Reproductive biology of *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae)

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Abstract

The ichneumonid *Diadegma semiclausum* Hellen has been recorded in many parts of the world as an important parasitoid of diamondback moth, *Plutella xylostella* (Linnaeus), a serious pest of brassica vegetable crops worldwide. Some aspects of reproductive biology were studied in controlled laboratory conditions of 21±1°C, 16:8 h (light:dark) and 50-60% RH. *Diadegma semiclausum* adults emerge only during the photophase. It has a protandrous emergence pattern because the male developmental time is shorter than the female. Most males emerge in the first half of the photophase whereas females emerge during the second half. Both males and females become sexually mature in <12 h after emergence. When paired with 3-d-old virgin mates, more newly emerged females (<12-h-old) mate, compared to newly emerged males. Females, immediately after eclosion (<1-h-old), do not carry mature eggs in their ovaries, and hence this is a strong synovigenic species. Maternal age affects the egg load, which reaches the maximum by 8 d after emergence. Egg resorption occurs in host deprived females and the number of mature eggs declines with age when >20 d. Females can mature eggs without a food supply, suggesting that it is an autogenous species. Host and plant cues do not enhance the initiation of the egg maturation process in newly emerged females. The longevity of adults *D. semiclausum* is immensely affected by food availability. Sugar fed individuals live 15-20 fold longer than those not provided with food or water after emergence. Females live longer than males. Body size does not affect the longevity of males, but large females live longer than small females, in the absence of food. A single mating does not affect the longevity of either males or females. Egg laying reduces the longevity of the females. Males show active courtship behaviour. Mating success increases with an increasing mate age. Female age is more important than male age, for mating success. Body size does not affect mating success. Neither age nor body size affects the premating and mating periods. Adults mainly mate during the photophase. Repeated mating occurs in continuously paired males and females. A single male can repeatedly mate the same female up to 5 times during 16 h of pairing. Mating duration and mating intervals decrease non-linearly in subsequent matings, after the first mating.

Females oviposit during the photophase. More eggs are laid in the first few oviposition bouts. More fertilised eggs are deposited in the first two oviposition bouts.

Host stage affects body size and developmental time of offspring. The developmental period of the parasitoid is the longest if the host is parasitied at the second instar. Males developing in the third and fourth instars and females in the third instar DBM larvae have the largest developmental periods. The host stage does not affect the sex ratio, parasitism rate and emergence rate. The sex ratio is highly male-biased. Parasitoids produce more female offspring, at higher host density conditions. Parasitism rate is inversely related to the host density. Fecundity is greater at a higher host density. At the density of 30 hosts, a *D. semiclausum* female can parasitise > 600 larvae in her lifetime. Longevity, host larvae mortality, parasitoid emergence rate and egg load at death, are not affected by host density. The parasitism rate reduces with the increase of the female's age. The sex ratio becomes increasingly male-biased, with the increase of age in parasitisng females.

Acknowledgements

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CHAPTER 1
GENERAL INTRODUCTION

1.1   Introduction

Diadegma semiclausum Hellen (Hymenoptera: Ichneumonidae) is one of the most important larval parasitoids of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Waterhouse & Norris 1987; Talekar & Shelton 1993). This parasitoid was introduced into New Zealand from Europe in 1936 (Muggeridge 1939; Beck & Cameron 1992; Fitton & Walker 1992; Talekar & Shelton 1993) and later into many other Asia-Pacific regions (Waterhouse & Norris 1987; Talekar & Shelton 1993). It is now a dominant parasitoid of DBM in Australia and New Zealand (Goodwin 1979; Beck & Cameron 1992; Furlong & Zalucki 2007), as well as in the relatively cooler highlands of other Asia-pacific regions (Talekar & Shelton 1993; Saucke et al. 2000). In the relatively hotter lowlands of many Asia-Pacific regions, *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) is the dominant larval parasitoids of DBM (Talekar & Yang 1991; Talekar & Shelton 1993; Saucke et al. 2000). *Cotesia plutellae* was also imported into New Zealand, but it was never released (Beck & Cameron 1992), since it possesses the ability to parasitise non-target species in several families of Lepidoptera (Cameron & Walker 1998). Compared to generalist species, such as *C. plutellae*, *D. semiclausum* is a specialist that attacks mainly DBM larvae, with a greater efficiency to locate hosts and a better adaptation to the host's defensive behaviour (Chua & Ooi 1986; Wang & Keller 2002). In East Africa, the introduction of *D. semiclausum* has displaced the existing indigenous species such as *Oomyzus sokolowskii* (Kurdjumov) (Hymeoptera: Eulophidae), *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichenumonidae) and *Apanteles* sp., suggesting that it is a superior parasitoid in DBM management (Gichini et al. 2008).

DBM is one of the most destructive cosmopolitan pests of crucifer crops. It shifts to new crops, such as sugar snap and snow peas and subsequent habituation, leading to host range expansion of this pest (Zhang & Liu 2006). It is the first pest that has been able to develop resistance to DDT (Shelton et al. 1993; Lingappa et al. 2004; Muhammad et al. 2005). It has now developed resistance against all classes of insecticides including biopesticides, such as crystal toxins from *Bacillus thuringiensis*

and spinosyns from *Saccharopolyspora spinosa* (Tabashnik et al. 1991; Tabashnik et al. 1997; Abhishek & Ashok 2005; Sarfraz & Keddie 2005). Reports have suggested that the New Zealand populations have already developed resistance to permethrin (Cameron et al. 1997).

In certain parts of the world, economic production of crucifers has become almost impossible, due to insecticidal failures to control this pest (Muhammad et al. 2005). DBM is causing a huge economic burden to crucifer growers. Apart from direct crop losses caused by DBM, an estimated cost of US$ 1 billion per year is required for its management (Talekar & Shelton 1993). The high resistance of DBM, to the major classes of insecticides and biopesticides, has led to increasing interest in the role of parasitoids, in the biologically based integrated management of DBM, which aims to reduce reliance on insecticides (Talekar & Shelton 1993; Liu & Yan 1998). The host plant's availability and the action of natural enemies are the two key biotic factors that regulate DBM populations in the field (Muckenfuss et al. 1990; Kfir 1997).

1.2   Role of Natural Enemies in DBM Management

Historically, DBM populations were suppressed below the economic threshold, in the United States and Europe, by natural enemies (Marsh 1917; Mustata 1992). Due to the absence of proper natural enemies, a problem with DBM is being reported in some countries (Talekar & Lin 1998). Natural enemies may significantly reduce the DBM populations (Muckenfuss et al. 1990; Ooi 1992), for example, in South Africa, parasitism of DBM by parasitoids may reach up to 90~100% (Kfir 1997). Due to the significant role of natural enemies, classical biological control against DBM has attracted a great deal of attention. The first effort in classical biological control of DBM took place in New Zealand, where a solitary larval endoparasitoid, *D. semiclausum*, was introduced from England in 1936 (Beck & Cameron 1992; Talekar & Shelton 1993). This parasitoid was established as a major larval parasitoid of DBM, in the field. In 1989, both *D. semiclausum* and *C. plutellae* were introduced into the Philippines, in order to manage DBM (Rejesus 2001). The successful establishment of parasitoids and the adoption of the parasitoid-based IPM technology thereafter, for DBM control, produced multiple benefits including an increase in yield and income (up to 75%) and a reduction in production and pesticide costs (up to 41% and 80%, respectively): As a result, environmental pollution and the destruction of beneficial arthropods were

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reduced in the parasitoid introduced areas (Rejesus 2001). Release of *D. semiclausum*, in order to manage DBM on cabbage production in Kenya, has resulted in an increased economic surplus of US$ 28.3 million, over 25 years (Macharia et al. 2005).

Despite some successful cases, the failure of *D. semiclausum*, in managing the DBM population, has also been reported. Populations of parasitoids and pests are dynamic in nature and they are affected by both biotic and abiotic factors. For example, asynchrony between DBM and *D. semiclausum* populations in New Zealand (Beck & Cameron 1992; Cameron et al. 1997) may be the factor that limits parasitoid population growth, making it difficult to successfully suppress these pest populations. Moreover, as reported in other countries, such as south-east Queensland, Australia, *D. semiclausum* achieved a high level of parasitism (≈ 85%) of DBM larvae, in the early crop season, but it failed to respond to the increasing DBM populations, later in the season (Beck & Cameron 1992; Cameron et al. 1997). Although the introduced parasitoids, together with native species, are currently suppressing the DBM populations, the control is not adequate and it still needs to be effectively incorporated into modern IPM programmes (Beck & Cameron 1992). The reason for this failure of DBM control is still poorly understood.

1.3   Relevance of the Study

A periodical release in the field may be necessary, if the existing parasitoids fail to control DBM. The biology and ecology of *D. semiclausum* have been studied to some extent, for example, the role of food sources in the longevity and fecundity (Winkler et al. 2006); their response to host-infested plants (Ohara et al. 2003b); patch leaving decision (Wang & Keller 2003; Wang & Keller 2004); and interaction with other parasitoids sharing the same host (Yang et al. 1994; Rossbach et al. 2005). However, knowledge in relation to effective mass rearing, such as reproductive behaviour, is still lacking for this parasitoid.

Knowledge of the reproductive biology of parasitoids is crucial to the development of integrated pest management (IPM) or biological control programmes, based on augmentative or inoculative releases (Waage & Ming 1984; Bautista et al. 1999; Persad & Hoy 2003). The reproductive behavioural strategies of insects are usually determined from the pre-adult stage. For example, the emergence pattern is

usually associated with the time needed for sexual maturation, and protandry (where males emerge earlier than females) and this may provide sufficient time for males to become sexually mature and inseminate the females, soon after emergence (del Castillo & Nunez-Farfan 2002; Doyon & Boivin 2006). Furthermore, egg production of parasitoids differs with species and it is related to their life-history strategies (Jervis et al. 2001). It is a dynamic process and is affected by several abiotic and biotic factors. A study of such reproductive traits may help to determine the ability of a parasitoid to suppress the pest population, based on the female's egg maturation capacity during her life time (Kapranas & Luck 2008); to match host supplies with egg availability; to determine a suitable age for parasitoids to be used in the laboratory or field release, in order to maximise their reproductive output (Riddick 2005; Bokonon-Ganta et al. 2007); and to provide a solid foundation for the study of parasitoid foraging and oviposition behaviour (Donaldson & Walter 1988; Minkenberg et al. 1992).

Most hymenopteran parasitoids are haplodiploid, i.e. males developing from unfertilised eggs and females from fertilised eggs. In such species, mating is essential in order to ensure the production of both male and female progeny (Quicke 1997). Thus, an understanding of the mating behaviour and a consideration of factors, which may increase mating success, might contribute to the development of efficient rearing techniques in the laboratory. From a biological control point of view, obtaining female progeny is more desirable, since they are directly involved in pest management. Host size may affect the fitness of parasitoids, such as body size (Nakamura & Noda 2002), development (Jenner & Kuhlmann 2006) and sex ratio (Ueno 1998). Thus, insect body size may be an important fitness trait, which is correlated with longevity, fecundity, progeny production, inseminating capacity and mating success (Ueno 1999b; Wang 2002; Jiménez-Pérez & Wang 2004; Wang & Zeng 2004; He & Wang 2008).

In New Zealand, emphasis has been given to the development of an integrated pest management (IPM) programme for brassica vegetables, in order to counter increasing levels of insecticide resistance in DBM (Beck & Cameron 1992). Development of IPM techniques, including the application of biological control agents, can reduce the reliance on chemical pesticides and it can also increase the value of harvested produce. For example, in the Philippines, the combination of *D. semiclausum* releases and BT applications has reduced the use of chemical insecticides sprays by at least 50-70% (Amend & Basedow 1997). The study of biology and the reproductive characteristics of *D. semiclausum* can help in the development of suitable IPM practices.

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However, very little is known about its basic biological and reproductive characteristics such as, emergence, sexual maturation, egg maturation and mating behaviour. In addition, an understanding of the role host size and density play, in parasitoid population growth, development, survival and sex allocation behaviour, will provide valuable information relating to host quality consideration, in order to increase the fitness of the parasitoids and the development of an efficient mass-rearing programme. This will also enhance the efficiency of field releases, by ensuring the quality of the released parasitoids.

