

POPULATION GENETICS AND MORPHOLOGICAL VARIATION IN MELON FLY, Zeugodacus cucurbitae (Coquillett) (DIPTERA: TEPHRITIDAE) IN THAILAND

CHONTICHA KUNPROM

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology at Mahasarakham University November 2017

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ABSTRACT

The melon fly, Zeugodacus cucurbitae (Coquillett) (Diptera: Tephritidae) is among the most economically important pests of fruits and fleshy vegetables. The genetic diversity, genetic structure, demographic history and morphological variation of Z. cucurbitae in Thailand were investigated. Mitochondrial cytochrome c oxidase I (COI) sequences were obtained from 155 specimens collected from 10 host plant species throughout Thailand. Low genetic variation was found in populations of Z. cucurbitae in Thailand, which is consistent with other studies of this species. Demographic history analysis detected a signal of population expansion dating back to 140,000 years ago, which possibly followed increases in host plants after climatic recovery of the penultimate Pleistocene glaciation. Population genetic structure analysis found that 51% of pairwise comparisons are genetically significantly different. Because populations that contributed markedly to genetic structuring possessed very low haplotype diversity, the effect of genetic drift could be a factor driving population differentiation. Comparisons of genetic differentiation between flies from different host plant species found no evidence of isolation. However, most haplotypes are unique for each host plant species, indicating that there are some degrees of isolation. Morphological variation analysis revealed that significant difference in wing variation between male and female. Morphological variation between sexes of the fly may also correlate with sexual behavior and flight performances.

Keywords: Gene flow, genetic structure, genetic variation, melon fly, population expansion.

ชื่อเรื่อง	พันธุศาสตร์ประชากรและความแปรผันของลักษณะสัณฐานวิทยาในแมลงวันแตง				
	Zeugodacus cucurbitae (Coquillett) (Diptera: Tephritidae)				
	ในประเทศไทย				
ผู้วิจัย	นางสาวชลธิชา ขุนพรม				
ปริญญา	ปรัชญาดุษฎีบัณฑิต สาขาวิชา ชีววิทยา				
กรรมการควบคุม	รองศาสตราจารย์ ดร. ไพโรจน์ ประมวล				
มหาวิทยาลัย	มหาวิทยาลัยมหาสารคาม ปีที่พิมพ์ 2560				

บทคัดย่อ

้แมลงวันแตง (Zeugodacus cucurbitae) (Coquillett) เป็นศัตรูพืชที่มีความสำคัญต่อพืชผัก ้ผลไม้และส่งผลเสียต่อระบบเศรษฐกิจ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความแปรผันทาง พันธุกรรม โครงสร้างทางพันธุกรรม ประวัติศาสตร์ประชากรและความแปรผันของลักษณะสัณฐานวิทยา ของแมลงวันแตงในประเทศไทยทั้งหมด 155 ตัวอย่าง จากพืชอาศัย 10 ชนิด โดยใช้ยืน cytochrome c oxidase subunit I (COI) ในไมโทคอนเดรียลดีเอ็นเอในการศึกษา พบมีความแปรผันทางพันธุกรรม ต่ำซึ่งสอดคล้องกับการศึกษาก่อนหน้านี้ การวิเคราะห์ประวัติศาสตร์ประชากรพบว่า แมลงวันแตงมีการ ้ขยายขนาดประชากรอย่างรวดเร็วมาแล้วเมื่อประมาณ 140,000 ปีที่ผ่านมา ซึ่งอาจเป็นผลจากการ เพิ่มขึ้นของพืชอาศัยจากการเปลี่ยนแปลงสภาพภูมิอากาศในยุคไพลสโทซีน การวิเคราะห์โครงสร้างทาง พันธุกรรมจากการเปรียบเทียบค่าความแตกต่างทางพันธุกรรม พบว่า ร้อยละ 51 ของคู่ประชากรที่ เปรียบเทียบมีความแตกต่างทางพันธุกรรมอย่างมีนัยสำคัญซึ่งอาจเป็นผลจากเจเนติกดริฟท์ส่งผลให้เกิด ้ความแตกต่างทางพันธุกรรมในบางประชากร จากการเปรียบเทียบความแตกต่างทางพันธุกรรมของ แมลงวันแตงในพืชอาศัยชนิดต่างๆ พบว่า ไม่มีความแตกต่างกัน อย่างไรก็ตาม รูปแบบของแฮพโพลไทด์ ้ส่วนใหญ่เป็นแฮพโพลไทป์ที่ไม่มีการใช้ร่วมกันในพืชอาศัยแต่ละชนิดซึ่งแสดงให้เห็นว่า แมลงวันแตงใน พืชอาศัยบางชนิดยังคงมีความแตกต่างทางพันธุกรรมกัน การวิเคราะห์ความแปรผันของลักษณะสัณฐาน วิทยา พบว่า มีความแตกต่างของขนาดและรูปร่างปีกระหว่างเพศผู้และเพศเมียอย่างมีนัยสำคัญทางสถิติ ทั้งนี้ความแปรผันของลักษณะสัณฐานวิทยาที่เกิดขึ้นอาจมีความสัมพันธ์กับความแปรผันของ สภาพแวดล้อม พฤติกรรมทางเพศที่แตกต่างกันรวมถึงศักยภาพในการบินระหว่างเพศผู้และเพศเมีย

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CHAPTER 1 INTRODUCTION

1.1 Background

The family Tephritidae (Diptera) commonly referred to true fruit flies (Aluja and Norrbom, 1999) which globally comprised of more than 5,000 species in 500 genera (Uchôa, 2012). Of these, 350 species in six genera, including *Anastrepha* Schiner, *Bactrocera* Macquart, *Ceratitis* Macleay, *Dacus* Fabricius, *Rhagoletis* Loew and *Zeugodacus* Hendel are significant agricultural pests (White and Elson-Harris, 1992; Van Houdt *et al.*, 2010; Plant Health Australia, 2011; Virgilio *et al.*, 2015). The genus *Bactrocera* Macquart is a largest genus comprise of approximately 600 described species (Vargas *et al.*, 2015) arranged in 10 subgenera (Drew and Roming, 2013) and least 50 species considered to be important pests and melon fly is one of them. The melon fly was placed in the subgenus *Zeugodacus* Hendel of the genus *Bactrocera* Macquart, its scientific name was *Bactrocera* (*Zeugodacus*) *cucurbitae* (Drew and Roming, 2013). However, in 2015, the subgenus *Zeugodacus* was separated from genus *Bactrocera* into the genus (Virgilio *et al.*, 2015). Therefore, *Bactrocera* (*Zeugodacus*) *cucurbitae*.

The melon fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) is one of the most important pest of fleshy fruits and vegetables. The melon fly is a multivoltine and poplyphagous fruit fly that infests more than 125 host plant species, especially the family Cucurbitaceae (Orian and Moutia, 1960; White and Elson-Harris, 1992; Vayssières, 1999, 2008; Dhillon *et al.*, 2005; White, 2006). Many important commercial fruits and vegetables of the family Cucurbitaceae such as pumpkins, watermelon, muskmelon, cantaloupe, squash, gourd and cucumber are host plants of *Z. cucurbitae*. Occasionally, this species also infest plants of the family Solanaceae including eggplant, tomato and other host plant species such as mango, orange, guava, papaya and peach (Pińero *et al.*, 2006; White and Elson-Harris, 1992). *Zeugodacus cucurbitae* has a large geographical distribution area, which India was presumed as a native range (Bezzi, 1913). The fly also invaded other geographic regions such as Southeast Asia (Li *et al.*, 2013), North America, Africa, Oceania (Virgilio *et al.*, 2010) and Hawaii (Bess *et al.*, 1961).



Zeugodacus cucurbitae is geographically widespread and attack numerous agricultural crops. However, knowledge of genetic structure, diversity and population history remains limited (Virgilio *et al.*, 2010). Understanding genetic structure and diversity is necessary for effective control and management strategies of the insect pests (Roderick *et al.*, 1996, Roderick and Navajas, 2003). Moreover, using molecular genetic markers could contribute to a better understanding of the pathway and intensity of genetic interchange (gen flow) among the populations. Previous studies, using COI sequences for investigating genetic variation and structure in *Z. cucurbitae* in China, Southeast Asia (Hu *et al.*, 2008) and India (Prabhakar *et al.*, 2012), revealed low genetic variation as a result of recent demographic history and high rate of gene flow. However, these studies using limited specimens from Thailand. Therefore, genetic structure and diversity of *Z. cucurbitae* in Thailand remain largely unknown.

In addition to the genetic variation and genetic structure, morphological variation is also important information regarding diversity and species recognition. In fruit flies, morphological variations can display in many ways, such as variation in body size, appendage length (are the simple characters), shape of the wing, number and arrangement of setae and leg or abdomen color patterns, or in attributes of developing stages (are the complex characters) (Drew and Roming, 2013; Boontop, 2016). Morphological variation analyses using wing shape is a useful technique to examine morphological differentiation among individuals or defined group of organism (Rohlf and Marcus, 1993; Rohlf, 1999). This technique has been used to distinguish closely related species and justify synonymies (Perero *et al.*, 1984; Reyment *et al.*, 1984; Willig *et al.*, 1986; McNamee and Dytham, 1993; Selivon, 1996; Adsavakulchai *et al.*, 1999; Schutze *et al.*, 2012b).

In this study, wing shape was used for morphological variation analysis and mitochondrial COI sequences were used to determine genetic variation, genetic structure and population history of *Z. cucurbitae* in Thailand. The mtDNA COI sequence was selected as a genetic marker because this gene was used as standard barcoding sequence (Hebert *et al.*, 2003) thus enable species identification from the immature stage (larva and pupa) in which morphological identification is problematic. In addition, the COI sequence could verify the genetic relationship between Thai specimens with those reported from other countries and also to use examined the

genetic diversity and genetic differentiation of *Z. cucurbitae* associated with different host plant species. This information is generally lacking in previous studies. The host plant plays an important role in the diversification of phytophagous insects (Boontop, 2016; Bush, 1969; Feder *et al.*, 1988; Guttman *et al.*, 1981; Waring *et al.*, 1990), including fruit flies. Differences in host plant usage could drive genetic differentiation leading to speciation (Bush, 1975; Carroll and Boyd, 1992; Diehl and Bush, 1984; Dingle and Winchell, 1997; Jamnongluk *et al.*, 2003a, b).

1.2 Objectives of the research

The objectives of the present study are:

1.2.1 To investigate genetic variation and population genetic structure of *Z. cucurbitae* in Thailand.

1.2.2 To investigate demographic history of Z. cucurbitae in Thailand.

1.2.3 To investigate morphological variation based on wing shape of *Z. cucurbitae* in Thailand.

1.3 Scope of the research

Specimens of *Z. cucurbitae* were collected from both natural forests and fruit orchards in Thailand. The infested fruits were reared in a laboratory under room temperature. After the adults emerged, the adult flies were preserved in 80% ethanol at -20 °C. Species was identified using adult morphology following Plant Health Australia (2011), Drew and Roming (2013) and Virgilio *et al.* (2015). DNA was extracted from individual adult fly. The COI gene was amplified using polymerase chain reaction (PCR). PCR products were checked, purified and sequenced. Population genetics structure of *Z. cucurbitae* in Thailand were analyzed using COI sequences.

All of right wing were dissected under stereomicroscope and mounted on glass slide with Hoyer mounting solution and air-dried prior to image photographed with a digital camera attached to a stereomicroscope. Landmark-based geometic morphometric method was applied to compare and visualize the morphological variation between sexes, host plants and geography among these *Z. cucurbitae* population in Thailand using MorphoJ software version 1.06d (Klingenberg, 2011).

CHAPTER 2 LITERATURE REVIEW

2.1 Classification of fruit fly

Phylum: Arthropoda Class: Insecta Order: Diptera Suborder: Brachycera Infraorder: Muscomorpha Superfamily: Tephritoidae Family: Tephritidae

Fruit flies belonging to the family Tephritidae of the order Diptera. These insects are one of the most diverse groups. The total number of species within this family are nearly 5,000 (Uchôa, 2012) that assigned into six subfamilies (Tachiniscinae, Blepharoneurinae, Phytalmyiinae, Trypetinae, Dacinae and Tephritinae). There are approximately 500 genera distributed throughout the tropical, subtropical and temperate regions (Christenson and foote, 1960; Weem *et al.*, 1999) and occupy habitats ranging from rainforests to open savanna (Drew, 1989a, b; Norrbom *et al.*, 1998; Michaux and white, 1999). The name "fruit fly" originates from larva feeding. Larva of some fruit flies species, especially those of the subfamilies Dacinae and Tephritinae are frugivorous and feed on fruit pulp of wild and cultivated plants, hence, bearing the name (Balagawi, 2006).

Fruit flies not only breed in fruit but can also breed in other living plant tissue as shoots, stems, leaves, buds, seeds and flowers (Christenson and foote, 1960; Diaz-Fleischer *et al.*, 2000). Blepharoneurinae feed in flowers, fruits, and make galls in Cucurbitaceae similarly with Trypetinae, larvae of this subfamily (e.g. *Coelotrypes*, *Acidoxantha* and *Macrotryptera* spp.) infest fruits, flowers and growing shoot tips, while others are also leaf miners. Dacinae feed in fruits or in seeds of a wide range of plant families, a few, especially those attacking species of Cucurbitaceae (e.g. *Z. diversa* (Coquillett) and *Z. scutellaris* (Bezzi)) can infest the flowers. Tephritinae, almost all species in this subfamily are associated with flowers, make gall, while a few

are stem miners or are leaf-miners in a wide array of plant taxa: Aquifoliaceae, Scrophulariaceae, and Verbenaceae, but mainly in flowerheads of Asteraceae (White and Elson-Harris, 1992; Norrbom, 2010; Uchôa and Nicácio, 2010). Although majority of species within family Tephritidae are phytophagous, except minority subfamilies are not. Fruit flies species in the subfamily Tachiniscinae are parasitoids of Lepidoptera, while most species of the subfamily Phytalmyiinae are saprophagous, which feed on live or dead bamboos (Poaceae) or on trees recently fallen of other plant families (Diaz-Fleischer and Aluja, 2000).

More than 800 species of fruit flies in the subfamily Dacinae are widespread in tropical and subtropical areas (Bellas, 1996). Dacini is one of three tribes in this subfamily (three tribes consisting Ceratitidini, Dacini, Gastrozonini). The Dacini are distribution concentrated in two regions, the Afrotropical region and Southeast Asia to northeastern Australia (Drew and Handcock, 1994). The tribe recognize four genera including *Bactrocera* Macquart, *Dacus* Fabricius, *Monacrostichus* Bezzi and *Zeugodacus* Hendel (Drew and Hancock 1994; Drew *et al.*, 1998; Virgilio *et al.*, 2015). Of these appears morphological similarities. The *Monacrostichus* Bezzi is small genera, there is members two species (*M. citricola* Bezzi and *M. malaysiae* Drew and Hancock). The majority of species belong in *Bactrocera* Macquart (more than 600 species), *Dacus* Fabricius (more than 200 species) (Thompson, 1998) and *Zeugodacus* Hendel (192 species) (Table 2.4) (Hancock and Drew, 2015; Virgilio *et al.*, 2015), which a large genera.

Most pest species of Tephritidae attack fruits and the great majority of them belong to the genera *Anastrepha* Schiner, *Ceratitis* Macleay, *Dacus* Fabricius, *Rhagoletis* Loew, *Bactrocera* Macquart (Figure 2.1) and *Zeugodacus* Hendel (Figure 2.15). The genus *Bactrocera* is large genus, with exceed 600 described species arranged in 10 subgenera which are divided into three groups including *Bactrocera* group, *Melanodacus* group and *Queenslandacus* group (Table 2.1). The genus *Zeugodacus* is a new genus separated from genus *Bactrocera* (Virgilio *et al.*, 2015) (detail showed in section 2.8) and arranged in 13 subgenera (Table 2.1) (Hancock and Drew, 2015; Virgilio *et al.*, 2015).

Most of them are known to infest host plants in the family Cucurbitaceae, however, subgenus *Diplodacus* May included non-cucurbit feeder (Drew and Hancock, 1999). The following 27 subgenera (Table 2.1) were distributed throughout the tropical and subtropical regions of Australasia and Oceania (Drew, 1989a), currently recognized from Africa, Southeast Asia, and the Pacific region (Drew and Hancock, 1994) and including the majority of the economically important fruit flies.





Figure 2.1 Representative members of each genus of fruit fly; (A) *Bactrocera*Macquart, (B) *Anastrepha* Schiner, (C) *Ceratitis* Macleay, (D) *Rhagoletis* Loew and(E) *Dacus* Fabricius.

(Source: https://www.flickr.com/photos/ruipara/15384635103/in/photostream/)

Genus	Group	Subgenus	
Bactrocera	Bactrocera group	Afrodacus Bezzi	
		Bactrocera Macquart	
		Bulladacus Drew and Hancock	
		Daculus Speiser	
		Gymnodacus Munro	
		Javadacus Hardy	
		Paratridacus Shiraki	
		Parazeugodacus Shiraki	
		Semicallantra Drew	
		Tetradacus Miyake	
	Melanodacus group	Hemisurstylus Drew	
		Hemizeugodacus Hardy	
		Melanodacus Perkins	
	Queenslandacus group	Queenslandacus Drew	
Zeugodacus	Zeugodacus group	Asiadacus Perkins	
		Austrodacus Perkins	
		Diplodacus May	
		Hemigymnodacus Hardy	
		Heminotodacus Drew	
		Hemiparatridacus Drew	
		Nesodacus Perkins	
		Niuginidacus Drew	
		Papuodacus Drew	
		Paradacus Perkins	
		Parasinodacus Drew and Roming	
		Sinodacus Zia	
		Zeugodacus Herdel	
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 Table 2.1 Classification of genus Bactrocera Macquart and genus Zeugodacus Hendel.

(From: Drew, 1989b; De Meyer *et al.*, 2015; Hancock and Drew, 2015; Virgilio *et al.*, 2015)

2.2 Biology of fruit fly

The fruit fly active throughout the year on several hosts. The life cycle undergoes complete metamorphosis that consists of four stages (Figure 2.2) similar to the other insects in the order Diptera. The developmental time from egg to adult takes between 14-27 days. The life cycle start at the female fruit flies lays the eggs into fresh fruit with a needle-sharp, pointed ovipositor. The oviposition sites show as discoloured, often blackish spots, which may exude distinctive blobs or filaments of gum. In the oviposition process may also inject fruit-rotting bacteria. Bactrocera species of fruit flies have a specific group of bacteria (family Enterobacteriaceae) (Drew and Lloyd, 1989) associated with them which are important with all stage of the life cycle (Lloyd et al., 1986) particularly very beneficial in larva stage. Three species of bacteria: Erwinia herbicola, Klebsiella oxytoca and Enterobacter cloacae were most associate with larva in infest fruit, which can result rotting tissue and drop prematurely. When the larva have finished feeding (grown complete with three instar larvae), they leave the fruits, fall to the ground birth to pupa. The prepupae emerge from the fruit and hop, burrow into the soil and development underneath it. This stage does not need food but their need pacification for growth. A short period, the pupa turns into an adult fly, it free-fly in the environment. After adult flies emergence, it require various resources to facilitate survival and reproduction. Key resources consist of protein to attain sexual maturity and in conjunction with lipids, egg production and moisture for metabolism and sugar for energy to sustain their highly moving habit (Fletcher, 1987). Most fruit flies are facultative breeders that will lay eggs whenever their host fruits are available, and so may have many generations per year depending on host fruit availability. Fruit flies use host plant or plant parts as refuges or for feeding, mating, oviposition and larval development. The selection of host plants with dense foliage may shelter the flies from the elements or predation by airborne predators such as dragonflies (Fletcher and Prokopy, 1991; Hendrichs et al., 1991). Moreover, females' fruit flies seek shaded and moist regions of the host plant in oviposition.





Figure 2.2 Life cycle of the fruit fly (Source: http://preventfruitfly.com.au/about-fruit-fly/life-cycle/)

All fruit flies develop by complete metamorphosis and their life cycle compose of four development stage including eggs, larva (three larval instar), pupa and adult. These stages may be separate into three parts, internal host plant (eggs and larva stage), under the ground (pupa stage) and free-living in the environment (adult stage).

2.2.1 Egg

Eggs are deposited by the adult flies (about 1-20 or more eggs) into the fresh fruit or vegetable (Figure 2.3A). Eggs are often laid up to eight weeks before the fruit is mature. The eggs are small (Figure 2.3B), about six or more creamy to white banana or grain shape eggs close to 1 mm long are deposited just beneath the skin fruits. Eggs hatch into larvae inside the fruit within two to four days at 25 °C. 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 sheltered within the fruit.



Figure 2.3 (A) The adult female fruit fly lays eggs into the maturing and ripening fruit of the host plant by ovipositor, (B) Eggs of the fruit fly.

