์โครงสร้างทางพันธุกรรม พบว่า แมลงวันผลไม้ *B. correcta* มีความแตกต่างทางพันธุกรรมโดยรวมของประชากรต่ำ เนื่องจากสามารถอพยพระหว่างประชากรได้สูงซึ่งเป็นผลจากความต่อเนื่องของแหล่งอาศัยตามธรรมชาติและพืชอาหารที่มีการเพาะปลูกอย่างแพร่หลายในประเทศไทยเป็นปัจจัยที่สำคัญที่ทำให้ประชากรของแมลงวันผลไม้ *B. correcta* ไม่มีความแตกต่างทางพันธุกรรม นอกจากนี้ประวัติศาสตร์ประชากรอาจเป็นปัจจัยที่สนับสนุนให้โครงสร้างทางพันธุกรรมโดยรวมของประชากรอยู่ในระดับต่ำ จากการวิเคราะห์ mismatch distribution และจากการทดสอบ Tajima's *D* และ Fu's F_S พบว่า ประชากรของแมลงวันผลไม้ *B. correcta* มีการขยายขนาดประชากรอย่างรวดเร็วในอดีตเมื่อสิ้นสุดยุคน้ำแข็งครั้งล่าสุด ซึ่งการเปลี่ยนแปลงสภาพภูมิอากาศในยุคไพลสโทซีนส่งผลให้โครงสร้างทางพันธุกรรมของประชากรต่ำ จากผลการศึกษาในครั้งนี้แสดงให้เห็นถึงความสำคัญของเครื่องหมายโมเลกุลที่สามารถใช้ตรวจสอบความหลากหลายที่ซ่อนเร้นในแมลงวันผลไม้นี้ได้ ซึ่งความหลากหลายดังกล่าวยังไม่มีการรายงานก่อนหน้านี้โดยใช้ลักษณะทางสัณฐานวิทยาและเซลล์วิทยา

คำสำคัญ: *Bactrocera correcta*, แมลงวันผลไม้, ความหลากหลายทางพันธุกรรม, Tephritidae

TITLE Population genetics of fruit fly *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) in Thailand
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ABSTRACT

Bactrocera correcta (Bezzi) is among the most destructive fruit fly pests of the genus *Bactrocera*. This species infested more than 60 plant species from 30 families, many of which are commercially important species. In this study, the genetic structure, diversity and demographic history of *B. correcta* in Thailand were inferred from mitochondrial cytochrome *c* oxidase subunit I (COI) sequences. High genetic diversity (0.0076 to 0.0325) was found among 171 samples collected from 15 locations. This is due largely to the existence of the two divergence lineages (I, II) revealed by median joining (MJ) network analysis. Genetic structure analysis revealed an overall low level of genetic differentiations between populations that suggests the flies can move freely across geographic regions. The continuous nature of the habitats, because of the host plants being commonly grown in Thailand, is the factor most likely responsible for the genetic homogeneity. In addition, the recent population history could also be a factor that contributed to the overall low level of the genetic structure. Mismatch distribution analysis as well as Tajima's *D* and Fu's F_S tests detected signals of recent demographic expansion dating back to the end of the last glaciations. The results of this study revealed a significant molecular marker to detected cryptic diversity in the fruit fly that has not been recognized previously using morphology and cytology.

Keywords: *Bactrocera correcta*, fruit fly, genetic diversity, Tephritidae

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List of Abbreviations

AMOVA	Analysis of molecular variance
bp	base pairs
°C	degree Celsius
COI	cytochrome *c* oxidase subunit I
DNA	deoxyribonucleic acid
$dNTP_s$	Deoxy nucleotide triphosphate
e.g.	exempli gratia
et al.	et alibi
etc.	et cetera
F_{CT}	the correlation of random haplotypes within a group of populations relative to that of random pairs of haplotypes drawn from the whole species.
F_{ST}	the correlation of random haplotypes within populations relative to that of random pairs of haplotypes drawn from the whole species.
F_{SC}	the correlation of the molecular diversity of random haplotypes within opulations relative to that of random pairs of haplotypes drawn from the region.
IBD	Isolation-by-distance
i.e.	id est
km	kilometer
K2P	Kimura's 2 parameter
MJ	Median joining
ml	milliliter
mm	millimeter
mM	millimolar
min	minute
mtDNA	mitochondrial deoxyribonucleic acid
PCR	Polymerase chain reaction
TBE	Tris-borate
US$	United States Dollar
UV	Ultraviolet

µg	microgram
µl	micro liter
µM	micro molar
%	percent

Chapter 1

Introduction

1.1 Background

The Tephritid fruit flies (ture fruit fly) belong to the family Tephritidae. This family is the most species rich of the fruit flies and is a serious pest of fruits and vegetables in the world. Tephritidae not only has a negative impact on economy of infested countries, but also has implication on the international trade due to the restrictions imposed by the importing countries on the export of fresh fruit and vegetables. Most pest species of Tephritidae belong to the genera *Anastrepha, Ceratitis, Bactrocera, Dacus* and *Rhagoletis*. Approximately 350 species of Tephritid fruit flies in the five genera those are most economically important, especially in the genus *Bactrocera*.

Fruit flies of the genus *Bactrocera* cause economic losses from direct fruit damage and from quarantine regulations that restrict the movement of fruits and vegetables from infested areas. Fruit flies of quarantine concern constitute an important barrier to the export of fresh fruits and vegetables host products, thereby limiting the trade potential of fruit and vegetable producing countries. This genus is one of the largest genera within Tephritidae with about 500 described species arranged in 28 subgenera (Drew, 1989; Drew and Hancock, 2000). Several species (e.g. the Oriental fruit fly, *B. dorsalis*; the Queensland fruit fly, *B. tryoni*; melon fly, *B. cucurbitae*) capable of attacking a wide variety of commercially produced fruit (White and Elson-Harris, 1992; Allwood *et al*., 1999; Clarke *et al*., 2005). In Thailand, many major pest species in this genus (e.g. *B. dorsalis* complex, *B. carambolae*, *B. pyrifoliae*, *B. cucurbitae*, *B. tau*, *B. diversa*, *B. latifrons* and *B. correcta*) are the most important economically.

The guava fruit fly, *B. correcta* (Bezzi), is one of the most destructive pests of the genus *Bactrocera* (Wang, 1996). In the past *B. correcta* is a minor pest but to date is a major serious pest of fruits (Kitthawee, 2000). This species is highly adaptable to new environments enabling its spreading rapidly. *Bactrocera correcta* was first recorded in

Bihar, India in 1916 (Bezzi, 1916) and is now distributed throughout South and South East Asia (Wang, 1996; Drew and Raghu, 2002) and in China (Liang *et al*., 1996). In Vietnam and central and northern Thailand, serious infestation by this fly causes great loss in fruit and vegetable production (Drew and Raghu, 2002). This fly infest a number of valuable commercial fruits and vegetables of more than 30 plant families (Allwood *et al*., 1999; Maynard *et al*., 2004) including guava (Kitthawee, 2000) and other fruits such as mangoes, peaches, melons, cashewnut, cherry, jujube, carambola, wax apple, banana and citrus fruits. Therefore, *B. correcta* is a serious pest for fruit production, considered as highly invasive, and is regarded as a key quarantine species by many countries (White and Elson-Harris, 1992; Liang *et al*., 1996; Allwood *et al*., 1999; Maynard *et al*., 2004).

Information regarding the genetic diversity, genetic structure and gene flow are crucial for pest control and management (Roderick, 1996; Roderick and Navajas, 2003). For example, sterile insect technique (SIT) that using sterile male to compete for mating with the wild males this method requires a great amount of sterile flies which should be in same proportions to the number of the wild flies (Itô *et al*., 2003). Information about effective population size and individual movement across populations (i.e. gene flow) which can be deduced from population genetic studies (Aketarawong *et al*., 2011; Karsten *et al*., 2013) is important for effective planning of releasing sterile insects correctly. Although considering as important pest species and contributed to significant economic lost, the detailed genetic structure of the species are poorly study.

In this study, mitochondrial cytochrome *c* oxidase I (COI) sequence were used to infer genetic structure and demographic history of *B. correcta* in Thailand. Several studies have shown that COI sequences could be used effectively for population genetic study of the fruit flies (Mun *et al*., 2003; Nardi *et al*., 2005; Shi *et al*., 2005; 2010; 2012; Hu *et al*., 2008; Meeyen *et al*., 2013) and investigated genetic relationship between *B. correcta* in Thailand with samples from other geographic regions also. The results provide significant information on genetic structure that could be used for pest management and control program. In addition, patterns of genetic structure and genetic diversity of *B. correcta* will increase our fundamental knowledge of biodiversity of Thailand.

1.2 Objectives of the research

The objectives of the present study are:

1.2.1 To investigate genetic variation of *B. correcta* in Thailand.

1.2.2 To investigate population genetic structure and demographic history of *B. correcta* in Thailand.

1.2.3 To determine the diversity of host-plant species of *B. correcta* in Thailand.

1.3 Scope of the research

Specimens of *B. correcta* were collected from natural habitats in Thailand. The infested fruits were reared in a laboratory under room temperature. Soon after the adults emerged, the adult flies were stored in 80% ethanol at -20 °C. Species was identified using adult morphology following White and Elson-Harris (1992) and Plant Health Australia (2011). DNA was extracted from individual adult fly. The mitochondrial cytochrome *c* oxidase I (COI) gene was amplified using polymerase chain reaction (PCR). PCR products were checked, purify and sequencing. Diversity of host-plants, genetic variation, population genetic structure and demographic history of *B. correcta* in Thailand were analyzed using COI sequences.

Chapter 2

Literature Reviews

2.1 Classification of the genus *Bactrocera* (Macquart)

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Suborder: Brachycera

Infraorder: Muscomorpha

Superfamily: Tephritoidae

Family: Tephritidae

Subfamily: Dacinae

Tribe: Dacini

Genus: *Bactrocera*

(Macquart, 1835)

Fruit flies are a group of insects forming the family Tephritidae of the order Diptera. Tephritidae is the most species rich family of fruit flies, with approximately 4,400 described species (Norrbom, 2004), in six subfamilies (Tachiniscinae, Blepharoneurinae, Phytalmyiinae, Trypetinae, Dacinae and Tephritinae); about 500 genera, and probably many undescribed species worldwide. Fruit fly are among the few groups of dipterans strictly phytophagous, except the Tachiniscinae, which are thought be parasitoids of Lepidoptera, and at least, some species of Phytalmyiinae that feed on live or dead bamboos (Poaceae) or on trees recently fallen of other plant families. Blepharoneurinae feed in flowers, fruits, and make galls in Cucurbitaceae; Trypetinae and Dacinae feed in fruits or in seeds of a wide range of plant families, and Tephritinae eat in flowers, make gall, or are leaf-miners in a wide array of plant taxa: Aquifoliaceae, Scrophulariaceae, Verbenaceae, but mainly in flowerheads of Asteraceae (Norrbom, 2010; Uchôa and Nicácio, 2010).

More than 800 species of fruit flies in the sub-family Dacinae which are the main species that infest soft fruits in tropical and sub-tropical areas (Bellas, 1996). Dacini is one of three tribes in the subfamily Dacinae. This tribe contains approximately 770 species arranged in four genera including *Bactrocera* (Macquart), *Dacus* (Fabricius), *Ichneumonopsis* (Hardy) and *Monacrostichus* (Bezzi) (Drew and Hancock, 1994; Drew *et al*., 1998).

The genus *Bactrocera* (Macquart) is large genus contains 528 described species arranged in 28 subgenera which are divided into four groups including *Bactrocera* group, *Melanodacus* group, *Queenslandacus* group and *Zeugodacus* group (Drew, 1989) (Table 2.1). Fruit flies in this genus are cause serious reduction in fruits and vegetables in many countries (Hardy, 1973).

Table 2.1 Four groups and their subgenera of *Bactrocera*.

Subgenera of *Bactrocera*	**Member of groups**
***Bactrocera* group**	*Afrodacus*
	Apodacus
	Bactrocera
	Bulladacus
	Gymnodacus
	Notodacus
	Semicallantra
	Tetradacus
	Trypetidacus
***Melanodacus* group**	*Hemisurstylus*
	Hemizeugodacus
	Melanodacus
***Queenslandacus* group**	*Queenslandacus*
***Zeugodacus* group**	*Asiadacus*
	Austrodacus
	Diplodacus
	Hemigymnodacus
	Heminotodacus
	Hemiparatridacus
	Javadacus
	Nesodacus
	Niuginidacus
	Papuodacus
	Paradacus
	Paratridacus
	Parazeugodacus
	Sinodacus
	Zeugodacus

(From: Drew, 1989)

2.2 Biological of the fruit fly

Member of subfamily Dacinae are multivoltine species (Fletcher, 1989). During their life cycle (Figure 2.1), fruit flies go through four development stages similar to the other insects in the order Diptera. The developmental time from egg to adult takes between 14-27 days. The duration of each stage and degree of survival depends on species, host plant and environmental conditions (Shaw *et al*., 1967). The female lays eggs into host fruits, and these eggs hatch to larvae. The larvae that hatch initially are small and delicate first instar larvae. They moult into slightly more robust second instar larvae, and these in turn moult into quite stout and tough third instar larvae. When the third instars have finished feeding, they leave the fruits, fall to the ground, and crawl away to a sheltered spot (usually in the soil) where they pupate. The larval skin becomes barrel-shaped, tanned brown and hard, and is known as the puparium. The true pupa is formed inside this puparium "shell". The pupa turns into an adult fly, which escapes from the puparium by splitting open the anterior end and squeezing out. Then, fruit with fruit fly larvae inside decay quickly if it as dried adequately. After emergence, adult feed for several days need sugars for survival, and need protein from the habitat to attain sexual maturity (Fletcher, 1987). Mating then occurs which subsequently triggers a behavioral switch in the female from mating behavior to oviposition behavior and the search for suitable host plants (Jang, 1995; Meats and Leighton, 2004).