1.4   Aim and Objectives

The overall aim of this research is to understand aspects of reproductive biology of *D. semiclausum* and the specific objectives are to:
  (1) investigate the emergence and sexual maturation period of *D. semiclausum*;
  (2) study egg maturation dynamics in *D. semiclausum*;
  (3) investigate factors affecting the longevity of *D. semiclausum*;
  (4) study the mating and oviposition behaviour of *D. semiclausum*;
  (5) understand the effect of host stage on body size, developmental time, emergence rate and sex ratio of *D. semiclausum*; and
  (6) to study the effect of host density in the reproductive behaviour of *D. semiclausum* such as fecundity, reproductive duration, parasitoid longevity, sex ratio, and parasitism rate.

CHAPTER 2
LITERATURE REVIEW

This chapter reviews the available literature and it primarily focuses on factors that can affect the reproductive fitness of parasitoids. Emphasis will be on the review of literature related to *D. semiclausum.*

2.1   Parasitoids as Potential Biocontrol Agents of DBM

Biological control represents the action of living organisms in suppressing the abundance or activity of pests (Mills & Wajnberg 2008). A number of natural agents are known to attack DBM, thus limiting its population growth. As shown in Figure 2.1, these natural enemies include parasitic wasps, predators and diseases (Alam 1992; Sarfraz et al. 2005). Amongst these, parasitoids are the most important group, in the context of the biological control of DBM.
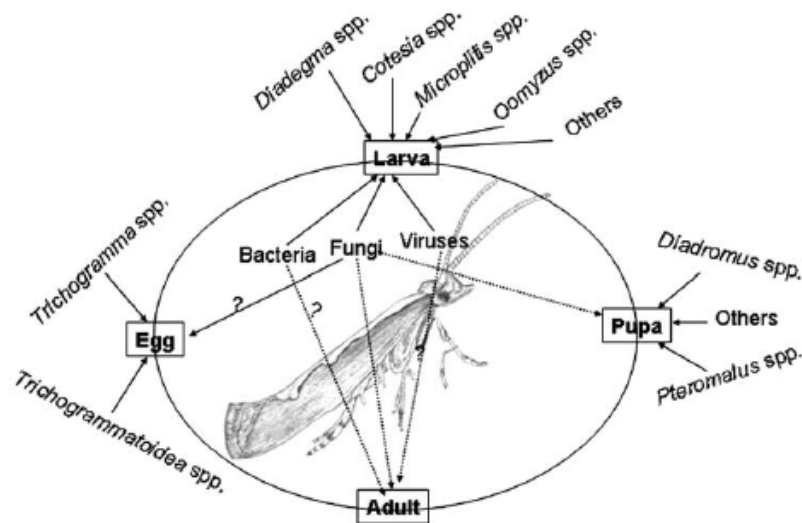
Figure 2.1 Biological control agents of DBM [From Sarfraz et al. (2005)].

Parasitoids are of immense importance in natural and agricultural ecosystems, where they influence or regulate the population density of many of their hosts (Godfray 1994). More than 135 parasitoid species have been recorded, which attack various stages of DBM throughout the world (Delvare 2004). The most important are six egg, 38 larval, and 13 pupal parasitoids (Lim 1986; Talekar & Shelton 1993). Amongst parasitoids that attack the DBM (Jankowska & Wiech 2006), only a few species

belonging to the ichneumonid genera *Diadegma* and *Diadromus*, braconid genera *Microplitis* and *Cotesia*, and eulophid genus *Oomyzus*, are considered to be predominant DBM parasitoids (Sarfraz et al. 2005).

2.2   *Diadegma* Species Attacking DBM

The genus *Diadegma* (Hymenoptera: Ichneumonidae, Campopleginae) represents a large group of parasitic wasps with 201 species known to occur worldwide (Yu & Horstmann 1997). *Diadegma* species are considered to be effective wasps that attack DBM larvae (Lim 1986).

Several *Diadegma* species, such as *D. fenestralis* (Holmgren), *D. insulare* (Cresson), *D. leontiniae* (Brethes), *D. mollipia* (Holmgren), *D. rapi* (Cameron), *D. chrysostictos* (Gmelin), *D. armillata* (Gravenhorst), *D. blackburni* (Cameron), and *D. semiclausum*, are reported to attack DBM (Rusinov 1977; Chua & Ooi 1986; Fitton & Walker 1992; Wagener et al. 2004; Wagener et al. 2006). However, many of these earlier studies on *Diadegma* were misleading, due to a lack of correct taxonomic identification and therefore, they need to be considered carefully (Azidah et al. 2000; Wagener et al. 2004). Poorly resolved taxonomy is still a major factor, which limits the exploitation of *Diadegma* species in a biological control (Azidah et al. 2000). A recent extensive study on *Diadegma* species, by Azidah et al. (2000), found seven distinct morphospecies (see section 2.5), together with their distribution: *D. fenestrale* from the Palaearctic and South-Asia to Philippines; *D. insulare* from the Nearctic to the northern Neotropical and some Pacific islands, including Hawaii; *D. leontiniae* in the southern Neotropical; *D. mollipla* in the Afrotropical, including some Indian Ocean and South Atlantic islands; *D. novaezealandiae* in New Zealand; *D. rapi* in Southeastern Australia; and *D. semiclausum* in the Palaeartic. Amongst *Diadegma* species, *D. semiclausum* is the best known parasitoid of DBM (Fitton & Walker 1992) and it has been introduced into many parts of the world. Recent progress has been made in the identification of *Diadegma* species, using molecular tools (Wagener et al. 2004). There is a wide geographic variation in the predominance of *Diadegma* species, which attack DBM larvae (Mustata 2001).

2.3 Host Range of *Diadegma* Species

Although many *Diadegma* species are formerly thought to have wide host ranges (Hardy 1938), most *Diadegma* species are relatively host-specific (Fitton & Walker 1992). This host range can be restricted to one or more species. For example, *D. semiclausum* parasitises only the larvae of DBM (Abbas 1988; Wang & Keller 2002), whereas *D. armillata* attacks several species from Plutellidae (Dijkerman 1990).

2.4 *Diadegma semiclausum* and its Role in DBM Management

2.4.1 Important Biological Characteristics

Diadegma semiclausum is a solitary larval endoparasitoid of DBM, where only one larvae can survive to adult, from each superparasitised larva (Yang et al. 1994). It is thought to have originated from Eurasia (Fitton & Walker 1992).

2.4.2 Use in Biological Control

Diadegma semiclausum has been released in many countries for the control of DBM and encouraging results have been obtained (Wang & Keller 2002). Due to its potential efficiency, *D. semiclausum* has been introduced from the UK to New Zealand (Muggeridge 1939) and subsequently to Indonesia (Vos 1953), Australia (Wilson 1960), Malaysia (Ooi 1992), Taiwan (Talekar 1996), the Philippines (Poelking 1992; Amend & Basedow 1997), Japan (Noda et al. 2000) and African countries, such as Kenya, Tanzania and Uganda (Gichini et al. 2008). Four parasitoid species including *D. semiclausum*, *D. collaris*, *Tetrastichus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) and *Macromalon orientale* Kerrich (Hymenoptera: Ichneumonidae), were introduced into the Cameron Highlands Malaysia, in the mid 1970s, from India, New Zealand, Australia and Indonesia (Ooi & Lim 1989). Only *D. semiclausum* and *D. collaris* were successfully established (Ooi 1986; Ooi 1992).

2.4.3 Pest Control and Parasitism

Being a host specialist, *D. semiclausum* has evolved sophisticated strategies to locate and parasitise DBM, making them more successful than generalists (Wang & Keller 2002). Following release, the successful control of a DBM population has been

reported in many studies. Field studies in Taiwan reported that *D. semiclausum* could provide an adequate control of DBM (Talekar & Yang 1991). In the Papua New Guinea Highlands, the release and successful establishment of *D. semiclausum* since 1995, have substantially reduced crop losses caused by DBM (Saucke et al. 2000). Ooi (1992) reported that the DBM populations in Malaysia were reduced by approximately 50% after the introduction and release of *D. semiclausum*. In most cases, the parasitism rate by *D. semiclausum* may be up to 70% (Lee et al. 1995; Iga 1997; Walker et al. 2002; Kwon et al. 2003; Furlong & Zalucki 2007). Parasitism rates up to 95% have been observed in south-east Queensland, Australia, during winter and early spring (Furlong & Zalucki 2007) and in the Philippines (Poelking 1992). In Japan, *D. semiclausum* was released into a cabbage field in Tokyo, in 1991, for the first time (Iga 1997). A single release of *D. semiclausum* produced 33% parasitism of DBM larvae in 1991 and four releases in 1992 and 1993 generated more than 70% parasitism (Iga 1997). Similar successful suppression of DBM populations, by multiple releases of *D. semiclausum*, has been reported in northern Japan (Noda et al. 2000). This parasitoid currently continues to suppress pest populations, together with the native *D. novaezealandiae,* in New Zealand, although not at a desirable level (Beck & Cameron 1992; Cameron et al. 1997).

2.5   Identification and Taxonomy of *D. semiclausum*

The taxonomic position of *D. semiclausum* is as follows:

Kingdom -- Animal
   Phylum -- Arthropoda
      Class -- Hexapoda
         Order -- Hymenoptera
           Family -- Ichneumonidae
             Subfamily-- Campopleginae
               Genus -------- *Diadegma*
                 Species ----------*Diadegma semiclausum*

Azidah et al. (2000) made a detailed taxonomic treatment of *Diadegma* species, based on morphometric measurements. They gave the following diagnostic characters of *D. semiclausum*:

- female adult has a distinct sharp ovipositior (Figure 2.2);

- females are larger than males, with head to body end length being 5.00-7.04 and 4.44-6.36 mm for females and males, respectively;

- ovipositor (the part exerted beyond the apex of the last metasomal tergite) is approximately 0.43 to 0.87 mm and 0.42-0.60 times hind tibial length;

- female and male antenna consist of 21~25 and 23~27 flagellomeres respectively;

- hind tibia is pale to yellow with subbasal and apical dark brown bands (Figure 2.3);