2.2.2 Larva

Larvae hatch from the eggs after about 48 hours or more (but not over 96 hours or 4 days) and feed on the fleshy fruit. Larva that has three stages (instars) (Figure 2.4) before they reach maturity, the larvae that hatch initially are small and delicate first instar (or first stage) larvae (Figure 2.4A) living beneath the fruit skin. They moult into slightly more robust second instar larvae (Figure 2.4B), and these in turn moult into quite stout and tough third instar larvae (Figure 2.4C). The second and third instar larvae gradually moving from beneath fruit skin towards the center of fruits respectively. The feeding activity of the larvae causes the fruit to prematurely ripen and rot. This usually takes between 10-14 days, by with time they are at least about seven mm or at most about 13 mm long of body lengt. The larvae are creamy white to pale yellow depend on the stage of the larva (Figure 2.5A, C), legless and taper towards the front end and the body consist 11 segments (Figure 2.5B, C). They have paired fine black mouth hooks (Figure 2.6) for tearing at the fruit tissue. When the larva is fully grown, it leave the fruit with a characteristic of jumping motion and burrows into the soil or organic matter. Infested fruit by fruit fly larvae will usually drop to the ground and very heavy losses can be incurred if control measures are not taken. However, the larval stage is the most difficult life stages to control because they are protected within the fruit.









(B)



Figure 2.4 Larva of the fruit fly; (A) first instar larva, (B) second instar larva, (C) third instar larva.





Figure 2.5 Larva and compositions of larva of the fruit fly, the body of fruit fly consist 11 segments.



Figure 2.6 A paired of black mouth hooks at the anterior end of fruit fly larva that use for tearing the fruit tissue.



2.2.3 Pupa

Larvae enter a post-feeding stage and commence "popping" or "jumping" out of the fruit. These larvae must be pupating in a moist substrate into the top 2-3 cm of the soil (Christenson and Foote, 1960; Prokopy and Roitberg, 1984; Fletcher, 1987). Pupa development occur in the soil underneath the host tree and is completed within about 12 days or several weeks depending on temperature (generally about 25 °C) (Bateman, 1967; Gibbs, 1967). In the soil, larvae become inactive and the larval skin becomes a brown barrel-shaped and change into oval, tanned brown and hard, and is known as the puparium about 5-8 mm long (Figure 2.7A). The true pupa is formed inside this puparium "shell" (Figure 2.7B) and does not need the nutrients for development. After stage fully, the pupa turns into an adult fly.





(A)



Figure 2.7 (A) Pupa of the fruit fly, (B) The puparium "shell" deposited true pupa.



2.2.4 Adult

Adult flies (Figure 2.8) emerge from the puparium throughout the year related to the suitable fruits availability. The degree of survival depends on species and environmental conditions (Shaw *et al.*, 1967). The adult fruit fly is capable of forcing its way through surprising depths of soil and fly into the foliage (Swan, 1949). The thorax length and width are about 2 mm and the abdomen width is about 3 mm (Arita and Kaneshiro, 1998). Females can be distinguished from males by the presence of an ovipositor (Figure 2.9), a dark-colored pointed structure at the end of the abdomen, which is used to pierce the fruit and lay eggs.



Figure 2.8 Adults of the fruit fly; (A) *Bactrocera carambolae*, (B) *Bactrocera papayae*, (C) *Bactrocera dorsalis*, (D) *Bactrocera correcta*, (E) *Bactrocera isolata*, (F) *Bactrocera cilifera*, (G) *Zeugodacus tau*, (H) *Zeugodacus apicalis*, (I) *Zeugodacus cucurbitae*.



Figure 2.9 (A) Ovipositor of female oriental fruit fly, *Bactrocera dorsalis*, (B) Female guava fruit fly, *Bactrocera correcta* using ovipositor to pierce the fruit and lay eggs.

A few days after emergence, the females require a source of protein for egg maturation. Adult fruit fly feed on secretion of plants from leaves, fruits, rotting fruits, nectar, pollen, bird feces, honeydew secreted by other insects (Christenson and Foote, 1960; Bateman, 1972; Fletcher, 1987) but also bacteria from fruit and leaf surfaces (Drew *et al.*, 1983; Courtice and Drew, 1984). Honeydew helps adult fly to reach a normal fertility and stimulates egg production. Adult female can lay eggs after mating one or two weeks. Female will continue to lay eggs throughout her life but mating only one time in her life. Mating behavior of fruit flies in tropical and subtropical regions occur in the host tree when light intensity decreases at nightfall (Bateman, 1979). However, some species in the genus *Bactrocera* prefer to mate in the morning and early afternoon (Alwood, 1997). After cohabite, adult female fruit flies are can fly many kilometers in searching for suitable host plants to oviposit. For example, some species of the genus *Bactrocera* can move up to 200 kilometer (Miyahara and Kawai, 1979).

The oviposition of female fruit fly depend on factors within the host plant, which commonly referred to as a stimulator, including olfactory, visual, tactile and gustatory (Smith, 1989). For examples, Queensland fruit fly, *B. tryoni* (Froggatt), Guava fruit fly, *B. correcta* (Bezzi) and Pumpkin fruit fly, *Z. tau* (Walker) respond to odor in quest of host plants to oviposit (Fitt, 1981; Poramarcom and Baimai, 1995). However, other species respond differently to stimulator. For example Olive fruit fly, *B. oleae* (Gmelin) was more attracted to the visual and odor (Fletcher, 1987). Generally, stimuli affect fruit flies in the short distance is the smell, visual and physical attribute (size,

shape and colour) of host plants. For examples, *B. dorsalis* (Hendel) and *Z. cucurbiate* (Coquillett) are respond to both circle shape and the reflectance of red and yellow color respectively. On the other hand, long distance female fruit flies respond to odor more than other stimuli (Poramarcom, 2000).

The oviposition preference of fruit fly determined by genetic characteristic (Fitt, 1981). Host plant attributes are significant influence on the abundance and behavior of fruit flies (Prokopy and Hendrichs, 1979; Kaspi and Yuval, 1999). Physical properties of the plants such as size, shape and color are the initial plant stimuli for oviposition. Host choice for female fruit fly to oviposit may be the suitable to developing of larva (Fletcher, 1987). Some fruit fly species such as *Rhagoletis* spp. (Prokopy et al., 1976), Ceratitis capitata (Wiedemann) (Prokopy et al., 1978) and Anastrepha suspense (Loew) (Prokopy et al., 1977) have evolved mechanisms to produce host marking pheromone (HMP) that reduce larval competition among the same or different fly species (Prokopy and Koyama, 1982; Roitberg and Prokopy, 1987). After oviposition, female fruit flies deposit HMP on the oviposition site to inhibit other females from ovipositing at the same site to avoid food competition and low offspring performane (Prokopy et al., 1984). Other Bactrocera spp. except B. oleae (Gmelin) (Girolami et al., 1981) has not been found to deposit HMP on the oviposition site. For these species, when the female fly arrives at the fruit, it explores the fruit surface before attempting to oviposit, if the female flies found the presence of larvae in the fruit, which to avoid ovipositing in the infested fruit for deter offspring density overmuch. The female flies may be change their oviposition preference to, non-infest host fruits (Cirio, 1971; Prokopy and Koyama, 1982; Fitt, 1981; Prokopy and Papaj, 1989). The fruit flies are regarded as the major insect pests of fruit and vegetable crops. Feeding damage can cause premature fruit drop and reduces both the quality and quantity of fruit and vegetable production. In addition to the direct losses, fruit fly infestation can cause serious losses of crop productions, decrease export trade value and increased pressure and cost on quarantine services (Drew and Roming, 1996).

2.3 Geographic distribution and diversity

More than 800 species of the Dacini assigned into genus *Bactrocera* Macquart, *Dacus* Fabricius, *Ichneumonopsis* Hardy, and *Monacrostichus* Bezzi. Of these, 68% are belong to *Bactrocera* and 32% to *Dacus*. Geographic distributions was are mainly in the tropical and subtropical rain forests of West Africa, coastal East Africa, Madagascar and the Mascarene Islands, southwest India, Southeast Asia from Nepal to southern China in the north to the Indonesian islands in the south, Papua New Guinea, northeastern Australia, and some South Pacific islands. Endemic species of Dacini occur in all of these areas (Drew and Hancock, 1994). Approximately 750 species of Dacini have been distribution of species in each of the genera. It is noteworthy that the prolific speciation in genus *Bactrocera* has occurred in Southeast Asia and Papua New Guinea while the greatest speciation in genus *Dacus* has occurred in Africa.

Papua New Guinea (PNG) has the greatest diversity of tropical fruit fly species in the world (Secretariat of the Pacific Community, 2001). Nearly 300 species have been recorded in this region. In the region east of Sulawesi and south of the equator and extending eastward to the Society Islands in French Polynesia found more than 290 species of the fruit flies (Waterhouse, 1993; Drew, 1989b). Approximately 63% (180 species) of the 290 species occur on the PNG mainland and major islands lying to the east (New Britain, New Ireland and Bougainville). This diversity is also reflected in the number of species known to infest commercial or edible fruits and vegetables; mainly pest species of economic significant including *B. frauenfeldi* (Schiner), *B. musae* (Tryon), *B. atrisetosa* (Perkins), *B. bryoniae* (Tryon), *B. umbrosa* (Fabricius), *B. moluccensis* (Perkins), *B. trivialis* (Drew), *B. papayae* Drew and Hancock, *B. neohumeralis* (Hardy), *Z. decipiens* (Drew), *Z. strigifinis* (Walker) and *Z. cucurbitae* (Coquillett) (Tenakanai, 1996)

The fruit flies in South America, especially in Brazil belong to six genera including *Anastrepha* Schiner, *Bactrocera* Macquart, *Ceratitis* McLeay, *Rhagoletis* Loew, *Dasiops* Rondani and *Neosilba* McAlpine deposited in two families (Tephritidae and Lonchaeidae) (Uchôa and Nicácio, 2010). The genus *Bactrocera* in Brazil is represented by only one species, *B. carambolae* Drew and Hancock which is economic importance in Southern part of Brazil. Likewise, genus *Ceratitis* occur only *C. capitata* which is 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 plant species (Uchôa *et al.*, 2002; Uchôa and Nicácio, 2010). For the genus *Rhagoletis*, in the Brazilian territory three species including *R. adusta* (Foote), *R. ferrugines* (Hendel) and *R. macquarti* (Loew) were record. In addition, genus *Anastrepha* is one of fruit fly genera that are widely distributed in South America, being 112 species recorded in Brazil (Nicácio and Uchôa, 2011) and able to attack grown fruit and/or vegetables of commercial value.

In tropical Asia, the region comprises the countries of India, Sri Lanka, Thailand, Laos, Vietnam, Kampuchea, Myanmar, Malaysia, Singapore, Philippines and Indonesia west of Irian Jaya. Tropical Asia possesses a warm, equable climate that allows for continuous cultivation. Coupled with the common practice of monoculture of fruits, this provides an abundant and uninterrupted supply of host fruits for fruit flies to breed in and multiply rapidly (Vijaysegaran, 1983). The major fruit flies pest species are Z. cucurbitae (Coquillett), Z. tau (Walker), B. albistrigata (deMeijere), B. correcta (Bezzi), B. latifrons (Hendel), B. zonata (Saunders), especially 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) (Vijaysegaran, 1996), it is now known that the species complex of at least 52 sibling species exists in the region, 40 of which are new species described, and eight of which are of economic importance (Table 2.2). These flies are responsible for extensive economic losses of the horticultural crops throughout the region (Drew and Hancock, 1994; Drew and Roming, 1996). In the Indian subcontinent, there are about 325 species of fruit flies occurring, of which 205 are endemic to India. The major pest species belong to the genus Bactrocera; B. dorsalis (Hendel), B. zonata (Saunders), B. correcta (Bezzi), B. latifrons (Hendel), B. versicolor (Bezzi), B. nigrofemoralis White and Tsuruta (Kapoor, 2005) and genus Zeugodacus; Z. cucurbitae (Coquillett) and Z. diversa (Coquillett).

Fruit flies are frequently active at all the time of the year, and their populations tend to build during the summer and becoming very abundant at harvest time. Because their quick development and ability to reproduce rapidly, fruit flies are cause large destruction of host fruits and vegetables. The developmental time and overall lifespan is largely influenced by environmental conditions such as host plant, temperature, humidity and season. Transportation of infested fruit is the main way the pest is introduced to a previously fly-free area. Larvae may leave fruit in transit or may pupate in packaging materials or vehicles, from which the adults later escape.

2.4 Economic impacts of fruit fly

Fruit flies are regarded as one of the most serious pests of fruit and vegetable and a major pest in term of trade barrier. Some fruit fly species such as B. dorsalis complex is a major pest group with more than 75 species and infest a wide range of hosts from many different plant species and families (about 200 plant species from 50 families) (Drew 1989, Fitt, 1990; Drew and Hancock 1994; Hollingsworth et al., 2003; Clarke et al., 2005; Kumar et al., 2011) and are "polyphagous". Many species are "oligophagous", breeding predominatly in plant species within the same family (Norrbom et al., 1998) such as Cucumber fruit fly, Z. cucumis (French) which primarily breed in plant species within the family Cucurbitaceae (Smith et al., 1988), On the other hand, minority species such as Olive fruit fly, B. oleae (Gmelin), infest only olive fruit and are "monophagous" in their host use pattern (White and Elson-Harris, 1992) but all of them can be enormous damage of fruits and vegetables. Fruit flies are breed multiple generations a year. For examples, melon fruit fly, Z. cucurbitae develop 8-10 generations each year (White and Elson-Harris, 1994; Weems and Heppner, 2001). This species is highly adaptable to new environments, enabling it to spread geographically rapidly. The guava fruit fly, *B. correcta* (Bezzi) is highly adaptable to new environments, spreading rapidly and causing serious economic damage to fruit production (Liu et al., 2013). Fruit flies can be high load eggs into the host plant. After female fruit flies laying the eggs under the fruit skin. The damage symptoms of fruit flies are showing fruit flies oviposition marks or fractures on fruits or vegetables (Figure 2.10). The peel fruit is breached, and bacteria enter and the fruit starts to decay. Infest fruits often rot and drop to the ground before harvest.

Nearly 5,000 described species of the 350 species are economically important. Almost half of them belong to the genus *Bactrocera* and the new genus *Zeugodacus* which show in Table 2.3 and Table 2.4 respectively. The damage start when the female fruit fly lay eggs in batches directly under the peel fruit and vegetables with their needle-sharp ovipositor. All of female fruit flies puncturing the fruit and vegetable,

Scientific name	Current known distribution	Commercial hosts	Pest status
B. caryeae (Kapoor)	Southern India, Sri Lanka	Ciitrus, guava, mango	Serious pest
B. dorsalis (Hendel)	Southern China, Taiwan, Sri Lanka, India, Myanmar, northern and central Thailand, Vietnam, Laos, Cambodia, Hawaii	Citrus, carambola, guava, mango, papaya, peach, pear	Major pest of international quarantine importance
B. occipitalis (Bezzi)	Philippines	Mango, guava	Serious pest of mango. Other host data lacking
B. carambolae Drew and	Andaman Islands, Indonesia,	Carambola, guava, mango,	Major pest
Hancock	Peninsular Malaysia, Singapore, Southern Thailand, Adventive in Surinam and French Guiana	breadfruit and several other fruits	
<i>B. papayae</i> Drew and Hancock	Peninsular Malaysia, Indonesia, southern Thailand, Borneo, Sulawese, Christmas Island	Banana, carambola, citrus, mango, papaya and others	Major pest
B. philippinensis	Philippines	Breadfruit, mango, papaya	Major pest. Host data lacking
Drew and Hancock			
B. kandiensis Drew and Hancock	Sri Lanka	Garcinia, mango	Serious pest. Host data lacking
<i>B. pyrifoliae</i> Drew and Hancock	Northern Thailand	Guava, peach, pear	Serious pest

Table 2.2 Fruit flies of economic significance in the Bactrocera dorsalis complex in Tropical Asia (Source: Drew and Hancock, 1994).
of these pushes bacteria from the peel into the fruit fresh. These bacteria cause fruit decay, which results in a substrate in which the larvae feed. Eggs hatch into larva that feed upon the fruit flesh (Figure 2.11), causing the fruit more decay to rot and fall.

The damage of fruits by fruit fly larvae is render about 90-100% to crop losses. For example, the world market for fresh fruit has been estimated more than US\$ 1 billion per year (Armstrong and Jang, 1997). In Hawaii, the direct impact of fruit flies on agriculture products was US\$ 15 million (Nakahara et al., 1997), which did not include the costs or impacts of insecticide use to control these pests. The main fruit fly species are important role for damage agriculture product is the melon fly, Z. cucurbitae (Coquillett). Hawaii has another pest in the form of the infamous Mediterranean fruit fly (medfly), *Ceratitis capitata* (Weidemann), the Oriental fruit fly, *B. dorsalis* (Hendel) and the Solanaceous or Malaysian fruit fly, B. latifrons (Hendel). These agricultural pests have had a major impact on Hawaii's agriculture due to of these attacks over 400 different species of fruits and vegetables, many of which are grown or could be grown in Hawaii. Therefore, reducing the types, quantity and quality of agricultural products of the islands (Jang, 2007). The fruit flies are estimated to cause an annual loss to fruit and vegetable over US\$ 200 million in Pakistan (Stonehouse et al., 1998) and over 75% of Australia's fruit and vegetable exports, valued at around US\$ 640 million in 2012-2013, are susceptible to fruit fly. Whereas, In Australia, not only horticultural losses but also the export trade bans. These bans have restricted the export of most horticultural produce from within the country to international and interstate markets because the produce were infest by fruit flies larva. Monetary estimates of fruit production and fruit flies damage in Australia are US\$ 100 million (Drew, 1996).

Eighteen fruit fly species in total record in PNG have been propagated from commercial or eatable host fruits and fleshy vegetables. Four of the most flies are significant damage commercial fruits including mango fly, *B. frauenfeldi* (Schiner), banana fly, *B. musae* (Tryon), Asian papaya fruit fly, *B. papayae* Drew and Hancock and melon fly, *Z. cucurbitae* (Coquillett). Levels infestation of these were highly destructive, high, moderate and attack fruits respectively, cause premature fruit drop and reduces quickly both the quality and quantity of fruit produced more than haft (Secretariat of the Pacific Community, 2001). Likewise, several countries in Pacific Island, hugely fruits infested by fruit flies often drop prematurely, such as carambola

(*Averrhoa carambola* L.) in Malaysia, mandarin (*Citrus reticulate* Blanco) infested by *B. minax* (Enderlein) in Bhutan, and capsicum and chili infested by *B. facialis* (Coquillett) in Tonga (Allwood and Leblanc, 1996) render horticultural production in this region were limit due to fruit fly larvae cause complete destruction of the fruits.

European, cherry fruit flies are the main cause of losses in cherries. In Romania and Bulgaria, 80-90% of late maturing cherries were damaged by the fly and 51% loss in Germany. Similarly, Jordan, Mediterranean fruit fly may cause 20-25% loss of citrus, 91% of peaches, 55% of apricots and 15% of plums. In addition, the fly was destroyed peaches up to 100% in Frankfurt. New Zealand, the monetary loss would be in excess of US\$ 8-10 million causing the outbreak of melon fly, *Z. cucurbitae*. The outbreak of Mediterranean fruit fly has already cost the New Zealand about US\$ 6 million (Allwood and Leblanc, 1996). Mediterranean region according to other region, losses caused by Olive fruit fly include 15-20%, 25%, 30-35%, 20-40% and 20-60% in Cyprus, Italy, Greece, Yugoslavia and Israel respectively (Fimiani, 1989). Moreover, two families; Tephritidae and Lonchaeidae are interesting of fruit flies in South America, especially in Brazil causing the fruit production lost (Uchôa and Nicácio, 2010).

The Asian region (East, Southeast and South) is among the top three regions worldwide for both exporters and importers of fresh fruits and vegetables. For example, in 2004, 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 and Vichitrananda, 2007). However, the region is equitable climate and rich diversity of plant life causing the greater species richness of damaging fruit flies (especially the Southeast Asia). Fruit fly infestation, to the direct losses, can result in serious losses in trade value and export opportunity due to strict quarantine. Tephritid fruit flies (especially, *B. dorsalis* (Hendel), *B. carambolae* Drew and Hancock, *B. correcta* (Bezzi) and *Z. cucurbitae* (Coquillett)) are causing direct damage to fruit and vegetables crops in this region which can lead to up to 90-100% yield loss.

In Thailand and neighborhood countries, the cost of losses due to infestation of fruit flies can be surprisingly high. Several species belong to the genus *Bactrocera* and *Zeugodacus* are great important pest and economic importance, such as *B. correcta*, *B. umbrosa*, *B. latifrons*, *B. tuberculata*, *B. carambolae*, *B. papayea*, *B. dorsalis*, *B. zonata*, *Z. diversa*, *Z. tau* and especially *Z. cucurbitae* (Drew and Romig, 1996).

There are crops losses have been up to 100% in cucumber and bitter gourd, of these are two of most popular vegetables from the cucurbit family in this region and infestation problem caused by the fly (Dhillon *et al.*, 2005). Moreover, the mango, guava and papaya are also were destruct by fruit flies larva (Mahmood, 2004; Aemprapa, 2007; Orankanok *et al.*, 2007), produce were losses at 12-60%, 40-90% and 20-60% respectively (Allwood and Leblanc, 1997), the destruction consist with India and Malaysia. The level of damage caused by fruit fly (*Carpomya vesuviana* Costa) ranged from 10-50% in India (Agrawal and Mathur, 1991) and fruit crop (guava and star fruit) were losses 100% in Malaysia (Signh, 1991).