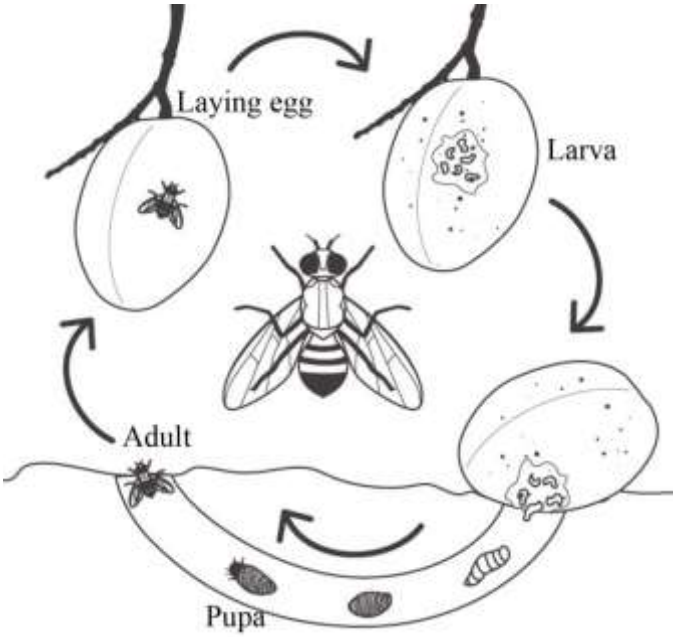

Figure 2.1 Life cycle of the fruit fly.
(Source: http://preventfruitfly.com.au/about-fruit-fly/life-cycle/)

Life cycle of the fruit fly is typical of higher flies. It undergoes complete metamorphosis that consists of four stages; these stages may be divided into three parts, host fruits, soil and aerial.

2.2.1 Egg

The female adult fruit fly lays eggs (1-20 eggs) into the maturing and ripening fruit of the host plants (Figure 2.2). The eggs (Figure 2.3) are small, usually 0.5-1.0 mm in length. The shape is spindle and colours are varying from creamy to white. The eggs hatch into larvae inside the fruit after a few days (2-4 days). At this stage, it is unlikely to be able to recognize the presence of fruit fly eggs in the fruit. The eggs are the most difficult life stages to control because they are protected within the fruit.

Figure 2.2 The female adult fruit fly lays eggs into the maturing and ripening fruit of the host plant by using ovipositor.

Figure 2.3 Eggs of the fruit fly.

2.2.2 Larva

The hatching larvae feed on the fresh of the fruit, gradually moving towards the centre of it. The feeding activity of the larvae causes the fruit to prematurely ripen and rot. Size of the larva are varying from 7.0-11.0 mm and color varies from creamy white to pale yellow depend on the stage of the larva (Figure 2.4 and Figure 2.5). The larva lived in host fruit about 10-14 days and when the larva is fully grown, it escapes from the fruit and drops onto the ground below, burrowing into the soil or organic matter (Lablanc *et al*., 2001). The larva can live and feed in the stalks, leaves, fruit, flower heads, or seeds (Christenson and Foote, 1960) and they can destroy 80-100% of fruit where it is not controlled. The main damage is done by the larvae while they develop inside fruit. The larval stage is the most likely stage that would recognize the presence of fruit fly in the fruit but the most difficult life stages to control because they are protected within the fruit as well as the eggs.

Figure 2.4 Larva of the fruit fly (head to the right).

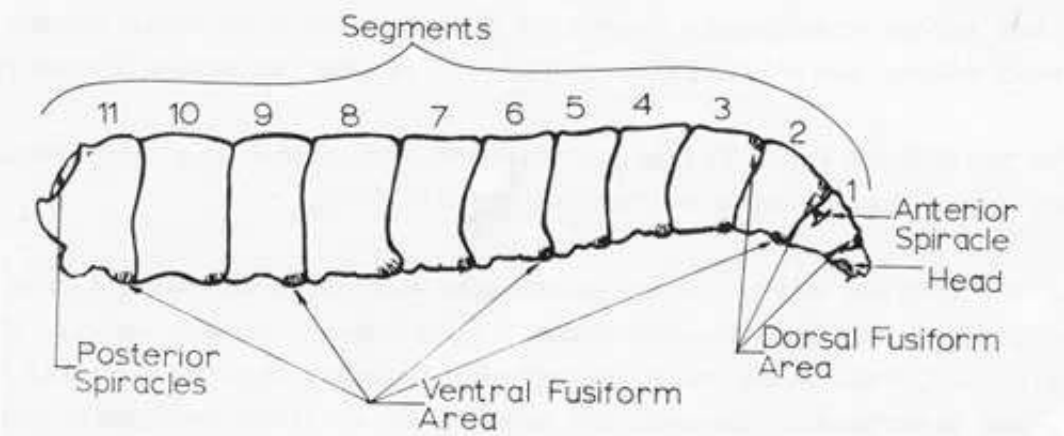

Figure 2.5 Compositions of larva of the fruit fly (head to the right).
(Source: http://entnemdept.ufl.edu/creatures/fruit/tropical/queensland_fruit_fly.htm)

2.2.3 Pupa

As the fruit ripens and rots, it falls to the ground. Fully mature larvae leave the fruit and burrow into the soil to pupate. In the soil, larvae become inactive and change into oval, light to dark brown or light brown, shining, seed like structure, about 5-8 mm long (Figure 2.6), hard pupae , in which adult flies develop. Similar to the other insect pupa does not need the nutrients for development.

Figure 2.6 Pupa of the fruit fly.

2.2.4 Adults

The adult fruit fly (Figure 2.7) may emerge from the pupae in as little as 12-14 days during the summer, or after several months over winter (at least approximately 25-50 days). The adult fruit fly is capable of forcing its way through surprising depths of soil and flies to the aerial (Swan, 1949). For the structure, the thorax length and width about 2 mm while abdomen width about 3 mm (Arita and Kaneshiro, 1998). The adult fly looks for the nourishment it needs to reach maturity, breed, and lay eggs in new season crops. Adult female fruit flies usually need to feed on a source of protein before eggs will mature for laying. The life spans of the adult fruit flies range between 2-300 days depend on various factors such as body size, food abundance, mating behavior, temperature, humidity, light etc. (Sivinski, 1993; Jaturat, 2007). At this stage of the lifecycle may be able to recognize adult flies landing on or hovering around fruit.

Figure 2.7 Adult of the fruit fly.

2.3 Biodiversity of the fruit fly

The worldwide approximation of 4,400 species in six subfamily; 27 tribes; 500 genera of the family Tephritidae are serious pest of crops especially tribe Dacini (White and Elson-Harris, 1992). Dacini contains approximately 770 describe fruit fly species in four genera including *Bactrocera, Dacus, Ichneumonopsis*, and *Monacrostichus* (Table 2.2). They are concentrated in two areas of the world i.e. Afrotropical Region and from Southeast Asia to northeastern Australia. Dacini are found in the tropical and subtropical rain forests in West Africa, coastal East Africa, Madagascar and the Mascarene Islands, southwest India, Southeast Asia, Nepal, Southern China, Indonesian islands, Papua New Guinea, northeastern Australia, and some South Pacific islands especially the rain forests of Southeast Asia possess the greatest species richness. Approximately 68% of the Dacini belong to genus *Bactrocera* and 32% to *Dacus*. It is noteworthy that the greatest speciation in genus *Dacus* has occurred in Africa while prolific speciation in genus *Bactrocera* has occurred in Southeast Asia and Papua New Guinea (Drew and Hancock, 2001) (Table 2.3).

In Queensland, fruit flies have been recorded from around 250 species in genus *Bactrocera* including those of native and introduced fruits. In Papua New Guinea, 100 and 88 described species have been recorded, the most damaging species are Asian papaya fruit fly (*Bactrocera papayae*), Melon fly (*B. cucurbitae*), Mango fly (*B. frauenfeldi*) and Banana fly (*B. musae*), India, there are about 325 species of fruit flies. The major pest species belong to the genus *Bactrocera* such as *B. cucurbitae, B. dorsalis* and *B. zonata*, while other species, such as *B. correcta, B. diversa* and *B. latifrons, Bactrocera versicolor* are still localized in their distribution (Kapoor, 2005).

In South America, especially in Brazil, the majority of fruit flies belong to two families, Tephritidae and Lonchaeidae (Uchôa and Nicácio, 2010), six genera (*Anastrepha* Schiner, *Bactrocera* Macquart, *Ceratitis* McLeay, *Rhagoletis* Loew (Tephritidae), *Dasiops* Rondani and *Neosilba* McAlpine (Lonchaeidae)). The genus *Bactrocera* in Brazil is represented by only one species, *B. carambolae* which is economic importance in Southern part of Brazil (Table 2.4). For the genus *Ceratitis,*

including *Ceratitis capitata* is one of the most important key pest of fruit and vegetable crops in Brazil and recorded in 60 species of host fruits from 22 families, of which 22 are native (Uchôa *et al*., 2002; Uchôa and Nicácio, 2010) (Table 2.4). In addition, genus *Anastrepha* is one of fruit flies are a wide distribution in South America, and able to attack grown fruit and/or vegetables of commercial value (Table 2.4).

In Southeast Asia, the countries such as Brunei, Cambodia, India, Sri Lanka, Laos, Vietnam, Kampuchea, Myanmar, Malaysia, Singapore, Philippines and Indonesia, and the Pacific region the major fruit fly pest species are of the genus *Bactrocera* (Drew, 1989; Drew and Hancock, 2000; Kittayapong *et al*., 2000; Plant Health Australia, 2011). The important pest species of this region, including *B. dorsalis* complex (*B. carambolae* Drew and Hancock, *B. dorsalis* Hendel, *B. occipitalis* Bezzi, *B. papaya* Drew and Hancock, *B. philippinensis* Drew and Hancock, *B. pyrifoliae* Drew and Hancock, *B. caryeae* Kapoor, *B. kandiensis* Drew and Hancock), *B. correcta* Bezzi, *B. latifrons* Hendel, *B. zonata* Saunders, *B. cucurbitae* Coquillett, *B. tau* Walker (Drew and Roming, 1996).

In Thailand and bordering countries, about 221 Dacini fruit fly species have been reported, of these, 182 species belong to the genus *Bactrocera*. Where at least 51 species are member of the *B. dorsalis* complex (Drew and Hancock, 2001; Clarke *et al*., 2001). In Thailand, seven species were geographically widespread including *B.dorsalis, B. papayea, B. carambolae, B. cucurbitae, B. latifrons, B. correcta* and *B. umbrosa* which are important pest species (Clarke *et al*., 2001).

The distribution and abundance of the fruit fly species depend on various factors such as seasonal, temperature, distribution of host plant etc., especially capability of fruit fly to feed various hosts. In addition, fruit fly movements are probably favored and increased by human activities and fruit transportations. The growing trades of exotic fruits, as well as the tourism industry (Shi *et al*., 2010) have been recognized as important factors influencing fly dispersal (Malacrida *et al*., 2007). Polyphagous usually have wider geographic distribution than the monophagous species because the ability to used wider host range (Drew and Roming, 2001).

Table 2.2 Worldwide geographic distribution of species of Dacini in each of the four genera.

Areas	**Total No. of Species**	**No. of Species of *Bactrocera***	**No. of Species of *Dacus***	***Ichneumonopsis***	***Monacrostichus***
Africa (including Madagascar and Mascarene Islands)	182	10	172	0	0
Southeast Asia	229	182	44	1	2
Papua New Guinea	168	155	13	0	0
Australia	87	75	12	0	0
Solomons (including Bougainville)	56	54	2	0	0
Vanuatu	13	12	1	0	0
New Caledonia	11	10	1	0	0
Fiji	4	4	0	0	0
Tonga	6	6	0	0	0
Samoa	7	7	0	0	0
Niue	2	2	0	0	0
Cook Islands	2	2	0	0	0
Austral Islands	2	2	0	0	0
Society Islands	2	2	0	0	0
Marquesas Islands	1	1	0	0	0
Tuamotu Archipelago	2	2	0	0	0
Micronesia/ North Pacific	2	2	0	0	0

(From: Drew and Hancock, 2001)

Table 2.3 The number of known species of Dacinae in zones within Southeast Asia and the Pacific region.

Zone	No. of species
Vanuatu/New Caledonia	20
East Indonesia	25
Solomon Islands	27
India	42
Philippines	47
South China/Southern Japan/Taiwan	48
Myanmar/Thailand to Vietnam	64
Australia	90
Malaysia/West Indonesia	106
Papua New Guinea	173

(From: Drew and Roming, 1997)

Table 2.4 Species of Fruit Flies (Diptera: Tephritoidea: Tephritidae) in South America.

Species	**Host Fruits**	**Plant Family**	**Distribution**
**Anastrepha antunesi* (Lima)	*Spondias cf. macrocarpa* Engl.	Anacardiaceae	Brazil
	Eugenia stipitata McVaugh	Myrtaceae	Peru
	Psidium guajava L.	Anacardiaceae	Venezuela
	Spondias purpurea L.		
**A. bahiensis* (Lima)	*Psidium guajava* L.	Myrtaceae	Brazil
	Myrciaria cauliflora (Mart.)	Moraceae	Colombia
	Brosimum potabile Ducke	Annonaceae	Brazil
	Helicostylis tomentosa (Poep. et Endl.)	Ulmaceae	
	Rollinia aff. *sericea* (Fries)		
	Ampelocera edentula Kuhlm.		
**A. bistrigata* (Bezzi)	*Pouteria gardneriana* (D.C.)	Sapotaceae	Brazil
	Psidium australe Cambess.	Myrtaceae	
	Psidium guajava L.		
***A. fraterculus* (Wiedemann)	*Rollinia laurifolia* Schltdl.	Annonaceae	Brazil
	Myrcianthes pungens (Berg.)	Myrtaceae	Argentina
	Psidium guajava L.		Bolivia
	P. kenedianum Morong		Colombia

Table 2.4 (continue).

Species	**Host Fruits**	**Plant Family**	**Distribution**
	Syzygium jambos (L.)		Ecuador Guyana Paraguay Peru Suriname Uruguay Venezuela
***A. grandis* (Mcquart)	*Citrullus lanatus* (Thunb.) *Cucumis sativus* L. *Cucurbita maxima* Duchesne *Cucurbita moschata* Duchesne *Cucurbita pepo* L.	Cucurbitaceae	Argentina Bolivia Brazil Colombia Ecuador Paraguay Peru Venezuela

Table 2.4 (continue).

Species	**Host Fruits**	**Plant Family**	**Distribution**
**A. leptozona* (Hendel)	*Anacardium occidentale* L. *Alibertia* sp. *Pouteria torta* (Martius) *Pouteria cainito* Radlk.	Anacardiaceae Rubiaceae Sapotaceae	Bolivia Brazil Guyana Venezuela
**A. macrura* (Hendel)	*Ficus organensis* (Miq.) *Schoepfia* sp. *Pouteria lactescens* (Vell.)	Moraceae Olacaceae Sapotaceae	Argentina Brazil Ecuador Paraguay Peru Venezuela
***A. oblique* (Macquart)	*Anacardium humile* St.Hil. *Anacardium othonianum* Rizzini *Spondias cytherea* Sonn. *Psidium kennedianum*	Anacardiaceae Myrtaceae	Argentina Brazil Bolivia Colombia

Table 2.4 (continue).