- vein 3rs-m of forewing usually present; vein M receives vein 2m-cu distinctly after the middle of the areolet (Figure 2.4).
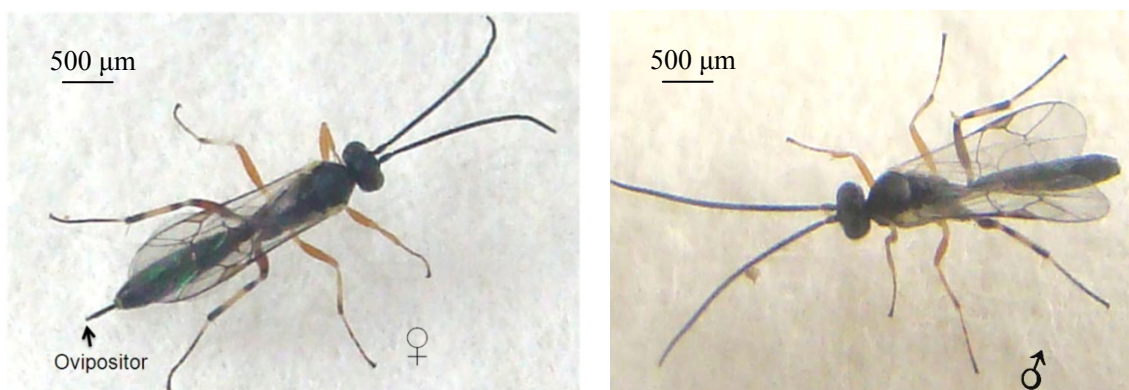
Figure 2.2 Adults of *D. semiclausum*. These pictures were taken in the present study.
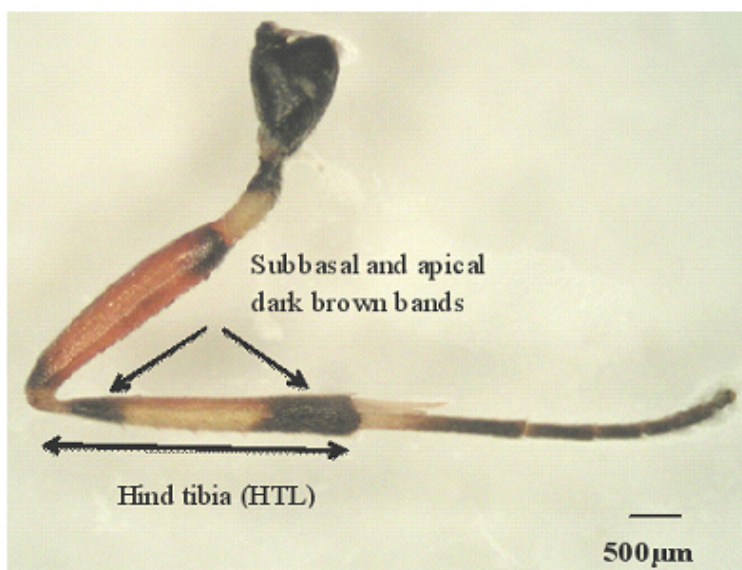
Figure 2.3 Hind tibia of *D. semiclausum*. This picture was taken in the present study.
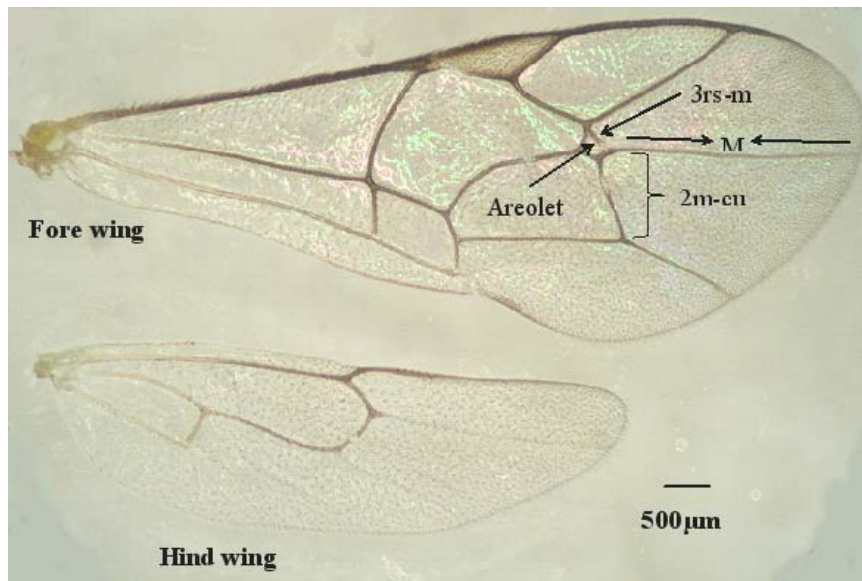
Figure 2.4 Wings of *D. semiclausum*. This picture was taken in the present study.

2.6   Developmental Stages of *D. semiclausum*

Diadegma semiclausum has complete metamorphosis, consisting of four distinct stages: egg, larva, pupa and adult (Figure 2.5).

2.6.1   Egg

Eggs are semicircular and cylindrical and approximately 0.22 mm long and 0.05 mm wide (Figure 2.5A). The newly laid egg is translucent. Eggs can be found in thoracic and abdominal segments of DBM larvae, immediately after oviposition (Talekar & Lin 1998). At 25ºC, the incubation period is approximately 38 hours (Abbas 1988).

2.6.2   Larva

As shown in Figure 2.5B, the newly emerged larva is transparent. The body is narrow and long with wider a anterior end and a narrow, pointed posterior end. It is approximately 0.67 mm long and 0.14 mm wide. The length of the tail tube is approximately 0.15 mm. When the larva is 4 to 5 d old, it becomes stout, its tail structure at the end of abdomen disappears (Figure 2.5C) and the digestive canal and body regions become clearly visible (Talekar & Lin 1998). There are five larval instars

(Abbas 1988) and the larval period is approximately 5.4 days at 25ºC (Abbas 1988). However, Huang et al. (2009) have reported only four instars.

2.6.3 Pupa

The cocoon is cylindrical and approximately 5 mm long and 1.64 mm wide (Figure 2.5F). Initially the cocoon is grey but later it turns dark and finally to brown, at the emergence time. The pre-pupa is light yellow in colour and approximately 4.6 mm long and 1.32 mm wide (Figure 2.5D). The pupa is approximately 4.5 mm long and 1.24 mm wide (Figure 2.5E) (Talekar & Lin 1998). At 25ºC, the pre-pupal and pupal periods are approximately 33 hours and 5.9 days, respectively (Abbas 1988).

2.6.4 Adult

Diadegma semiclausum is a small black wasp, approximately 5-7 mm long (Figures 2.2 and 2.5H). The female has a long ovipositor (Talekar & Lin 1998).
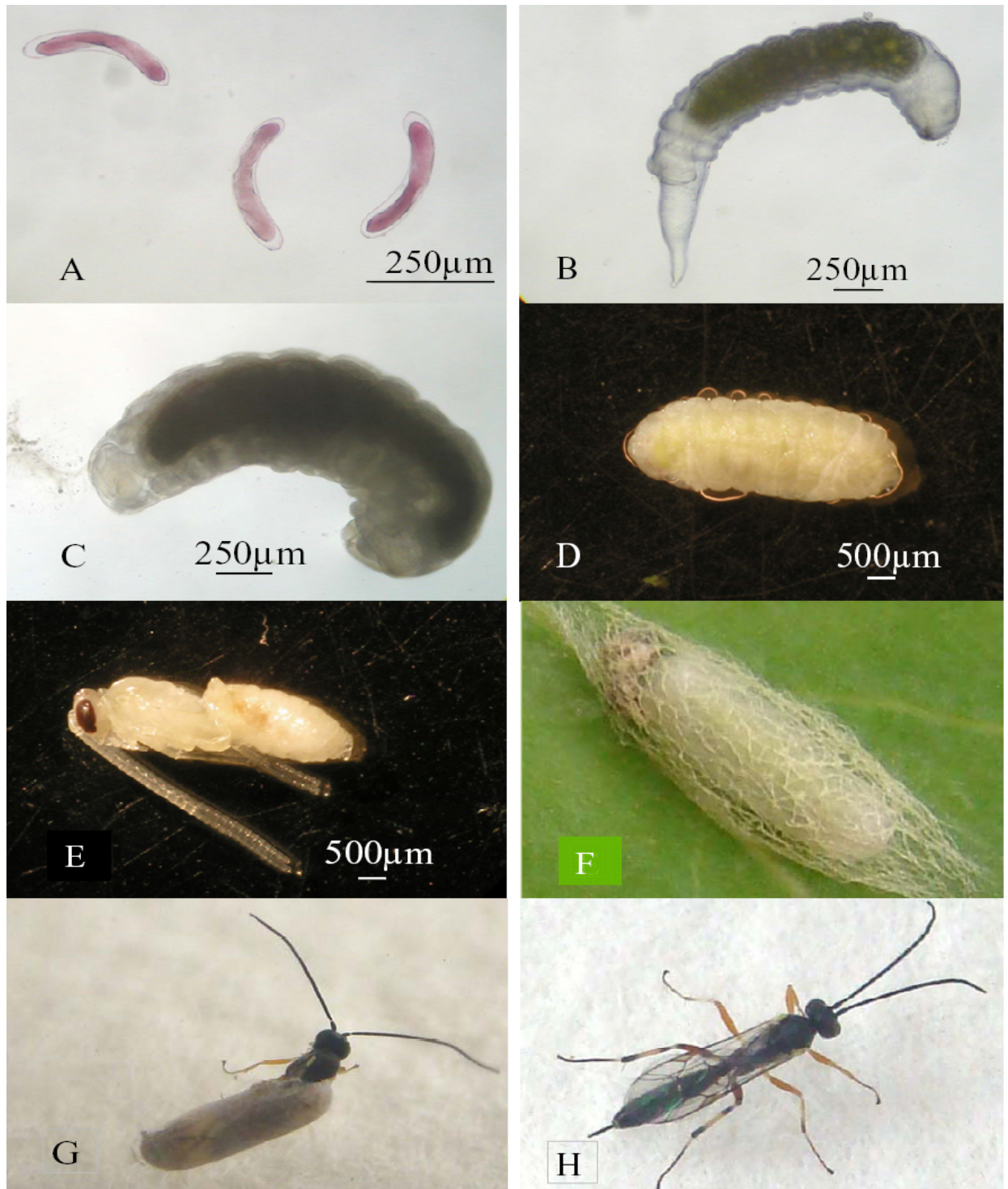
Figure 2.5 Life stages and development of the *D. semiclausum*: (A) eggs; (B) larva (4 days after oviposition); (C) larva (6 days after oviposition); (D) prepupa (8 days after oviposition); (E) pupa in cocoon (9 days after oviposition); (F) cocoon (12 days after oviposition); (G) adult emerging from the cocoon; and (H) adult. All pictures were taken in the present study.

2.7   General Biology of *D. semiclausum*

Diadegma semiclausum females usually lay one egg into the host larva and can deposit 14 eggs per day, with a total number of 164 eggs during their lifetime (Abbas 1988). However, Winkler et al. (2006) reported that nectar fed females parasitise an average of 390 ± 31 DBM larvae. After hatching, larvae are active and they move easily inside the DBM larvae (Talekar & Lin 1998). Parasitoid larvae consume the hosts' tissue and leave a cuticle before pupation (Huang et al. 2008a). Parasitism results in decreasing growth, feeding, excretion, assimilation and respiration of the DBM larvae. It also reduces the efficiency of the conversion of ingested and digested food to the body substance of DBM larvae (Huang et al. 2008a). When host larva becomes prepupa, the parasitoid larva eats the prepupa and larval skin, and the excreta of the parasitoid larva is pushed to the posterior end of the prepupa, in order to prepare for pupation (Talekar & Lin 1998). Later, it forms its own cocoon inside the webbing of the DBM cocoon (Talekar & Lin 1998). The adult gnaws a small circular hole at the anterior end, climbs out from the hole, flutters its wings and flies away (Talekar & Lin 1998).

Temperature plays a significant role in the growth and development of *D. semiclausum* (Yang et al. 1993; Lee et al. 1995; Golizadeh et al. 2008). The developmental period, from egg to adult, is approximately 21.5, 21.1, 16.6 and 13.3 days, at 20, 22, 25 and 28ºC, respectively (Lee et al. 1995) and the lower temperature threshold for development of eggs, larvae, pupae and adults is 5.7, 3.8, 5.9 and 6.6ºC, respectively (Yang et al. 1993).

Availability of food plays a significant role in the longevity and reproductive capacity of *D. semiclausum*. At 25ºC, parasitoid adults survive and lay eggs for 28 days when provided with diluted honey as food, but for only three days when deprived of food (Yang et al. 1993). Abbas (1988) reported that (at the same temperature) the longevity is 11.2 and 14.6 days for males and females, respectively, when fed honey.

2.8   Host Location and Searching Behaviour of *D. semiclausum*

The foraging behaviour of *D. semiclausum* has been well studied (Wang & Keller 2002; Wang & Keller 2003; Wang & Keller 2004; Bukovinszky et al. 2007). *Diadegma semiclausum* is highly attracted to both uninfested and infested crucifer plants: However, females are more attracted to host infested plants than uninfested

plants and the origin of attraction is believed to be plant odour, rather than the host and their associated products, such as faeces, silk and exuviae (Ohara et al. 2003b). Rossbach et al. (2005) also reported that they are only attracted to crucifer host plant odours, with no response to peas infested with DBM. Upon arrival onto the host infested plants, the females initiate antennal contact with host-damaged sites and search for hosts (Ohara et al. 2003a). Females employ both antennae and ovipositor in their host searching and they also use visual perception, when in the immediate vicinity of hosts (Wang & Keller 2002). The searching females show a stereotype sequence of behaviour (antennate-groom-still-fly), whilst moving over the plant, before encountering the hosts (Wang & Keller 2002). The host location and searching strategies of *D. semiclausum* females are more adaptive to the defensive behaviour of the hosts, and they are more effective in detecting and parasitising the hosts, than other important parasitoids, such as *C. plutellae* (Wang & Keller 2002). *Diadegma semiclausum* females show aggregative behaviour and they spend more time in high host density patches, although density dependent parasitism has not been observed (Waage 1983). This patch leaving tendency decreases with increasing interpatch distance; unsuccessful host encounter; the presence of host damage; increasing host density; and clustered host distribution, but it increases with the successful oviposition and oviposition rate (Wang & Keller 2003; Wang & Keller 2004).