(A)

(B)

Figure 2.10 Fruit flies oviposition sites; (A) on the peel fruit, (B) inside the fruit.





Figure 2.11 Eggs hatch into larva that feed upon the flesh fruit, causing the fruit decay to rot and fall; (A) Guava, *Psidium guajava* L., (B) Chili, *Capsicum annuum* L., (C) Rose apple, *Syzygium samarangense* (Blume) Merr. & L.M.Perry, (D) Mangosteen, *Garcinia mangostana* L., (E) Cherry, *Malpighia glabra* L., (F) Jack fruit, *Artocarpus heterophyllus* Lam., (G) Papaya, *Carica papaya* L., (H) Susung-kalabaw, *Uvaria rufa* Blume, (I) Custard Apple, *Annona squamosa* L., (J) Carambola, *Averrhoa carambola* L., (K) Mango, *Mangifera indica* L., (L) Gac fruit, *Momordica cochinchinensis* (Lour.), (M) Hog plum, *Spondias pinnata* (L.f.) Kurz, (N) Barking deer's mango, *Irvingia malayana* Oliv. ex A.W. Benn., (O) Sponge gourd, *Luffa cylindrical* (L.) M. Roem., (P) Pomegranate, *Punica granatum* L., (Q) Santol, *Sandoricum koetjape* (Merr.), (R) Fig, *Ficus carica* L., (S) Eggplant, *Chionanthus parkinsonii* (Hutch.) Bennet & Raizada, (T) Rambeh Bambi, *Baccaurea ramiflora* Lour.

Pest status	Species	Host Plant	Distribution
Highly destructive to edible fruits	B. carambolae Drew and	Polyphagous fruit pest	Vietnam to Indonesia. Introduced
	Hancock		into South America
	B. correcta (Bezzi)	Polyphagous fruit pest	Pakistan to Vietnam
	B. dorsalis (Hendel)	Polyphagous fruit pest	Tropical Asia (widespread)
			Introduced into Africa and
			Oceania
	B. latifrons (Hendel)	Mainly Solanaceae	Pakistan to Taiwan; south to
			Sulawesi. Introduced into Hawaii
			and Africa
	B. neohumeralis (Hardy)	Polyphagous fruit pest	Australia, New Guinea
	B. oleae (Gmelin)	Olive	Africa. Introduced into southern
			Europe, the Middle East and
			California
	B. tryoni (Froggatt)	Polyphagous fruit pest	Australia. Introduced in Oceania
	B. zonata (Saunders)	Polyphagous fruit pest	India to Vietnam
High damage to edible fruits	B. aquilonis (May)	Polyphagous fruit pest	Australia. May be conspecific
			with <i>B. tryoni</i> .

Table 2.3 Pest status and distribution of economic important species of fruit flies ranked by category of severity.

Table 2.3	b (Continued)
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Pest status	Species	Host Plant	Distribution
	B. caryeae (Kapoor)	Oligophagous fruit pest	Southern India
	B. curvipennis (Froggatt)	Polyphagous fruit pest	New Caledonia
	B. facialis (Coquillett)	Polyphagous fruit pest	Tonga
	B. frauenfeldi (Schiner)	Polyphagous fruit pest	Australia, Micronesia (except
			Marianas), New Guinea,
			Solomon Islands
	B. jarvisi (Tryon)	Polyphagous fruit pest	Australia
	B. kandiensis Drew and Hancock	Oligophagous fruit pest	Sri Lanka
	B. kirki (Froggatt)	Polyphagous fruit pest	French Polynesia, Fiji (Rotuma),
			Niue, Samoa (American and
			Western), Tonga
	B. kraussi (Hardy)	Polyphagous fruit pest	Australia
	B. melanotus (Coquillett)	Polyphagous fruit pest	Cook Islands
	B. minax (Enderlein)	Citrus	Bhutan, China, Nepal
	B. musae (Tryon)	Banana	Australia, New Guinea
	B. occipitalis (Bezzi)	Oligophagous fruit pest	Kalimantan, Philippines
	B. passiflorae (Froggatt)	Polyphagous fruit pest	Fiji, Wallis and Futuna, Niue
	B. psidii (Froggatt)	Polyphagous fruit pest	New Caledonia

Table 2.3 (Continued)

Pest status	Species	Host Plant	Distribution
	B. trilineola Drew	Polyphagous fruit pest	Vanuatu
	B. tsuneonis (Miyake)	Citrus	China, Japan
	B. xanthodes (Broun)	Polyphagous fruit pest	Cook Islands, Fiji, French
			Polynesia (Austral group), Niue,
			Samoa (American and Western),
			Tonga, Wallis and Futuna
Moderate damage	B. albistrigata (deMeijere)	Oligophagous fruit pest	Indonesia, Malaysia
	B. bryoniae (Tryon)	Banana, chili pepper	Australia, New Guinea
	B. distincta (Malloch)	Sapotaceae	Fiji, Samoa (American and
			Western), Tonga, Wallis Is
	B. halfordiae (Tryon)	Oligophagous fruit pest	Australia
	B. melas (Perkins and May)	Polyphagous fruit pest	Australia. May be conspecific
			with B. tryoni.
	B. moluccensis (Perkins)	Inocarpus fagifer	Java to New Guinea, Solomon
			Islands
	B. obliqua (Malloch)	Guava, Syzygium	New Guinea
	B. passiflorae (sp. nr.)	Oligophagous fruit pest	Fiji, Tokelau, Tonga (Niuas
			Group), Tuvalu

Table 2.3 (Continued)

Pest status	Species	Host Plant	Distribution
	B. pyrifoliae Drew and Hancock	Guava, peach, pear	Thailand, Vietnam (Member of
			<i>B. dorsalis</i> complex)
	B. trivialis (Drew)	Oligophagous fruit pest	New Guinea (Member of
			<i>B. dorsalis</i> complex)
	B. tuberculata (Bezzi)	Oligophagous fruit pest	Bangladesh to Vietnam
	B. umbrosa (Fabricius)	Breadfruit, jackfruit	Widespread from southern
			Thailand through New Guinea to
			New Caledonia
Attacks edible fruits	B. arecae (Fabricius)	Betel nut	Malaysia (Peninsular), Singapore,
			Thailand
	B. atramentata (Hering)	Pometia pinnata	New Guinea
	B. bancroftii (Tryon)	Mulberry	Australia
	B. expandens (Walker)	Mangosteen	Australia, Indonesia (Moluccas),
			New Guinea
	B. hastigerina (Hardy)	Spondias	New Guinea, Solomon Islands
	B. hochii (Zia)	Luffa cylindrica (fruit)	Bangladesh to Vietnam; south to
			Sumatra
	<i>B. lineata</i> (Perkins)	Pometia pinnata	New Guinea

Table 2.3 (Continued)

Pest status	Species	Host Plant	Distribution
	B. mesomelas (Bezzi)	Guava	Africa
	B. mucronis (Drew)	Guava, sweetsop	New Caledonia
	B. munda (Bezzi)	Squash (fruit)	Philippines, Taiwan
	B. murrayi (Perkins)	Mango, Surinam cherry	Australia, New Guinea
	B. mutabilis (May)	Guava, kumquat	Australia
	B. nigrofemoralis White and	Pomelo, mamey sapote	Indian subcontinent, including
	Tsuruta		Sri Lanka
	B. nigrotibialis (Perkins)	Guava, rose-apple	India to Vietnam; south to
			Indonesia (Lesser Sundas)
	B. ochroma Drew and Romig	Mango	Indonesia
	B. perfusca (Aubertin)	Mango, rose-apple	French Polynesia (Marquesas
			only)
	B. pruniae Drew and Romig	Peach	Vietnam
	B. quadrisetosa (Bezzi)	Pometia pinnata	Solomon Islands, Vanuatu
	B. speculifera (Walker)	Breadfruit	New Guinea
	B. versicolor (Bezzi)	Sapodilla	India, Sri Lanka

Note: polyphagous; use of plants from several plant families, oligophagous; use of plants from a single family.

(Source: modified from Vargas et al., 2015).

2.5 Fruit fly control

Fruit flies are known for their short lifespan and rapid reproduction (Sarwar, 2015) render the fly can be distributed throughout the world. Currently, the fruit flies are widespread throughout all regions of the globe and can damage the economic enormously because the fruit flies are significantly infesting almost every commercial fruit and vegetable farm. A result of extensive distribution of pest make it difficult to control. Moreover, the adult female fruit flies are able to lay their eggs inside the fruit tissue with their ovipositor, it prefers young, green and soft fruits for eggs laying. After their eggs hatch to larva internal the fruit tissue, the fruits attacked in this stage (larva stage), which fail to develop properly and rot on the plant or fall to the ground. The newly emerged larva is now sheltered from the external environment (the larva damage the fruits internally), making difficult to control pest with pesticides (Uchôa, 2012; Vargas *et al.*, 2015). In addition, their presence inhibits the export of horticultural produce. Therefore, there is a need to explore alternative methods for control and develop an integrated control tactic for effective management of pest and reduce loss of productivity.

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).

Various strategies have been develop for using to control fruit flies. All of them suitable for area-wide control which congruent with the large distribution of the fruit fly. These include: Insecticide-based suppression tools (cover sprays; Roessler, 1989, protein bait sprays; Vargas *et al.*, 2008; Prokopy *et al.*, 1992, 2003; Peck and McQuate,

2000; Piñero *et al.*, 2009) and soil drenches; Stark and Vargas, 2009; Stark *et al.*, 2013, 2014), Male annihilation; Steiner *et al.*, 1965; Koyama *et al.*, 1984; Vargas *et al.*, 2000, 2014), Sterile insect releases (Steiner *et al.*, 1970; Koyama, 1996; McInnis *et al.*, 2007), Releases of natural enemies (Vargas *et al.*, 2007, 2012) and Cultural controls (fruit wrapping or bagging, crop hygiene or sanitation measures, early harvesting and resistant crops/non-host status) (Allwood, 1996; Vijaysegaran, 1996).

2.5.1 Insecticide-based suppression tools

2.5.1.1 Cover sprays

The use of insecticides applied as cover sprays to the affected crops to inhibit fruit fly damage is common practice in several countries. The history of insecticide sprays to control fruit fly initiate by Back and Pemberton (1918a, b), their use of inorganic insecticides such as lead arsenate and sodium fluorsilicate for sprayed on the plants. Subsequently, a wide range of insecticides are being preferred used such as the carbamate, organophosphate and synthetic pyrethroid types. These insecticides are usually applied at the time the fruit commence ripe, which the respective fruits become susceptible to oviposition. Spraying were continued at weekly intervals until about 1-2 weeks before the fruits are harvested (Rejesus et al., 1991; Meksongsee et al., 1991; Isnadi, 1991). Although, this method has high level of protection fruit flies infestation, but this method has several disadvantages including very expensive in cost of pesticide, very time-consuming in labour, adversely affect beneficial organisms, including biological control agents and pollinating agents, especially borers (that are hidden from the spray), undesirable for the environment generally and can cause health problems for person applying the spray and may also leave chemical residues in the fruits, therefore, users should be careful to use due to when misused, can lead to a number of problems, but cover sprays, when used properly, are extremely useful compounds. However, this technique should only be used as a last resort if all other measures fail.

2.5.1.2 Protein bait sprays

Protein bait sprays are diluting protein bait concentrate with water and mixing it with an insecticide (Figure 2.12). This method displayed the effectiveness of hydrolysed protein in poison bait formulations for fruit fly control firstly by Steiner (1952). Since then, protein bait sprays have turn into a significant method of suppressing or eradicating fruit fly populations in many parts of the world. Previously, Malathion was to be used, but more recently chlorpyrifos, fipronil or Spinosad have been instead used and more popular in gardener. Several countries reported the use of protein baits for fruit fly control such as in Thailand (Meksongsee *et al.*, 1991), Philippines (Rejesus *et al.*, 1991) and Malaysia (Vijaysegaran, 1989). The control in both Thailand and Philippines were concentrated in mango and carambola. Despite, the use of proteinbased bait sprays in many area, but this method is not as widespread as it should be because the protein bait is more expensive due to of these have to be imported from foreign sources and inaccessible to a large number of fruit growers. However, protein baits spraying offers many advantages including suitable to use in integrated pest management programs due to less harmful to beneficial insects, less material was used, so costs are reduced and it is more friendly environment because of the much reduced pesticide usage resulting to minimize fruit residue problems included little drift effect may be happen especially, a hazard to the operator is considerably reduced.



Figure 2.12 Protein bait sprays using motorcycle. (Source: bugsforbugs.com.au)

2.5.1.3 Soil drenches

This method has been used against Mediterranean fruit fly in the past by contact insecticides drenched into the soil. The goal is to directly kill larvae entering the soil into pupae, pupae in the soil, and adults emerging from pupae by drenching the soil surrounding host plants. Previously, the organophosphate insecticide diazinon using for this purpose contains whereas in California has not been popular since 2001 because of its environmental toxicity, difficulty in disposing all ground clutter and debris and a potential lack of effectiveness in the varied soil types found in urban environments.

2.5.2 Male annihilation technique (MAT)

This method is mostly used for eradication of fruit flies from areas like islands where natural obstacle diminish the occasion of re-infestation. It involves attractant Methyl eugenol mixed with the pesticide naled for strongly male attractant because it is needed for proper production of their sex pheromone. The male flies in the area responding to the methyl eugenol, come to feed on the blocks and are killed resulting the female flies in the area remain unfertilized, and so cannot breed and the population dies out (Figure 2.13). Not only Methyl eugenol (ME) but also Cue-lure (CUE) and Trimedlure (TME) is known about the parapheromones. Additionally, Spiroketal (SK) is commonly known to as pheromones. Kind of these can be attractant different fruit flies species for example, Methyl eugenol (ME) used for attractant mostly fruit flies in genus Bactrocera such as Oriental fruit fly, B. dorsalis (Hendel), Peach fruit fly, B. zonata (Saunders), Carambola fruit fly, B. carambolae Drew and Hancock, Philippine fruit fly, B. philippinensis Drew and Hancock and Banana fruit fly, B. musae (Tryon); Cuelure (CUE) used for attractant mostly fruit flies in genus Zuegodacus such as a melon fly, Z. cucurbitae (Coquillett), pumpkin flower fruit fly, Z. caudata (Fabricius) and pumpkin fruit fly, Z. tau (Walker); Trimedlure (TML) used for attractant Mediterranean fruit fly (Medfly) and Natal fruit fly; and the last one pheromone is Spiroketal (SK) used for attractant Olive fruit fly, B. oleae (Gmelin). Generally, MAT widely used by growers because of the impressive catches of flies and makes use of small amounts of the attractant pheromones and parapheromones. In California and Florida, for example, the use of male attractant technique (MAT) has been successfully developed in the 1970's to eradicate introduced populations of Mediterranean fruit flies. 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). Similar with Japan attempts to use Cue-lure to eradicate melon fly populations, the result of experiment reveal that Cue-lure baits can reduced the male population in islands of Japan by 99% after 5 months' treatment (Iwaizumi et al., 1994).



Figure 2.13 Fruit flies trap using methyl eugenol for male attractant.

2.5.3 Sterile insect releases or sterile insect technique (SIT)

This technique is a powerful biological control method, it involves mass-rearing and sterilization of male fruit flies. The sterile males are released into the wild in the target area, and they compete with fertile males in mating with wild females. The efficacy of this method depends on mating between sterile male fruit flies (biological control agent) and their wild couple in order to reduce reproductive potential. If there are many more sterile males' fruit flies than fertile ones, most of the females will remain unfertilized, and the population will eventually die out due to these females will lay sterile eggs, leading to population disruption, which reduces overall pest damage. Thus, sterilize male fruit flies for this method requires a great amount of sterile flies which should be in same proportions to the number of the wild flies for good effectiveness to control. Irradiation to pupa of fruit flies using gamma radiation from a Co60 Gamma (Kumar *et al.*, 2011).

SIT is effective because it is a species-specific approach and one of the most environmentally friendly solutions to insect pest management, but it is a multi-million dollar operation requiring major facilities and much manpower, and is not suitable for general fruit fly control of the individual farmer level. This method is widely using pest control programs in many parts of the world. It has seen the successful application with various fruit fly species. For example, mass release of sterile flies to suppress natural populations of *B. dorsalis* (Hendel) and *B. correcta* (Bezzi) from 1984-1987 can reduce fruit demage in the experimental fields in Thailand (Orankanok *et al.*, 2007). The Mediterranean fruit fly from the northern part of Chile and the southern part of Peru, and the melon fly from Japan are also decreasing from using the SIT method. However, with the species complexes, often two or three species may infest a single fruit and elimination of one species may result in resurgence of another, such as SIT may be unpractical with fruit fly species complex (*B. dorsalis* complex). Therefore, to maximize SIT effectiveness, the mating success among the sterile males and wild females should be enhanced to cover all of the species.

2.5.4 Releases of natural enemies

The use of natural enemies (parasites and predators) to overcome pest populations is pleasurable because it is relatively safe, permanent and frugal. The use of biological control to control fruit flies started already in 1902 (Wharton, 1989). There are numerous examples in several countries where reductions of infestation from fruit fly species. In Hawaii, introduce of larva parasitoid belonging to family Eulophidae, Braconidae and Chalcididae (Allwood *et al.*, 2001), of these are released to control Oriental fruit fly, Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and melon fly (*Zeugodacus cucurbitae* (Coquillett)). *Psyttalia fletcheri* (Hymenoptera: Braconidae), for example, is one of the parasitoids that had showed a high parasitism degree in *Z. cucurbitae* (Coquillett). *Fopius arisanus* (Sonan) is another one that has a tendency parasitoid tested in Hawaii to control *B. latifrons* (Hendel) (Bokonon-Gatan *et al.*, 2007). Additionally, releases of a suite of parasitoids resulted in reduction of infestation in populations of Mediterranean and Oriental fruit flies of up to 95% (Waterhouse, 1993). Likewise, in Australia, *Fopius arisanus* is native parasitoid of Queensland fruit fly, *B. tryoni* (Froggatt) (Waterhouse, 1993). Southeast Asia and surrounding countries are doubtlessly a rich center of diversity of parasites and predators of fruit flies. Several species of which have been report in Thailand (Meksongsee *et al.*, 1991), Malaysia (Vijaysegaran, 1983; Serit *et al.*, 1986; Palacio, 1990) and India (Agrawal and Mathur, 1991). *Diachasmimorpha longicaudata, Fopius arisanus, B. vandenboshi* and *Psyttalia insici* is a high parasitism to control *B. correcta* (Bezzi) in Thailand (Ramadan and Messing, 2003).

Predators may include ants, spiders, assassin bugs, lygaeid bugs, carabid beetles, staphylinid beetles and probably others. For example, olive fly, *Bactrocera oleae* (Gmelin) population was reduced by birds that ate 81% of infested fruits (Bigler *et al.*, 1986). Similarity with the local forest habitat, predation by fruit-eating vertebrates such as birds and primates results in marked reductions in fruit fly numbers. Therefore, these predators that feed on infested fruits in the field are very important for the reduction of fruit fly populations because sometimes that kind of predation has been more successful than the control of fruit flies by parasitoids.

2.5.5 Cultural controls

2.5.5.1 Fruit wrapping or bagging

Wrapping or bagging of individual fruits on the tree with paper bags made from double layers of newspaper or brown paper and calico to prevent oviposition from female fruit fly (Figure 2.14). The technique is available in several areas and several counties because easy to practice. Country in Asia such as Malaysia carambola or star fruit (*Averrhoa carambolae* L.) has been cultivated for over 70 years using this technique. In 1989, damage levels of carambola may be reduced from nearly 100% to 15-25% about 17,000 tonnes worth US\$ 8 million were exported to Europe, Hong Kong and Singapore using the bagging technique (Vijaysegaran, 1989), similarly with Thailand and Taiwan. This technique is used in both countries to protect mangoes from fruit fly attack and melons from melon fly respectively (Wen, 1988). In addition, fruit wrapping is also carried out for mango production in the Philippines, particularly in Cebu Island are as well (Hapitan and Castillo, 1976). In Thailand this method is used in particular in mango orchards (Allwood *et al.*, 2001). The wrapping or bagging technique is environmentally safe, it is effective, and can be used for a number of fruits such as guava, mango, carambola and some gourds but is not possible for others like citrus, papaya and sapodilla. Additionally, this method may possibly promote the dispersal of female flies in seek oviposition site.



Figure 2.14 Bagging method to protect the fruits from insect pests and save the fruits fall to the ground; wrapped in (A) brown paper bags, (B) newspaper, (C) calico, (D) plastic bag.