Species	**Host Fruits**	**Plant Family**	**Distribution**
			Ecuador Paraguay Peru Venezuela
***A. pseudoparallela* (Loew)	*Mangifera indica* L. *Psidium guajava* *Passiflora alata* Curtis *Passiflora edulis* Sims. *Passiflora quadrangularis*	Anacardiaceae Myrtaceae Passifloraceae	Argentina Brazil Ecuador Peru
***A. serpentine* (Wiedemann)	*Spondias purpurea* L. *Mammea americana* L. *Salacia campestris* Walp. *Alibertia* sp. *Coffea canephora* L.	Anacardiaceae Clusiaceae Hippocrateaceae Rubiaceae Moraceae	Argentina Brazil Colombia Ecuador Guyana

Table 2.4 (continue).

Species	**Host Fruits**	**Plant Family**	**Distribution**
	Ficus gomelleira Kunth and Bouché *Achras sapota* L. *Chrysophyllum cainito* L. *Cotia* sp. *Manikara* spp. *Pouteria* spp.	Sapotaceae	Peru Suriname Venezuel
	Pouteria torta *Pouteria ramiflora* (Martius) *Mimusops coriacea* (A. DC.) *Mimusopsis commersonii* (G. Don.)		
***A. sororcula* (Zucchi)	*Spondias purpurea* L. *Licania tomentosa* Fritsch *Terminalia catappa* L. *Casearia sylvestris* Swartz	Anacardiaceae Chrysobalanaceae Combretaceae Fabaceae	Brazil Colombia Ecuador Paraguay

Table 2.4 (continue).

Species	Host Fruits	Plant Family	Distribution
	Byrsonima orbignyana A. Jussieu	Flacourtiaceae	
	Mouriri elliptica Martius	Oxalidaceae	
	Psidium cattleianum Sabine	Malpighiaceae	
	Psidium kennedyanum Morong	Melastomataceae	
	Schoepfia sp.	Myrtaceae	
	Physalis angulata L.	Olacaceae	
		Oxalidaceae	
		Rosaceae	
		Rubiaceae	
		Solanaceae	
***A. striata* (Schiner)	*Spondias mombin* L.	Anacardiaceae	Bolivia
	Spondias purpurea L.	Annonaceae	Brazil
	Rolinia mucosa Jacq.	Araceae	Colombia
	Attalea excelsa Martius	Chrysobalanaceae	Ecuador

Table 2.4 (continue).

Species	Host Fruits	Plant Family	Distribution
	Chrysobalanacus icaco	Lauraceae	Guyana
	Persea americana L.	Malpighiaceae	Peru
	Byrsonima crassifolia L. Rich.	Moraceae	Suriname
	Artocarpus heterophyllus Lam.	Myrtaceae	Venezuela
	Campomanesia cambessedeana O. Berg.	Rutaceae	
	Eugenia stipitata McVaugh	Passifloraceae	
	Psidium acutangulum DC	Sapotaceae	
	Psidium austral Cambess.		
	Psidium guajava L.		
	Psidium guineense SW		
	Citrus sinensis L.		
	Passiflora edulis		
	Pouteria cainito L.		

Table 2.4 (continue).

Species	Host Fruits	Plant Family	Distribution
****A. turpiniae** (Stone)	*Andira cuyabensis* Benthan	Fabaceae	Brazil
	Andira humilis Martius	Myrtaceae	
	Psidium kennedyanum	Caricacea	
	Psidium guajava	Combretaceae	
	Psidium guineense	Anacardiaceae	
	Eugenia dodoneifolia Cambess.	Rosaceae	
	Syzygium jambos L.	Rutaceae	
	Jacaratia heptaphylla (Vell.)		
	Terminalia catappa L.		
	Mangifera indica L.		
	Spondias purpurea L.		
	Prunus persicae L.		
	Citrus sinensis		

Table 2.4 (continue).

Species	**Host Fruits**	**Plant Family**	**Distribution**
***A. zenildae* (Zucchi)	*Licania tomentosa*	Chrysobalanaceae	Brazil
	Terminalia catappa	Combretaceae	
	Andira cuyabensis	Fabaceae	
	Banara arguta Briquel	Flacourtiaceae	
	Mouriri elleptica	Melastomataceae	
	Sorocea sprucei saxicola (Hassler)	Moraceae	
***Bactrocera carambolae* (Drew and Hancock)	*Benincasa hispida* (Thunb.)	Cucurbitaceae	Brazil
	Cucumis sativus L.	Myrtaceae	Guyana
	Cucurbita pepo L.	Rosaceae	Suriname
	Lagenaria siceraria (Molina)	Rutaceae	
	Luffa acutangula (L.)	Sapotaceae	
	Luffa aegyptiaca (Mill.)	Solanaceae	
	Momordica charantia L.		
	Trichosanthes cucumerina L.		
	Psidium guajava		

Table 2.4 (continue).

Species	**Host Fruits**	**Plant Family**	**Distribution**
	Syzygium samarangense (Blume) *Prunus persica* (L.) *Citrus aurantium* L. *Citrus maxima* Merr. *Manilkara zapota* (L.) *Capsicum annuum* L. *Lycopersicon esculentum* Mill.		
***Ceratitis capitata* (Wiedemann)	*Juglans australis* Grisebach *Hancornia speciosa* Gomez *Licania tomentosa* *Terminalia catappa* *Mouriri elliptica* *Inga laurina*	Juglandaceae Apocynaceae Chrysobalanaceae Combretaceae Melastomataceae Mimosaceae	Argentina Brazil Bolivia Chile Colombia Ecuador Paraguay

Table 2.4 (continue).

Species	Host Fruits	Plant Family	Distribution
	Syzygium jambos	Myrtaceae	Peru
	Chrysophyllum gonocarpum Engler	Sapotaceae	Uruguay
	Pouteria ramiflora		Venezuela

* potential or ** real economic importance (From: Norrbom and McAlpine, 1997; Katiyar *et al*., 2000; Uchôa *et al*., 2002; Ovruski *et al*., 2003; Oliveira *et al*., 2006; Uramoto *et al*., 2008; Zucchi, 2008; Costa *et al*., 2009; Silva *et al*., 2010; Castañeda *et al*., 2010; Uchôa, 2012)

2.4 Economic impact of the fruit fly

Fruit flies belonging to the family Tephritidae (Order: Diptera) are considered a very destructive group of insects that cause enormous economic losses in agriculture, especially in a wide variety of fruits, vegetables and flowers (Diamantidis, 2008). Approximately 10% of a total number of species within this family are serious pests distributed around the world in temperate, subtropical and tropical areas (Christenson and Foote, 1960; Weems *et al*., 1999). The damage starts when the female fruit fly punctures the fruit with its long and sharp ovipositor and laying eggs under the soft skin in both mature and green fruits (Hollingsworth and Allwood, 2000). The fruit skin is breached, and bacteria enter and the fruit starts to decay. The larvae that hatch from the eggs feed on the decaying fruit tissue, and on the yeasts and bacteria that multiply in it causing the fruits to rot (Phillon, 2005) resulting in unmarketable fruits. Due to the larva is three instars the fruits can be totally destroyed (Ye and Liu, 2005). It is believed that some fruit fly females carry bacteria with them that they inject into the fruit at ovipositor so that the fruit decays faster. Fruits with fruit fly larvae inside decay quickly. It is sometimes possible to cut out the damage for home consumption of the remaining part of the fruit but infested fruit are generally unsalable and can certainly not be exported. Crop losses can vary from a few per cent to 100%, and losses of 90% over are common.

The fruit flies are great economic importance because they are considered the key pests that most adversely affect the production and marketing of fruits and vegetables around the world. They are able of inserting the ovipositor to drop their eggs into the living tissues of host plants, such as green fruit, fruit in process of maturation or ripe fruits. According Lourenção *et al*., (1996), *Neosilba perezi* (Romero and Ruppel) is a key pest in shoots of cassava clones. The families Tephritidae are causing direct and indirect damages. The direct ones are because their eggs hatch and the larvae eat the underlying flesh of the fruits. The indirect damage is due to depreciation of the fruits in the market retailers; opening holes through which can penetrate pathogenic microorganisms or decomposers, or yet, causing the early fall of fruits attacked in the

field. Some species of fruit flies are also the major bottleneck in the exports of fresh fruits and vegetables between nations. This is because the importing countries generally impose stringent quarantine barriers to the producing and exporting countries where fruit flies do occur, fearing the entry exotic species inside the imported products in their territories (Uchôa and Nicácio, 2010; White and Elson-Harris, 1992). In addition, the fruit flies are impose a significant cost on horticultural production every year. The world market for fresh fruit has been estimated at US$ 722 billion in 1995 (Armstrong and Jang, 1996). Especially the genus *Bactrocera* cause economic losses from direct fruit damage as well as from quarantine regulations.

Several countries that have the horticultural industry were loss of economic revenue due to fruit fly infestion. For example, In Hawaii the direct impact of fruit flies was 15 million dollars (Nakahara *et al*., 1977), which did not include the costs or impacts of insecticide use to control these pests. Twenty-four years later, Staples and Cowie (2001) reported potential impacts of 300 million dollars due to fruit flies in Hawaii (Jang, 2007). For South Africa, the fruit export was dropped by 80% in 2008 (Ekesi *et al*., 2009).

The Asian region is among the top three regions worldwide for both exporters and importers of fruits and vegetables. In 2004, for example, Asian countries produced 178 million tons of tropical fruits which amounted to 66% of the total global production and earned US$ 2.5 billion (Somsri *et al*., 2007). Tephritid fruit flies (especially, *B. dorsalis, B. cucurbitae* and *B. correcta*) (Table 2.5) are cause direct damage to fruit and vegetables crops in this region which can lead to up to 90-100% yield loss. The Asian region is equitable climate and rich diversity of plant life, is home to several species of highly damaging fruit flies (especially the Southeast Asia). In addition, to the direct losses, fruit fly infestation can result in serious losses in trade value and export opportunity due to strict quarantine.

In Southeast Asia, the major fruit fly pest species are *Bactrocera albistrigata* de Meijere, the *B. dorsalis* complex (*B. carambolae* Drew and Hancock, *B. dorsalis* Hendel, *B. occipitalis* Bezzi, *B. papaya* Drew and Hancock, *B. philippinensis* Drew and Hancock, *B. pyrifoliae* Drew and Hancock, *B. caryeae* Kapoor, *B. kandiensis* Drew and

Hancock), *B. correcta* Bezzi, *B. latifrons* Hendel, *B. zonata* Saunders, *B. cucurbitae* Coquillett, *B. tau* Walker. These pest species account for damage to most fruit crops and many vegetable crops. Some species have a number of specific host fruits while also overlapping with other species in the same hosts. For example, *B. carambolae* is the primary pest of carambola, is also found as the major pest of mangoes and papaya, and guavas (Drew and Roming, 1997).

In Thailand and bordering countries, the cost of losses due to infestation of fruit flies can be surprisingly high especially the mango, guava and star fruit (Mahmood, 2004; Aemprapa, 2007; Orankanok *et al*., 2007). In Thailand, there are examples where losses have been up to 100% in cucurbit species, caused by Melon fly (*B. cucurbitae*) (Phillon *et al*., 2005). Crop losses in mango (12-60%), guava (40-90%) and papaya (12-60%) (Allwood and Leblanc, 1997). In Malaysia, fruit flies cause severe damage to certain potential fruit crops like guava and starfruit (Tobin, 1990). Fruit crops may suffer 100% damage if not protected owing to the fact that these are the polyphagous pests and losses can run several million dollars annually (Signh, 1991).

Table 2.5 Fruit flies in the genus *Bactrocera* (Diptera: Tephritidae) of economic importance in Asia.

Species	**Common name**	**Distribution**	**Host range**
B. albistrigata	Asian Terminalia Fruit Fly	Andaman islands, central to southern Thailand, Malaysia, Kalimantan (Borneo, Inodonesia east to Sulawesi, Christmas Is.	11 host plant species
B. carambolae	Carambola Fruit Fly	Southern Thailand, Malaysia, Kalimantan (Borneo, Inodonesia east to Sumbawa. Adventive in Andaman Is, Surinam, Frenh Guiana, Brazil	78 host plant species from 27 plant families
B. caryeae	Indian Fruit Fly	Southern India and Sri Lanka	Guava, mango, citrus, Barbados cherry
B. caudata	-	Widespread across S.E. Asia	Flowers of commercial /edible
B. correcta	Guava Fruit Fly	Sri Lanka, India, Nepal, Pakistan, Myanmar, northern Thailand, southern Vietnam, Cambodia, southern China	62 host plant species from 30 plant families

Table 2.5 (Continue).

Species	Common name	Distribution	Host range
B. cucurbitae	Melon Fly	S.E. Asia and Asia. Adventive in Hawaiian Islands, P.N. Guinea to Solomon Is, Nauru, African continent, Mauritius, Reunion, Egypt	A very wide range of Cucurbitaceae, but also recorded on other fruits of economic importance
B. diversa	-	Sri Lanka, India, Nepal, Bhutan, China, Thailand	Flowers of commercial /edible Cucurbitaceae
B. dorsalis	Oriental fruit Fly	Cambodia, Laos, Vietnam, Myanmar, Thailand, southern China, Taiwan, Sri Lanka, India, Nepal, Bhutan	123 host plant species from 41 plant families
B. invadens	-	Sri Lanka, southern India. Adventive in Africa	A wide range of commercial and edible fruits. Severe in Africa
B. kandiensis	Sri Lankan Fruit Fly	Sri Lanka	21 host plant species

Table 2.5 (Continue).

Species	Common name	Distribution	Host range
B. latifrons	Solanum Fruit Fly	Cambodia, Laos, Vietnam, Myanmar, Vietnam, Thailand, Malaysia, Sri Lanka, India, Pakistan to southern China, Taiwan. Adventive in Hawaii	17 host plant species primarily in the family Solanaceae
B. minax	Chinese Citrus Fruit Fly	Northeast India, Sikkim, Bhutan, southern China	Major pest of citrus and *Fortunella* species (Rutaceae)
B. occipitalis	Bezzi Fruit Fly	Philippines, Sabah (east Malaysia), Brunei, Kalimantan (Borneo)	8 known host plant species. Needs more host surveys
B. papayae	Asian Papaya Fruit Fly	Southern Thailand, Malaysia, Kalimantan (Borneo), Indonesia. Now in P.N. Guinea, Irian Jaya and northern Torres Strait Islands	About 200 host plant species from 50 plant families. Considered the most virulent and serious fruit fly species

Table 2.5 (Continue).