Learning by females could change their foraging behaviour (Li & Liu 2003). *Diadegma semiclausum* females show significant preference for host larvae with plant volatiles, which they had previously experienced (Li & Liu 2003). Searching for hosts and oviposition occurs only during the photophase and parasitism does not occur during darkness (Talekar & Yang 1991) and thus, the duration of day length could have a significant impact on their parasitism and sex allocation behaviour (Sagarra et al. 2000).

2.9   Reproductive Biology

2.9.1   Emergence, Sexual Receptivity and Mating

Most *D. semiclausum* adults emerge during the first few hours of the photophase (Yang et al. 1993). Abbas (1988) suggested that *D. semiclausum* can mate immediately after the emergence and mating can occur during the day and/or night, but scarce information is available, regarding the sexual maturation and mating of *D. semiclausum*.

2.9.2 Reproductive System

The reproductive organs of most parasitoid females comprise a pair of ovaries, each with several ovarioles, and oviducts, spermatheca, vagina, ovipositor and associated glands, including venom glands (Jervis et al. 2005). The female reproductive organs of *D. semiclausum* were preliminarily described by Huang et al. (2008b) and they are shown in Figure 2.6. The ovary is of the polytrophic type. Each ovary is composed of 12 ovarioles and each ovariole is differentiated into a terminal filament, germarium, and vitellarium. Most mature oocytes are located nearest to the common oviduct and the others, which are progressively less mature, are located towards the germinal epithelium where division of the germ cells occurs (Figure 2.6). Mature oocytes are encased in a continuous single layer of cells within the separate oviducts. When the mature oocytes are ready to oviposit, secretions from the venom gland and Dufour's gland are added (Huang et al. 2008b).

The male reproductive system of *D. semiclausum* has not been reported in any literature. A preliminary dissection of the male reproductive system of *D. semiclausum* (Figure 2.7) (in the present study) shows similarity to the caterpillar-hunting wasp *Ancistrocerus antilope* (Panzer) (Hymenoptera: Vespidae), as described by Bushrow et al. (2006). It consists of testes, seminal vesicles, deferent ducts, accessory glands, and ejaculatory ducts (Figure 2.7). Sperm produced from the testis becomes mixed with the contents of the accessory gland from the vas deferens before ejaculation (Quicke 1997).
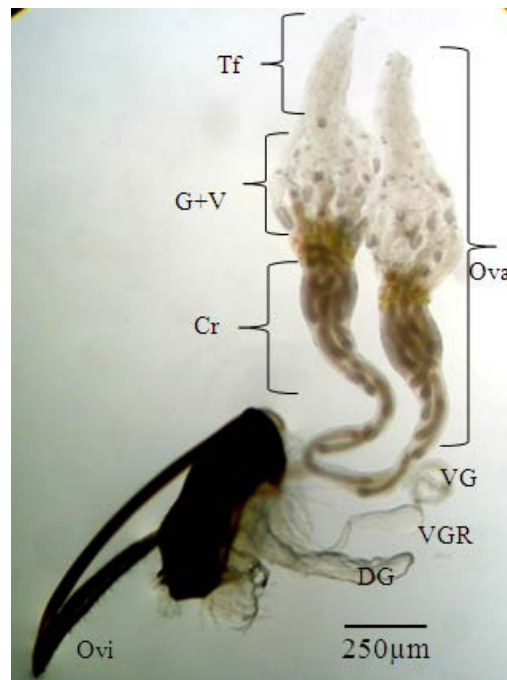
Figure 2.6 Female reproductive system of *D. semiclausum*. Ova – ovary; Tf - terminal filament; G – germarium; V – vitellarium; Cr – calyx; VG - venom gland; VGR - venom gland reservoir; DG - Dufour's gland; and Ovi - ovipositor. This picture was taken in the present study.

Figure 2.7 Male reproductive system of *D. semiclausum*. T – testis; VD - vas deferens; AG - accessory gland; and ED - ejaculatory duct. This picture was taken in the present study.

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2.9.3   Egg Maturation

Parasitoids differ in the proportion of their potential life time complement of eggs, which the females carry at emergence. In pro-ovigenic species, females have all their mature eggs at emergence whilst, in synovigenic species, females mature eggs during their adult stage (Flanders 1950; Quicke 1997; Boivin et al. 2005). However, some species probably fall in between these two extremes and they are termed pro-synovigenic, i.e. females carry some mature eggs at emergence but continue to produce and mature more eggs throughout their adult lifespan (Quicke 1997; Jervis et al. 2001). Jervis et. al. (2001) extensively examined the egg maturation characteristics of 638 parasitoid wasp species belonging to 28 families. They reported that only 1.8% of these species were strictly pro-ovigenic but synovigeny is, so far, the predominant egg maturation pattern (98.12% of species). Huang et al. (2008b) reported that *D. semiclausum* is a synovigenic species and females need several days to fill their ovaries with mature eggs after eclosion. However, they have not precisely discussed the egg maturation dynamics in *D. semiclausum*. Another important *Didegma* species, *D. insulare* Cresson (Hymenoptera: Ichneumonidae), is a pro-synovigenic species (Lee et al. 2004; Lee & Heimpel 2008), wich emerges with approximately 24 mature eggs in the ovaries (Lee et al. 2004).

2.9.3.1 Factors Affecting Egg Maturation

Egg maturation is a dynamic process in most insect parasitoids and is affected by a number of factors (Papaj 2000; Wu & Heimpel 2007). Several factors, such as age (Bokonon-Ganta et al. 2007), body size (Eliopoulos et al. 2003), host stimuli (Papaj 2000; Bokonon-Ganta et al. 2007), oviposition (Ramadan et al. 1995; Wang & Messing 2003), food supply (Riddick 2007; Kapranas & Luck 2008) and mating status (Lee et al. 1998) are known to affect the egg maturation process. Riddick (2006) suggested that egg load is positively related to the body size of lab-cultured *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). Host stimuli (host and host infested plant odours), for example, as in the egg-larval parasitoid of the Mediterranean fruit fly, *Fopius ceratitivorus* Wharton (Hymenoptera: Braconidae), are known to induce egg maturation (Bokonon-Ganta et al. 2007). The act of oviposition, which significantly increases the rate of egg maturation, has been reported in *F. arisanus* (Sonan)

(Hymenoptera: Braconidae) (Wang & Messing 2003; Wu & Heimpel 2007). *Metaphycus flavus* Howard and *M. luteolus* Timberlake (Hymenoptera: Encyrtidae), parasitoids of soft scale insects, are strictly synovigenic and they mature eggs only with a carbohydrate supply (Kapranas & Luck 2008). The egg maturation process in *D. semiclausm* has been poorly addressed, so far. There is some evidence that food supply may promote egg maturation of *D. semiclausum* (Wratten et al. 2003). However, detailed information on the potential factors, which influences the egg maturation dynamics of this species, is lacking.

2.9.3.2 Egg Maturation Period

The period required to mature their eggs varies in different species. In *F. arisanus*, females require 4-6 days to mature eggs, after emergence (Wang & Messing 2003). In *Biosteres vandenboschi* (Fullaway) (Hymenoptera: Braconidae), a parasitoid of the early-instar of oriental fruit fly, newly emerged females have an average of 4.2 mature eggs and their ovarian maturation peaks 12 days after emergence, with 41.2 mature eggs (Ramadan et al. 1995). In *F. ceratitivorus*, ovarian maturation peaks 5 days after emergence, with 61.6 mature eggs and it then declines thereafter (Bokonon-Ganta et al. 2007). An understanding of parasitoid egg maturation period is very important, in relation to biological control programmes, because it provides the necessary guidelines on when the parasitoids should be used for augmentative release, or used in laboratory mass rearing, for the parasitising of host larvae. Egg morphology and its subsequent development, after oviposition of *D. semiclausum* eggs, has been studied (Huang et al. 2009). Females need several days after eclosion to fill their ovaries with mature egg (Huang et al. 2008b). However, there have not been any studies undertaken to particularly gain an understanding of egg maturation dynamics and reabsorption in *D. semiclausum.*

2.9.4 Factors Affecting the Reproductive Fitness

The parasitoid's fitness is the integral outcome of the behaviours performed by a parasitoid in its lifetime (van Baalen & Hemerik 2008). The lifetime reproductive success of parasitoids has important implications for our understanding of the evolution of life-history strategies and the demographics of host–parasitoid interactions (Godfray

1994). The parasitoid's fitness is affected by several important factors, which I review as follows:

2.9.4.1 Host Stage

Parasitoid larvae feed on the bodies of other arthropods and they eventually kill their hosts. Parasitoid adults are free-living and the main task of females is to find and parasitise hosts, for the next generation (Quicke 1997; Godfray 2004). Thus, hosts provide the entire nutritional and physiological environment for the immature development of the parasitoids (Sequeira & Mackauer 1992). Foraging parasitoid females may encounter hosts of different sizes or stages during their reproductive lifespan, and thus, host quality evaluation by females plays a significant role in their reproductive fitness. Smaller hosts may contain insufficient resources for the parasitoids to complete their development (Elzinga et al. 2003) , thus reducing their fitness (Harvey et al. 1995). Whilst larger hosts are expected to be more profitable than smaller hosts in terms of parasitoid fitness, as larger hosts contain a greater quantity of resources than smaller hosts (Harvey 1996). Consequently, parasitoids developing in larger hosts will benefit in terms of increasing adult size (King 1988; Ueno 1998); the proportion of female progeny produced (Charnov 1979; Godfray 1994; Ueno 1999b; He & Wang 2006b); and egg load at emergence (Visser 1994; Mills & Kuhlmann 2000; He & Wang 2006b). However, larger hosts may be able to better defend themselves immunologically, than smaller ones (Strand & Pech 1995). As a result, female parasitoids must develop strategies in order to find a balance between their fitness return and oviposition costs (Chau & Mackauer 2001).

Several studies have shown that *D. semiclausum* females prefer second and third over first and fourth instars of DBM larvae, for oviposition (Lee et al. 1995; Monnerat et al. 2002; Cai et al. 2005). However, information is lacking about the role of host quality in the reproductive fitness of this species. Moreover, the role of host quality, in sex ratios, is not univocally supported (Abbas 1988; Yang et al. 1993; Kwon et al. 2003).

2.9.4.2 Host Density

Host density influences different fitness components of parasitoids, by affecting their sex ratio (Asante & Danthanarayana 1993), longevity (Bai & Smith 1993) and parasitism rate (Ferreira De Almeida et al. 2002). The relationship between host density and sex ratio is not consistent across species, or even within species across studies (King 1987). Izraylevich & Gerson (1995) reported a male-biased sex ratio in *Hemisarcoptes coccophagus* Meyer, in higher host density, because the immature female progeny suffered higher mortality. van Dijken et al. (1991) demonstrated that, in *Epidinocarsis lopezi* (DeSantis), the proportion of male progeny increases from 44% at low host density, to 70% at high host density. In analytical host-parasitoid models, changes in the density-dependent sex ratio influence the level of equilibrium populations, in addition to the stability of the host-parasitoid relationship (Hassell & Waage 1984) and (in turn) it determines the success or failure of the biological control (Hassell 1986). General life-history theory predicts that reproduction has a cost against survival (Roff 2002). A trade-off between reproduction and survival is likely to occur, when the parasitoid encounters a different host density (Pexton & Mayhew 2002).

2.9.4.3 Body Size

Body size is an important determinant of parasitoid fitness (Bai et al. 1992; Visser 1994; Kazmer & Luck 1995; Ode et al. 1996; Cloutier et al. 2000; He & Wang 2006a). For example, larger females may have greater longevity (Visser 1994; Ueno 1999b; He & Wang 2006a); better male attraction through sex pheromone (Cloutier et al. 2000); higher fecundity (Bai et al. 1992; Visser 1994; He & Wang 2006a); higher dispersal and host searching ability (Visser 1994; Ellers et al. 1998; He & Wang 2006a); greater oviposition success (Ueno 1999b); and an innate capacity for increase (Cloutier et al. 2000). Larger males may live longer (Ode et al. 1996; He & Wang 2006a) and thus gain advantage in competition for mate access, in addition to having a higher insemination capacity (Ode et al. 1996; He & Wang 2006a). Although less studied, the body size of parasitoids is also known to affect the sex ratio of the offspring. For example, larger *Anaphes nitens* Girault (Hymenoptera: Mymaridae) females produced more female offspring, compared to the smaller ones (Serena Santolamazza-Carbone 2007). Similarly, larger males of *Aphidus ervi* Haliday resulted in the production of a

female-biased population, for longer periods than the smaller males (He & Wang 2006a).