(Source; http://www.pestnet.org/fact_sheets/fruit_flies__solomon_is._021.htm)

2.5.5.2 Crop hygiene or sanitation measures

Because of the wide host ranges and large numbers of alternate hosts enabling a high population density of adult fruit flies, particularly of the *B. dorsalis* complex. There are about 75 species members of this complex that can infested more than 200 host plant species in 50 families (Drew 1989, Fitt, 1990; Drew and Hancock 1994; Hollingsworth *et al.*, 2003; Clarke *et al.*, 2005; Kumar *et al.*, 2011). In orchards that are uncontrolled for breeding of fruit flies or poorly managed, the crop hygiene or orchard sanitation were need. To avoid of infestation of fruit flies in orchard, the infested fruit should be removed. Collection and destruction of all unwanted fruits or the infested fruits, in particular the fruit on the tree that present signs of attack and on the ground for reducing damaging fly populations (Vijaysegaran, 1985). However, infested fruits on

the tree should be first destruction before falling fruits on the ground because sometime the infested fruit on the ground, the larvae have already left the fruit, the dispersal or removed it may be there was not any benefit. Several countries apply the technique for control fruit flies, for example, In China, the destruction of host fruits to reduce fruit fly damage was impressive (Yang, 1991). *Bactrocera citri*, a serious pest of citrus in this country, was controlled by orchard sanitation. Similarly with Jangjen region, Sichuan province, exceed 8 million infested fruits were destroyed during the operation which lasted from 1951-1952. Infestation was reduced from 25% in previous years to 0.5% in 1953. In Chengdu and Shaanxi provinces, the infested fruits were destroyed during 1953 worth more than 17 million. The following year infestation decreases from 80% to 5%. Hence, in orchard where sanitation measures or crop hygiene are practice the level of fruit flies decreases significantly (Verghese *et al.*, 2004)

2.5.5.3 Early harvesting

Avoidance of fruit fly infestation is possible by harvesting crops because development of fruit flies does not appear to occur in certain fruit such as banana, papaya and sapodilla when they are 100% green (Vijaysegaran, 1996). Therefore, early harvesting is an important technique in the production of these fruit. Papaya, for example, a major export variety developed in Malaysia, if cautiously harvested when a hue of yellow appears on the skin, is completely free of fruit fly damage. The papaya was produced about 24,000 tonnes worth US\$ 5 million were exported using this early harvesting technique in 1989. Likewise, banana have been exported around the world due to they are free of fruit flies 100% at the mature green stage, except in countries where Banana fruit fly, *B. musae* (Tryon) and Papaya fruit fly, *B. papaya* Drew and Hancock occur may be not avoidance. Banana fruit flies lay eggs in banana fruits when the fruit are very young and their eggs do not hatch until the fruit commences to ripen. Papaya fruit fly are also, they may infest green banana. Thus, no facilitate using this option in countries abovementioned.

2.5.5.4 Resistant crops/non-host status

The production of crop varieties that are less attractive for fruit flies has shown good effects such as in Fiji Island, there are some chilli (*Capsicum annuum* L.) varieties that are classified as non-hosts for fruit flies. In Thailand there are some fruit crops that are not susceptible to fruit fly attacks for instance rambutan (*Nephelium lappaceum* L.), mangosteen (*Garcinia mangostana* L.) and duku/langsat (*Lansium domesticum* Corrêa). However, occasional damage may be observed when the fruit are over-ripe and/or cracked or damaged on the tree and such fruits support complete larval development.

2.6 Population genetics of fruit fly

In the broad sense, population genetics is the study of evolutionary processes, gene frequencies in and among subdivided populations, comprehensive estimations of variation in terms of allelic and genotypic frequencies for examine genetic variation and genetic structure of the population. Because of all evolutionary changes is accompanied by change in gene frequencies, population and evolutionary genetics overlap, therefore, population genetic data using modern molecular techniques are important to understanding of living systems including the colonization success, estimating history dispersal rate and gene flow within and among populations, estimates of effective (i.e. reproductive) population sizes, tests for rapid expansions of population size and pairwise genetic distances included determine pest origin and to recognize migration routes (Reyes and Ochando, 1998; Davies *et al.*, 1999; Gilchrist *et al.*, 2006)

The data foregoing, fruit flies are widespread around the world and have a member nearly 5,000 described species. Many species show very similar morphology and is a render limitation on the morphological study of the fruit fly. In addition, the population size of the fruit fly increased quickly and the distribution area expanded rapidly informative to reconstruct historical processes are very important. Then, if given appropriate population genetic data, it can be inferred the origin of outbreaks or reintroductions and supporting the quality control of fruit flies.

The knowledge on genetic variability and its distribution among populations of tephritid species are useful for pest management strategies (Molona-Nery *et al.*, 2014). 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) are important for this SIT (Aketarawong *et al.*, 2011; Karsten *et al.*, 2013).

Population genetic study can be used for the management of agricultural pest because the data of this study used to evaluate invasion pest risks, especially, fruit flies. Virgilio *et al.* (2010) analyzing the macrogeographic population structure of the agriculture pest *Z. cucurbitae* in cosmopolitan using microsatellite analysis to identify the geographic origin of the species and reconstruct its range expansion and regional colonization pathways the result reveal that high levels of genetic diversity in population from Pakistan, India and Bangladesh. It has been suggest that *Z. cucurbitae* originated in Central Asia and expanded its range to East Asia and Hawaii and to Africa and into the islands of the Indian Ocean. A number of outliers expose high levels of admixture (Q>0.70) with populations from different regions. The result indicated that gene flow could play significant role on inter-regional. Anthropogenic transport is the most plausible promoter for the dispersal of *Z. cucurbitae*.

Population genetic study of the melon fly, *Z. cucurbitae*, in Reunion Island was used to infer its geographical origin. Microsatellites uncover the occurrence of three different genetic clusters of *Z. cucurbitae* in Reunion Island including African and Asian are clearly distinguishable relatives. High level of gene flow between clusters were found and also occurs with populations from the African continent and, to a lesser extent, from Asia. The *Z. cucurbitae* clusters show distinct distributions across eastern and western locations in Reunion Island and their abundance is also following with the average amount of rainfall. Moreover, the microsatellite and sequence analyses in this study suggest Africa as the most probable source area for populations of *Z. cucurbitae* in Reunion Island (Jacquard *et al.*, 2013).

Seven populations of *B. carambolae* from the known geographical areas including Southeast Asia (i.e., Indonesia, Malaysia, Thailand) and South America (i.e., Suriname) were examine for genetic variation, genetic structure and genetic relationships between populations using eight microsatellite DNA markers. The result demonstrated that the Suriname samples were genetically differentiated from Southeast Asian populations because genetic drift during the colonization process, local adaptation and condition of habitats such as sufficient time for genetic drift to take effect. The source populations of *B. carambolae* in Suriname were presume from West Sumatra and Java. The result of this study indicated that population genetics could be a powerful tool for the identification of the region of origin of *B. carambolae* and the pathways of

invasion and historical demography can play an important role in estimating dispersal rates and understand the colonization of *B. carambolae* (Aketarawong *et al.*, 2015).

2.7 Morphological variation and taxonomy of fruit fly

Many economically important fruit fly pest species belong to *Bactrocera dorsalis* complex that comprise of more than 75 sibling species. Among these, *B. dorsalis* (Hendel), *B. carambolae* Drew and Hancock, *B. occipitalis* (Bezzi), *B. papayae* Drew and Hancock and *B. philippinensis* Drew and Hancock are morphologically (White and Elson-Harris, 1992; Drew and Hancock, 1994; Iwahashi, 2001; Ebina and Ohto, 2006), genetically (Smith *et al.*, 2003; Armstrong and Ball, 2005), physiologicalaly (Fletcher and Kitching, 1995) and behaviorally very similar (Medina *et al.*, 1998; Tan, 2003) thus presenting difficulty for species identification (Van *et al.*, 2010). Additionally, some studies reveal that development of adult flies of the same species from different localities show different morphological characters (Riska, 1986; Azevedo *et al.*, 1998; Klingenberg and Zaklan, 2000; Hoffmann *et al.*, 2002). Adult flies of the different species from same fruit and locality, also possessed different morphology (Kitthawee and Rungsri, 2011).

Morphometric analysis has been used for population structure and historical migration inference in fruit flies, especially closely related species. For example, Adsavakulchai *et al.* (1999) used morphometric study for identification of the *B. dorsalis* complex including *B. dorsalis*, *B. arecae*, *B. propinqua*, *B. pyrifoliae*, *B. verbascifoliae*, species E, species K and species P used wing shape analysis. The result revealed that all of 424 wing specimens were separated of "grouped" with 89.6% accurate identification. The result after clustering, the percentage of "grouped" between the *B. dorsalis* complex and *Z. tau*; *B. arecae* and species E; *B. dorsalis* and *B. verbascifoliae*; *B. propinqua* and *B. pyrifoliae*; and species K and species P is 100.0%, 98.9%, 98.1%, 95.2% and 84.6% accurate identification respectively. The results are satisfying and this method of numerical taxonomy (from wing image) may be handy for the practical identification of other groups of insect pests. Similarly, a study in *B. dorsalis* complex (Khamis *et al.*, 2012) using wing veins and tibia length analysis for identify of the invasive fruit fly pest, *B. invadens* population from other closely related *Bactrocera* species. The result of the principal component analysis demonstrated

that 15 components which correspond to the 15 morphometric measurements. The first two principal axes (PC1 and PC2) encouraged to 90.7% of the total variance and display partial separation of these populations. In the Canonical discriminant analysis indicated that only the first five canonical variates were statistically significant. The first two canonical variates (CV1 and CV2) encouraged a total of 80.9% of the total variance clustering *B. invadens* with other members of the *B. dorsalis* complex while distinctly separating *Z. cucurbitae*, *B. correcta*, *B. oleae* and *B. zonata*. In addition, the classification tree constructed by the Neighbor-Joining method revealed that the *Bactrocera* species populations were group into four clusters. First cluster consisted of the *B. dorsalis* complex (*B. invadens*, *B. kandiensis* and *B. dorsalis* s.s.), branching from the same node while the second cluster was paraphyletic clades of *B. correcta* and *B. zonata* and the last two clusters consisting of *Z. cucurbitae* and *B. oleae*, respectively, which monophyletic clades.

Apart from *B. dorsalis* complex study, the geometric morphometric analysis also used for identification in other Bactrocera species complex. Kitthawee and Dujardin (2010) using the geometric approach to explore the Bactrocera tau complex in Thailand. The result shown that two non-overlapping clusters within both males and females in exploratory analysis using kernel density. The clusters analysis separated into two group and were not congruent with geography. One cluster (cluster I) contained only one plant, Momordica cochinchinensis (Lour.) Spreng., the other one (cluster II) contained five different plants including Coccinia grandis (L.) Voigt, Cucurbita moschata Duchesne, Trichosanthes tricuspidata Lour., Cucumis sativus L. and *M. cochinchinensis* (Lour.). The study shows that host plants influence the variation of wing shape of the fruit fly. Likewise, the differentiation in wing shape in the Bactrocera tau complex (Z. tau A and C) on a single fruit species of Thailand were determine by Kitthawee and Rungsri (2011). The result indicated that Z. tau A and C possessed greater wing variation in size and shape in male than in female. The wing shape of the female has no different between species that could be related to the complex relationship with the environment. It is possible that female in the larval stage of the two species (Z. tau A and C) are managed using the same kind of plants in the area properly. The difference of the wing shape in males as a result of using the different area inside a host plant of the larval stage. Both species may be a separate

species by sympatric differentiation process in the same kind of host plant with the microhabitat. These phenomena cause an accumulation of different sub-populations within the large population and lead to discrimination reproduction, resulting in separation of a different species. In addition, intraspecific analysis were found difference in wing shape of both male and female in *Z. tau* A and C as a result of physical factor, is high temperature. High temperatures leading to the development of physiological of fruit flies to adapt to environmental change that may help to increase performance in flight resulting of increased of fitness.

Geometric morphometric analyses using wing shape was used to study population structure in fruit flies species complex. The wing shape and mitochondria DNA were using determine population genetic structure and population history of *B. dorsalis* complex consist *B. dorsalis*, *B. papayae* and *B. philippinensis* by Schutze *et al.* (2012b). The result demonstrate that difference of wing shape in all of them. Beast analysis show *B. dorsalis* complex are probably migrated from northern Thailand, about 540kya for colonization to Malaysia and Sumatra, about 470 kya and 270 kya respectively. Isolation-by-distance analysis revealed that *B. dorsalis* complex are likely to be migrated across to the Philippines, but no migrated between the Philippines and Taiwan. The information about the distribution and migration of the fruit fly *B. dorsalis*, *B. papayae* and *B. philippinensis* can be used to manage these pests.

Wing shape could also used as a possible tool for the diagnosis of the other genus of fruit flies. The genus *Ceratitis*, especially complex species including *C. fasciventris*, *C. anonae* and *C. rosa* complex were identified of morphospecies and genotypic clusters using the potential of wing shape. The result indicated that morphospecies and genotypic clusters for both males and females in *Ceratitis* complex are significant differences. This study suggest that wing shape might represent a possible tool for the diagnosis of species within the *C. fasciventris*, *C. anonae* and *C. rosa* complex (Cann *et al.*, 2015). In addition, geometric morphometric method using wing shape and wing size variation was applied to investigate morphological variation within and between populations in the other insects (honeybee; Tofilski, 2008; *Calopteryx splendens*; Sadeghi *et al.*, 2009; *Myzus persicae*; Zhang *et al.*, 2016; *Diaphorina citri*; Paris *et al.*, 2016; *Poecilobothrus regalis*; Chursina and Negrobov, 2016;) and fish (*Rutilusrutilus caspicus*) (Ghojoghi *et al.*, 2014).

2.8 Melon fly, Zeugodacus cucurbitae (Coquillett)

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Suborder: Brachycera

Infraorder: Muscomorpha

Superfamily: Tephritoidae

Family: Tephritidae

Subfamily: Dacinae

Tribe: Dacini

Genus: Zeugodacus

Species: Z. cucurbitae (Coquillett)

The melon fly, Zeugodacus cucurbitae (Coquillett) (Diptera: Tephritidae) was first described as Dacus cucurbitae by Coquillett (1899) based on specimens reared from in cucumbers collected in Honolulu, Hawaii (USA). Zeugodacus cucurbitae was first placed in the subgenus Dacus (Drew, 1973), and then transferred to genus Bactrocera (Drew, 1989). Subsequently, Drew (1989) submits a classification recognizing both taxa (Dacus and Bactrocera) as genera, based on the abdominal tergites being fused, (in *Dacus*), or not fused (in *Bactrocera*). Later, the species has been move to subgenus Zeugodacus of the genus Bactrocera by Drew (1989). This subgenus characterized by the posterior lobe of the male lateral surstylus being long and the male abdominal sternite 5 being slightly concave along the posterior margin (rather than having a deep V shaped indentation) (Drew and Hancock 1999). Zeugodacus *cucurbitae* has also been placed in other genera after originally described as genus Dacus (Dacus cucurbitae Coquillett) including Chaetodcus (Chaetodacus cucurbitae (Coquillett)), Strumeta (Strumeta cucurbitae (Coquillett)) and Bactrocera (Bactrocera cucurbitae (Coquillett)) (Drew, 1989) and, most recently, Zeugodacus (Zeugodacus cucurbitae (Coquillett)) (De Meyer et al., 2015, Virgilio et al., 2015). Virgilio et al. (2015) propose that subgenus Zeugodacus should be separated into different genus based on molecular phylogenetic analysis that found the separation of Zeugodacus and Bactrocera and Dacus (Virgilio et al., 2015). Additionally, species within Zeugodacus

commonly use plants of the family Cucurbitaceae as host that strong different from *Bactrocera* (White, 2006). Because of *Zeugodacus* and *Dacus* had a common ancestry, but very different of morphological characters (White, 2006). Hence, evidence from morphological (Drew and Hancock, 1999), ecological (White, 2006) and molecular data (Virgilio *et al.*, 2015) is sufficient to elevate the *Zeugodacus* group of subgenera to be placed in a separate genus. Therefor, in this study chosen to refer to new generic to this species as *Zeugodacus* (*Zeugodacus*) *cucurbitae*, although the most existing literature refers to it under the former combination, *Bactrocera* (*Zeugodacus*) *cucurbitae*.

The genus *Zeugodacus* currently includes 192 species (De Meyer *et al.*, 2015; Hancock and Drew, 2015; Virgilio *et al.*, 2015) (Table 2.4). However, there has not been universal acceptance of this change among scientists familiar with tephritid fruit fly taxonomy (Mcquate *et al.*, 2017). Most species within this genus are restricted to the Oriental and Australasian Regions, with a few species reaching into the eastern Palearctic in China and Japan, except for *Z. cucurbitae* which was introduced into other parts of the world. The melon fly is presumed Indian origin (Bezzi, 1913) and currently widely distributed across the world (Figure 2.17) including more than 30 tropical and subtropical countries and regions for example North America, South America, West Africa, East Africa, Oceania and Asia (Table 2.5).

The melon fly is one of the most destructive pests of fleshy fruits and vegetables and a major, highly polyphagous, agricultural pest that attacks more than 125 plant species (Table 2.6). Most of them belong to *Cucurbitaceae* and *Solanaceae* (Bezzi 1913; Orian and Moutia, 1960; Doharey, 1983; White and Elson-Harris, 1992; Vayssières, 1999, 2008; Dhillon *et al.*, 2005; White, 2006) including commercial crops such as pumpkin, cantaloupe, watermelon, squash, gourd, cucumber, tomatoes, eggplant, peppers and bean as well as soft fruits such as mango, orange, papaya and peach (White and Elson-Harris, 1992). The fly prefers to infest young, soft skinned ovaries even before anthesis (Maharjan *et al.*, 2015).

In many countries, the melon fly is responsible for high infestation rates in cucurbits crops and induces significant losses. The extent of crop loss varies from 30% to 100% (Gupta and Verma, 1992) such as Bangladesh, losses due to melon fly infestation were estimated 30% of annual agricultural produces in the country (Naqvi, 2005). Fruits of cucurbits were losses are infested by this pest species including melon

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(*Cucumis melo* L.), sweet gourd (*Cucurbita maxima* Duchesne), snake gourd (*Trichosanthes cucumerina* L., *Benincasa hispida* (Thunb.) Cogn.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), ivy gourd (*Coccinia grandis* (L.) Voigt), cucumber (*Cucumis sativus* L., *Cucumis trigonus* Roxb.), white-flowered gourd (*Lagenaria siceraria* (Molina) Standl.), luffa (*Luffa aegyptiaca* Mill.) balsam-apple (*Momordica balsamina* L.) and bitter gourd (*Momordica charantia* L.) etc. (Wadud *et al.*, 2005; Khan *et al.*, 2007; Saha *et al.*, 2007). According to the reports in China, the fly often caused losses of 30% to 90%. Naura and Solomon in India country, *Z. cucurbitae* had caused a reduction in production of watermelon, bitter gourd and towel gourd and pumpkin by 28.55%, 95%, 90% and 87%, respectively (Dhillon *et al.*, 2005). In Hawaii, pumpkin and squash have been heavily attacked even before the fruit had set as same as Thailand, the melon fly was found throughout and can destroy commercial fruit about 100%.

Zeugodacus cucurbitae is a medium-sized species has a characteristic is face fulvous with large black oval spot (Figure 2.16A); notopleura and postpronotal lopes yellow; scutum red-brown with or without fuscous marking (Figure 2.16C); medial post-sutural vitta and lateral post-sutural vitta yellow present; a yellow spot anterior to notopleural suture; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior notopleural seta (npl) dorsal; scutellum entirely yellow; legs with all segment fulvous with apical 1/3 of all femora, fore and hind tibiae dark fulvous (Figure 2.16D); wing with cells basal costal (bc) and costal (c) colourless, microtrichia in outer corner of cell costal only; a broad fuscous costal band paler spot in apex of wing, pale infuscation along radial-medial (r-m) crossvein and dark fuscous along discal medial-cubital (dm-cu) crossvein; a broad fuscous anal streak (Figure 2.16E); abdominal terga III-V orange-brown with a 'T' pattern consisting of a narrow black transvers band across anterior margin of tergum III and a medium to broad medial longitudinal band over all three terga, anterolateral corners of terga IV and V fuscous to dark fuscous (Figure 2.16B).

The melon fly developed from egg to adult stage takes 12-28 days, eggs stage of the fly was about 1-2 days, 4-9 days and 7-11 days for larvae and pupa stage respectively (Weems and Heppner, 2001) depending on temperature and host plant. In general, all of fruit fly species are pupate in the soil, the melon were included. After the full-grown larvae come out of the fruit for pupae in the soil. The larvae pupate in the soil at a depth of 0.5-15 centimeter. The depth up to which the larvae move in the soil for pupation, and survival depend on soil texture and moisture (Jackson et al., 1998; Pandey and Misra, 1999), a short time after that (stage fully) the pupa turns into an adult fly. The adult melon fly has a life cycle lasts from 21-179 days (Fukai, 1938; Narayanan and Batra, 1960). The females and males survived for 27-133 days and 65-249 days respectively. However, seasonal, host plant and temperature influence the melon fly abundance for example during winter season, the Z. cucurbitae hide under dry leaves of trees and bushes and not have activity, different from the hot and dry season, these seasons have abundance of the host plant of this fly and high temperature render the melon fly have actively such as infesting the fruit in trees, feed on honeydew. Commonly, the melon fruit fly actively when the temperature below about 32 °C and the relative humidity ranges between 60-70% (Dillon et al., 2005). However, the temperature higher than 32 °C the fly is not ideal for its growth and reproduction. This species there are 8-10 generations in a year (White and Elson-Harris, 1994; Weems and Heppner, 2001).