Species	Common name	Distribution	Host range
B. philippinensis	-	Philippines	Mango, papaya, breadfruit, *Syzygium* species. Full host surveys lacking
B. pyrifoliae	-	Northern Thailand, northern Vietnam	7 host plant species, importantly in the family Rosaceae
B. scutellaris	-	China, India, Myanmar, Nepal, Pakistan, Thailand, Vietnam, Bhutan	Flowers of 4 species of commercial/edible Cucurbitaceae
B. scutellata	-	Bhutan, China, Japan, Taiwan, Thailand, Vietnam	Flowers of commercial /edible Cucurbitaceae

Table 2.5 (Continue).

Species	**Common name**	**Distribution**	**Host range**
B. tau	-	Southern China, Taiwan, Thailand, Malaysia, Indonesia (Kalimantan)	24 host plant species, primarily in the family Cucurbitaceae.
B. tuberculata	-	China, Myanmar, Thailand, Vietnam, Cambodia	A range of commercial fruit incl. peach, mango papaya and *Syzygium* species
B. umbrosa	-	Widespread across S. E. Asia. P.N. Guinea, south Pacific islands to Vanuatu and New Caledonia	A range of edible *Artocarpus* species, especially, jackfruit and breadfruit
B. zonata	Peach Fruit Fly	Sri Lanka, India, Pakistan, Thailand, Vietnam, Mauritius and Egypt	20 host plant species from 15 plant families

(From: Drew and Hancock, 1994; Kumar *et al*., 2011)

2.5 Fruit flies management

Fruit flies of the family Tephritidae are considered to be the most important insect pest species of fruits worldwide. Losses of soft fruit and vegetables as a result of fruit fly infestation occur across all countries and their presence inhibits the export of horticultural produce. Several agencies, for example the Regional Management of Fruit Fly Projects (RMFFP) (Allwood, 2000) funded by the Food and Agricultural Organization (FAO), the Australian government through the Australian Agency for International Development (AusAID), the United Nations Development Programme (UNDP), New Zealand government Aid (NZAID), the Secretariat of the Pacific Community (SPC), national governments of Pacific Island countries and territories and fruit fly projects funded by Australian Centre for International Agricultural Research (ACIAR). Both the ACIAR-funded and RMFFP activities aimed to provide improved fruit fly management tools for growers, to improve prospects for entering export markets and to support horticultural exports. These projects capably help several countries such as Fiji, Tonga, Samoa, the Cook Islands and Vanuatu to solve the fruit fly problems and can export commercial crops again (Mcleod, 2005).

Fruit fly management can be divided in 4 different categories: chemical, cultural, biological and genetic (Sarango, 2009).

2.5.1 Chemical control

The application of insecticides is done by spray cover on the entire crop or trees. Insecticides can also be used in a mix with attractants like cue-lure and methyl-eugenol. This is a technique called Male Annihilation Technique (MAT) (Figure 2.8) and consists of many bait stations throughout the field. This method reduces the male proportion in a population to a low level and therefore mating does not occur. Experience in field demonstrated that the level of infestation in mango in India decrease to 5% from levels of infestation between 17% and 66% by using this technique (Verghese *et al*., 2006). This method is very important in the control of both female and male fruit flies in distinction when using insecticide and attractants that is specific for male fruit flies. The advantage of this method is generally effective in killing fruit flies but this method has the disadvantage is very expensive in cost of pesticide and very time-consuming in labour. This method also kill beneficial organisms that keep other

orchard pest in check-cover-spraying often results in increased damage by other pest, especially borers and can cause health problems for person applying the spray and also leave chemical residues in the fruits. In addition, Insecticides can even be used together with protein baits.

Protein bait spraying, this involves diluting protein bait concentrate with water and mixing it with an insecticide. Adult fruit flies needs protein for their reproductive functions; beer waste based protein baits or other mixed with insecticide have been successfully used in Vietnam. This method has the advantage because it cheap in terms of materials, greatly reduced health impact on operators, less pesticide put into the environment, no risk of residues in the produce if applied correctly and virtually no impact on non-target organisms because only fruit flies are attracted to the bait but this method has the disadvantage is still requires a certain amount of labour to apply, though much less than cover spraying or bagging and need to be repeated more than once during a crop cycle, especially if weather is very wet.

Figure 2.8 Male Annihilation Techniques (MAT)
(Source: http://www.fvdp.gop.pk/oacts.html)

2.5.2 Cultural control

These techniques are the most successful sanitation measures, growing crops that better can withstand fruit flies attacks, early harvest, and bagging.

Sanitation measures, the infested fruits should be removed; in particular, the fruit on the tree that present signs of attack should be removed instead of removing fallen fruits on the ground were the larvae have already left the fruit. In fields where sanitation measures are practice the level of fruit flies decreases significantly (Verghese *et al*., 2004).

Resistant crops, the production of crop varieties that is less attractive for fruit flies have shown good effects. Some chili varieties are classified as non-hosts for fruit flies in Fiji Islands. In Thailand, there are some fruit crops that are not susceptible to fruit fly attacks (Allwood *et al*., 2001).

Early harvest, fruit flies prefer to attack fruits and vegetables depending on the stage of maturity. In some crops there is the possibility to harvest fruits early to avoid fruit fly infesting.

Bagging, this is a kind of exclusion. A single fruit or a cluster or even a whole tree can be covered by a bag (Figure 2.9). The bags prevent fruit flies from infesting the fruits. Often the bag is made of paper but also cloth can be a material resistant enough. Bags made of old newspaper can be an economic and effective way to protect the fruits. In Thailand, this method is used in particular in mango orchards (Allwood *et al*., 2001). Even plant leaves can be an appropriate material for bagging fruits (e.g. banana). The advantage of this method is effective when applied properly, increases the fruit quality, which also increases the price and materials are very cheap but this method has the disadvantage is very laborious to apply.

Figure 2.9 Cover crop or trees by bag, paper or calico.
(Source: http://www.daleysfruit.com.au/forum/fruit-fly-control/)

2.5.3 Biological control

Introduction of parasitoids to infested fields has given good results in management of fruit flies (Figure 2.10). The use of biological control for fruit flies has started since 1902 (Wharton, 1989). There are examples where reductions of infestation have been nearly 95% as the experiment in Hawaii showed when larvae parasitoids belonging to the families Eulophidae, Braconidae and Chalcididae were introduced (Allwood *et al*., 2001). *Psyttalia fletcheri* (Hymenoptera: Braconidae) is one of the parasitoids that had showed a high parasitism degree in *B. cucurbitae*, *Fopius arisanus* (Sonan) is other promising parasitoid tested in Hawaii to control *B. latifrons* (Bokonon-Gatan *et al*., 2007), *Diachasmimorpha longicaudata*, *Biosteres arisanus, B. vandenboshi* and *Psyttalia insici* is a high parasitism in Thailand to control *B. correcta*. A biological control can also be conducted 15 via measures that favour the established parasitoids in a kind of conservation of biological control agents. The biological control agents are often reared in different localizations than the place where they will be released. In Thailand, it is reported that the potential to find Eulophidae parasitoids that can be used in Hawaii is great, especially in the north region (Ramadanb *et al*., 2003).

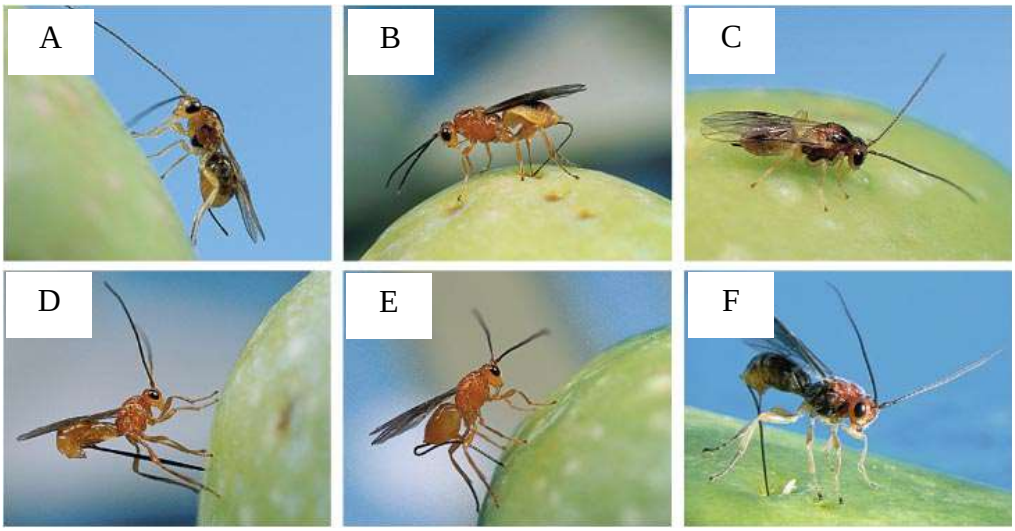

Figure 2.10 Parasitoids of fruit flies; (A) *Psyttalia lounsburyi* (B) *Bracon celer* (C) *Utetes africanus* (D) *Diachasmimorpha longicaudata* (E) *Diachasmimorpha kraussii* and (F) *Fopius arisanus*.
(Source: http://californiaagriculture.ucanr.edu/landingpage.cfm?article=ca.v065n01p21&fulltext=yes)

2.5.4 Genetic control

Sterile Insect Technique (SIT) is based on the release of sterilized male fruit flies into the field. Competition between sterile and wild males for females will end up with females mating with sterile male flies and therefore no offspring will be generated. Irradiation to pupa of fruit flies using gamma radiation from a Co60 Gamma cell 220 source (Figure 2.11) (Kumar *et al*., 2011). Radiation is used to sterilize the flies for this method requires a great amount of sterile flies which should be in same proportions to the number of the wild flies but also an appropriate rearing of flies that carry many of the genetic characteristics presented in the population that will be controlled (Itô *et al*., 2003).

In Thailand, area wide integrated pest management (AW-IPM) using Sterile Insect Technique (SIT) was used to control fruit flies, especial *B. dorsalis* and *B. correcta* which are considered to be the key insect pest of fruit production in Thailand. The result revealed that in Ratchaburi Province (western of Thailand) the integrated approach has been effective in controlling fruit flies by reducing damage from over 80%

before programme implementation to an average of less than 3.6% in the past five years (2000-2004). And in Pichit Province (northern of Thailand) where the control programme has been carried out for only two year (2003 and 2004), the infestation has been reduced from 42.9 to 15.5%. That clearly shows that fruit fly control in Thailand using an integrated area-wide approach with an SIT component could be expanded to other production areas with significant economic returns (Orankanok *et al*., 2006, 2007).

A new concept for plant protection emerged in the late 1950 as a reaction to the sole reliance on pesticides. At that time, the negative aspects of chemical control became better known, such as environmental contamination, residue problems, the killing of non-target organisms, the development of manmade pests because natural enemies were eliminated, the development of resistance to pesticides and the increasing cost of pesticides. Consequently, a more integrated approach to pest control was advocated, giving due consideration to ecological factors such as natural mortality which may keep insect pest populations below economic damage levels. This new concept called Integrated Pest Control (IPC) and later Integrated Pest Management (IPM).

Integrated Pest Management (IPM) is the careful integration of a number of available pest control techniques that discourage pest population development and keep pesticide and other interventions to levels that are economically justified and safe for human health and the environment. IPM emphasizes the growth of a healthy crop and the least disruption of agro-ecosystems, there by encouraging natural pest control mechanisms to play their role. Pesticides are applied on a need basis only, with the necessary precautions to avoid negative side effects.

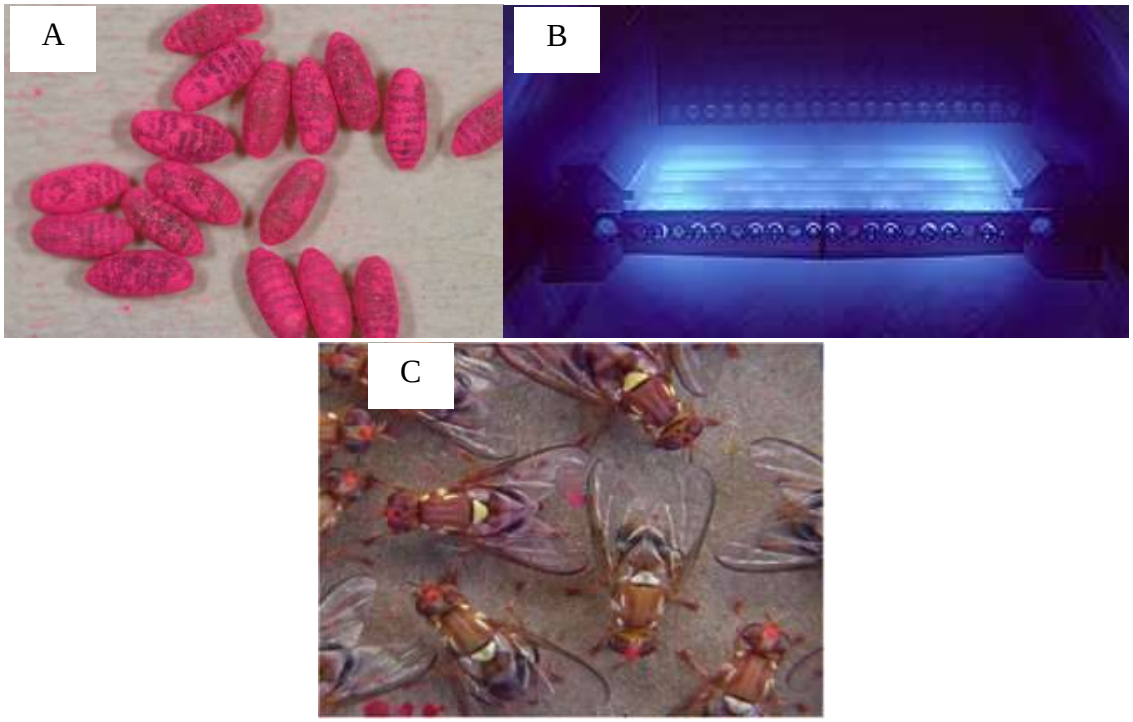

Figure 2.11 Sterile Insect Technique (SIT); (A) fruit flies pupae dyed with powder that transfers to adults for identification of sterile flies in the field (B) Cobalt 60 source used to irradiate fruit flies pupae to induce reproductive sterility and (C) Sterilize male fruit flies for release to target areas.
(Source: http://bio.mq.edu.au/research/groups/behavbiol/Fruitflies.html)

2.6 Population genetic study

Population genetic is the study of evolutionary processes, change the frequency of genes or alleles, investigated the genetic variation and genetic structure of the organism because of evolutionary changes occurring on the basis of population dynamics and genetic variation. Therefore, population genetic study using modern molecular techniques are crucial for a better understanding of living systems. Basic information of population genetic is useful in academic evolutionary biology, use in the management or control insect pests, insect vectors, including various creatures that importance of agriculture and medical (e.g. plants, animals and microorganisms) and conservation planning rare and endangered animal and plant species.