2.9.4.4 Age and Mating History

As mentioned earlier, most hymenopteran insects, including *D. semiclausum*, are haplo-diploid. Therefore, mating plays an essential role in the production of female offspring. Besides the direct impact on the carryover of the generation, mating may also affect other fitness components, such as longevity, lifetime fecundity and progeny production. Mating can stimulate oogenesis and egg maturation, leading to a significant reduction in the female lifespan (Ellers 1996; Wheeler 1996; Jacob & Evans 2000). In fact, several studies have reported the negative impact of mating on the longevity of both male (Sagarra et al. 2002; Burton-Chellew et al. 2007) and female parasitoids (Li et al. 1993b; Jacob & Evans 2000).

Mating may also influence the foraging behaviour of parasitoids. For example, mated females may have higher fecundity (Tagawa et al. 1987) and more actively search for hosts (Sousa & Spence 2000), thereby reducing superparasitism rate (Sagarra et al. 2002). In parasitoids, females are usually monandrous and males are polygynous (Teder 2005; He & Wang 2008). After several matings, males may suffer sperm depletion, thus ensuring their mates produce more male offspring (King 2000).

CHAPTER 3

EMERGENCE, SEXUAL MATURATION AND ADULT LONGEVITY OF *DIADEGMA SEMICLAUSUM*

3.1   General Introduction

The biology and ecology of *D. semiclausum* have been studied, to some extent (Abbas 1988; Yang et al. 1993; Kwon et al. 2003; Winkler et al. 2006). The developmental biology of the parasitoid is affected by the ambient environmental conditions and laboratory methods (Chong & Oetting 2006b) and the strains of the insects (Ellers & van Alphen 1997). The significant impact of temperature on the biological characteristics, such as the development and sex allocation behaviour of this species, has been reported in many studies (Yang et al. 1993; Lee et al. 1995; Liu et al. 2001; Golizadeh et al. 2008). *Diadegma semiclausum* is a potential biocontrol agent in the management of DBM. The ability of this specialist parasitoid, to control DBM may provide a great opportunity in the mass culture and field releases of this species, when necessary. Although emphases have been placed on the use of this species, in DBM management, less attention has been given to an understanding of its biological characteristics, which are vital for laboratory mass rearing and field releases (Bautista et al. 1999).

This chapter describes the general methodology applied throughout this research and it investigates the emergence and sexual maturation, egg maturation dynamics and longevity of *D. semiclausum* with the aim to improve our understanding of its fundamental biology and efficiency, as a primary biological control agent of DBM.

3.2   General Methodology

The materials, procedures, environmental conditions and definitions, detailed in this section, were used throughout this thesis.

3.2.1   Materials and Methods

Breeding colony: The general methods for maintaining breeding colony are shown in Figure 3.1. The breeding colonies of DBM and *D. semiclausum* were initiated

from parasitised and healthy DBM larvae, which were collected from a commercial farm in Palmerston North, New Zealand, during January 2008.
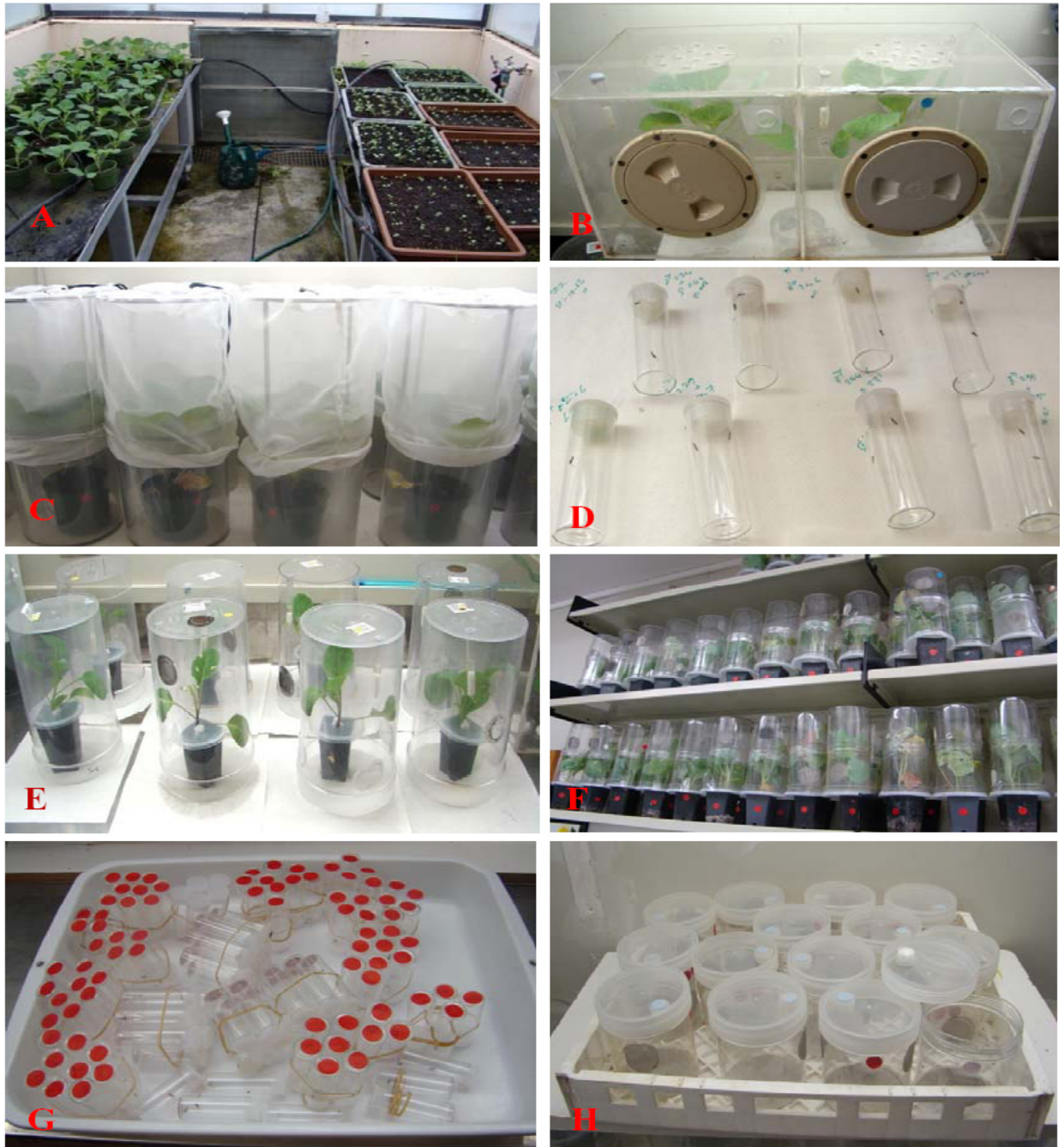
Figure 3.1 Rearing and maintenance of DBM and *D. semiclausum* colonies: (**A**) cabbage seedlings grown in glasshouse; (**B**) plexiglass cages maintaining cabbage seedlings for DBM oviposition; (**C**) plastic jars for rearing DBM larvae; (**D**) glass vials as mating arenas for *D. semiclausum*; (**E**) oviposition chamber maintaining cabbage seedlings infested by DBM larvae for *D. semiclausum* oviposition; (**F**) rearing of parasitised DBM larvae on cabbage seedlings in plastic cylinders for pupation; (**G**) *D.*

semiclausum pupae maintained individually in glass vials for emergence; and (**H**) DBM and *D. semiclausum* adults maintained separately in plastic containers and fed on a honey solution. All these pictures were taken during the present study.

The DBM host plants, cabbages (Summerglobe hybrid, *Brassica oleracea* var *capitata*) (Terranova seeds Pty Limited, Australia), were grown in the glasshouse (Figure 3.1A). The DBM larvae were reared on potted cabbage seedlings in plexiglass cages (30 × 30 × 30 cm), with two holes (13 cm in diameter) made on the top and bottom of the cage and covered with a metal mesh (aperture size = 0.25 mm) for ventilation (Figure 3.1B). One side of the cage was fitted with a circular opening (16 cm in diameter) for handling the seedlings and insects in the cage (Figure 3.1B). 20 newly emerged DBM adults (10 males and 10 females) were released into a plexiglass cage containing a potted cabbage seedling (6-8 weeks old). After 24 h, the seedling, together with the DBM eggs was removed and maintained in a transparent plastic jar (25 cm in height × 17 cm in diameter) (Figure 3.1C). A circular metal frame covered with a nylon net (aperture size = 0.25 mm) was kept on top of the jar, in order to prevent the newly emerged larvae from moving away from the plant (Figure 3.1C).

Virgin males and females of *D. semiclausum* were paired in glass vials (7.5 cm in height × 2.5 cm in diameter) for mating (Figure 3.1D). In order to maintain the parasitoid colony, a once-mated parasitoid female was released into a plastic chamber (25 cm height × 17 cm diameter), with two circular openings (5 cm in diameter) covered with fine metal mesh (aperture size = 0.25 mm) for ventilation (Figure 3.1E). Each chamber contained a cabbage seedling, together with 10 3rd or 4th instar DBM larvae. After 24 h, the parasitised larvae were removed and reared in the same infested seedling until pupation, in a plastic container (10 cm in height × 8.5 cm in diameter) with two circular holes (5 cm in diameter) covered with fine metal mesh (aperture size = 0.25 mm) for ventilation (Figure 3.1F). Pupae were maintained in individual glass vials (4 cm in height × 2.5 cm in diameter) until emergence (Figure 3.1G) and then used for further parasitisation of the DBM larvae. Both DBM and parasitoid adults were fed with a 10% honey solution soaked in cotton balls (0.5 cm in diameter) (Figure 3.1H).

Electronic scale: The pupae of both species were weighed individually, using an electronic balance (Mettler Toledo, AG135, Switzerland) with a readability of 0.01 mg.

Microscope: A stereomicroscope (Leica MZ12, Germany) equipped with a micrometer eyepiece was used for measuring morphological measurement and egg count.

Photographs: Unless stated otherwise, all photographs presented in this thesis were taken using a digital camera (Canon Power Shot A360, China).

3.2.2   Environmental Conditions

The insects were maintained and all experiments carried out in bioassay rooms in the Entomology and IPM Laboratory, Massey University, at $21 \pm 1°C$, $50 \pm 10\%$ RH and 16:8 h (light:dark) (lights on at 08:00 h and off at 24:00 h). Lighting was provided by high frequency broad-spectrum biolux tubes (Osram, Germany).

3.2.3   Definitions of Biological Parameters for *D. semiclausum*

Fecundity is the total number of parasitised DBM larvae.

Egg load is the number of mature eggs in the ovaries.

Fertility is the total number of female progeny produced.

Sex ratio is the proportion of male progeny.

Reproductive period is the number of days during which the female lays eggs.

Adult longevity is the number of days in which the adult lives.

3.2.4   Statistical Analysis and Reported Values

Rejection level was set at $P < 0.05$ for all data. All analyses were carried out using SAS STAT (SAS Institute 2006). Unless stated otherwise, all reported values are mean ± SE.

3.3   Emergence Pattern and Sexual Maturation

3.3.1   Introduction

Insect emergence events are usually rhythmic and often species-specific (Beck 1980; Saunders 1982). This temporal rhythm is controlled by an intrinsic circadian

oscillation, such that emergence occurs at a particular time of day or night. The emergence of insects is mediated by exogenous factors, such as light and photoperiod (Kamm 1974) or endogenous factors, such as a hormone, which is stimulated by exogenous factors (Truman 1972). In insects, such emergence is sex-biased, resulting in either protandry (males emerging before females) (Bulmer 1983; Sawada et al. 1997; Letters 2001) or potogyny (females emerge before males) (Buck 2001). For example, males of *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae), a larval parasitoid of the tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), emerge two days before females in a generation, and approximately 1 h before females, on any given day (Hirose & Vinson 1988). The emergence pattern of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is protognous, where the female emergence skews towards being earlier, than that of the male emergence (Van Timmerman et al. 2001). Nevertheless, in some species, the emergence patterns of male and female adults are remarkably similar, with no evidence for protandry or protogyny, such as that in the psyllid, *Diaphorina citri* Kuwayama (Wenninger & Hall 2007). Reasons why insects show such different emergence patterns is the subject of interests to behavioural ecologists. Most predominantly, such behaviour is considered to be an evolutionary strategy of insects, which enhances their reproductive fitness (Wiklund & Fagerström 1977; Wang et al. 1990; Wedell 1992; Doyon & Boivin 2006).