Subgenus	Species	Species status	Host Plant	Distribution
(Asiadacus)	absolutus (Walker)	Combination nova	**	Indonesia
	apicalis (Meijere)	Combination nova	Trichosanthes wawraei	Brunei, China,
			Cogn. (flowers)	Malaysia, Thailand,
				Vietnam
	apiciflavus (Yu, He and Chen)	Combination nova	**	China, Thailand
	atypicus(White and Evenhuis)	Combination nova	**	**
	<i>bakeri</i> (Bezzi)	Combination nova	**	Philippines
	careomacula (Drew and Romig)	Combination nova	**	Philippines
	maculifacies (Hardy)	Combination nova	Siphonodon celastrineus	Thailand, Southern
			Griff.	Veitnam
	melanopsis (Hardy)	Combination nova	**	Indonesia
(Austrodacus)	alampetus (Drew)	Combination nova	**	**
	atrisetosus Perkins*	Status revision	**	Thailand
	cucumis (French)*	Combination nova	Cucurbitaceae (fruit)	Australia, Thailand
	unichromatus (Drew)	Combination nova	**	**
(Diplodacus)	signatifer (Tryon)	Combination nova	**	**

Table 2.4 List of 192 valid species in genus Zeugodacus Hendel.

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
(Hemigymnodacus)	diversus (Coquillett)*	Combination nova	See Allwood et al., 1999	India, Sri Lanka,
				China, Nepal,
				Thailand, Southern
				Veitnam, Pakistan
	mukiae (Drew and Romig)	Combination nova	Zehneria wallichii	Thailand, Northern
			(C.B. Clarke) C. Jeffrey,	Vietnam, Bhutan
			Z. maysorensis Wight	
			& Arn.	
(Heminotodacus)	dissidens (Drew)	Combination nova	**	**
(Hemiparatridacus)	abdoaurantiacus (Drew)	Combination nova	**	**
(Nesodacus)	atrichus (Bezzi)	Combination nova	**	Philippines,
(Niuginidacus)	singularis (Drew)	Combination nova	**	**
(Papuodacus)	complicatus (White)	Combination nova	**	Indonesia
	fereuncinatus (Drew and Romig)	Combination nova	**	Thailand
	maculifemur Hering	Status revision	**	Myanmar
	neopallescentis (Drew)	Combination nova	**	**
	ochrosterna (Drew and Romig)	Combination nova	**	Vietnam

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	sinensis (Yu, Bai and Chen)	Combination nova	**	China, Peninsular
				Malaysia
(Paradacus)	abdopallescens (Drew)	Combination nova	**	**
	angustifinis (Hardy)	Combination nova	**	Indonesia
	areolatus (Walker)	Combination nova	**	Indonesia
	aurantiventer (Drew)	Combination nova	**	**
	citroides (Drew)	Combination nova	**	**
	decipiens (Drew)*	Combination nova	Cucurbita pepo L.	Papua New Guinea
	duplicatus (Bezzi)	Status revision	**	Central and
				Southern India, Sri
				Lanka
	fulvipes (Perkins)	Combination nova	**	East Malaysia,
				Indonesia
	hancocki (Drew and Romig)	Combination nova	**	Indonesia
	magnicauda (White and Evenhuis)	Combination nova	**	**
	urens (White)	Combination nova	**	Indonesia
(Parasinodacus)	ablepharus (Bezzi)	Combination nova	**	Philippines,
				Southern Vietnam

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	binoyi (Drew)	Combination nova	**	Southern India
	brevivitta (Drew and Romig)	Combination nova	Melastoma	Peninsular Malaysia
			malabathricum L.	
	cilifer (Hendel)	Combination nova	See Allwood et al., 1999	Taiwan, China,
				Loas, Thailand,
				Vietnam, Peninsular
				Malaysia,
				Indonesia, Sumatra,
				Pasaman
	citrifuscus (Drew and Romig)	Combination nova	**	Central Thailand
	eurylomatus (Hardy)	Combination nova	**	Indonesia
	incises (Walker)	Combination nova	**	Myanmar,
				Andaman Island,
				China, India,
				Thailand,
				Peninsular
				Malaysia, Northern
				Vietnam

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	longicaudatus (Perkins)	Combination nova	**	East Malaysia,
				Thailand
	pahangiae (Drew and Romig)	Combination nova	<i>Liasea</i> sp.	Peninsular Malaysia
	pantabanganiae (Drew and Romig)	Combination nova	**	Philippines
	pseudocucurbitae (White)	Combination nova	**	East Malaysia,
				Indonesia,
				Thailand,
				Peninsular Malaysia
	vinnulus (Hardy)	Combination nova	**	Indonesia,
				Thailand,
				Peninsular Malaysia
	waimitaliae (Drew and Romig)	Combination nova	**	Indonesia
(Sinodacus)	angusticostatus (Drew)	Combination nova	**	**
	bogorensis (Hardy)	Combination nova	**	Indonesia
	buvittatus (Drew)	Combination nova	**	**
	disturgidus (Yu, Deng and Chen)	Combination nova	**	**
	emarginatus (Perkins)	Combination nova	**	**
	hamaceki (Drew and Romig)	Combination nova	**	**

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	hochii (Zia)	Combination nova	See Allwood et al., 1999	China, Indonesia,
				Thailand,
				Peninsular
				Malaysia, Vietnam
	infestus (Enderlein)	Combination nova	**	Indonesia,
				Thailand,
				Peninsular
				Malaysia, Northern
				Vietnam
	longivittatus (Chua and Ooi)	Combination nova	**	Peninsular Malaysia
	paululus (Drew)	Combination nova	**	**
	perpusillus (Drew)	Combination nova	**	**
	sepikae (Drew)	Combination nova	**	**
	speciosus (Drew and Romig)	Combination nova	**	East Malaysia
	spectabilis (Drew and Romig)	Combination nova	**	Philippines
	strigifinis (Walker)*	Combination nova	Cucurbitaceae (fruit)	Australia, Papua
				New Guinea
	surrufulus (Drew)	Combination nova	**	**

 Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	transversus (Hardy)	Combination nova	**	Indonesia
	triangularis (Drew)*	Combination nova	Cucurbitaceae (fruit)	Papua New Guinea
	univittatus (Drew)	Combination nova	**	**
	whitei (Drew and Romig)	Combination nova	**	East Malaysia
(Zeugodacus)	abdoangustus (Drew)	Combination nova	**	Papua New Guinea
	abnormis (Hardy)	Combination nova	**	Indonesia, East
				Malaysia,
	aithonota (Drew and Romig)	Combination nova	**	Northern Vietnam
	ambigua Shiraki	Status revision	**	Taiwan,
	amoenus (Drew)	Combination nova	**	**
	anala (Chen and Zhou)	Combination nova	**	**
	anchitrichotus (Drew)	Combination nova	**	**
	apicofemoralis (Drew and Romig)	Combination nova	**	Indonesia
	armillatus (Hering)	Combination nova	**	**
	assamensis (White)	Combination nova	**	India, Bhutan,
				Northern Vietnam

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	atrifacies Perkins	Status revision	**	Peninsular and East
				Malaysia, Thailand,
				Bhutan, Northern
				Vietnam, China
	baliensis (Drew and Romig)	Combination nova	**	Indonesia
	baoshanensis (Zhang, Ji, Yang and Chen)	Combination nova	**	**
	bezzianus Hering	Status revision	**	China
	biguttatus (Bezzi)	Combination nova	**	India, Bhutan
	borongensis (Drew and Romig)	Combination nova	**	Indonesia
	brachus (Drew)	Combination nova	**	**
	buruensis (White)	Combination nova	**	Indonesia
	calumniatus (Hardy)	Combination nova	**	Philippines,
				Indonesia
	caudatus (Fabricius)*	Status revision	See Allwood et al., 1999	Indonesia, Brunei,
				China, India,
				Peninsular and East
				Malaysia,
				Myanmar,

Table 2.4	(Continued)
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Subgenus	Species	Species status	Host Plant	Distribution
				Sri Lanka, Taiwan,
				Thailand, Vietnam
	choristus May	Status revision	**	**
	connexus (Hardy)	Combination nova	**	Indonesia
	cucurbitae (Coquillett)*	Status revision	See Allwood et al., 1999	See Table 2.5
	curtus (Drew)	Combination nova	**	**
	daclaciae (Drew and Romig)	Combination nova	**	Southern Vietnam,
				Thailand
	daulus (Drew)	Combination nova	**	**
	depressus Shiraki*	Status revision	See Allwood et al., 1999	Taiwan, Japan,
				Ryukyu Island,
				Korea
	diaphoropsis Hering	Status revision	**	Indonesia
	diaphorus (Hendel)	Combination nova	**	Taiwan, India, Sri
				Lanka, China,
				Indonesia,
				Peninsular
				Malaysia,
Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
				Thailand, Vietnam,
				Bhutan
	dorsirufus (Drew and Romig)	Combination nova	**	Bhutan
	dubiosus (Hardy)	Combination nova	**	Indonesia
	elegantulus (Hardy)	Combination nova	**	Philippines, East
				Malaysia, Indonesia
	emittens (Walker)	Status revision	**	Indonesia
	exornatus Hering	Status revision	**	Indonesia,
				Peninsular Malaysia
	fallacis (Drew)	Combination nova	**	**
	flavipilosus (Hardy)	Combination nova	**	Indonesia
	flavolateralis (Drew and Romig)	Combination nova	Drypetes sp.	Southern Thailand
	flavopectoralis Hering	Status revision	**	Indonesia
	flavoverticalis (Drew and Romig)	Combination nova	Strychnos nux-vomica L.	Thailand
	freidbergi (White)	Combination nova	**	India
	fulvoabdominalis (White and Evenhuis)	Combination nova	**	**
	fuscipennulus (Drew and Romig)	Combination nova	**	**
	fuscoalatus (Drew and Romig)	Combination nova	**	India

Subgenus	Species	Species status	Host Plant	Distribution
	gavisus (Munro)	Status revision	**	India, Sri Lanka
	gracilis (Drew)	Combination nova	**	**
	hatyaiensis (Drew and Romig)	Combination nova	**	Southern Thailand
	havelockiae (Drew and Romig)	Combination nova	Trichosanthes sp.,	India
			Momordica dioica	
			Roxb. ex Willd.	
	heinrichi Hering	Status revision	**	Indonesia, Brunei,
				Peninsular and East
				Malaysia, Thailand,
				Northern Vietnam
	hekouanus (Yu, He and Yang)	Combination nova	**	China
	hengsawadae (Drew and Romig)	Combination nova	Ocimum sp.,	Thailand
			Siphonodon sp.	
	hoabinhiae (Drew and Romig)	Combination nova	**	India, Thailand,
				Northern Vietnam
	hodgsoniae (Drew and Romig)	Combination nova	Hodgsonia macrocapa	Indonesia,
			(Blume) Cogn. var.	Peninsular Malaysia
			capniocarpa (Ridl.) S.L.	

Table 2.4	(Continued)
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Subgenus	Species	Species status	Host Plant	Distribution
			Tsai ex A.M.Lu & Z.Y.	
			Zhang, T. tricuspidata	
			Lour.	
	hoedi (White)	Combination nova	**	**
	hululangatiae (Drew and Romig)	Combination nova	**	Peninsular Malaysia
	indentus (Hardy)	Combination nova	**	Philippines
	iriomotiae (Drew and Romig)	Combination nova	**	Japan
	ishigakiensis Shiraki	Status revision	**	Japan
	isolatus (Hardy)	Combination nova	See Allwood et al., 1999	Thailand, China,
				Loas, Southern
				Vietnam
	javadicus (Mahmood)	Combination nova	**	Indonesia
	juxtuncinatus (Drew and Romig)	Combination nova	**	Philippines
	kaghanae (Mahmood)	Combination nova	**	Pakistan
	khaoyaiae (Drew and Romig)	Combination nova	**	Peninsular
				Malaysia, Thailand
	laguniensis (Drew and Romig)	Combination nova	**	Philippines

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	laocaiae (Drew and Romig)	Combination nova	**	Northern Vietnam,
				Thailand
	lipsanus (Hendel)	Status revision	**	Taiwan
	liquidus (Drew and Romig)	Combination nova	**	Thailand
	luteicinctutus (Ito, 2011)	Combination nova	**	**
	macrophyllae (Drew and Romig)	Combination nova	**	Malaysia, Thailand
	macrovittatus (Drew)	Combination nova	**	**
	maculatus Perkins	Status revision	**	Peninsular
				Malaysia, Thailand,
				China
	melanofacies (Drew and Romig)	Combination nova	**	Peninsular and East
				Malaysia, Thailand
	menglanus (Yu, Liu and Yang)	Combination nova	**	China, Bhutan,
				Thailand
	mesonotaitha (Drew)	Combination nova	**	**
	minimus (Hering)	Combination nova	**	Indonesia
	mundus (Bezzi)	Status revision	Cucurbita maxima	Philippines, Taiwan
			Duchesne	

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	nakhonnayokiae (Drew and Romig)	Combination nova	**	Peninsular
				Malaysia, Thailand,
				Vietnam
	namlingiae (Drew and Romig)	Combination nova	**	Bhutan
	neoelegantulus (White)	Combination nova	**	Indonesia
	neoemittens (Drew and Romig)	Combination nova	**	Indonesia
	neoflavipilosus (Drew and Romig)	Combination nova	**	Indonesia
	neolipsanus (Drew and Romig)	Combination nova	**	Indonesia
	nigrifacies Shiraki	Status revision	**	Taiwan
	okunii Shiraki	Status revision	**	Taiwan, Indonesia
	pemalangiae (Drew and Romig)	Combination nova	**	Indonesia
	perplexus (Walker)	Combination nova	**	Indonesia
	persignatus (Hering)	Combination nova	**	Indonesia
	platamus (Hardy)	Combination nova	**	Southern Thailand,
				Indonesia,
				Peninsular and East
				Malaysia
	proprescutellatus (Zhang, Chen and Gao)	Combination nova	**	China, Bhutan

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	pubescens (Bezzi)	Combination nova	**	Philippines
	purus (White)	Combination nova	**	**
	quasiinfestus (Drew and Romig)	Combination nova	**	Thailand
	reflexus (Drew)	Combination nova	**	**
	rubellus (Hardy)	Combination nova	T. wawraei Cogn.	Thailand
	sabahensis (Drew and Romig)	Combination nova	**	East Malaysia
	sandaracinus (Drew)	Combination nova	**	**
	sasaotiae (Drew and Romig)	Combination nova	See Allwood et al., 1999	Indonesia
	scutellaris (Bezzi)*	Status revision	See Allwood et al., 1999	India, China,
				Myanmar, Nepal,
				Thailand, Bhutan,
				Northern Vietnam,
				Peninsular Malaysia
	scutellatus (Hendel)*	Status revision	See Allwood et al., 1999	Taiwan, China,
				Japan, Bhutan,
				Thailand, Vietnam
	scutellinus (Bezzi)	Combination nova	**	Philippines
	semongokensis (Drew and Romig)	Combination nova	**	East Malaysia

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
	signatus Hering	Status revision	**	India, Bhutan,
				Thailand
	sonlaiae (Drew and Romig)	Combination nova	**	Bhutan, Northern
				Vietnam
	sumbensis Hering	Status revision	**	Indonesia
	synnephes (Hendel)	Status revision	**	Taiwan, Indonesia,
				Peninsular
				Malaysia,
				Philippines,
				Thailand
	tapervitta (Mahmood)*	Combination nova	Luffa cylindrical (L.) M.	Philippines
			Roem.,	
			M. cochinchinensis	
			(Lour.) Spreng.	
	tappanus (Shiraki)	Combination nova	**	Taiwan
	tau (Walker)*	Combination nova	See Allwood et al., 1999	China, India,
				Sikkim, Sri Lanka,
				Taiwan, Indonesia,

Table 2.4 (Continued)

Subgenus	Species	Species status	Host Plant	Distribution
				Bhutan, Brunei,
				Peninsular and East
				Malaysia, Thailand,
				Vietnam, Pakistan,
				Philippines
	tebeduiae (Drew and Romig)	Combination nova	**	Brunei, Peninsular
				and East Malaysia,
				Indonesia, Thailand
	timorensis Perkins	Status revision	**	Timor, Koepang,
				Indonesia
	trichosanthes (Drew and Romig)*	Combination nova	Bittergourd (fruit)	Indonesia,
				Peninsular and East
				Malaysia, Thailand
	trichotus May	Status revision	**	**
	tricuspidatae (Drew and Romig)	Combination nova	T. tricuspidata Lour.	Thailand
	trimaculatus (Hardy and Adachi)*	Combination nova	M. cochinchinensis	Philippines
			(Lour.) Spreng.,	
			<i>M. charantia</i> L.	

Subgenus	Species	Species status	Host Plant	Distribution
	trivandrumensis (Drew and Romig)	Combination nova	**	India
	ujungpandangiae(Drew and Romig)	Combination nova	**	Indonesia
	uncinatus (Drew and Romig)	Combination nova	**	Philippines
	unilateralis (Drew)	Combination nova	**	**
	vargus (Hardy)	Combination nova	**	Indonesia
	vultus (Hardy)	Combination nova	**	Thailand, China,
				Laos, Bhutan,
				Peninsular and East
				Malaysia,
				Indonesia,
				Philippines,
				Vietnam
	watersi (Hardy)	Combination nova	Bryonia sp., T. primata	India
	yalaensis (Drew and Romig)	Combination nova	Fagraea ceilanica	Thailand
			Thunb.	

Table 2.4 (Continued)

Species	Species status	Host Plant	Distribution
yoshimotoi (Hardy)	Combination nova	**	Northern and
			Southern Vietnam,
			Bhutan, Nepal,
			Thailand
zahadi (Mahmood)	Combination nova	**	Southern India
	Species yoshimotoi (Hardy) zahadi (Mahmood)	SpeciesSpecies statusyoshimotoi (Hardy)Combination novazahadi (Mahmood)Combination nova	SpeciesSpecies statusHost Plantyoshimotoi (Hardy)Combination nova**zahadi (Mahmood)Combination nova**

* Economic important species

** No known record

(Source: modified from Drew and Roming, 2013; De Meyer et al., 2015; Hancock and Drew, 2015; Vargas et al., 2015; Virgilio et al.,

2015)



(A)



(B)

Figure 2.15 Adults of melon fly, Zeugodacus cucurbitae; (A) female, (B) male.





Figure 2.16 Morphological characteristics of *Zeugodacus cucurbitae*; (A) head, (B) scutum, (C) abdomen, (D) legs, (E) wing.



Figure 2.17 Geographic distributions of melon fly, Zeugodacus cucurbitae.

(Source: Boontop, 2016)

Country	References	
South America		
South Pacific Island	Fletcher, 1987	
Africa		
Cameroon	Fontem et al., 1999; Weem and Heppner, 2001; De Meyer et al., 2015	
Cote d'Ivoire	Weem and Heppner, 2001	
Egypt	Weem and Heppner, 2001	
Gambia	Weem and Heppner, 2001; De Meyer et al., 2015	
Kenya	Christenson and Foote, 1960; Weems and Heppner, 2001;	
	De Meyer et al., 2015	
Mali (it was found in 2000)	Weem and Heppner, 2001; De Meyer et al., 2015	
Mauritius	Christenson and Foote, 1960; Weems and Heppner, 2001;	
	De Meyer et al., 2015	
Reunion (identified in 1972)	Weem and Heppner, 2001; De Meyer et al., 2015	
Seychelles	Weem and Heppner, 2001; De Meyer et al., 2015	
Somalia	Weem and Heppner, 2001	
Sudan	De Meyer et al., 2015	
Tanzania	Christenson and Foote, 1960; Weems and Heppner, 2001;	
	De Meyer <i>et al.</i> , 2015	

 Table 2.5 Geographic distributions of melon fly, Zeugodacus cucurbitae.