Populations can be structured for molecular, morphological, ecological, or behavioral characters (Aluja and Norrbom, 2001). Knowledge of the genetic structure of fruit fly populations could provide a basis for studying the molecular evolution of locally adaptive traits in natural populations. In addition, population genetic structures make understand genetic differences within, and especially among, different populations of a single species (Aluja and Norrbom, 2001) which will lead to the study of evolution due to the changes occurring in the evolution have a fundamental change of population genetic structure.

Population genetic study could also provide crucial information for pest control and management. Understanding genetic differentiation among populations, colonization structure, migration routes, and patterns of distribution across the natural landscape are very important for the success of pest management strategies (Wu *et al*., 2011). For example, sterile insect technique (SIT), use of this method can greatly reduce the need for environmentally and medically hazardous pesticides. Information gathered from a population genetic study would be very useful in designing this method because this method requires a great amount of sterile flies that should be in same proportions to the number of the wild flies (Itô *et al*., 2003). Consequently, information about effective population size and individual movement across populations (i.e. gene flow) is important to know for this method and to plans of releasing sterile insects correctly (Aketarawong *et al*., 2011; Karsten *et al*., 2013).

Mun *et al*. (2003) analyzing the genetic structure of Asian populations of *B. depressa* in Korea and Japan. The result reveals that high levels of genetic subdivision between Korea and Japan. Theses population has isolated for approximately 1 million years ago. There is also signature of more recent range expansions within each country (Mun *et al*., 2003). The results of this study indicated that historical environmental change could play significant role on genetic structure and diversity of fruit fly.

Population genetic study of the melon fly, *B. cucurbitae* from China and Southeast Asia, in contrast, reveal low level of genetic structuring. The genetic diversity and genetic differentiations between populations is low. The result indicates that *B. cucurbitae* movement across populations in a high rate because no major geographic barrier (big rivers and high mountains) between populations. Therefore, the fly is

expected to disperse freely, considering its high dispersal ability leads to high rate of gene flow among populations (Hu *et al*., 2008).

Genetic structure and diversity of highly diverse taxa, *B. dorsalis* have also been investigate. As would be expected, high level of genetic structure and diversity were found. It has been suggested that this could be a result of geographically widespread combined with geographic barrier such as large mountain ranges as a major obstacle to gene flow (Shi *et al*., 2005; 2010; Liu *et al*., 2007; Hu *et al*., 2008).

Recent advanced in molecular techniques and analytical methods allow complicate genetic structure and diversity, and population history inference. Nardi *et al*. (2005) examined colonization and demographic history of olive fly, *B. oleae*. The result reveals that evolutionary processes which led to the historical range expansion of the species might have been tightly linked to the evolution and distribution of the olive tree. Colonization history analysis indicated that invasion of the olive flies in the American region most likely originated from the Mediterranean area (Nardi *et al*., 2005).

Aketarawong *et al*. (2007) investigated the population structure and genetic variability in 14 geographical populations across the four areas of the actual species range: Far East Asia, South Asia, Southeast Asia and the Pacific Area of *B. dorsalis* complex. The colonization process of this fly associated with a relatively stable demographic structure of the adventives populations. The scattering of a population has implications for the enrichment and the maintenance of genetic variability. The overall genetic profile of the considered populations suggests a Western orientated migration route from China to Bangladesh probably across the Northern area of the Indian subcontinent.

Wu *et al*. (2012) investigated population genetic structures of *B. cucurbitae* in Southeast Asia and China. They found that the western regions showed higher haplotype diversity than eastern regions (China-east). Yunnan province showed highest levels of genetic diversity among China populations. Haplotype diversity decreased with longitude from west to east suggest that *B. cucurbitae* has expanded from west to east within a limited geographic scale.

Population genetics of fruit fly in Thailand is scantly examined. There are only two species (*B. dorsalis*; Aketarawong *et al*., 2007; and *B. latifrons*; Meeyen *et al*. 2013) that have been studied at the population genetic level. Population genetic

structure and demographic history of the *B. latifrons* were inferred from mitochondrial COI barcoding sequences. The results indicated overall low level of genetic structure but considerable level of genetic diversity. Population genetic structure and demographic history of this species were influenced by the combination of continuous distribution of the host plants and historical climatic change in the Pleistocene period. Recent demographic expansion (16,000 year ago) has been recorded in this species (Meeyen *et al*., 2013).

2.7 *Bactrocera correcta* (Bezzi)

The guava fruit fly, *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) was first recorded in Bihar, India in 1916 (Bezzi, 1916) and is now distributed throughout South and Southeast Asia (Wang, 1996; Drew and Raghu, 2002) and in China (Liang *et al*., 1996). *Bactrocera correcta* is one of the most destructive pests of the genus *Bactrocera* (Wang, 1996; Kitthawee, 2000). This species is highly adaptable to new environments enabling it to spread geographically rapidly. *Bactrocera correcta* infested 62 plant species from 30 families (Allwood *et al*., 1999; Maynard *et al*., 2004) (Table 2.6) including several commercially important fruits such as mangoes, guava, peaches, melons, cashewnut, cherry, jujube, carambola, wax apple, banana and citrus fruits (White and Elson-Harris, 1992). Large damage to commercial fruit crops have been reported in Thailand and Vietnam (Drew and Raghu, 2002). Because *B. correcta* causing serious economic damage to fruit production and considered as highly invasive thus it is considered as a key quarantine species by many countries (White and Elson-Harris, 1992; Maynard *et al*., 2004).

Bactrocera correcta is a small sized species (Figure 2.12). Face was fulvous with a pair of transverse elongate black spots almost meeting in centre; scutum black with dark red-brown along lateral and posterior; postpronotal lobes and notopleura yellow; mesopleural stripe reaching almost to anterior notopleura seta dorsally; broad parallel sided lateral postpronotal vittae ending behind intra-alar seta; medial postpronotal vita absent; scutellum yellow with narrow black basal band; legs with all segments entirely fulvous except hind tibiae pale fuscous; wings with cells basal-costal band and costal band colourless, both cells entirely devoid of microtrichia, a narrow

pale fuscous costal band confluent with R_{2+3} and ending at apex of this vein, a small oval fuscous spot across apex of R_{4+5}, anal streak absent but with a pale fuscous tint within cell cup; supernumerary lobe of medium development; abdominal terga III-V red-brown with a 'T' pattern consisting of a narrow transverse black band across anterior margin of tergum III and a narrow medial longitudinal black band over all three terga, narrow black anterolateral corners on terga IV and V, a pair of oval red-brown shining spots on tergum V (Figure 2.13); posterior lobe of male (Figure 2.14) surstylus short; female (Figure 2.15) with aculeus tip needle shaped (Plant Health Australia, 2011) .

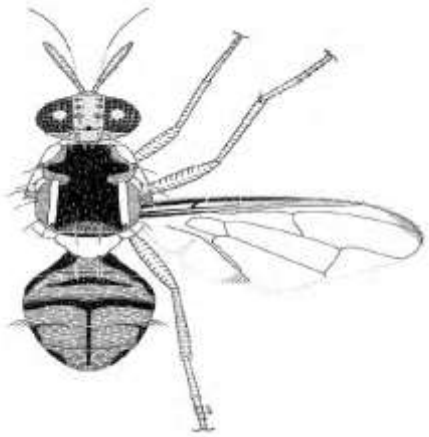

Figure 2.12 Morphology of fruit fly *Bactrocera correcta*.
(Source: Plant Health Australia, 2011)

Figure 2.13 Character of head, thorax, legs, abdomen and wings of guava fruit flies, *Bactrocera correcta*.

Figure 2.14 Adult male guava fruit flies, *Bactrocera correcta*.

Figure 2.15 Adult female guava fruit flies, *Bactrocera correcta.*

Table 2.6 Host-plants species of fruit fly *Bactrocera correcta*.

Family	**Scientific Name**
Anacardiaceae	*Anacardium occidentale* *Bouea macrophylla* *B. oppositifolia* *Mangifera indica* (White and Elson-Harris, 1992) *Spondias cytherea* *S. pinnata*
Annonaceae	*Polyalthia longifolia*
Apocynaceae	*Carissa caramdus* (Hoa *et al.*, 2006)
Arecaceae	*Areca catechu*
Cactaceae	*Opuntia vulgaris*
Capparaceae	*Capparis sepiaria* *C. thorellii* *Maerue siamensis*
Caricaceae	*Carica papaya*
Combretaceae	*Terminalia catappa* (White and Elson-Harris, 1992)
Cucuritaceae	*Coccinia grandis* *Cucumis melo*
Dipterocarpaceae	*Dipterocarpus obtusifolius*
Elaeocarpaceae	*Elaeocarpus madopetalus* *Muntingia calabura*
Euphorbiaceae	*Baccaurea racemosa* *Phyllanthus acidus* *Securinega virosa*
Flacourtiaceae	*Flacourtia indica* *F. jangomas*

Table 2.6 (continue).

Family	**Scientific Name**
Lecythidaceae	*Careya arborea*
	C. sphaerica
Malpighiaceae	*Malpighia emarginata*
Meliaceae	*Sandoricum kortjape*
	Walsura intermedia
Moraceae	*Artocarpus integer*
Musaceae	*Musax paradisiaca*
Myristicaceae	*Knema angustifolia*
Myrtaceae	*Eugenia paniala*
	E. pseudosubtilis
	Psidium guajava
	(White and Elson-Harris, 1992*;*
	Hoa *et al.*, 2006)
	Syzygium aqueum
	S. cumini
	S. jambos (Hoa *et al.*, 2006)
	S. malaccense
	S. samarangense
Olacaceae	*Olax scandens*
	Schoepfia fragrams
Oxalidaceae	*Averrhoa carambola*
Rhamnaceae	*Ziziphus jujuba*
	Z. mauritiana
	(White and Elson-Harris, 1992)
	Z. oenoplia
	Z. royundifolia

Table 2.6 (continue).

Family	**Scientific Name**
Rosaceae	*Prunus avium* *P. cerasus* *P. persica* (White and Elson-Harris, 1992)
Rutaceae	*Citrus grandis* *C. reticulate*
Sapindaceae	*Dimocarpus longan* *Lepisanthes fruticosa*
Sapotaceae	*Manilkara zapota* (White and Elson-Harris, 1992) *Mimusops elengi*
Simaroubaceae	*Irvingia malayana*

(From: White and Elson-Harris, 1992; Allwood *et al*., 1999; Hoa *et al.*, 2006)

Chapter 3

Research Methodology

3.1 Sample collection

Specimens of *B. correcta* were collected from natural habitats in Thailand (Figure 3.1, Table 3.1 and Table 3.2). Adult flies were obtained from 11 host plants (Figure 3.2 and Table 3.3) including Climbing Llang-llang (*Artabotrys siamensis* Miq.), Rose apple (*Syzygium samarangense*), Guava (*Psidium guajava*), Mango (*Mangifera indica*), Ivy gourd (*Coccinia grandis*), Acerola (*Malpighia emarginata*), Calabula (*Muntingia calabura*), Kayu (*Irvingia malayana*), Jujube (*Zizyphus mauritiana* Lam.), Eggplant (*Solanum melongena* L.) and Starfruit (*Averrhoa carambola*). The infest fruits were collected from natural habitat and bring back to laboratory where they were placed in a plastic box containing sawdust at the bottom and covered by calico (Figure 3.3) and kept under room temperature (Figure 3.4). Soon after the adults emerged, the adult flies were stored in 80% ethanol at -20 °C for further studies.

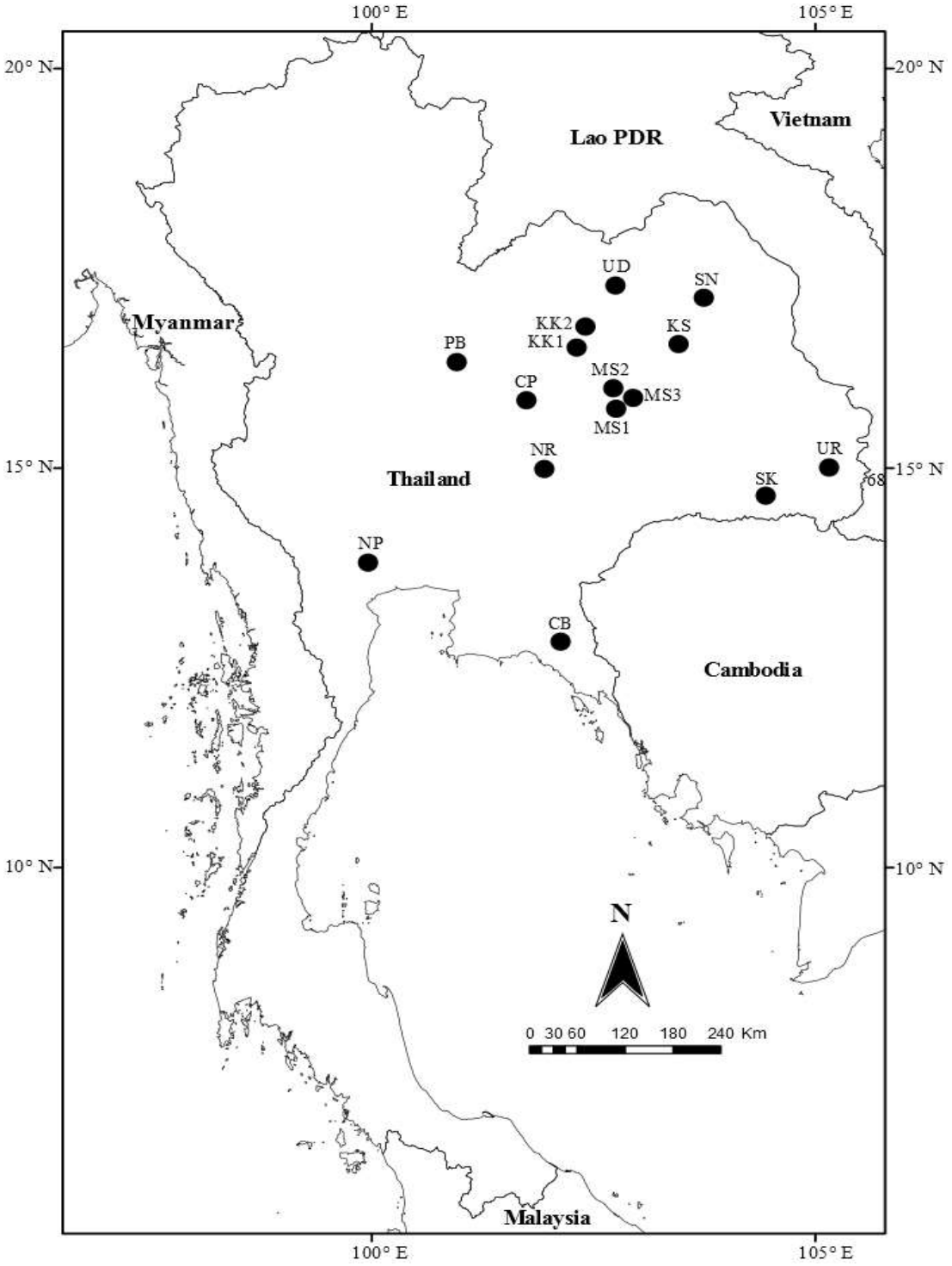

Figure 3.1 Sampling locations of *Bactrocera correcta* in Thailand; MS-Maha Sarakham, KS-Kalasin, KK-Khon Kaen, CP-Chiyaphum, UD-Udon Thani, SK-Si Sa Ket, UR-Ubon Ratchatani, NR-Nakhon Ratchasima, SN-Sakon Nakhon, NP-Nakhon Pathom, PB-Phetchabun and CB-Chanthaburi. Details of the sampling site were given in table 3.1.