Most studies, which focus on this emergence pattern, have been undertaken in lepidopteran species, particularly butterflies (Wiklund & Fagerström 1977; Iwasa et al. 1983; Forsberg & Wiklund 1988; Baughman 1991), with a handful of studies on hymenopteran parasitoids (He et al. 2004). In the parasitic hymenopterans, such rhythmicity is often synchronised with mating (Gordh & DeBach 1976; Nadel & Luck 1985). Many opine braconid species mate immediately after emergence (Hagen 1953), whilst in others such as, *Fopius arisanus*, there is compulsory premating period (Hagen 1953; Quimio & Walter 2000).

An understanding of parasitoid emergence and sexual maturation patterns may enhance our knowledge about the reproductive biology, which is instrumental when considering *D. semiclausum* for the biological control of DBM. This study, therefore, aims to gain an understanding of the emergence pattern and sexual maturation of *D. semiclausum* adults.

3.3.2   Materials and Methods

3.3.2.1 Emergence

In order to determine the daily emergence pattern of *D. semiclausum* adults, a mated female was released into a jar (Figure 3.1E) containing 30 third instar DBM larvae, which were feeding on a cabbage seedling. The female was allowed to stay for 24 hour for oviposition and then removed. Parasitised DBM larvae fed on the cabbage seedling until pupation. The seedling was replaced with an uninfested one, when necessary. Once the parasitoids pupated, they were individually maintained in a glass vial (4 cm in height × 2 cm in diameter) for emergence. Emergence was recorded hourly during the photophase and the emerged adults were sexed. In order to determine whether the parasitoids emerged during the scotophase, or not, emergence was observed immediately after lights on. The emergence pattern was determined by recording the 603 adults (457 males and 146 females) that emerged during the photophase period. The developmental duration, from oviposition to emergence, was calculated for both sexes.

3.3.2.2 Sexual Maturation

In order to investigate the sexual maturation period of *D. semiclausum*, two treatments were set up: (1) 3-day-old virgin females were paired individually with newly emerged males (<12 h) and (2) 3-day-old virgin males were paired individually with newly emerged females (<12 h). Each pair was maintained and observed in the above mentioned glass vial, for 12 h continuously. The premating period (time period between pairing and copulation) and mating duration (time period from genitalia connection to disconnection) were recorded using a stop watch. In order to determine the possibility of the parasitoids remating, each pair was maintained in the same glass vial, after first mating, until completion of 12 h observation period. The mating duration of multiple mating(s) was also recorded. There were 26 and 34 pairs (replicates) for treatments (1) and (2), respectively. Parasitoids, which had emerged from DBM parasitised at third instar larvae, were used in these experiments.

3.3.2.3 Statistical Analysis

Data on the emergence and mating success were analysed using a Chi-square test. Data on the developmental duration of adults were not normally distributed and they were analysed using a Kruskal-Wallis test, followed by Dunn's procedure for multiple comparison. Data on the premating period and mating duration were analysed by ANOVA, followed by a Tukey's studentised range test. The relationship between the number of matings and mating duration was analysed with a linear regression.

3.3.3 Results

3.3.3.1 Emergence

Diadegma semiclausum adults emerged only in the photophase. Both males and females displayed a daily pattern of emergence. Male emergence peaked in the first 2 h, following lights on, with significantly higher number of males emerging during the first half of the photophase, than during the second half (Chi-square test: $\chi^2 = 42.28 > \chi^2_{1,0.05} = 3.8$, P < 0.001) (Figure 3.2). Female emergence concentrated between 9 and 14 h, after lights on, with significantly higher number of females emerging during the second half of the photophase, than during the first half (Chi-square test: $\chi^2 = 6.17$, P < 0.05) (Figure 3.2).
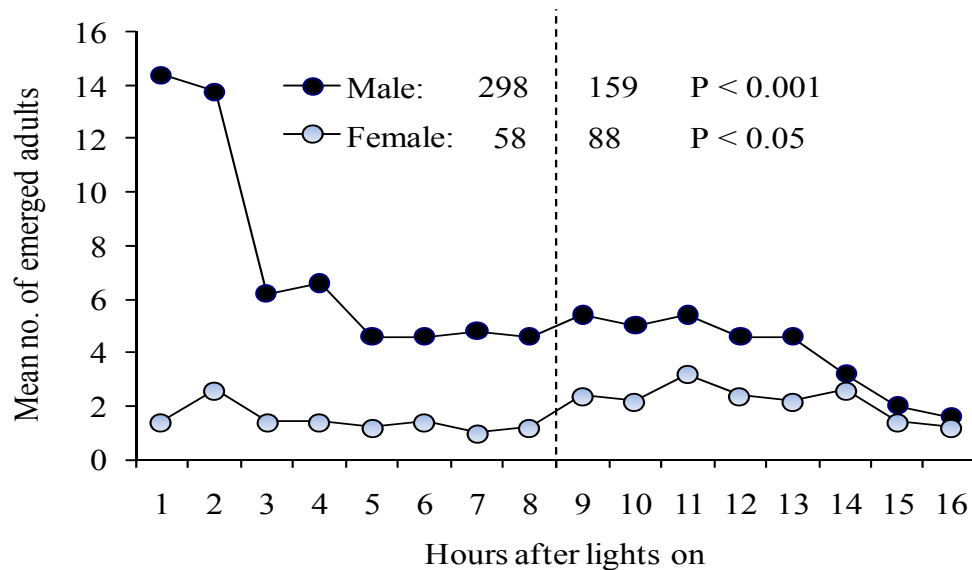
Figure 3.2 Mean hourly number of *D. semiclausum* males and females emerging throughout the photophase.

The developmental duration from eggs to adults was significantly shorter for males (mean ± SE = 15.63 ± 0.05 d), than that for females (16.27 ± 0.08 d) (Kruskal-Wallis test: $\chi^2 = 30.28 > \chi^2_{1,0.05} = 3.84$; P < 0.001).

3.3.3.2 Sexual Maturation

Both males and females were able to mate <12 h, after emergence. After perceiving the presence of a female, the male started chasing and grasping her in order to mount and achieve genital contact. Once the male was able to connect his genitalia with that of the female, mating occurred. Results show that mating success was significantly higher in newly emerged females, than in newly emerged males, when paired with 3-day-old mates (Chi-square test: $\chi^2 = 22.11 > \chi^2_{1,0.05} = 3.8$, P < 0.001) (Table 3.1). However, mate age did not significantly affect the premating period (ANOVA: $F_{1,16} = 0.11$, P > 0.05) and mating duration (ANOVA: $F_{1,10} = 0.03$, P > 0.05) (Table 3.1).

Table 3.1 Mean premating period (h) and mating duration (min); and mating success (%) of *D. semiclausum* adults

	Premating period	Mating duration	Mating success
3-day-old female × <12-h-old male	5.09 ± 3.99 a	6.70 ± 0.40 a	6.25 b
3-day-old male × <12-h-old female	5.84 ± 0.69 a	7.00 ± 0.22 a	57.70 a

Means (± SE) followed by the same letters in each column are not significantly different (P > 0.05).

When females were allowed to remain continuously with the same males, after the first mating, 3-day-old females failed to remate, whilst approximately 45% (5 out of 11) of newly emerged females (<12 h) remated, with a mean number of 2.60 ± 0.24 matings. The mating duration of newly emerged females increased significantly with the increasing number of matings (Linear regression: $F_{2,11} = 12.40$, P = < 0.05) (Figure 3.3).
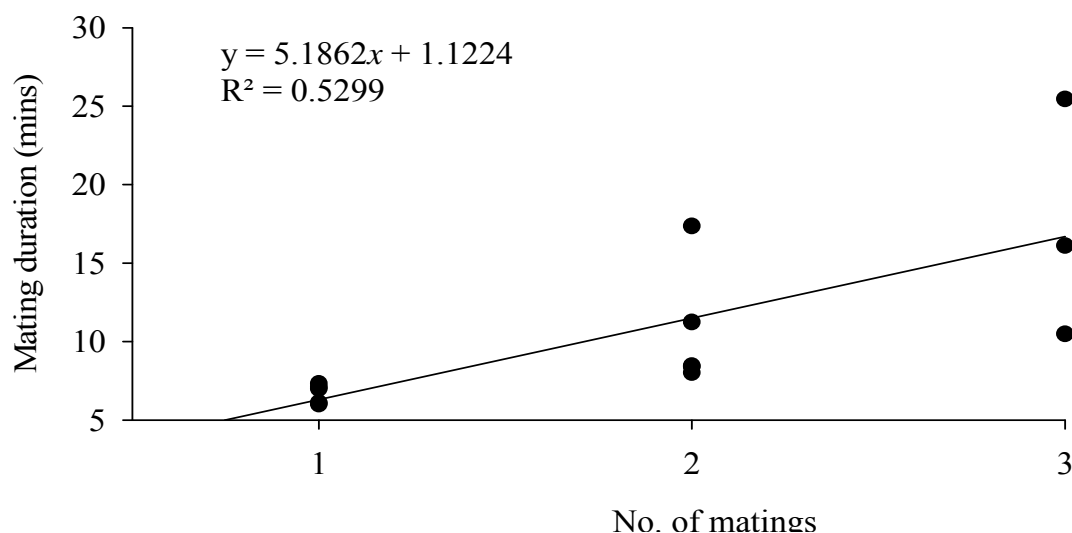
Figure 3.3 Relationship between mating duration (mins) and number of matings in newly emerged *D. semiclausum* females.

3.3.4 Discussion

The emergence pattern of parasitoids usually links to behavioural adaptations and reproductive strategies, such as feeding, mating and oviposition (He et al. 2004). Results from the present study and another study by Yang et al. (1993) show that *D. semiclausum* only emerges during the photophase. The rhythmicity of adult emergence, during the day, is well known amongst hymenopteran wasps, such as *A. ervi* (He et al. 2004) and several *Trichogramma* species (Pompanon et al. 1995). My study also demonstrates that the rhythmical emergence of *D. semiclausum* is stimulated by light. This emergence pattern is apparently linked to mating (Section 4.2.4.4) and oviposition (Section 4.3.4), which mostly occurs during the day time.

My study indicates that *D. semiclausum* is a protandrous species, as reported in many Hymenopteran parasitoids (Pompanon et al. 1995; Doyon & Boivin 2006). Several studies have assessed the adaptive value of protandry in the reproductive success of males, in insects. For example, protandry may increase the reproductive fitness of males, by providing males with more mating opportunities, if the reproductive success of males is correlated with which the number of females they mate (Waage & Ming 1984). The main advantage of protandry for males is the early access to females, as the latter emerge (Doyon & Boivin 2006). Quicke (1997) suggested that the protandry is common in many parasitoids, because a late-emerging male is likely to encounter already mated females and therefore he is genetically doomed, since he can

not find any matings. A similar advantage of protandry is expected to occur in *D. semiclausum*.

For some parasitic hymenopterans, a period of sexual maturation is necessary, for example, the fruit fly parasitoid, *F. arisanus* (Quimio & Walter 2000). My study indicates that both males and females (<12-h old) were able to mate and produce female progeny (unpublished data). However, compared to newly emerged females, only a small number of newly emerged males were able to mate. This suggests that *D. semiclausum* males probably require a longer time than the females, to become sexual mature. Pompanon et al. (1995) reported that the earlier emerged males in *Trichogramma* species show the maximum activity, at the time of the female emergence. He et al. (2004) also demonstrated that newly emerged males of *A. ervi* are able to perform their courtship display, but they fail to mate until they are 4 h old. In the current study, newly emerged males displayed courtship behaviour, but such behaviour was not as rigorous as that performed by 3-d-old males. Earlier emergence of males may allow them to have sufficient time to become sexual mature and thus enhance their courtship ability. Therefore, a shorter developmental duration and longer sexual maturation period in males might be adaptive strategies, used to synchronise with the availability of sexually mature mates.