Table 2.5 (Continued)

Country	References	
Benin	De Meyer et al., 2015	
Burkina	De Meyer et al., 2015	
Burundi	De Meyer et al., 2015	
Guinea	De Meyer et al., 2015	
Senegal	De Meyer <i>et al.</i> , 2015	
Togo	De Meyer <i>et al.</i> , 2015	
Nigeria	De Meyer <i>et al.</i> , 2015	
Ivory Coast	De Meyer <i>et al.</i> , 2015	
Ghana	De Meyer et al., 2015	
Uganda	De Meyer et al., 2015	
East Africa	Narayanan and Batra, 1960; Weems and Heppner, 2001	
Australic-Oceania		
Australia	Narayanan, 1953; Narayanan and Batra, 1960; Fletcher, 1987;	
	Osmelak, 1920	
Hawiian Islands	Back and Pemberton, 1917; Narayanan, 1953; Christenson and Foote,	
	1960; Narayanan and Batra, 1960; Uchida et al., 1920;	
	Weems and Heppner, 2001	
Solomon Islands (it was discover in 1984)	Eta, 1985; Hollingsworth et al., 1997; Weems and Heppner, 2001	

Table 2.5 (Continued)

Country	References
Northern Mariana Islands (it was detected in 1943)	Steiner et al., 1965; Mitchell, 1980; Wong et al., 1989; Weems and
	Heppner, 2001
Papua New Guinea	Hollingsworth et al., 1997; Weems and Heppner, 2001
Guam	Christenson and Foote, 1960; Wong et al., 1989; Weems and
	Heppner, 2001
Nauru	Hollingsworth and Allwood, 2002; Weems and Heppner, 2001
Kirbali	Weems and Heppner, 2001
Island of Rota	Wong et al., 1989; Weems and Heppner, 2001
Asia	
Afghanistan	Weems and Heppner, 2001
Brunai	Weems and Heppner, 2001
Bangladesh	Weems and Heppner, 2001
Cambodia	Weems and Heppner, 2001
China (numerous provinces such as Yunnan, Guangdong, Hainan,	Narayanan, 1953; Narayanan and Batra, 1960; Liang et al., 1993;
Guangxi, Fujian and Zhejiang)	Weems and Heppner, 2001; Wu et al., 2011; Li et al., 2013
Christmas Island	Weems and Heppner, 2001
East Timor	Weems and Heppner, 2001

Table 2.5 (Continued)

Country	References	
India (numerous states)	Shah et al., 1948; Narayanan, 1953; Narayanan and Batra, 1960;	
	Fletcher, 1987; Vargas et al., 1920; Gupta and Verma, 1992;	
	Pareek and Kavadia, 1995; Weems and Heppner, 2001	
Indonesia (numerous islands)	Hardy, 1949; Narayanan, 1953; Christenson and Foote, 1960;	
	Narayanan and Batra, 1960; Weems and Heppner, 2001	
Iran	Weems and Heppner, 2001	
Laos	Weems and Heppner, 2001	
Malaysia	Hardy, 1949; Narayanan, 1953; Narayanan and Batra, 1960; Tan and	
	Lee, 1982; Weems and Heppner, 2001	
Myanmar	Narayanan, 1953; Narayanan and Batra, 1960; Weems and Heppner,	
	2001	
Nepal	Narayanan, 1953; Narayanan and Batra, 1960; Weems and Heppner,	
	2001	
Oman	Weems and Heppner, 2001	
Pakistan	Shah et al., 1948; Narayanan, 1953; Narayanan and Batra, 1960;	
	Qureshi et al., 1974; Weems and Heppner, 2001	
Philippines	Hardy, 1949; Narayanan, 1953; Christenson and Foote, 1960;	
	Narayanan and Batra, 1960; Weems and Heppner, 2001	

Table 2.5 (Continued)

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Table 2.6 Host plants of melon fly, Zeugodacus cucurbitae.

Type of host plant	Common Name	Scientific Name	References
Cucurbitaceous vegetable	Squash melon	Benincasa hispida (Thunb.) Cogn.	Allwood et al., 1999; Narayanan
			and Batra, 1960
	Colocynth	Citrullus colocynthis (L.) Schrad.	Allwood et al., 1999
	Water melon	C. lanatus (Thunb.) Matsum. & Nakai	Narayanan, 1953; Narayanan and
			Batra, 1960; Pareek and Kavadia,
			1994; Allwood et al., 1999;
			Weems and Heppner, 2001
	Ivy gourd	Coccinia grandis (L.) Voigt	Allwood et al., 1999; Narayanan
			and Batra, 1960
	Muskmelon	Cucumis melo L.	Allwood et al., 1999; Narayanan,
			1953; Narayanan and Batra,
			1960; Wen, 1985; Pareek and
			Kavadia, 1994; Weems and
			Heppner, 2001
	Snap melon	C. momordica Roxb.	Narayanan, 1953; Narayanan and
			Batra, 1960; Allwood et al.,
			1999; Weems and Heppner, 2001

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References
	Cucumber	C. sativus L.	Allwood et al., 1999; Narayanan,
			1953; Narayanan and Batra,
			1960; Pareek and Kavadia, 1994;
			Weems and Heppner, 2001
	Wild cucurbits	C. trigonus Roxb.	Allwood et al., 1999
	Long melon	C. utilissimus Roxb.	Narayanan, 1953; Narayanan and
			Batra, 1960; Pareek and Kavadia,
			1994; Weems and Heppner, 2001
	Wild cucurbits	C. trigonus Roxb.; C. pubescens Wall;	Narayanan, 1953; Narayanan and
		C. anguria L.; Citrullus colocynthis (L.)	Batra, 1960; Uchida et al., 1990;
		Schrad.; Sycos pachycarpus Hooker and Arnott;	
		Lagenaria amebicana Ser.; Coccinia grandis	White and Elson-Harris, 1994;
		(L.) Voigt; C. dipsaceus; Momordica charantia	Weems and Heppner, 2001;
		L. var. muricata Willd.	Dhillon et al., 2005
	Pumpkin	Cucurbita maxima Duchesne	Allwood et al., 1999

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References
	Pumpkin	C. moschata Duchesne	Allwood et al., 1999; Back and
			Pemberton, 1917; Narayanan,
			1953; Narayanan and Batra,
			1960; Wen, 1985; Pareek and
			Kavadia, 1994; Hollingsworth
			et al., 1997; Weems and
			Heppner, 2001
	Pumpkin	C. pepo L.	Allwood et al., 1999
	Balsam apple	Diplocyclos palmatus (L.) C.Jeffrey	Weems and Heppner, 2001
	Redball snakegourd	Gymnopetalum integrifolium (Roxb.) Kurz	Allwood et al., 1999
	Calabash	Lagenaria siceraria (Molina) Standl.	Allwood et al., 1999; Narayanan
			and Batra, 1960; Wen, 1985;
			Weems and Heppner, 2001
	Bottle gourd	L. vulgaris Ser.	Narayanan, 1953; Narayanan and
			Batra, 1960; Pareek and Kavadia,
			1994; Allwood et al., 1999;
			Weems and Heppner, 2001

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References
	Ribbed gourd	Luffa acutangula (L.) Roxb.	Allwood et al., 1999; Narayanan,
			1953; Narayanan and Batra,
			1960; Pareek and Kavadia, 1994;
			Weems and Heppner, 2001
	Sponge gourd	L. cylindrical (L.) M.Roem.	Narayanan, 1953; Narayanan and
			Batra, 1960; Pareek and Kavadia,
			1994; Allwood et al., 1999;
			Weems and Heppner, 2001
	Sponge gourd	L. aegyptiaca Mill.	Allwood <i>et al.</i> , 1999
	-	Melothria wallichii C. B. Clarke	Allwood <i>et al.</i> , 1999
	Cantaloupe	M. liukiuensis Nakai	Weems and Heppner, 2001;
			Iwaizumi, 1993
	Balsam apple	Momordica balsamina L.	Allwood <i>et al.</i> , 1999
	Bitter gourd	M. charantia L.	Allwood et al., 1999; Narayanan
			1953; Narayanan and Batra,
			1960; Wen, 1985; Wong et al.,
			1989; Uchida et al., 1990;

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References
			Pareek and Kavadia, 1994;
			Hollingsworth et al., 1997;
			Weems and Heppner, 2001
	Gac fruit	M. cochinchinensis (Lour.) Spreng.	Allwood et al., 1999
	Balsam Pear	M. dioica Roxb. ex Willd.	Allwood et al., 1999
	-	Mukia maderaspatana (L.) M.Roem.	Allwood et al., 1999
	Snake gourd	Trichosanthes anguina L.	Narayanan, 1953; Narayanan and
			Batra, 1960; Hollingsworth et al.,
			1997; Allwood et al., 1999;
			Weems and Heppner, 2001
	Wild snake gourd	T. cucumerina L.	Allwood et al., 1999; Narayanan,
			1953; Narayanan and Batra,
			1960; Hollingsworth et al., 1997;
			Weems and Heppner, 2001
	Pointed gourd	T. dioica Roxb.	Narayanan, 1953; Narayanan and
			Batra, 1960; Allwood et al.,
			1999; Weems and Heppner, 2001
	Snake gourd	T. ovigera Blume	Allwood et al., 1999

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References
	-	T. tricuspidata Lour.	Allwood et al., 1999
	-	T. wallichiana (Ser.) Wight	Allwood et al., 1999
	-	T. wawraei Cogn.	Allwood et al., 1999
Other vegetable	Okra	Abelmoschus esculentus (L.) Moench	Narayanan and Batra, 1960;
			Kumagai et al., 1996
	Dry onion	Allium cepa L.	McBride and Tanda, 1949
	Kohl rabi	Brassica culorapa L.	Narayanan and Batra, 1960;
			Ranganath and Veenakumari,
			1996
	Cauliflower	B. oleracea L. var. botrytis L.	Narayanan and Batra, 1960
	Broccoli	B. oleracea L. var. capitate L.	McBride and Tanda, 1949
	Chilly/green pepper	Capsicum frutescens L.	Narayanan, 1953; Narayanan and
			Batra, 1960
	Kundru	Cephalendra indica (Wight & Arn.) Naudin	Narayanan, 1953; Narayanan and
		nom. illeg.	Batra, 1960
	Tomato	Lycopersicon esculentum Mill.	Allwood et al., 1999
	Grenadille	Passiflora edulis Sims; P. seemanni Griseb;	Narayanan and Batra, 1960;
		P. quandrangularis L.	Weems and Heppner, 2001

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References
	Brinjal	Solanum melongena L.	Narayanan, 1953; Narayanan and
			Batra, 1960; Weems and
			Heppner, 2001
	Brinjal	Solanum melongena L.	Narayanan, 1953; Narayanan and
			Batra, 1960; Weems and
			Heppner, 2001
	-	S. trilobatum L.	Allwood <i>et al.</i> , 1999
	Zingerone	Bulbophyllum patens King ex Hook.f.	Hong and Nishida, 2000
Grian legumes	Pigeonpea	Cajanus cajan (L.) Millsp.	Narayanan and Batra, 1960
	Hyacinth bean	Dolichos lablab L.	Narayanan and Batra, 1960
	Lime bean	Phaseolus limensis Macfad.	Narayanan and Batra, 1960
	Green gram	P. radiates L.	Narayanan and Batra, 1960
	French bean	P. vulgaris L.	Narayanan and Batra, 1960;
			Wong et al., 1989; Allwood
			et al., 1999; Weems and
			Heppner, 2001

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References		
	Long bean	Vigna unguiculata (L.) Walp. subsp.;	Narayanan and Batra, 1960;		
		V. sinensis (L.) Savi ex Hassk.;	Wong et al., 1989; Allwood		
		V. sesquipedalis (L.) Verdc.	et al., 1999; Weems and		
			Heppner, 2001		
Other field crop	Sun flower	Helianthus annus L.	White and Elson-Harris, 1994		
	Sweet corn	Zea mays L.	White and Elson-Harris, 1994		
Fruits	-	Abelmoscus moschatus Medik. subsp.	Allwood et al., 1999		
	Sour soap	Anona muricata L.	Narayanan and Batra, 1960		
	Custard apple	A. reticulate L.; A. squamosa L.	Narayanan and Batra, 1960		
	Starfruit/carambolas	Averrhoa carambola L.	Wen, 1985; Armstrong et al.,		
			1995		
	Papaya	Carica papaya L.	Narayanan, 1953; Narayanan and		
			Batra, 1960; Wong et al., 1989;		
			Vargas et al., 1990; Weems and		
			Heppner, 2001		
	Pummelo	Citrus grandis (L.) Osbeck	Narayanan, 1953; Tan and Lee,		
			1982		

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References		
	Tangerine	C. reticalata Blanco	McBride and Tanda, 1949;		
			Narayanan and Batra, 1960;		
			Weems and Heppner, 2001		
	Orange	C. sinensis (L.) Osbeck	Narayanan and Batra, 1960;		
			Weems and Heppner, 2001		
	Longan	Euphoria longan Lour.	McBride and Tanda, 1949		
	Fig	Ficus carica L.	Narayanan, 1953; Narayanan and		
			Batra, 1960; Weems and		
			Heppner, 2001		
	-	F. chartacea Wall. Ex King	Allwood et al., 1999		
	Strawberry	Fragaria chiloensis (L.) Duchesne	Narayanan and Batra, 1960		
	Litchi	Litchi chinensis Sonn.	Wen, 1985		
	Mango	Mangifera indica L.	Narayanan and Batra, 1960;		
			Weems and Heppner, 2001		
	Blue field banana	M. paradisiaca (L.) sp. sapientum (L.) Kuntze	McBride and Tanda, 1949		
	Guava	Psidium guajava L.	Allwood et al., 1999; Narayanan,		
			1953; Narayanan and Batra,		
			1960; Wen, 1985		

Table 2.6 (Continued)

Type of host plant	Common Name	Scientific Name	References		
	Peach	Prunus persica (L.) Bastsch	Narayanan, 1953; Narayanan and		
			Batra, 1960; Weems and		
			Heppner, 2001		
	European pear	Pyrus communis L.	Narayanan and Batra, 1960		
	Apple	P. malus L.	Narayanan and Batra, 1960; Wen,		
			1985		
	Date palm	Phoenix dactylifera L.	Narayanan, 1953; Narayanan and		
			Batra, 1960		
	Avocado	Persea Americana Mill.	Narayanan, 1953; Narayanan and		
			Batra, 1960		
		Tetrastigma lanceolarium (Roxb.) Planch.	Allwood et al., 1999		
	Galls grape vine	Vitex trifolia L.	Narayanan, 1953; Narayanan and		
			Batra, 1960		
	Jujub	Ziziphus jujube Mill.	Allwood et al., 1999		
	Sapodilla	Manilkara zapota (L.) P.Royen	Allwood et al., 1999		
	Banana	Musa sp.	White and Elson-Harris, 1949		
	Blue field banana	M. paradisiaca L.	McBride and Tanda, 1949		
Other host	-	Dracaena curtissii Ridl.	Allwood et al., 1999		

Table 2.6	(Continued)
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Type of host plant	Common Name	Scientific Name	References		
	-	Capparis sepiaria L.	Allwood et al., 1999		
	-	C. thorellii Gagnep.	Allwood et al., 1999		
	-	Maerua siamensis (Kurz) Pax	Allwood et al., 1999		
	Leech lime,	Citrus hystrix DC.	Allwood et al., 1999		
	Mauritus papeda				



CHAPTER 3 RESEARCH METHODOLOGY

3.1 Specimens collection

Zeugodacus cucurbitae were collected from 23 populations throughout Thailand (Table 3.1, Figure 3.1) between March 2012 and September 2015.

All the fruit fly samples were either gathered from forest or collected from fruit orchard which were obtained from 10 host plants (Table 3.2, Figure 3.2) including Ivy gourd (*Coccinia grandis* (L.) Voigt), Sponge gourd (*Luffa cylindrical* (L.) M. Roem.), Redball snakegourd (*Trichosanthes tricuspidata* Lour.), Cucumber (*Cucumis sativus* L.), Wax gourd (*Benincasa hispida* (Thumb.) Cogn.), Muskmelon (*Cucumis melo* L.), Bitter gourd (*Momordica charantia* L.), Gac fruit (*Momordica cochinchinensis* (Lour.)), Pumpkin (*Cucurbita moschata* Duchesne) and Mango (*Mangifera indica* L.). Fruits that were infested with larva were bring back to laboratory, and placed in a plastic box containing sawdust at the bottom and covered by calico for trapping emerging flies and the lid with a hole on the top (Figure 3.3) and kept under room temperature (generally about 25±4 °C) (Gibbs, 1967; Bateman, 1967; Dhillon *et al.*, 2005) (Figure 3.4). Adult specimens emerging from reared fruits were preserved in 80% ethanol and kept at -20 °C for further study.





Figure 3.1 Sampling locations of *Zeugodacus cucurbitae* in Thailand (see detail in Table 3.1).





Figure 3.2 Host plant species were infested by melon flies, *Zeugodacus cucurbitae* in this study; (A) Ivy gourd (*Coccinia grandis* (L.) Voigt), (B) Sponge gourd (*Luffa cylindrical* (L.) M. Roem.), (C) Redball snakegourd (*Trichosanthes tricuspidata* Lour.), (D) Cucumber (*Cucumis sativus* L.), (E) Wax gourd (*Benincasa hispida* (Thumb.)
Cogn.), (F) Muskmelon (*Cucumis melo* L.), (G) Bitter gourd (*Momordica charantia* L.), (H) Gac fruit (*Momordica cochinchinensis* (Lour.)), (I) Pumpkin (*Cucurbita moschata* Duchesne) and (J) Mango (*Mangifera indica* L.).





Figure 3.3 Rearing of fruit flies; (A) infested fruits, (B) plastic box contained sawdust, (C) placed the infested fruits into the plastic box, (D) covered the plastic box by calico, (E) 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.



		Geographic	Latitude/	Altitude	Host-plant species	No. of	No. of No. of	Collection
Location	Code					COI	right	date
		region	Longitude	(111)		sequence	wing	uute
Khet Lat Krabang	BKK	Central	13° 43′ 14″ N	4	Coccinia grandis (L.)	5	6	10/09/2015
Bangkok			100° 45′ 00″ E		Voigt ^a			
Mueang	KPT	Central	16° 26' 04" N	98	Trichosanthes	4	10	20/02/2014
Kamphaeng Phet			99° 22′ 06″ E		tricuspidata Lour. ^b			
Fang	CMI1	North	19° 52′ 11″ N	1886	C. grandis (L.) Voigt ^a	-	1	22/11/2012
Chiang Mai			99° 03′ 18″ E					
Chom Thong	CMI2	North	18° 24′ 46″ N	286	C. grandis (L.) Voigt ^b	5	3	16/01/2015
Chiang Mai			98° 41′ 31″ E					
Long	PRE	North	18° 00′ 53″ N	156	Luffa cylindrica (L.) M.	3	1	10/12/2013
Phrae			99° 42′ 55″ E		Roem. ^a			
					C. grandis (L.) Voigt ^a	5	8	10/12/2013
Mae La Noi	MSN	North	18° 22′ 39″ N	315	C. grandis (L.) Voigt ^b	5	6	16/01/2015
Mae Hong Son			97° 56′ 30″ E					

Table 3.1 Sampling locations, host plant species and number of specimens' information of Zeugodacus cucurbitae in Thailand at 23 locations.