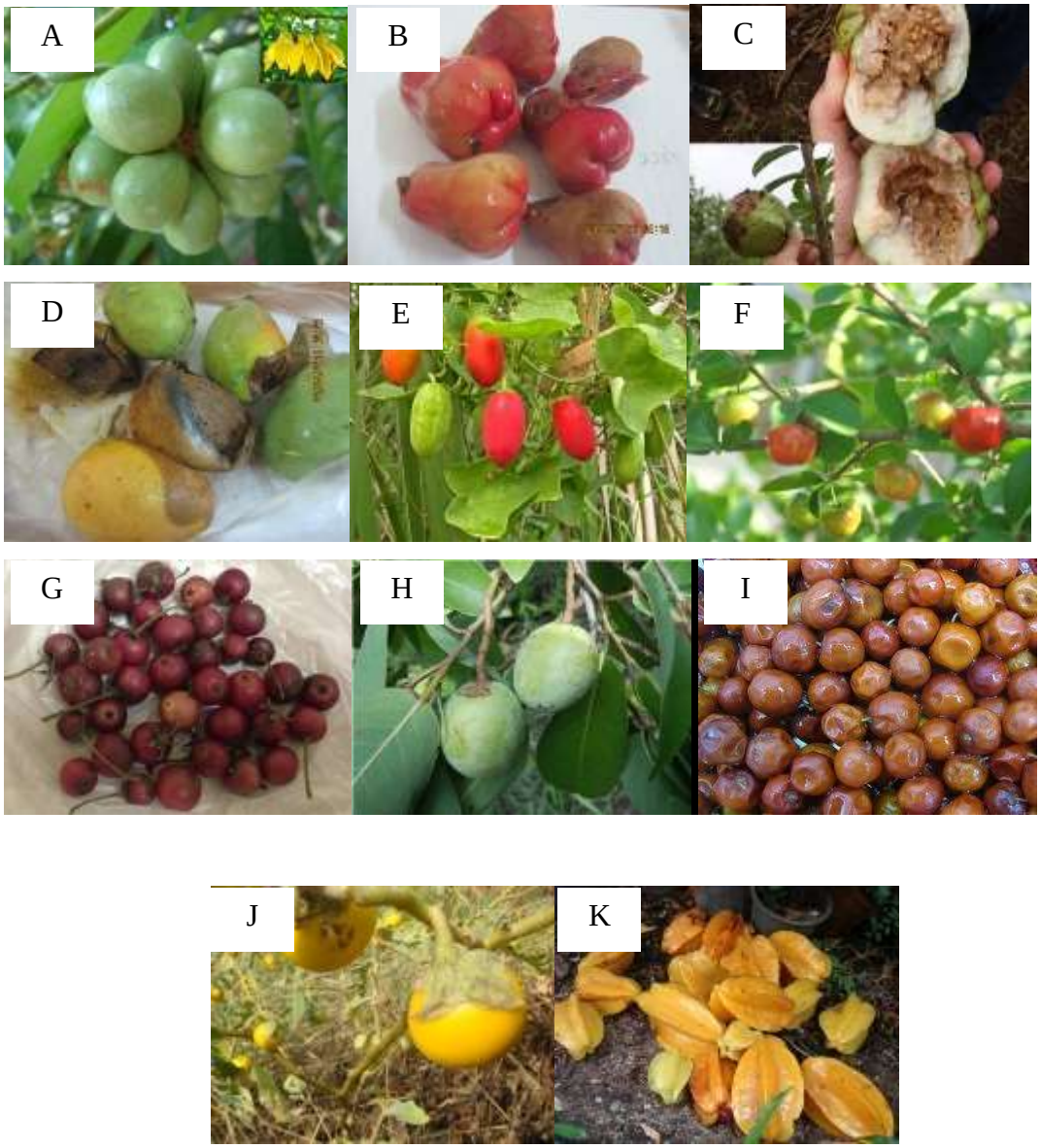

Figure 3.2 Host plant species were infested by fruit flies *Bactrocera correcta* in this study; (A) Climbing Llang-llang (*Artabotrys siamensis* Miq.), (B) Rose apple (*Syzygium samarangense*), (C) Guava (*Psidium guajava*), (D) Mango (*Mangifera indica*), (E) Ivy Gourd (*Coccinia grandis*), (F) Acerola (*Malpighia emarginata*), (G) Calabula (*Muntingia calabura*), (H) Kayu (*Irvingia malayana*), (I) Jujube (*Zizyphus mauritiana* Lam.), (J) Eggplant (*Solanum melongena* L.) and (K) Star fruit (*Averrhoa carambola*).

Figure 3.3 Rearing of fruit flies; (A) a plastic box contained sawdust (B) place the infested fruits into the plastic box (C) cover the plastic box by calico (D) placed the plastic box that contained infested fruit covered by calico in the shelf at room temperature.

Figure 3.4 Infested fruits were reared in a laboratory under room temperature.

Table 3.1 Sample collection sites and host-plant species of *Bactrocera correcta* used in this study.

Location	**Code**	**Geographic region**	**Latitude/ Longitude**	**Altitude (m)**	**Host-plant species**	**No. of Sample**	**Collection date**
Kantharawichai, Maha Sarakham	MS1	Northeast	16° 14′ 58″ N 103° 15′ 52″ E	166	*Artabotrys siamensis Mig.*	1	08/05/2013
					Syzygium samarangense	1	22/04/2013
					S. samarangense	2	23/04/2013
					S. samarangense	1	23/04/2013
					S. samarangense	3	25/04/2013
					Psidium guajava	5	28/07/2012
					P. guajava	2	01/03/2013
					Mangifera indica	1	20/07/2012
					M. indica	1	20/07/2012
					M. indica	1	20/07/2012
					Coccinia grandis	1	29/05/2013

Table 3.1 (continued).

Location	**Code**	**Geographic region**	**Latitude/ Longitude**	**Altitude (m)**	**Host-plant species**	**No. of Sample**	**Collection date**
Mueang, Maha Sarakham	MS2	Northeast	16° 9′ 50″ N 103° 19′ 45″ E	138	*S. samarangense* *S. samarangense* *Malpighia emarginata* *Muntingia calabura* *P. guajava*	1 2 2 1 2	13/06/2013 22/04/2013 05/05/2013 05/05/2013 29/04/2013
Na Dun, Maha Sarakham	MS3	Northeast	15° 42′ 50″ N 103° 13′ 37″ E	162	*Irvingia malayana* *Zizyphus mauritiana* Lam.	2 1	08/08/2012 19/05/2013
Kham Muang, Kalasin	KS	Northeast	16° 55′ 24″ N 103° 37′ 54″ E	201	*Z. mauritiana* Lam.	4	13/08/2012
Mueang, Khon Kaen	KK1	Northeast	16° 32′41″ N 102°50′37″ E	173	*P. guajava* *Solanum melongena* L.	8 8	07/04/2013 07/04/2013

Table 3.1 (continued).

Location	**Code**	**Geographic region**	**Latitude/ Longitude**	**Altitude (m)**	**Host-plant species**	**No. of Sample**	**Collection date**
Si Chomphu, Khon Kaen	KK2	Northeast	16° 49′30″ N 102°11′17″ E	226	*M. marginata*	11	23/07/2013
Khon San, Chaiyaphum	CP	Northeast	16° 36′ 25″ N 101° 54′ 35″ E	260	*S. samarangense*	10	19/01/2013
Kut Chap, Udon Thani	UD	Northeast	17°27′14″ N 102°25′ 35″ E	233	*S. samarangense* *P. guajava*	9 9	20/05/2013 20/05/2013
Prang Ku, Si Sa Ket	SK	Northeast	14° 48′ 59″ N 104° 04′ 00″ E	139	*Averrhoa carambola* *Z. mauritiana* Lam.	7 9	11/08/2012 11/08/2012
Mueang, Ubon Ratchatani	UR	Northeast	15° 13′ 44″ N 104° 51′ 15″ E	121	*S. samarangense* *P. guajava*	1 10	04/08/2012 04/08/2012

Table 3.1 (continued).

Location	**Code**	**Geographic region**	**Latitude/ Longitude**	**Altitude (m)**	**Host-plant species**	**No. of Sample**	**Collection date**
Pak Chong, Nakhon Ratchasima	NR	Northeast	14°39′19″ N 101°26′02″ E	457	*S. samarangense*	15	17/04/2013
Phanna Nikhom, Sakon Nakhon	SN	Northeast	17°19′23″ N 103°52′04″ E	211	*M. indica*	10	12/06/2013
Mueang, Nakhon Pathom	NP	Central	13°48′51″ N 100°02′24″ E	7	*S. samarangense* *S. samarangense*	1 12	07/07/2013 07/08/2013
Bueng Sam Phan, Phetchabun	PB	North	15°40′50″ N 100°56′07″ E	205	*P. guajava*	14	04/06/2013
Mueang, Chanthaburi	CB	East	12°37′03″ N 102°05′ 54″ E	10	*S. samarangense*	3	24/05/2013

Table 3.2 Sample collection site and number of COI sequence of *Bactrocera correcta* in Thailand.

Location	**Code**	**Geographic region**	**Latitude/ Longitude**	**Altitude (m)**	**No. of samples**
Kantharawichai, Maha Sarakham	MS1	Northeast	16° 14′ 58″ N 103° 15′ 52″ E	166	19
Mueang, Maha Sarakham	MS2	Northeast	16° 9′ 50″ N 103° 19′ 45″ E	138	8
Na Dun, Maha Sarakham	MS3	Northeast	15° 42′ 50″ N 103° 13′ 37″ E	162	3
Kham Muang, Kalasin	KS	Northeast	16° 55′ 24″ N 103° 37′ 54″ E	201	4
Mueang, Khon Kaen	KK1	Northeast	16° 32′41″ N 102°50′37″ E	173	16
Si Chomphu, Khon Kaen	KK2	Northeast	16° 49′30″ N 102°11′17″ E	226	11

Table 3.2 (continued).

Location	**Code**	**Geographic region**	**Latitude/ Longitude**	**Altitude (m)**	**No. of samples**
Khon San, Chaiyaphum	CP	Northeast	16° 36′ 25″ N 101° 54′ 35″ E	260	10
Kut Chap, Udon Thani	UD	Northeast	17°27′14″ N 102°25′ 35″ E	233	18
Prang Ku, Si Sa Ket	SK	Northeast	14° 48′ 59″ N 104° 04′ 00″ E	139	16
Mueang, Ubon Ratchatani	UR	Northeast	15° 13′ 44″ N 104° 51′ 15″ E	121	11
Pak Chong, Nakhon Ratchasima	NR	Northeast	14°39′19″ N 101°26′02″ E	457	15
Phanna Nikhom, Sakon Nakhon	SN	Northeast	17°19′23″ N 103°52′04″ E	211	10

Table 3.2 (continued).

Location	**Code**	**Geographic region**	**Latitude/ Longitude**	**Altitude (m)**	**No. of samples**
Mueang, Nakhon Pathom	NP	Central	13°48′51″ N 100°02′24″ E	7	13
Bueng Sam Phan, Phetchabun	PB	North	15°40′50″ N 100°56′07″ E	205	14
Mueang, Chanthaburi	CB	East	12°37′03″ N 102°05′ 54″ E	10	3

Table 3.3 List of host-plants species of *Bactrocera correcta* found in this study.

Family	Scientific Name	Common Name
Annonaceae	*Artabotrys siamensis* Mig.	Climbing Llang-llang
Myrtaceae	*Syzygium samarangense*	Rose apple
Myrtaceae	*Psidium guajava*	Guava
Anacardiaceae	*Mangifera indica*	Mango
Cucurbitaceae	*Coccinia grandis*	Ivy Gourd
Rosaceae	*Prunus cerasus*	Acerola
Elaecocarpaceae	*Muntingla calabura*	Calabula
Irvingiaceae	*Irvingia malayana*	Kayu
Rhamnaceae	*Ziziphus mauritiana* Lam.	Jujube
Solanaceae	*Solanum melongena* L.	Eggplant
Oxalidaceae	*Averrhoa carambola*	Starfruit

3.2 Species identification

Species were identified using adult morphology. The major characteristics including wing vein shape and detail (black band and point terminal the wings), overall colour and colour patterning (colour of legs and colour patterns of thoracic and abdomen), shape and size. Only adult specimens were used identify by morphology because other stages (egg, larva and pupa) are very difficult or cannot be identify based on morphological characters (Houdt *et al*., 2010; Asoka *et al*., 2011). Species were identified following White and Elson-Harris (1992) and Plant Health Australia (2011).

3.3 DNA extraction, amplification and sequencing

Genomic DNA was extracted from individual adult flies using the GF-1 Tissue DNA Extraction Kit (Vivantis, Selangor Darul Ehsan, Malaysia). A 584-bp fragment of the mitochondrial cytochrome *c* oxidase I (COI) gene was amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAG GGTGACCAAAAAATCA-3′) (Folmer *et al*., 1994). PCR amplifications were carried out in a final volume of 50 µl containing 2 µl of DNA template, 2 µl of each primer (10 µM), 3 µl of 50 mM $MgCl_2$, 5 µl of 10x PCR buffer, 1.6 µl of 10 µM dNTPs, 0.4 µl of *Taq* DNA polymerase (5 U/ µl). Temperature profile of the PCR including an initial denaturation at 94 °C for 2 min followed by 36 cycles of 94 °C for 30 min, 45 °C for 45 min and 72 °C for 45 min with the final extension at 72 °C for 5 min.