In the present study, older females failed to remate, when paired with the newly emerged males. This may be due to two reasons: Firstly, the newly emerged male of *D. semiclausum* may fail to produce a chemical and lack the sufficient courtship display, which are necessary to induce receptivity of females, because males need more time to become sexually mature. Secondly, 3-day-old females may have switched off their receptivity, after the first mating. A reduced level of pheromone production and a decreased level of females' receptivity for copulation have been cited as the reasons for such behaviour (Schwörer et al. 1999). However, chemicals produced by *D. semiclausum* males and females and their role in mating behaviour are unknown and thus, this is worthy of further study.

3.4   Factors Affecting Egg Maturation and Egg Load

3.4.1   Introduction

The reproductive success of an insect female is considered as the function of the number of eggs laid and the survival and fecundity of each resulting offspring (Minkenberg et al. 1992). Oviposition decisions in parasitoids are dynamic, in response to a physiological state, such as egg load (Mangel 1989a; Rosenheim & Rosen 1991; Hughes et al. 1994; Randen & Roitberg 1996; Ueno & Ueno 2007) and an information state, such as host availability and previous foraging experience (Hughes et al. 1994; Ueno 1999a; van Baaren et al. 2005), or a combination of both (Mangel 1989b). The state-dependent oviposition model often assumes that egg load, as a dynamic variable, is an important factor, which influences oviposition decisions by parasitoids (Mangel 1987; Sirot et al. 1997). Females with higher egg loads are known to search more intensively (Minkenberg et al. 1992; Hughes et al. 1994); encounter more hosts per foraging bout (Rosenheim & Rosen 1991; Hughes et al. 1994); be less selective over the host quality (Minkenberg et al. 1992; Fletcher et al. 1994; Bjorksten & Hoffmann 1998); spend less time in handling hosts (Rosenheim & Rosen 1991); lay larger clutches (Rosenheim & Rosen 1991; Minkenberg et al. 1992); and superparasitise the hosts more often (Völkl & Mackauer 1990; Sirot et al. 1997). Thus, knowledge relating to the dynamics of egg load is vital, in order to understand egg load associated behaviour.

Diadegma semiclausum is a synovigenic species (Huang et al. 2008b). However, it is unclear whether this parasitoid is a strong or weak synovigenic species. Synovigenic species continue to mature eggs throughout their life time (Jervis et al. 2001). Several factors, including food supply (Kapranas & Luck 2008), age (Sandanayaka et al. 2009), body size (Eliopoulos et al. 2003) and host-associated stimuli cues (Papaj 2000; Wang & Messing 2003), are known to affect such a process. Knowledge of such information can help increase the fitness of the parasitoids, by increasing their production potential. For example, body size is usually related to a with the higher egg load (Pexton & Mayhew 2002; Eliopoulos et al. 2003). The selection of larger females, in such species may result in a higher reproductive rate. In addition to this applied aspect, such information may help in future studies on host foraging and oviposition behaviour of the parasitoid.

Egg load and egg maturation dynamics in *D. semiclausum* (and factors affecting them) are still poorly understood. The goal of this research is to identify whether this process is affected by parasitoid age; body size; adult food supply; and host/host plant cues.

3.4.2   Materials and Methods

3.4.2.1 Effect of Parasitoid Age on Egg Load

In order to determine the egg maturation period, females of different ages (0, 0.5, 1, 2, 3, 5, 6, 7, 8, 10, 20, 30, 40 and 50 days old after emergence) were killed (by freezing at -20°C) and dissected in a droplet of Ringer's solution (15 g NaCl, 0.7 g KCl, 0.4 g CaCl2 in one litre of sterile water), on a slide under the stereomicroscope. The ovaries were covered with a cover slip and gently pressed. The mature eggs (fully chorionated) present in the ovaries (Figure 2.5A) were counted and measured under a compound microscope (Olympus, GH, Japan), which was equipped with transmitted light and a micrometer eyepiece. All parasitoids (except 0 h old ones) used in the experiment were fed with a 10% honey solution and individually maintained in a plastic container, to ensure their virginity. DBM larvae were not provided for oviposition in this experiment. There were 48, 21, 25, 21, 22, 31, 32, 32, 45, 35, 29, 21, 14 and 15 female adults (replicates) for the above age groups, respectively. The parasitoids that emerged from pupae parasitized, at third instar DBM larvae, were used in this study.

3.4.2.2 Effect of Parasitoid Body Size on Egg Load

This experiment was designed to test whether parasitoid body size had any effect on egg load. Newly emerged (0 day old, immediately after emergence) and 8-day-old females were used for this experiment. The hind tibia length of 35 0-day-old and 35 8-day-old females was measured under the stereomicroscope as the index of parasitoid size. Since newly emerged females did not contain any mature eggs, only the developing oocytes were counted: Whereas, in 8-day-old females, mature eggs were counted as mentioned above. Eight-d-old females were fed with a 10% honey solution immediately after emergence, but they were not provided with host larvae for oviposition.

3.4.2.3 Egg Load at Death

40 females were individually maintained in a plastic container (Figure 3.1H), fed with a 10% honey solution and not provided with no host larvae, during their entire lifespan. The females, which died naturally at different ages, were immediately dissected, in order to determine whether they still had mature eggs in their ovaries.

3.4.2.4 Effect of Food Supply on Egg Load

In order to determine whether and how food supply affects the egg maturation and egg load, two treatments were set up: (1) A 10% honey solution was provided, as food for females immediately after emergence; and (2) no food was provided for females. Parasitoids were individually maintained in a plastic container (Figure 3.1H) and not allowed to lay eggs. They were killed by freezing at -20°C 48 h after emergence and dissected for an egg count as described above. There were 21 and 19 replicates for honey-fed and starved parasitoids, respectively.

3.4.2.5 Effect of Host and Host Plant Cues on Egg Maturation

A previous study has shown that *D. semiclausum* adults are highly attracted to host-infested plants, compared to uninfested ones (Ohara et al. 2003b). The experiment in this study was undertaken to investigate whether host larvae and a host plant could enhance the egg maturation process in *D. semiclausum*. Due to females aged 48 h or older having a substantial number of eggs in their ovaries (Section 3.4.3.1), newly emerged females, without any mature eggs were used in this experiment. Nineteen newly emerged females were individually released into a plexiglas cage (Figure 3.1E), maintaining a cabbage seedling infested with 10 third instars DBM larvae. The females had access to food (10% honey solution) inside the cage. After 24 h, the females were removed, frozen and dissected for an egg count. Twenty-five 24-h-old females, which are deprived of any host larvae but fed on a honey solution, were used as control.

3.4.2.6 Statistical Analysis

Data on egg load affected by parasitoid age and food supply and egg maturation affected by host/host plant cues were normally distributed and hence they were analysed using ANOVA, followed by a Tukey's studentised range test. Data on the egg load affected by parasitoid age were square-root transformated, before ANOVA. The relationships between parasitoid body size and number of developing oocytes and mature eggs, and between parasitoid longevity and egg load at death, were analysed using linear regression.

3.4.3   Results

3.4.3.1 Effect of Parasitoid Age on Egg Load

Newly emerged females did not contain any mature eggs (Figure 3.4A). Mature eggs were first detected when females were 12 h old (Figure 3.4B). Two days after emergence, all females had mature eggs in their ovaries (Figure 3.4D).

Mature eggs were semicircular, sausage-shaped and about 0.32 mm long and 0.05 mm wide (Figures 3.4B-F). The mean number of mature eggs in the ovaries significantly increased, from 0.86 ± 0.32 in 12-h-old females, to 40.49 ± 1.98 in 8-d-old ones and then decreased afterwards (ANOVA: $F_{12,330} = 94.21$, $P < 0.001$) (Figure 3.5). A maximum number of 73 mature eggs were found in the ovaries of an 8-day-old female.
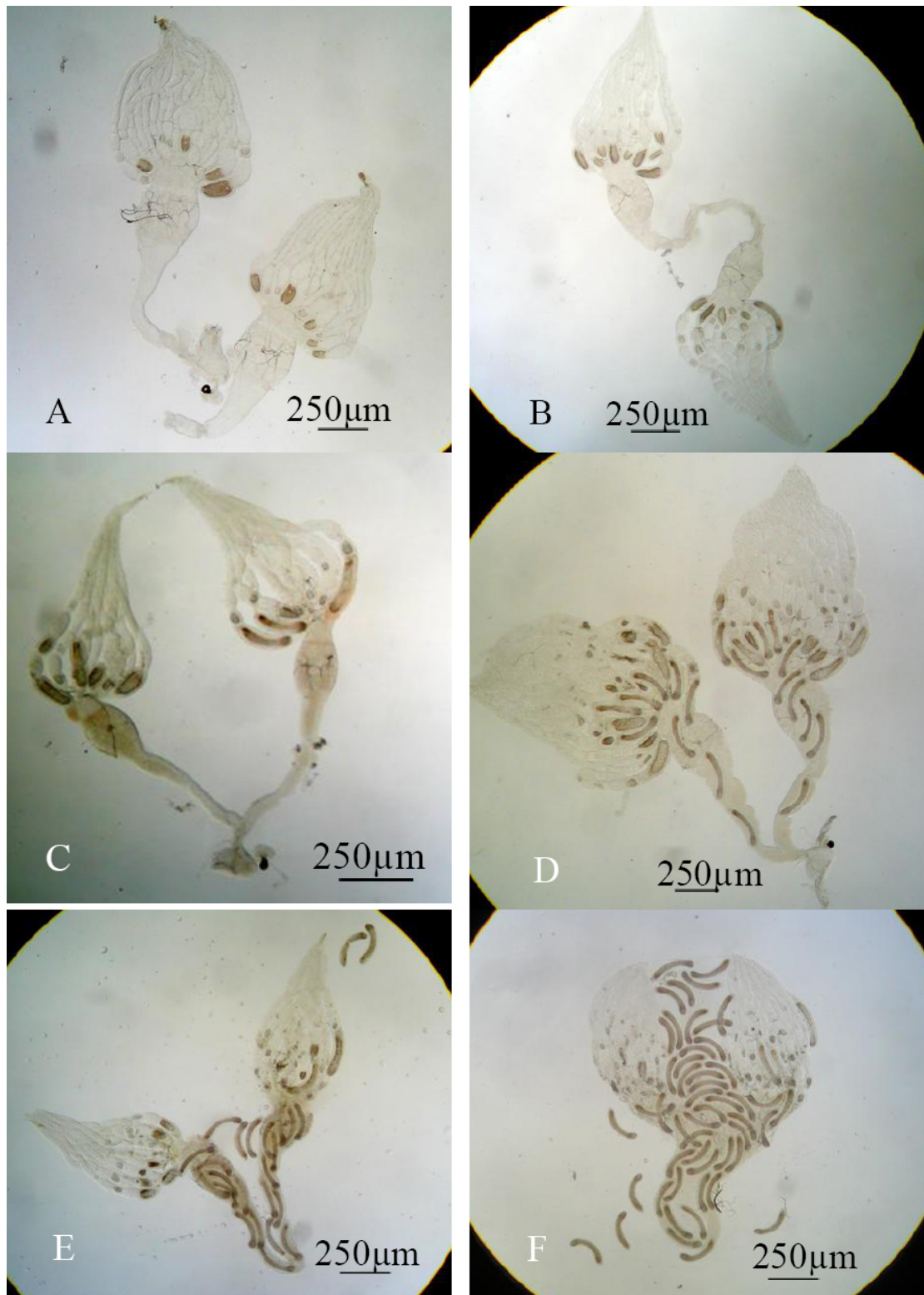
Figure 3.4 Mature eggs in the ovaries of *D. semiclausum* females of different ages: (A) 0-h-old; (B) 12-h-old; (C) 1-d-old; (D) 2-d-old; (E) 3-d- old; (F) 10-d-old. All these pictures were taken during the present study.
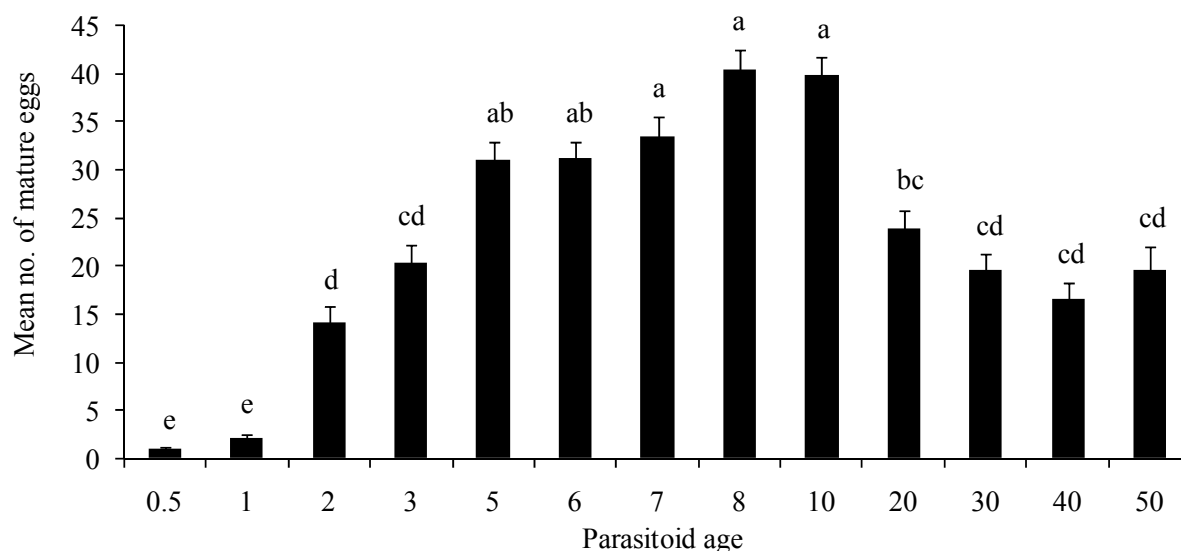
Figure 3.5 Mean (± SE) number of mature eggs in *D. semiclausum* females of different ages (days). Columns with the same letters are not significantly different (P > 0.05).