Table 3.1 (Continued)

	Coographic	Latitudo/ A	Altitudo		No. of	No. of	Collection
Code	region	Langitudo	(m)	Host-plant species	COI	right	doto
	region	Longitude	(m)		sequence	wing	ualt
PBI	West	13° 06′ 20″ N	9	C. grandis (L.) Voigt ^a	10	15	14/09/2015
		99° 57′ 02″ E					
CPN	South	09° 48′ 33″ N	309	C. grandis (L.) Voigt ^a	12	6	09/07/2015
		98° 48′ 51″ E		L. cylindrica (L.) M.	4	-	09/07/2015
				Roem. ^a			
RNG	South	09° 13′ 25″ N	215	C. grandis (L.) Voigt ^a	15	8	05/07/2015
		98° 26′ 25″ E					
KBI1	South	07° 37′ 17″ N	20	C. grandis (L.) Voigt ^a	4	1	15/05/2015
		99° 01′ 51″ E					
KBI2	South	08° 04' 09" N	13	Benincasa hispida	12	14	06/07/2015
		98° 59′ 58″ E		(Thumb.) Cogn. ^a			
MKM1	Northeast	16° 14′ 58″ N	166	C. grandis (L.) Voigt ^a	3	77	13/12/2012
		103° 15′ 52″ E		Cucumis sativus L. ^a	5	22	06/06/2013
				T. tricuspidata Lour. ^a	3	1	06/04/2013
				C. sativus L. ^a	3	-	25/06/2013
	Code PBI CPN RNG KBI1 KBI2 MKM1	CodeGeographic regionPBIWestCPNSouthRNGSouthKBI1SouthKB12SouthMKM1Northeast	CodeGeographic regionLatitude/ LongitudePBIWest13° 06' 20" N 99° 57' 02" ECPNSouth09° 48' 33" N 98° 48' 51" ERNGSouth09° 13' 25" N 98° 26' 25" EKBI1South07° 37' 17" N 99° 01' 51" EKBI2South08° 04' 09" N 98° 59' 58" EMKM1Northeast16° 14' 58" N 103° 15' 52" E	CodeGeographic regionLatitude/ LongitudeAltitude (m)PBIWest13° 06' 20" N9PBIWest13° 06' 20" N9PO° 57' 02" E99° 57' 02" E309PSNSouth09° 48' 33" N309PS° 48' 51" E98° 48' 51" E215RNGSouth09° 13' 25" N215RNGSouth09° 13' 25" N20PS° 26' 25" E98° 26' 25" E309KBI1South07° 37' 17" N20PS° 59' 58" E98° 59' 58" E166MKM1Northeast16° 14' 58" N166103° 15' 52" E160103° 15' 52" E	CodeGeographic regionLatitude/ LongitudeAltitude (m)Host-plant speciesPBIWest13° 06' 20" N9C. grandis (L.) Voigta99° 57' 02" E99° 57' 02" E99° 57' 02" E100° 48' 33" N309C. grandis (L.) VoigtaCPNSouth09° 48' 33" N309C. grandis (L.) Voigta98° 48' 51" EL. cylindrica (L.) M. Roem.aRNGSouth09° 13' 25" N215C. grandis (L.) Voigta98° 26' 25" E98° 26' 25" E99° 01' 51" E13KBI1South07° 37' 17" N20C. grandis (L.) Voigta99° 01' 51" E98° 04' 09" N13Benincasa hispida886 29' 58" E(Thumb.) Cogn.a103° 15' 52" ECucumis sativus L.a103° 15' 52" E166C. grandis (L.) Voigta103° 15' 52" E166C. grandis (L.) Voigta103° 15' 52" E166C. grandis (L.) Voigta	CodeReographic regionLatitude/ LongitudeAltitude (m)Host-plant speciesNo. of CDI lequencePBIWest 13° 06' 20" N9C. grandis (L.) Voigt ^a 10PBIWest 13° 06' 20" N9C. grandis (L.) Voigt ^a 10PWSouth09° 57' 02" E12121298° 57' 02" E 20° 6' 25" E 20° 6' 25" E1212RNGSouth09° 13' 25" N215C. grandis (L.) Voigt ^a 1598° 26' 25" E 8° 26' 25" E 8° 26' 25" E1515KBI1South07° 37' 17" N20C. grandis (L.) Voigt ^a 499° 01' 51" E 13° 86' 26' 25" E1212KBI2South08° 04' 09" N13Benincasa hispida1298° 59' 58" E(Thumb.) Cogn. ^a 1233MKM1Northeast16° 14' 58" N166C. grandis (L.) Voigt ^a 3103° 15' 52" E 13° 15' 52" E 15° 15' 15'' 15'' 15'' 15'' 15'' 15'' 15''	$ \begin{array}{c c c c c } \mbox{Code} & \begin{tabular}{ c c c } \mbox{Fegion} & \begin{tabular}{ c c } \mbox{Fegion} & \begin{tabular}{$
Table 3.1 (Continued)

		Geographic	Latitude/	Altitude		No. of No. of		Collection
Location	Code		Langitudo	(m)	Host-plant species	COI	right	doto
		region	Longitude	(111)		sequence	wing	uate
Mueang	MKM2	Northeast	16° 09′ 50″ N	138	C. grandis (L.) Voigt ^a	3	-	13/12/2012
Maha Sarakham			103° 19′ 45″ E		C. grandis (L.) Voigt ^a	2	8	09/03/2015
					C. melo L. ^a	5	12	05/05/2013
					<i>Momordica charantia</i> L. ^a	3	-	05/05/2013
Khon San	CPM	Northeast	16° 18′ 14″ N	520	C. grandis (L.) Voigt ^a	4	1	10/06/2015
Chaiyaphum			101° 46′ 41″ E					
Na Haeo	LEI	Northeast	17° 21′ 17″ N	794	C. grandis (L.) Voigt ^a	9	36	19/09/2015
Loei			101° 03′ 42″ E					
Na Kae	NPM	Northeast	16° 55′ 10″ N	156	M. cochinchinensis	5	4	22/07/2013
Nakhon Phanom			104° 33′ 01″ E		(Lour.) ^a			
Si Bun Rueang	NBP	Northeast	16° 54′ 24″ N	200	<i>C. melo</i> L. ^a	3	2	23/07/2013
Nong Bua Lam Phu			102° 15′ 45″ E					
Si Chiang Mai	NKI	Northeast	17° 56′ 28″ N	252	Cucurbita moschata	3	-	07/11/2014
Nong Khai			102° 35′ 43″ E		Duchesne. ^a			

Table 3.1 (Continued)

		Geographic	I atituda/	Altitudo		No. of	No. of	Collection
Location	Code	nogion	Langitudo	(m)	Host-plant species	COI	right	doto
		region	Longhude	(111)		sequence	wing	uale
Chaturaphak Phiman	RET	Northeast	15° 51′ 07″ N	147	<i>M. charantia</i> L. ^a	5	6	08/08/2013
Roi Et			103° 38′ 02″ E					
Phanna Nikhom	SNK	Northeast	17° 19′ 23″ N	211	Mangifera indica L. ^a	4	1	12/06/2013
Sakon Nakhon			103° 52′ 04″ E					
Prang Ku	SKK	Northeast	14° 48′ 59″ N	139	C. grandis (L.) Voigt ^a	5	21	11/08/2012
Si Sa Ket			104° 04′ 00″ E		C. grandis (L.) Voigt ^a	3	-	18/09/2012
					C. moschata Duchesne. ^a	3	1	11/08/2012
Nam Som	UND1	Northeast	17° 44′ 44″ N	218	C. grandis (L.) Voigt ^a	-	4	02/08/2014
Udon Thani			102° 14′ 13″ E		L. cylindrica (L.) M.	-	12	02/08/2014
					Roem. ^a			
Si That	UND2	Northeast	17° 00′ 11″ N	156	C. grandis (L.) Voigt ^a	-	18	25/08/2014
Udon Thani			103° 09′ 42″ E					
Total						155	305	

^a Fruit orchards

^b Wild fruits

Family	Scientific name	Common name
Cucurbitaceae	Benincasa hispida (Thumb.) Cogn.	Wax gourd
	Coccinia grandis (L.) Voigt	Ivy gourd
	Cucumis sativus L.	Cucumber
	Cucumis melo L.	Muskmelon
	Cucurbita moschata Duchesne.	Pumpkin
	Trichosanthes tricuspidata Lour.	Redball snakegourd
	Luffa cylindrical (L.) M. Roem.	Sponge gourd
	Momordica charantia L.	Bitter gourd
	Momordica cochichinensis (Lour.)	Gac fruit
Anacardiaceae	Mangifera indica L.	Mango

Table 3.2 List of host plant species used in this study.

3.2 Species identification

Specimens were identify to species following Plant Health Australia (2011), Drew and Roming (2013) and Virgilio *et al.* (2015) using development suitable adult flies based on morphological characters including head, wing (vein, cells and shape), thorax (color and present or absent of Medial post-sutural vitta), legs (color) and abdomen (color, size and shape). Only adult specimens were used 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).

3.3 DNA extraction, PCR amplification and DNA sequencing

Total DNA was extracted from all over individual adult flies using the GF-1 Tissue DNA Extraction Kit (Vivantis, Selangor Darul Ehsan, Malaysia). DNA fragments (684 bp) of the mitochondrial COI gene was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAAATCA-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 MgCl₂, 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 loaded on 1% agarose gel. Five micro liters of PCR product were mixed with 1 µl of Novel Juice. 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 were visualized with blue light and photographed using digital camera. PCR products were purified with HiYieldTM Gel/PCR DNA Extraction Kit (RBC Bioscience) followed the manufacturing protocol before DNA sequencing which performed at Macrogen (Seoul, Korea), using the same primers as in PCR.

3.4 Morphological procedure

Right wing was dissected under stereomicroscope (OLYMPUS SZ-PT) and mounted on glass slide with Hoyer mounting solution and air-dried prior to image photographed with a digital camera attached to a stereomicroscope (OLYMPUS SZ-X7). Each wing was captured with the same accessories and to the same scale. Twelve landmarks were digitized for each wing (Figure 3.5) using TPSDig2 version 2.19 (Rohlf, 2015) and TPSUtility program version 1.60 (TPSUtil) (Rohlf, 2013). These landmarks are homologous landmark-point which have a placement identical within and between populations. The landmark was defined by the functional concordance of wing structure all of the samples called "homology" (Dryden and Mardia, 1998; Zelditch *et al.*, 2004).



Figure 3.5 Twelve homologous landmarks-points on the right wing of *Zeugodacus cucurbitae* used in the morphological variance analysis; 1. terminal of vein R_{2+3} , 2. terminal of vein R_{4+5} , 3. terminal of vein M, 4. junction of vein M and dm-cu, 5. junction of vein CuA₁ and dm-cu, 6. junction of veins A₁ and CuA₂, 7. junction of CuA₁ and CuA₂, 8. junction of vein CuA₁ and dm-bm cross vein, 9. junction of vein M and dm-bm cross vein, 10. junction of vein M and r-m cross vein, 11. junction of vein R_{4+5} and r-m cross vein, 12. junction of vein R_1 and costal vein.

3.5 Data analysis

3.5.1 Genetic variation

Haplotype diversity (*h*) and nucleotide diversity (π) were estimated using Arlequin Version 3.5.1.2 (Excoffier and Lischer, 2010). Genealogical relationships between haplotypes (Bandelt *et al.*, 1999) were inferred using median joining (MJ) network method in the program Network v5.0 (http://www.fluxus-engineering.com). The MJ network was calculated based on 155 sequences were obtained in this study and 157 sequences were obtained from Genbank (Table 3.3).

The Haplotype diversity (h) or gene diversity 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).



Country	Number of	Genbank	BOLD		
Country	sequences	Accession Numbers	Process ID		
Thailand	14	KT588325-338	-		
India	10	GQ154106-109	-		
		-	MVTBI200-08		
		-	MVTBI202-08		
		-	MVTBI203-08		
		KP233798	-		
		KP851001	-		
		JF776376	-		
USA	8	GQ154102-105			
		-	MVTBI184-08		
		-	MVTBI185-08		
		-	MVTBI186-08		
		-	MVTBI187-08		
China	35	GQ1541000-101	-		
		KF660029-030	-		
		KF660032	-		
		KF660034	-		
		KF660037-038	-		
		KF660040-048	-		
		KF660054-058	-		
		JN635562	-		
		JX266418-421	-		
		HM590447	-		
		-	GBDPT801-14		
		-	GBDPT802-14		
		-	GBDPT803-14		
		-	GBDPT804-14		
		-	GBDPT805-14		
		-	MVTBI198-08		
		-	MVTBI199-08		
Cambodia	6	GQ154097-099	-		
		-	MVTBI197-08		
		-	MVTBI303-09		
		-	MVTBI304-09		
Bangladesh	9	GQ154090-096	-		
C		-	MVTBI190-08		

Table 3.3 Genbank accession numbers and BOLD process ID of the Cytochrom *c* oxidase subunit I (COI) gene sequences of *Zeugodacus cucurbitae* from public data included in this study.

Table 3.3 (Continued)

<u> </u>	Number of	Genbank	BOLD
Country	sequences	Accession Numbers	Process ID
		-	MVTBI195-08
Tanzania	10	GQ154129-130	-
		GQ154132	-
		GQ154134	-
		-	MVTBI181-08
		-	MVTBI182-08
		-	MVTBI183-08
		-	MVTBI221-08
		-	MVTBI222-08
		-	MVTBI223-08
Philippines	6	GQ154125-126	-
		DQ116248	-
		-	MVTBI178-08
		-	MVTBI179-08
		-	MVTBI180-08
Kenya	7	GQ154110-113	-
		-	MVTBI204-08
		-	MVTBI205-08
		-	MVTBI206-08
Indonesia	8	DQ116245-247	-
		KF660063-067	-
Reunion	14	DQ116242	-
		JX162208	-
		AY788409	-
		GQ154114	-
		GQ154116	-
		GQ154118	-
		GQ154120	-
		-	MVTBI208-08
		-	MVTBI209-08
		-	MVTBI210-08
		-	MVTBI211-08
		-	MVTBI212-08
		-	MVTBI213-08
		-	MVTBI214-08
Malaysia	6	GQ154122	-
		GQ154124	-
		-	MVTBI215-08



Table 3.3 (Continued)

Country	Number of	Genbank	BOLD		
Country	sequences	Accession Numbers	Process ID		
		-	MVTBI216-08		
		-	MVTBI217-08		
		-	MVTBI218-08		
Sudan	2	GQ154128	-		
		-	MVTBI219-08		
Unknown	22	FJ903491	-		
		DQ006865	-		
		DQ006875-876	-		
		KJ753952-953	-		
		KC662201-203	-		
		HQ664519-522	-		
		HQ664525-526	-		
		HQ664530	-		
		HQ664532	-		
		HQ664535-536	-		
		HQ664538	-		
		HQ664545	-		
		HQ664547	-		
Total	157				

3.5.2 Population genetic structure

Analysis of genetic differentiation indices (F_{ST}) based on Kimura 2-parameter model (K2P) were used to estimate population genetic structure. The population pairwise F_{ST} was calculated in Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010). 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 which also runs in Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010).

Determination of the relationships between genetic distance (F_{ST} from Arlequin) and geographic distance (km) (i.e. testing for an isolation-by-distance (IBD) model) were performed by Mantel test (Mantel, 1967). The Mantel test was computed in IBD v1.52 (Bohonak, 2002) using 1000 randomizations.

3.5.3 Demographic history

Mismatch distribution analysis was used to examine the demographic history of the populations coupled with population equilibrium test and neutrality test. The unimodal mismatch distribution indicated that population that has undergone recent past demographic expansion (Roger and Harpending, 1992). Expected demographic parameter, the sum-of-squares deviation (SSD) and Harpending's raggedness index (*Hri*) (Harpending, 1994) were used to test detraction based on the sudden expansion model (Roder and Harpending, 1992). Population expansion time was calculated from $\tau = 2$ ut (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). Furthermore, Fu's *Fs* test (Fu, 1997) and Tajima's *D* (Tajima, 1989) statistical tests were also used to investigate the population equilibrium. Large negative values of these tests were expected from demographic population expansion. Mismatch distribution and associated demographic expansion parameters were estimated in Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010).

3.5.4 Morphological variation

A total of 305 (144 males and 161 females) right wings from *Z. cucurbitae* specimens collected from 23 populations (Table 3.1) were obtained for morphological variation analysis.

The input file was prepared as follows, the TPS file building from image using TPSUtil version 1.60 (Rohlf, 2013) and then use for defining and digitizing twelve landmark point in TPSDig2 version 2.19 (Rohlf, 2015). The NTS file was converted from TPS file using TPSUtil version 1.60 (Rohlf, 2013) and this file was used for morphological variation analysis in the software MorphoJ version 1.06d (Klingenberg, 2011)

Generalized procrustes analysis (GPA) was performed to superimpose landmark configurations in order to remove non-shape data variation including size, orientation and translocation for extracted variation from shape for accurate examine differences in the wing shape (Adams *et al.*, 2004), which using the generalized procrustes superimposition method (Chursina and Negrobov, 2016). Procrust-transformed coordinated data were then subjected to principal component analysis (PCA). The PCA was used to determine individual variability and measurements contributed most to variability on morphology. Analysis of variances (ANOVA) was estimated the effected of sex, host plants and geography that contributed to morphological variations. When a statistical significance was found by ANOVA, the discriminant function analysis (DFA) was used to examine the separation between groups of specimens and canonical variate analysis (CVA) was used to distinguish among multiple groups of specimens to comfirm statistical significance for group separation. The DFA was performed to compare wing shape between sexes (male and female), a cross-validation matrix was used to estimate error essociated with group assignment by DFA using leave-one-out cross-validation method (Lachenbruch, 1967) and visualization of shape difference for each landmark as wireframe graph and transformation grids. The difference in wing size between male and female visualized using box plot in PAST (Paleontological Statistics) software version 3.16 (Harmmer et al., 2001). The CVA was used to investigate difference in wing shape of host plants and geography. The variance at each landmark were analyzed using consensus analysis using the Relative warps (tpsRelw) version 1.68 (Rohlf, 2017). The GPA, PCA, CVA, DFA and ANOVA were conducted using the open-source morphometric software MorphoJ version 1.06d (Klingenberg, 2011).



CHAPTER 4 RESULTS

4.1 Genetic variation

A fragment of 457 bp of the mitochondrial COI gene was obtained from 155 individuals of *Z. cucurbitae* from 20 locations in Thailand collected from 10 host plant species. Sequences were deposited in GenBank under the accession numbers KY113161 - KY113315. A total of 46 haplotypes was identified among 155 sequences. Of these, 30 haplotypes were unique, and 16 haplotypes were shared by at least two individuals. The haplotype diversity in each population ranged from 0 in Chaiyaphum (CPM), Nong Bua Lam Phu (NBP) and Nong Khai (NKI) to 1.000 in Sakon Nakhon (SNK) with an average of 0.8820 (Table 4.1). Nucleotide diversity in each population ranged from 0 in Chaiyaphum (CPM), Nong Bua Lam Phu (NBP) and Nong Khai (NKI) to 0.0064 in Roi-Et (RET) with an average of 0.0044 (Table 4.1).

For the host plant species, the haplotype diversity ranged between 0.2857 in sponge gourd (*Luffa cylindrica*) and 1.000 in mango (*Mangifera indica*). Nucleotide diversity ranged between 0.0009 in sponge gourd (*Luffa cylindrica*) and 0.0057 in bitter gourd (*Momordica charantia*) (Table 4.2). Among 46 haplotypes identified, 39 were unique, and seven haplotypes were shared by at least two host plant species. Ivy gourd (*Coccinia grandis*) the between possessed and highest number (21 haplotypes) of unique haplotypes (Table 4.2). Genetic divergence within the host plant species based on the K2P model ranged from 0% to 1.40%, with a mean of 0.25% (Table 4.2). Greatest within host plant genetic divergence (1.40%) was found in bitter gourd (*Momordica charantia*). The K2P genetic divergence between flies from different host plant species ranged from 0% to 2.23%, with a mean of 0.28%. Maximum genetic divergence (2.23%) was found between bitter gourd (*Momordica charantia*) and redball snakegourd (*Trichosanthes tricuspidata*), but there is large overlap for genetic divergent within and between host plant species (Table 4.2).



Location code	No. of samples	Haplotype diversity	Nucleotide diversity
		$(h) \pm SD$	$(\pi) \pm SD$
BKK	5	0.7000 ± 0.2184	0.0027 ± 0.0022
СРМ	4	0	0
CMI	5	0.7000 ± 0.2184	0.0015 ± 0.0014
CPN	16	0.6000 ± 0.1267	0.0011 ± 0.0010
KPT	4	0.5000 ± 0.2652	0.0038 ± 0.0031
KBI1	4	0.5000 ± 0.2652	0.0008 ± 0.0009
KBI2	12	0.6667 ± 0.1409	0.0020 ± 0.0015
LET	9	0.8056 ± 0.1196	0.0022 ± 0.0017
MSN	5	0.8000 ± 0.1640	0.0024 ± 0.0020
MKM1	14	0.7912 ± 0.0894	0.0028 ± 0.0020
MKM2	13	0.9359 ± 0.0507	0.0030 ± 0.0020
NPM	5	0.9000 ± 0.1610	0.0033 ± 0.0025
NBP	3	0	0
NKI	3	0	0
PBI	10	0.2000 ± 0.1541	0.0003 ± 0.0005
PRE	8	0.7857 ± 0.1127	0.0040 ± 0.0027
RNG	15	0.1333 ± 0.1123	0.0002 ± 0.0003
RET	5	0.9000 ± 0.1610	0.0064 ± 0.0045
SNK	4	1.0000 ± 0.1768	0.0039 ± 0.0031
SKK	11	0.9455 ± 0.0535	0.0061 ± 0.0037
Total	155	0.8802 ± 0.0174	0.0044 ± 0.0026

Table 4.1 Haplotype diversity (*h*) and nucleotide diversity (π) of the 20 populations of *Zeugodacus cucurbitae* based on mitochondrial cytochrome *c* oxidase I (COI) gene sequence. Abbreviated sample site (location code) name refer to Table 3.1.

Table 4.2 Haplotype diversity (*h*), nucleotide diversity (π) and genetic divergence of *Zeugodacus cucurbitae* population separated from 10 host plant species in this study.

			H	aplotyp	e			Percent genetic divergences		
Host Plants	No. of samples	Number of sampling location	No. of haplotypes	Shared haplotypes	Unique haplotype	Haplotype diversity $(h) \pm SD$	Nucleotide diversity $(\pi) \pm SD$	within host plant (mean)	between host plant (mean)	
Ivy gourd (Coccinia grandis)	90	13	27	6	21	0.8809 ± 0.0213	0.0043 ± 0.0026	0-0.79 (0.22)	0-1.82 (0.27)	
Redball snakegourd (Trichosanthes tricuspidata)	7	2	4	3	1	0.8095 ± 0.1298	0.0035 ± 0.0025	0-1.20 (0.27)	0-2.23 (0.29)	
Sponge gourd (Luffa cylindrica)	7	2	2	2	-	0.2857 ± 0.1964	0.0009 ± 0.0009	0-0.39 (0.10)	0-1.60 (0.24)	
Wax gourd (Benincasa hispida)	12	1	5	2	3	0.6667 ± 0.1409	0.0028 ± 0.0019	0-0.79 (0.26)	0-2.00 (0.31)	
Cucumber (<i>Cucumis sativus</i>)	8	1	5	3	2	0.8571 ± 0.1083	0.0043 ± 0.0029	0-0.59 (0.18)	0-1.6 (0.24)	
Muskmelon (Cucumis melo)	8	2	3	2	1	0.7500 ± 0.0965	0.0023 ± 0.0017	0-0.39 (0.19)	0-1.60 (0.28)	
Bitter gourd (Momordica charantia)	8	2	6	2	4	0.8929 ± 0.1113	0.0057 ± 0.0037	0-1.40 (0.64)	0-2.23 (0.47)	

Table 4.2 (Continued)

		Number of	I	Iaplot	уре	Hanlatyna	Nucleotide	Percent genetic divergences	
Host Plants	No. of samples	sampling location	No. of hanlotynes	Shared	haplotypes Unique	$\begin{array}{c} \text{Haplotype} \\ \text{diversity} \\ (h) \pm \text{SD} \end{array}$	diversity $(\pi) \pm SD$	within host plant (mean)	between host plant (mean)
Gac fruit									
(Momordica cochinchinensis)	5	1	4	1	3	0.9000 ± 0.1610	0.0033 ± 0.0026	0-1.20 (0.12)	0-1.40 (0.19)
Pumpkin (Cucurbita moschata)	6	2	3	2	1	0.7333 ± 0.1552	0.0022 ± 0.0018	0-0.39 (0.15)	0-1.40 (0.22)
Mango (Mangifera indica)	4	1	4	1	3	1.0000 ± 0.1768	0.0048 ± 0.0037	0-0.79 (0.40)	0-1.81 (0.30)
Total	155	27	63	24	39	0.9550 ± 0.0086	0.0044 ± 0.0026	0-1.40 (0.25)	0-2.23 (0.28)

4.2 Mitochondrial genealogy

The MJ network was calculated from all available COI sequences of *Z. cucurbitae* in the public database (i.e. GenBank, BOLD). The mitochondrial genealogy of 312 sequences (155 sequences obtained in this study and 157 sequences obtained from public database) revealed no major divergence lineage (Figure 4.1). There is no association of haplotype cluster with geographic region (Figure 4.1) or host plant species (Figure 4.2). Overall, the network has a star-like shape, characteristic of population expansion (Slatkin and Hudson, 1991). Specimens from all host plant species and all geographic regions (except those from Sudan) shared the central haplotype.