PCR products were checked with 1% agarose gel which contained 0.5 µg/ml of ethidium bromide (Sambrook *et al*., 1987; Pramual *et al*., 2005). Five micro liters of PCR product were mixed with 1 µl of 6x loading buffer. The mixture loaded into a well of the submerged (in 0.5x TBE buffer; 89 mM Tris Base, 89 mM Boric Acid, 2 mM EDTA) was carefully. A voltage of 100 volt was applied for 30-40 minutes and the gel was examined under ultraviolet (UV) light and photographed using digital camera. PCR products were purified by using the HiYield™ Gel/PCR DNA Extraction Kit (RBC Bioscience) followed the manufacturing protocol. Sequencing was performed at Macrogen (Seoul, Korea), using the same primers as in PCR.

3.4 Data analysis

3.4.1 Genetic variation

Haplotype diversity (*h*) and nucleotide diversity (π) were calculated using Arlequin v3.5.1.2 (Excoffier and Lischer, 2010). The median joining (MJ) network (Bandelt *et al*., 1999) was used to estimate the genealogical relationships between haplotypes. The MJ network was calculated based on 209 COI sequences of *B.correcta*. Of these, 171 sequences were obtained in this study and 38 sequences were obtained from Genbank (Accession nos. DQ116262 and JX 297530 from Viet Nam; JX297522-25, JX 297527- 28, JX 456552 from China; JX 297529 from Laos; JX 297531 from Myanmar; KF289766, GU323781-82 from India; JQ692631, JQ692641, JQ692676, JQ692711, JQ692753, JQ692756, JQ692784, JQ692787, JQ692832, JQ692856 from Sri Lanka; and DQ116263- 65, HM590450-51, JX 297532-38, AB568102, AB720881 from Thailand). MJ network analysis was performed in NETWORK v4.6.1.2 (http://www.fluxus-engineering.com).

The Haplotype diversity (*h*) was calculated following the equation:

$$h = \frac{n}{n-1}\left(1 - \sum_{i=1}^{k} p_i^2\right)$$

where n is the number of gene copies in the sample, k is the number of haplotypes and p is the sample frequency of the i haplotype (Nei, 1987).

The nucleotide diversity (π) was calculated following the equation:

$$\pi_n = \frac{\sum_{i=1}^{k} \sum_{j<i} P_i P_j \hat{d}_{ij}}{L}$$

Where $\hat{d}_{ij}$ is the number of mutations having occurred since the divergence of haplotype i and j, k is the number of the haplotype, P_i is the frequency of the haplotype i and P_j is the frequency of the haplotype j (Tajima, 1993; Nei, 1987).

3.4.2 Population genetic structure

Population genetic structure was estimated by population pairwise F_{ST}. The significance test statistic was obtained by 1023 permutations. Analysis of molecular variance (AMOVA) was used to test the genetic differentiation among groups of populations from different host-plants and geographic regions. Both population pairwise F_{ST} and AMOVA analyses were performed in Arlequin using Kimura 2-parameter model (K2P).

A Mantel test (Mantel, 1967) was used to determine the relationship between genetic distance (F_{ST} from Arlequin) and geographic distance (km) to test an isolation-by-distance (IBD) model. The Mantel test was implemented in IBD v1.52 (Bohonak, 2002) using 1000 randomizations.

3.4.3 Demographic history

Mismatch distribution was used to test the demographic history of the populations. Population that has undergone recent past demographic expansion shows unimodal mismatch distribution (Roger and Harpending, 1992). The sum-of-squares deviation and Harpending's raggedness index (Harpending, 1994) were used to test deviation from the sudden expansion model. Mismatch distribution was estimated using Arlequin. Population expansion time was calculated from $\tau = 2ut$ (where $u = m_T\mu$, m_T is the length of nucleotide sequences under study, μ is the mutation rate per nucleotide and t is the generation time; Roger and Harpending, 1992), assuming a divergence rate of 2.3% per million years for insect mtDNA (Brower, 1994). In addition, Fu's F_S test (Fu, 1997) and Tajima's D (Tajima, 1989) statistical tests were also used to test the population equilibrium. Large negative values of these tests were expected from demographic population expansion.

Chapter 4

Results and Discussion

4.1 Results

4.1.1 Genetic variation

A 584 bp fragment of the mitochondrial COI gene were sequenced from 171 specimens of *B. correcta* from 15 locations in Thailand. Sequences were deposited in Genbank with the accession numbers KJ879751-KJ879921. There were 180 base substitutions of these, 91 were transitions and 89 were transversions. A total of 83 haplotypes were identified of these 72 haplotypes were unique and 11 haploytypes were shared by at least two individuals. The most common haplotype was found in all sampling locations except Phetchabun and Chanthaburi. *Bactrocera correcta* specimens from India, Sri Lanka, Laos, Viet Nam and China also shared this haplotype. Haplotype diversity (*h*) and nucleotide diversity (π) for each population are show in Table 4.1. Haplotype diversity in each population ranged from 0.3455 in Ubon Ratchatani (UR) to 1.000 in Maha Sarakham (MS3) and Chanthaburi (CB) with an average of 0.9337. Nucleotide diversity in each population range from 0.0076 in Nakhon Ratchasima (NR) to 0.0325 in Chaiyaphum (CP) with an average of 0.0132

The MJ network (Figure 4.1 and Figure 4.2) was calculated from 209 COI sequences (171 sequences obtained in this study and 38 sequences obtained from Genbank including accession no. DQ116262 and JX 297530 from Viet Nam; JX297522-25, JX 297527- 28, JX 456552 from China; JX 297529 from Laos; JX 297531 from Myanmar; KF289766, GU323781-82 from India; JQ692631, JQ692641, JQ692676, JQ692711, JQ692753, JQ692756, JQ692784, JQ692787, JQ692832, JQ692856 from Sri Lanka; and DQ116263- 65, HM590450-51, JX 297532-38, AB568102, AB720881 from Thailand) of *B. correcta.* The MJ network revealed two distinct genetically lineages (I, II) among the members of *B. correcta*. Most specimens (204 sequences) belong to the lineages I. Five specimens (two specimens from Maha Sarakham and Kalasin and one specimen from Si Sa Ket) from Thailand forming lineage II with connected to the lineage I by 36 mutation steps. The haplotype cluster in

the MJ network associated neither with host-plants nor with geographic origins (Figure 4.1 and Figure 4.2). Overall, the network has a star-like shape with central haplotype shared by globally distributed populations (Thailand, India, Sri Lanka, Laos, Viet Nam and China), characteristic of recent demographic expansion population (Slatkin and Hudson, 1991).

Genetic relationships between Thai *B. correcta* and sequences from other geographic regions are as follows. Three mitochondrial COI sequences from India shared the central haplotype. Two sequences from Viet Nam were made up of two haplotypes, of which one were unique and link up with a short branch length to the haplotype group I, one sequence shared the central haplotype. One sequence from Laos was shared the central haplotype. Seven sequences from China were made up of six haplotypes with one haplotype were unique and connected to the central haplotype, three sequences being shared with the central haplotype and three sequence shared haplotypes with Thai specimens and clustered to the haplotype group I. Ten sequences from Sri Lanka were included in the MJ network analysis. Three sequences shared the central haplotype, seven sequences were unique and join to the haplotype group I. One sequence from Myanmar were made up of one unique haplotype and directly connected to the central haplotype.

Table 4.1 Haplotype diversity (h) and nucleotide diversity (π) of 15 populations of *Bactrocera correcta* in Thailand.

Location	**No. of samples**	**Haplotype diversity (h) ± SD**	**Nucleotide diversity (π) ± SD**
MS1	19	0.9532 ± 0.0358	0.0079 ± 0.0046
MS2	8	0.9643 ± 0.0772	0.0125 ± 0.0075
MS3	3	1.0000 ± 0.2722	0.0174 ± 0.0137
KS	4	0.8333 ± 0.2224	0.0193 ± 0.0134
KK1	16	0.7000 ± 0.1274	0.0093 ± 0.0053
KK2	11	0.9636 ± 0.0510	0.0186 ± 0.0105
CP	10	0.9722 ± 0.0640	0.0325 ± 0.0181
UD	18	0.9739 ± 0.0293	0.0216 ± 0.0115
SK	16	0.9191 ± 0.0438	0.0094 ± 0.0054
UR	11	0.3455 ± 0.1722	0.0118 ± 0.0068
NR	15	0.8000 ± 0.0771	0.0076 ± 0.0044
SN	10	0.7778 ± 0.0907	0.0160 ± 0.0091
NP	13	0.9615 ± 0.0412	0.0149 ± 0.0083
PB	14	0.9890 ± 0.0314	0.0118 ± 0.0066
CB	3	1.0000 ± 0.2722	0.0115 ± 0.0093
Total	**171**	**0.9337 ± 0.0118**	**0.0132 ± 0.0069**

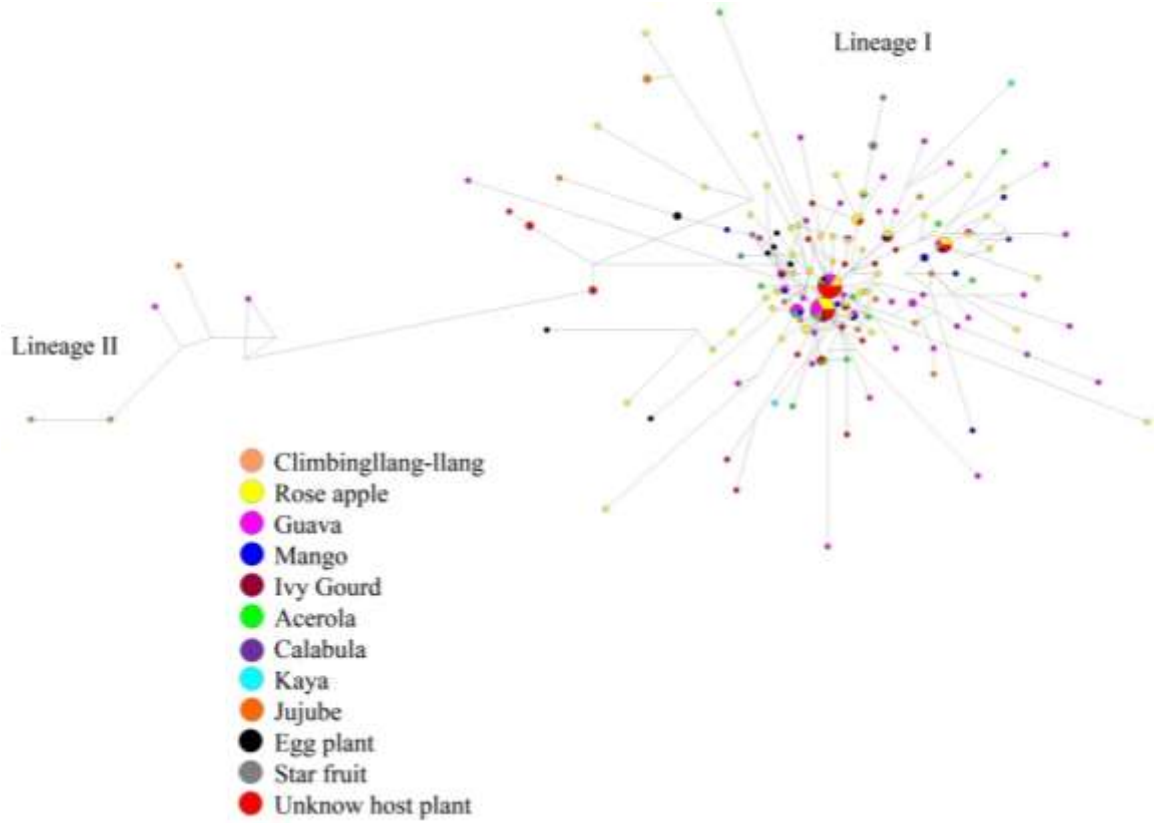

Figure 4.1 Median Joining network of 209 COI sequences (171 sequences from Thailand and 38 sequences from other geographic regions) of *Bactrocera correcta*. Each circle represents a haplotype and sizes are relative to the number of individuals sharing the specific haplotype. Haplotypes are labeled according to different host plants.

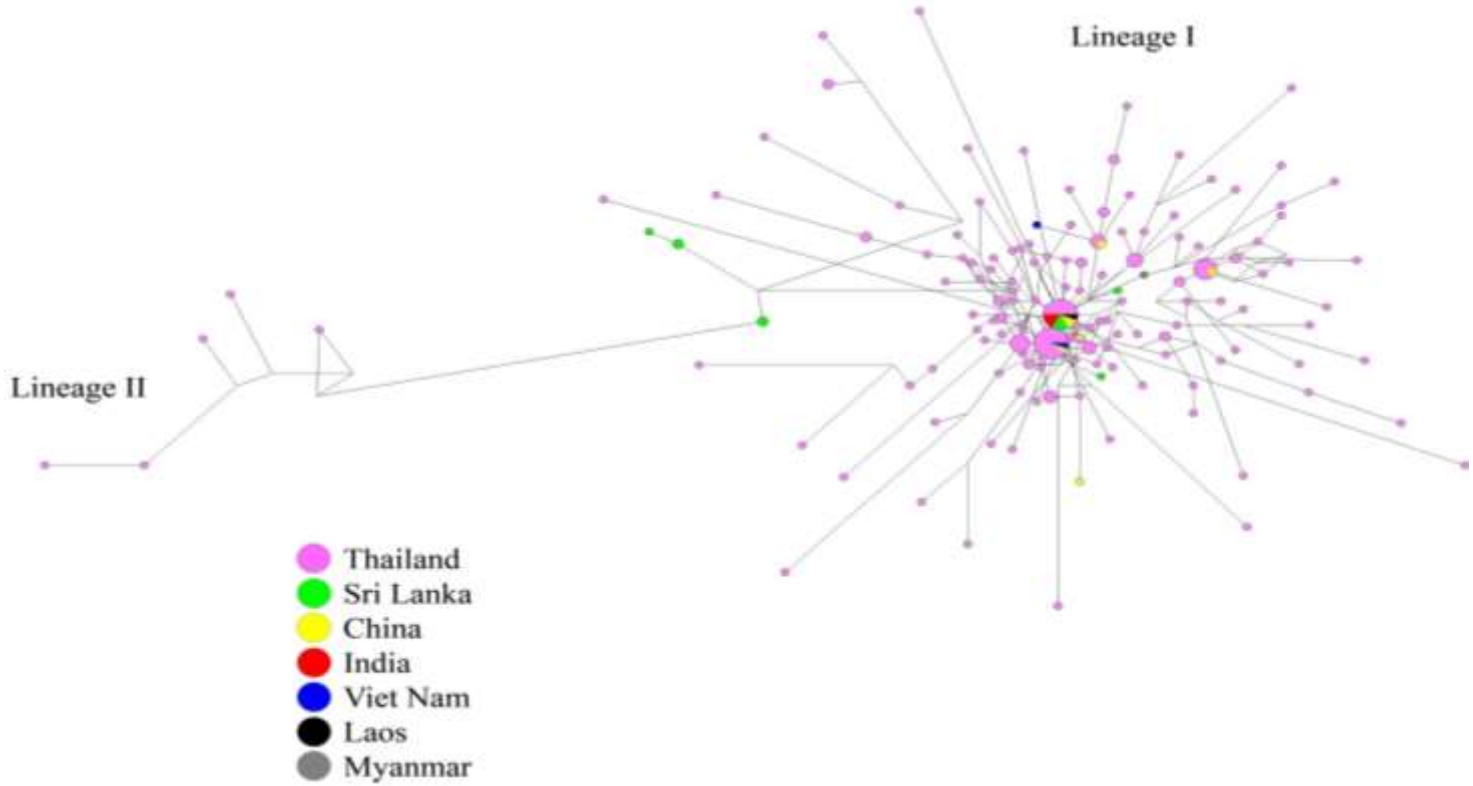

Figure 4.2 Median Joining network of 209 COI sequences (171 sequences from Thailand and 38 sequences from other geographic regions) of *Bactrocera correcta*. Each circle represents a haplotype and sizes are relative to the number of individuals sharing the specific haplotype. Haplotypes are labeled according to country of specimen origins.