Genetic relationships between Thai *Z. cucurbitae* and sequences from other geographic regions are as follows. All of COI sequences from Thailand (155 sequences were obtained in this study and 14 sequences were obtained from previous publication (Kunprom and Pramual, 2016) were made up of 46 haplotypes. Only two haplotypes (first and second common haplotypes) were shared with sequences from other geographic regions. Forty-five sequences from Thailand shared the first common haplotype with specimens from all countries included in this study, except for those from Sudan. Forty-two sequences from Thailand shared the second common haplotype which directly connected to the central haplotype with sequences from India, Bangladesh, Reunion, China, Cambodia, Philippines, Indonesia and Malaysia. Remaining sequences (82 sequences) were unique to Thailand.

4.3 Population genetic structure

Population pairwise F_{ST} analysis revealed that 51% of the total comparisons were genetically statistically different, and the remaining (49%) were not (Table 4.3). Populations from Ranong (RNG) and Phetchaburi (PBI) made major contributions to genetic structure. With few exceptions, comparisons of these populations with others are all highly significantly different (Table 4.3). Mantel's test found no significant relationships ($R^2 = 0.0190$, P = 0.9000) between genetic (pairwise F_{ST}) and geographic distances. AMOVA analysis by grouping populations according to the geographic and host plant species revealed no significant differences among groups (Table 4.4).



Figure 4.1 Network for the 312 COI sequence (155 sequences for Thailand and 157 sequences for other geographic regions) of *Zeugodacus cucurbitae* haplotypes constructed by the method median joining network. Circle of the same color indicate haplotypes from the same country. Size are relative to the number of individuals with a specific haplotype. Haplotypes are labelled according to country of origin.



Figure 4.2 Network for the 155 sequences for Thailand and 157 sequences for other geographic regions of *Zeugodacus cucurbitae* haplotypes constructed by the method median joining network. Circle of the same color indicate haplotypes from the same host plant. Size are relative to the number of individuals with a specific haplotype. Haplotypes are labelled according to host plant species.

Table 4.3 Population pairwise F_{ST} values of 14 populations of *Zeugodacus cucurbitae* based on mitochondrial cytochrome *c* oxidase I (COI) gene sequence. Abbreviated sample site (location) name described in Table 3.1. The bold numbers indicated statistical significance following standard Bonferroni correction (P < 0.05).

Populations	BKK	CMI	CPN	KBI	LEI	MSN	MKM1	MKM2	NPM	PBI	PRE	RNG	RET	SKK
BKK	0.000													
CMI	0.738	0.000												
CPN	0.043	0.805	0.000											
KBI	0.010	0.558	0.127	0.000										
LEI	-0.034	0.707	0.082	0.088	0.000									
MSN	0.632	0.508	0.725	0.453	0.622	0.000								
MKM1	0.232	0.518	0.335	0.151	0.196	0.375	0.000							
MKM2	-0.023	0.647	0.039	0.083	0.053	0.565	0.251	0.000						
NPM	0.150	0.680	0.170	0.203	0.188	0.539	0.298	0.117	0.000					
PBI	0.627	0.898	0.483	0.424	0.523	0.802	0.514	0.383	0.135	0.000				
PRE	0.315	0.443	0.410	0.217	0.358	0.161	0.179	0.321	0.197	0.383	0.000			
RNG	0.739	0.927	0.600	0.517	0.637	0.855	0.603	0.489	0.293	0.007	0.479	0.000		
RET	0.242	0.608	0.429	0.322	0.328	0.510	0.425	0.281	0.175	0.420	0.280	0.516	0.000	
SKK	0.108	0.361	0.248	0.092	0.129	0.238	-0.008	0.174	0.170	0.369	0.090	0.462	0.227	0.000

Source of variation	Чf	SSD	Percentage	E statistic
Source of variation	u. 1.	55D	of variation	F-statistic
Geographic regions				
Among groups	4	28.639	8.82	$F_{\rm CT} = 0.08819$
Among populations within	15	56.673	32.02	$F_{\rm ST} = 0.40839^*$
groups				
Within populations	135	101.849	59.16	$F_{\rm SC} = 0.35117^*$
Host plants				
Among groups	9	26.701	-9.04	$F_{\rm CT} = -0.09038$
Among populations within	10	02 025	59 07	$E_{} = 0.40028*$
groups	18	03.033	38.07	$F_{ST} = 0.49028^{+1}$
Within populations	127	79.044	50.97	$F_{\rm SC} = 0.53253^*$
* P<0.05				

Table 4.4 Results of the AMOVA analyses of 20 populations of *Zeugodacus cucurbitae* from Thailand, with grouping according to geographic regions and host plants.

4.4 Demographic history

Mismatch distribution analysis revealed a unimodal mismatch graph (Figure 4.3), a characteristic of recent population expansion. Both sum-of-squares deviation (SSD = 0.0018, P = 0.1100) and Harpending's raggedness index (0.0639, P = 0.0800) were not significantly different from the simulated data under the sudden population expansion model. This is congruent with the star like shape of the median joining network. Population expansion was also supported by highly significant negative values for both Tajima's D (-2.3573, P < 0.001) and Fu's F_S (-27.5748, P < 0.001) tests. The population expansion time was estimated based on 2.3% sequence divergence for insect mitochondrial DNA (Brower, 1994) and the assumed nine generations per year for *Z. cucurbitae* (White and Elson-Harris, 1992; Weems and Heppner, 2001), was 140,000 years ago (95% confidence intervals was 110,000-160,000 years ago).



Figure 4.3 Mismatch distribution of the 312 COI sequences of *Zeugodacus cucurbitae* demonstrating observed and expected pairwise differences based on the predictions of sudden population expansion model. Mismatch distribution of *Zeugodacus cucurbitae* is consistent with predictions of the sudden population expansion model (SSD = 0.0018, P = 0.1100; Harpending's raggedness index = 0.0639, P = 0.0800).

4.5 Morphological variation

The two principal components (PC1 and PC2) were accounted for 94% of the general shape variation in sexes (Figure 4.4), particularly in PC1, which alone accounted for 90% of the variation.

The results of ANOVA analysis revealed that the differences in the shape and centroid size between sexes were significant (Table 4.5) but no significant different in wing from both between host plants and geography. Additionally, the interaction between sex, host plants and geography from both centroid size and shape were statistic significant, which indicated that the difference in wing was affected by sexes.

The result of the discriminant analysis exposed there to be distinct discriminant wing shape between the *Z. cucurbitae* populations of male and female (Figure 4.5). The discriminant function score shows 97.92% correct classification between sexes, whereas best cross-validation score shown 96.53% correct, indicating that DFA good quality results. Transformation grid indicated the source of wing shape variation (wing shape change) different landmark in two sexes (Figure 4.6). Wireframe graph depicting the average wing shapes of male and female (Figure 4.7). The box plot showed different

wing size of female larger than male of *Z. cucurbitae* population (Figure 4.8). Variances at each landmark in consensus configuration analysis demosatrated that landmark 6 $(S^2=0.000301)$ was is the highest contribution to the different between male and female and landmark 12, 1, 5 and 10 (S²; LM12=0.000146, LM1=0.000145, LM5=0.000103, LM10=0.000091) introduced the largest contribution on wing shape difference (Table 4.6).



Figure 4.4 Scatter plot of the first two principal component (PC1 and PC2) of wing shape variations base on 12 landmarks. The light blue circle was represents male and dark blue circle represent female.



Figure 4.5 A discriminant scores between male and female of *Zeugodacus cucurbitae* constructed using wing shape.



Figure 4.6 Transformation grid shows wing shape change for each landmark.



Figure 4.7 Wireframe graph depicting the average wing shapes of male (light blue) and female (dark blue) of *Zeugodacus cucurbitae*.



Figure 4.8 Box plot of centroid size between male and female of *Zeugodacus cucurbitae*.



Table 4.5 The effect of sex, host plant, geography and their interaction on centroid size and wing shape of Zeugodacus cucurbitae in Thailand.

Character	Effect	SS	MS	df	F	Р
Centroid size	Sexes	9.6x10 ⁻⁵	9.6x10 ⁻⁵	1	91.22	< 0.0001
	Host plants	2.2x10 ⁻⁵	2.5x10 ⁻⁴	9	0.44	0.8966
	Geography	2.0x10 ⁻⁴	5.0x10 ⁻³	4	0.09	0.9832
	Sexes and host plants and geography	1.2x10 ⁻⁶	4.6x10 ⁻⁴	27	22.32	< 0.0001
	Error	2.8x10 ⁻⁵	1.1×10^{-4}	26		
Shape	Sexes	0.1035	0.0052	20	66.26	< 0.0001
	Host plants	0.0250	0.0001	180	0.42	1.0000
	Geography	0.0117	0.0002	80	0.51	0.9998
	Sexes and host plants and geography	0.1441	0.0003	540	6.64	< 0.0001
	Error	0.0406	0.0001	520		

SS: sum of squares; MS: mean squares; df: degree of freedom; F: F-criterion; P: P-value

Landmark	S^2X	S ² Y	S^2
1	0.00011849	0.00002642	0.00014491
2	0.00003153	0.00003410	0.00006563
3	0.00004941	0.00003354	0.00008296
4	0.00007136	0.00001820	0.00008955
5	0.00007541	0.00002783	0.00010324
6	0.00019660	0.00010474	0.00030134
7	0.00001384	0.00001287	0.00002671
8	0.00001415	0.00001218	0.00002633
9	0.00002290	0.00002907	0.00005197
10	0.00007688	0.00001452	0.00009140
11	0.00005084	0.00002705	0.00007789
12	0.00010711	0.00003917	0.00014628

Table 4.6 Variances at each landmark of Zeugodacus cucurbitae in Thailand

Canonical variate analysis (CVA) revealed no separation between groups based on wing shape variation of *Z. cucurbitae* from different host plants and geographic locations. The first canonical variable (CVA1) accounted for 24.62%, the first and second together (CVA1+CVA2) showed 46.93% variance among hosts plants (Figure 4.9) and geographic locations. The result of CVA indicated that morphological variation was not separated according to geographic location. The first and second canonical variable axes describe 60.90% (CV1=34.23% and CV2=26.70%) (Figure 4.10).





Figure 4.9 CVA scatter pot of canonical variable based on wing shape of *Zeugodacus cucurbitae*. Each circle represents a specimens and was labeled according to the host plant (BA: Sponge gourd, FF: Wax gourd, FK: Gac fruit, FT: Pumpkin, KD: Redball snake gourd, MA: Bitter gourd, MG: Mango, TA: Muskmelon, TG: Cucumber, TL: Ivy gourd) (full detail of host plants indicated in Table 3.2).





Figure 4.10 CVA scatter pot of canonical variables based on wing shape variation analysis of *Zeugodacus cucurbitae*. Each circle represents a specimen and was labeled according to geographic origin (C: Central, N: Northern, S: Southern, NE: Northeastern, W: Western).

CHAPTER 5 DISCUSSION AND CONCLUSION

5.1 Discussion

5.1.1 Genetic variation and demographic history

Zeugodacus cucurbitae in Thailand exhibited low genetic variation (maximum nucleotide diversity = 0.0064). Nucleotide diversity in Thai *Z. cucurbitae* is much lower than other fruit fly species, such as *B. dorsalis* complex (0.0200) (Shi *et al.*, 2012), *B. tryoni* (0.0180) (Blacket *et al.*, 2012), *B. latifrons* (0.0086) (Meeyen *et al.*, 2013) and *B. correcta* (0.0325) (Kunprom *et al.*, 2015). Low genetic diversity observed in *Z. cucurbitae* in Thailand is consistent with those reports from other geographic regions, including China, Southeast Asia (Hu *et al.*, 2008) and India (Prabhakar *et al.*, 2012) which found low genetic variation in this species.

Genetic diversity of the species could be affected by population history (Hewitt, 1996, 2000). Demographic history analysis revealed that Z. cucurbitae has undergone recent population demographic expansion date back to the late Pleistocene (approximately 140,000 years ago). During the Pleistocene glaciations, although the ice sheet did not directly covered Southeast Asia but the climatic conditions were cooler and drier (Voris, 2000; Penny, 2001). Most of the rainforest was wiped out and replaced by savannah grassland and pine forest (Brandon-Jones, 1998). Previous studies in forest-dependent insects revealed the impact of this historical climatic change on genetic structure and diversity of the species including anopheles mosquito, Anopheles dirus (O'Loughin et al., 2008; Morgan et al., 2011) and black fly, Simulium tani (Pramual et al., 2005). Because life cycle of the fruit flies was depend on availability of the host plants, thus the Pleistocene climatic change could also affect their population. The most recent glaciations ended about 18,000 years ago. However, many species in Southeast Asia respond to the penultimate glaciations (about 135,000 years ago) (Cannon and Manos, 2003; O'Loughin et al., 2008; Morgan et al., 2011) because they had a more severe impact than the most recent (Brandon-Jones, 1998). Thus, signal of the population expansion in Z. cucurbitae dates back to 140,000 years ago could be the respond of this fruit fly to the increasing of the host plants after the recovery of the climate from cool and dry to warm and moist conditions.

5.1.2 Population genetic structure

Population genetic structure analysis revealed that 51% of the comparisons were genetically significantly different. Significant genetic differentiation between populations suggest a limitation of gene flow. Geographic barriers, such as large mountain ranges and habitat fragmentation, and species dispersal have been found to be important factors that limit gene flow in fruit flies (Aketarawong et al., 2007; Hu et al., 2008; Wu et al., 2011; Shi et al., 2014; Molina-Nery et al., 2014). Zeugodacus cucurbitae occupies a wide range of elevations from sea level up to 1,600 m above sea level (Vayssières et al., 2008). Zeugodacus cucurbitae is a multivoltine, polyphagous fruit fly that utilizes more than 125 host plant species (White and Elson-Harris, 1992). Most of these plants are widely distributed in Thailand in both natural forest and agricultural areas (Allwood et al., 1999). An experimental study found that Z. cucurbitae moved over 200 km, presumable in search of host plants and mates (Miyahara and Kawai, 1979). Considering its broad habitat range and high dispersal ability, physical barriers, such as mountains and fragmented land use patterns, seem to be unlikely explanations for the genetic structure detected among Z. cucurbitae populations in Thailand. Shared haplotypes between geographically largely isolated populations with no significant relationship between genetic and geographic distances also suggest a minor role for physical barriers.

The most likely explanation for genetic differentiations of some populations of *Z. cucurbitae* in Thailand is genetic drift. The random sampling of the alleles from the source population of the colonizer could lead to the genetic differentiation of the population by the effect of genetic drift (Excoffier and Ray, 2008). Two populations (RNG and PBI) that contributed markedly to the genetic structuring of *Z. cucurbitae* in Thailand possessed very low haplotype diversity, supporting the idea that genetic drift is driving genetic differentiation. Effects of genetic drift on population differentiation have been reported in other fruit fly species, such as *B. carambolae*, *B. dorsalis* (Boykin *et al.*, 2014; Aketarawong *et al.*, 2007, 2015), *Ceratitis capitata* and *Anastrepha fraterculus* (Morgante *et al.*, 1985; Reyes and Ochando, 1997, 1998; Alberti *et al.*, 1999, 2002; Gilchrist *et al.*, 2006).

Two northern populations (CMI and MSN) also showed high degrees of genetic differentiation. These populations possessed haplotypes that are highly diverge from the

main haplotype group as revealed in the MJ network analysis. Although these haplotypes were collected from the same host plant species as many other populations, they were from different sources. Ivy gourd (*Coccinia grandis*), the host plant for populations CMI and MSN occurred from natural forests, while for other populations this plant occurred in fruit orchards. A similar situation has been reported in other fruit fly species in Thailand. Meeyen *et al.* (2013) found that *B. latifrons* collected from fruit orchard and forest areas show significant genetic differentiation. Thus, it is possible that populations CMI and MSN were significantly different from other populations because they derived divergent haplotypes from the natural forest host plant species which is now invaded the *Coccinia grandis*, the host plant that widely distributed in both forest and agricultural areas.

Comparisons of genetic divergent among host plant species found no evidence of genetic isolation. All host plant species including in this study shared at least one common COI haplotype. However, many COI haplotypes are unique to particular host plant species. For example, 21 haplotypes out of 27 found in Ivy gourd (*C. grandis*), three out of four haplotypes in mango (*M. indica*) and gac fruit (*M. cochinchinensis*) are unique. The high degree of haplotype uniqueness suggests that there are some limitations of gene flow among the host plant species.

5.1.3 Morphological variation

Landmark-based geometric morphometric method based on wing shape was applied to compare and visualize the morphological variation between sexes, host plants and geography among *Z. cucurbitae* populations in Thailand. The results only indicated significant morphological differentiation between sexes. Maximum differentiation was related to the junction of veins A_1 and CuA_2 (Landmark 6), junction of vein R_1 and costal vein (Landmark 12), terminal of vein R_{2+3} (Landmark 1), junction of vein CuA_1 and dm-cu (Landmark 5) and junction of vein M and r-m cross vein (Landmark 10), respectively. Sivinski and Dodson (1992) revealed that females of tephritidae fly are larger than males, therefor wing size of females were larger than that of males in *Z. cucurbitae* populations (result is shown). However, several previous studies demonstrated that body size and wing size of fruit flies are influenced by environmental variables such as temperature and larval food sources (Hoooper, 1978; Krainacker *et al.*, 1987; Kitthawee and Rungsri, 2011). In present study, *Z. cucurbitae* population was collected from natural habitats in different times of the year that exhibit variation in environmental conditions (see detail in Table 3.1). Thus, seasonal variations of environmental conditions could also contributed to morphological variation. Kitthawee and Rungsri (2011) investigated morphological variation in *Z. tau* complex on *momordica cochinchinensis* using wing geometric morphometric analysis and found that both wing size and wing shape of *Z. tau* complex collected from different seasons were different (Kitthawee and Rung sri, 2011).

In the present study, wing shape of *Z. cucurbitae* was differences between male and female based on relative maximum area of the junction of veins A₁ and CuA₂ (Landmark 6). Similar results were reported in *B. dorsalis* complex, where the junction of veins A₁ and CuA₂ is the most contributed to wing shape variation and may play a functional role in the mating systems of the fly (Schutze *et al.*, 2012a). Therfore, wing shape variation in *Z. cucurbitae* possibly related to a functional role of wing in the mating systems as in *B. dorsalis* complex. In Neotropical tephritidae genus *Blepharoneura*, wing shape variations may influence audible signals produced during courtship (Marsteller *et al.*, 2009).

5.2 Conclusion

Analysis of mitochondrial DNA sequences revealed that *Z. cucurbitae* in Thailand has undergone population expansion possibly in respond to the increasing of the host plant following climatic recovery of penultimate Pleistocene glaciations. Some populations show high degree of genetic differentiation due to effect of genetic drift. No genetic isolation among flies from different host plant species, but many haplotypes are unique to the host species. This indicates that there are some degrees of genetic isolation between *Z. cucurbitae* from different host plant species.

Geometric morphometric analysis of morphological variation found no differentiation for wing shape and size between host plants and geography. However, male and female of *Z. cucurbitae* show significant different in wing shape size. Maximum differentiation was related to the junction of veins A₁ and CuA₂ (Landmark 6) on the wing of the fly. Sexual dimorphism in wing shape and size of *Z. cucurbitae* possibly related to environmental conditions during the collection time. Environmental variables, behavior and flight performance could also play a role in wing variation. However, morphological variation in *Z. cucurbitae* and other fruit fly species requires additional studies.



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