4.1.2 Population genetic structure

Population pairwise F_{ST} values revealed that most populations were genetically not significantly different (Table 4.2).The exception is comparisons between Phetchabun (PB) with the other populations where almost all F_{ST} were statistically significant different. AMOVA analyses by grouping populations according to the host-plant species and geographic regions found no significant genetic differentiation among groups (Table 4.3). Mantel's test revealed no significant relationships (r^2 = 0.0074, P=0.3030) between genetic distance (pairwise F_{ST}) and geographic distance.

4.1.3 Demographic history

Mismatch distribution analysis revealed a unimodal mismatch graph (Figure 4.3), a characteristic of recent population demographic expansion. This is consistent with the star-like shape of the mtDNA genealogy. Both sum-of-squares deviation (SSD=0.0049, P=0.8300) and Harpending's raggedness index (0.0075, P=0.9200) were not significantly different from the simulated data under the sudden population expansion model (Figure 4.3). Population expansion was also supported by highly significant negative values of both Tajima's D (−2.3708, P< 0.001) and Fu's F_S (−24.7529, P< 0.001) tests. Population expansion time estimated based on 2.3% sequence divergence for insect mitochondrial DNA (Brower, 1994) and assuming eight generations per year for fruit fly based on the rearing information for *B. correcta* (Liu and Ye, 2009) the expansion time was estimated to be approximately 15,000 years ago.

Table 4.2 Population pairwise F_{ST} values between 15 populations of *Bactrocera correcta* in Thailand.

population	**MS1**	**MS2**	**MS3**	**KS**	**KK1**	**KK2**	**CP**	**UD**	**SK**	**UR**	**NR**	**SN**	**NP**	**PB**	**CB**
MS1	0.000														
MS2	0.035	0.000													
MS3	0.202	0.120	0.000												
KS	0.152	0.119	0.120	0.000											
KK1	0.068	**0.106**	0.294	0.355	0.000										
KK2	0.103	-0.013	0.144	0.150	0.085	0.000									
CP	**0.145**	0.014	-0.011	0.015	**0.164**	0.022	0.000								
UD	**0.075**	0.003	0.120	0.124	0.032	-0.009	0.062	0.000							
SK	0.020	-0.034	0.155	0.169	0.056	0.047	0.072	0.037	0.000						
UR	0.114	0.034	0.213	**0.333**	**0.095**	0.062	**0.129**	0.053	0.078	0.000					

Table 4.2 (continue).

population	MS1	MS2	MS3	KS	KK1	KK2	CP	UD	SK	UR	NR	SN	NP	PB	CB
NR	**0.105**	0.041	**0.366**	0.409	0.038	0.054	**0.118**	0.022	-0.001	0.071	0.000				
SN	**0.211**	0.042	0.353	0.175	**0.254**	0.018	0.037	0.029	0.121	**0.197**	0.214	0.000			
NP	**0.119**	-0.017	0.224	0.130	**0.154**	0.006	0.030	0.017	0.028	0.145	0.081	-0.032	0.000		
PB	**0.087**	0.120	0.042	0.187	**0.172**	**0.197**	**0.169**	**0.162**	**0.102**	**0.206**	**0.244**	**0.318**	**0.221**	0.000	
CB	0.075	0.058	0.145	0.202	0.030	-0.010	0.003	-0.037	0.046	0.034	0.119	0.247	0.121	0.168	0.000

Bold characters indicate statistical significance at $P<0.05$

Table 4.3 Results of the AMOVA analyses of 15 populations of *Bactrocera correcta* from Thailand, with grouping according to geographic regions and host-plants.

Source of variation	**d.f.**	**SSD**	**Percentage of variation**	***F*-statistic**
geographic regions				
Among groups	4	30.0950	0.95	F_{CT} = 0. 0095
Among populations within groups	10	67.1930	8.94	F_{ST} = 0.0989*
Within populations	156	493.7880	90.11	F_{SC} = 0. 0903*
Host plants				
Among groups	7	51.6120	1.96	F_{CT} = 0.0196
Among populations within groups	14	83.2570	11.00	F_{ST} = 0.1297*
Within populations	144	445.1580	87.03	F_{SC} = 0.1123*

*P<0.05

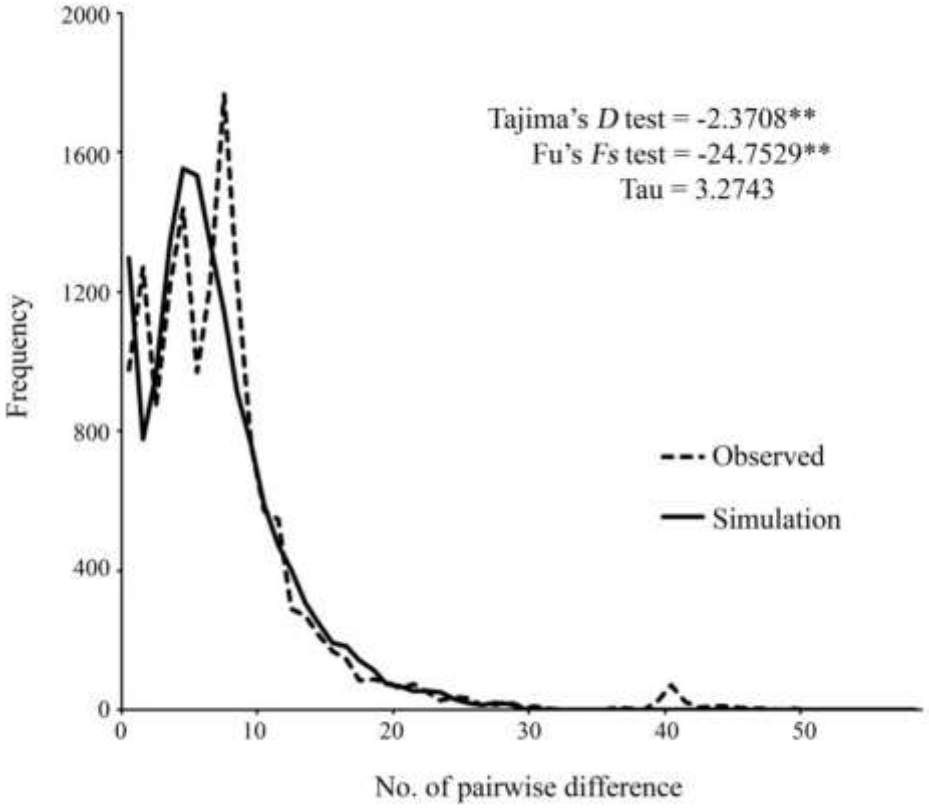

Figure 4.3 Mismatch distribution of 171 COI sequence of *Bactrocera correcta* from Thailand representing the observed and expected pairwise differences under the sudden population expansion model. Mismatch distribution of *Bactrocera correcta* is consistent with the sudden population expansion model (SSD=0.0049, *P*=0.8300; Harpending's raggedness index=0.0075, *P*=0.9200).

4.2 Discussion

4.2.1 Genetic variation

Genetic variation of *B. correcta* in Thailand (range between 0.760% and 3.250% with an average of 1.312%) base on COI sequence was higher than other fruit fly species including *B. cucurbitae* (0.1%-0.3%) (Hu *et al*., 2008), *B. oleae* (0.09%-0.48%) (Dogac *et al*., 2013) and *B. latifrons* (0.09-0.86%) (Meeyen *et al*., 2013). Level of genetic variation in *B. correcta* is also higher than those of fruit fly species complexes such as *B. dorsalis* (0.7%-2.0%) (Shi *et al*., 2012) and *B. tryoni* (0.5%-1.8%) (Blacket *et al*., 2012). High level of genetic variation in *B. correcta* is due largely to existent of divergence lineages revealed by haplotype network analysis. These lineages are not associated with host plant species or geographic origins. Although all individuals of lineage II were from the northeastern Thailand but many other specimens from this region were clustered in lineage I. When checked the species identification with COI barcoding sequences in the BOLD systems and found that all members of lineage II were 100% matching with *B. correcta* in the database. Thus, the possibly of the divergence lineage bing due to misidentification is unlikely.

Genetic divergence between the two lineages based on K2P model is 2.08% that fall in the range of 3% cut-off value for DNA barcode sequences (Hebert *et al*., 2003). However, some studies have suggested that this cut-off value is not appropriate due to large variations of the intraspecific genetic divergence (Meier *et al*., 2006). The values of interspecific genetic divergence estimated for 60 fruit fly species range between 0.1% and 5.3% with a mean of 0.9% (Armstrong and Ball, 2005). Therefore, it is unable to decide here that the two lineages of *B. correcta* observed in this study represent different species or not. However, because the two lineages show great genetic distinction with low genetic divergence within each lineage thus suggested that there was no genetic exchange (i.e. gene flow) between the two lineages.

Previous study has also recognized two divergence lineages of *B. correcta* in Thailand. Jamnongluk *et al*. (2003) found that two specimens of *B. correcta* collected from the same host plant (*Syzygium samarangense*) show considerable genetic distance based on COI sequences and the authors claimed that these two specimens represented two sibling species of *B. correcta*. Genetic divergence of these specimens was related to

the *Wolbachia* infection as one specimen found infected by *Wolbachia* but the other was not infected (Jamnongluk *et al*., 2003). Unfortunately, in this study unable to include these specimens in analysis because the COI fragment used by Jamnongluk *et al*. (2003) was not overlap with this study sequence and also not test the *Wolbachia* infection thus it is interesting topic for further investigation.

4.2.2 Population genetic structure

Population pairwise F_{ST} values indicate overall low level of genetic structure between populations of *B. correcta*. The results are consistent with many other population genetic studies in fruit flies which often detect low genetic structuring (Meeyen *et al*., 2013). Two factors are most likely the explanations for genetically homogenous among populations of *B. correcta* in Thailand. First, *B. correcta* utilized wide host range with more than 60 plant species from 30 families reported (Clarke *et al*., 2001). Many host plants (e.g. mango, rose apple, guava) are commonly grow in Thailand thus make the populations geographically continuous. This could promote genetically exchange (i.e. gene flow) between populations (Meeyen *et al*., 2013) that counter effect of genetic drift or selection to lowering level of genetic differentiation. The second factor that could contribute to low level of genetic structuring in *B. correcta* is the recent population history.

4.2.3 Demographic history

Mismatch distribution analysis and the Fu's F_s and Tajima's *D* tests indicate recent demographic expansion in this species. The expansion time estimated to be at the end of Pleistocene glaciations (approximately 20,000 years ago). The results consistent with previous studies in another fruit fly species, *B. latifrons* (Meeyen *et al*., 2013) and other insects such as mosquitoes (O'Loughlin *et al*., 2008; Morgan *et al*., 2011) and black flies (Pramual *et al*., 2005, 2011). Climatic condition of the Pleistocene glaciations in tropical Asia including Thailand was thought to be drier with lower temperature (Penny, 2001). These conditions leading to contraction of the tropical forest couple with the expansion of broad leaf, dry dipterocarp species (Penny, 2001). Climatic condition was recovered into the warm and humid at about 18,000 years ago that allows the tropical forest to expand. Thus, signal of demographic expansion in *B. correcta* is most likely associate with the host-plant species expansion. Population expansion detected in the *B. correcta* after the climatic condition recovery to warm and

humid conditions is consistent with the present-day investigation on seasonal abundance of this species. Clarke *et al*. (2001) found that seasonal dynamic of the *B. correcta* reach its peak of fly abundance between May to September which is the mid of the rainy season which might be related to host plant fruiting time.

The exception to overall genetically homogeneity is the significant differentiations of the Phetchabun (PB) population. Most comparisons revealed significant F_{ST} values indicate limit gene flow between PB with other populations. Geographically, this population was isolated from others by large mountain range (Phetchabun range). Ecological study of the *B. correcta* indicated that mountain range is an effective geographic barrier because this species preferred low altitude area (Liu *et al*., 2013). Mountain range also found as an important geographic barrier for gene flow in other fruit flies (Shi *et al*., 2005; Meeyen *et al*., 2013).

Chapter 5

Conclusion

In this study, mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequences were used to investigate genetic variation, population genetic structure and demographic history of *B. correcta* in Thailand. High genetic diversity in *B. correcta* due to the existent of divergence lineages (lineages I and II) was found. Detection of genetically divergence lineages indicated the usefulness of molecular marker to reveal hidden diversity because this divergence has not been observed at morphological level.

Despite overall high genetic diversity, genetic structure of *B. correcta* was low. The exception is populations from Pethchabun (PB) province which the F_{ST} values are significantly in all comparisons with other populations indicate limit gene flow between PB with other populations. Geographically, this population was isolated from others by large mountain range (Phetchabun range) that play significant role as gene flow barrier.

Demographic history analysis found a signal of recent population expansion dating back to the end of Pleistocene. Because this pattern has been found in other co-geographically distributed species in Thailand thus pointed out the important of this historical event on genetic structure and diversity of tropical mainland Asia. The results will be useful for prediction of the fly is occurrence, invasive and geographical distribution, which would help develop better management strategies for this important pest.

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