3.4.3.2 Effect of Parasitoid Body Size on Egg Load

Most females (24 of 29) contained developing oocytes, at emergence. The number of developing oocytes, in the ovaries of newly emerged females, significantly increased with their body size (Linear regression: $F_{1,33}$ = 9.55, P < 0.05) (Figure 3.6). Similarly, a positive correlation was found between the body size and number of mature eggs, in the ovaries of 8-d-old females (Linear regression: $F_{1,34}$ = 9.55, P < 0.05) (Figure 3.7). The number of developing oocytes, in newly emerged females, varied from 0 to 13, with a mean of 4.26 ± 0.65 oocytes and mature eggs in 8-d-old females ranged from 19 to 67, with a mean of 39.96 ± 2.04 eggs.

$y = 26.24x - 30.80$
$R^2 = 0.22$
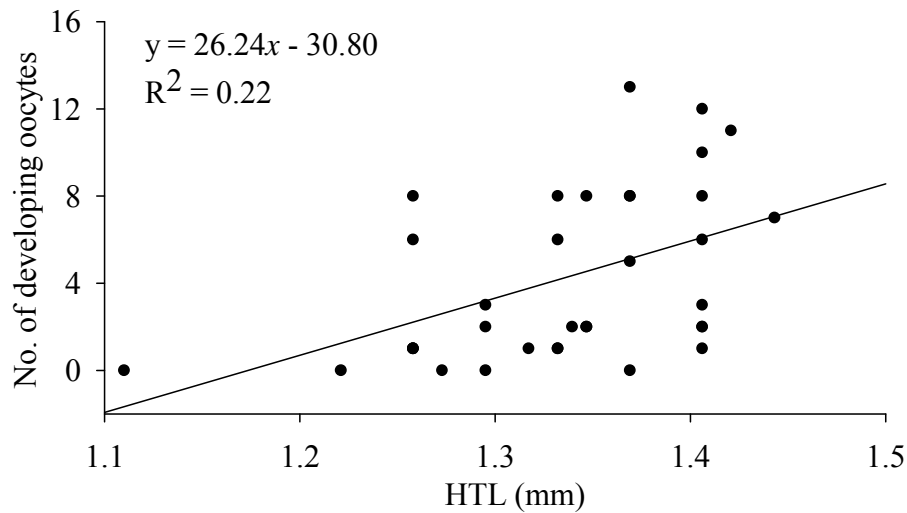
Figure 3.6 Relationship between the number of developing oocytes and body size (hind tibia length, HTL) of newly emerged females.

$y = 73.57x - 59.96$
$R^2 = 0.19$
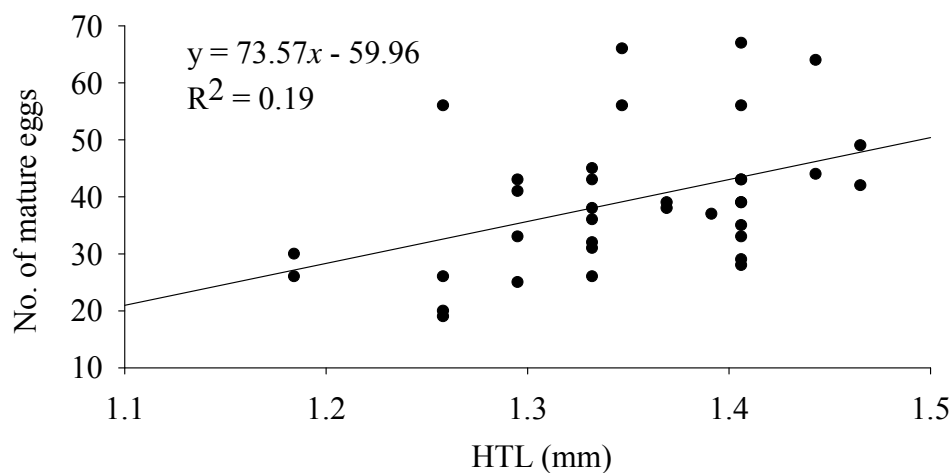
Figure 3.7 Relationship between the number of mature eggs and body size (hind tibia length, HTL) of 8-day-old females.

3.4.3.3 Egg Load at Death

The females lived for 25-81 days, with the mean longevity being 40.3 ± 1.64 days. All females at death contained mature eggs in their ovaries, with a mean egg load of 18.02 ± 1.24, which ranged from 3-37 eggs. Female age, at death, had no effect on the number of eggs that remained in their ovaries (Linear regression: $F_{1,38} = 0.51$, $P > 0.05$) (Figure 3.8).

$$y = 13.84 + 0.09x$$
$$R^2 = 0.01$$
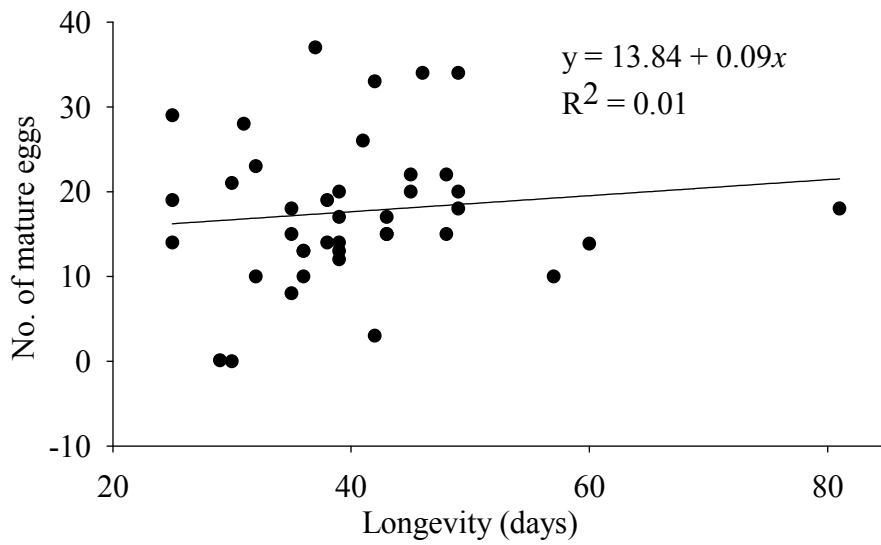
Figure 3.8 Egg load at death in *D. semiclausum* females.

3.4.3.4 Effect of Food Supply on Egg Load

Egg maturation occurred without food supply. However, the food supply for females significantly stimulated egg maturation (ANOVA: $F_{1,38} = 18.04$, $P < 0.001$) (Figure 3.9).
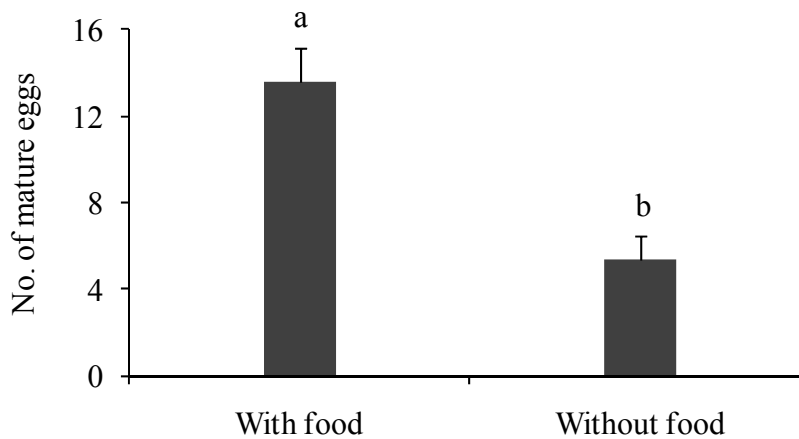
Figure 3.9 Mean ($\pm$ SE) number of mature eggs in 2-d-old *D. semiclausum* females, with or without food supply. Columns with the same letters are not significantly different ($P > 0.05$).

3.4.3.5 Effect of Host and Host Plant Cues on Egg Maturation

Among 19 parasitoids used in this experiment, only one female was able to parasitise a host larva. The number of mature eggs in 24-h-old females was similar, no matter whether or not they were provided with host larvae fed on the host plant (Mean ± SE: 1.47 ± 0.28) or not (2.00 ± 0.42) (ANOVA: $F_{1,42} = 0.23$, $P > 0.05$).

3.4.4   Discussion

Newly emerged females did not contain any mature eggs and they matured their eggs over time, suggesting that *D. semiclausum* is a highly synovigenic species (Jervis et al. 2001). In other ichneumonids, such as *Aptesis nigrocincta* Gravenhorst (Babendreier 2007) and *Diadromus collaris* (Gravenhorst) (Liu et al. 2001), newly emerged females do not contain mature eggs in their ovaries, but they have many immature eggs. The duration needed to achieve a maximum egg load, following emergence, is species specific. For example, in *F. arisanus*, mature egg-load peaks in 4 to 6 days posteclosion (Wang & Messing 2003). Similarly, in *Microplitis croceipes* (Cresson), egg production peaks 3 to 6 days after emergence (Navasero & Elzen 1992). *D. semiclausum* females required approximately 8 days in order to achieve the maximum mature egg load.

Results show that, if hosts were not provided for oviposition, the number of mature eggs in *D. semiclausum* ovaries significantly decreased following the peak, which occurred 8 days after emergence. This suggests that egg re-absorption occurrs in *D. semiclausum* females, if they are deprived of hosts, even though a honey solution has been supplied. It has been found that some synovigenic species have the ability to re-absorb eggs, given a state of host or food deprivation (Rosenheim 2000). For example, in *M. flavus* and *M. luteolus*, strictly synovigenic parasitoids of soft scale insects, eggs are gradually re-absorbed, following peak ovarian maturation until they have almost no eggs and finally die in the absence of hosts (Kapranas & Luck 2008). It also has been reported that egg re-absorption can result in egg limitation in synovigenic parasitoids and thus, females may be temporarily incapable of ovipositing, when hosts are available (Jervis & Kidd 1986). However, although *D. semiclausum* females may re-absorb their mature eggs, they always have some mature eggs in their ovaries, even at the end of their life. Therefore, it is unlikely that *D. semiclausum* females become egg limited, due

to egg re-absorption. Re-absorbing reproductive materials appears to improve the survival of host-deprived *D. semiclausum*, since the mean longevity of host-deprived females is significantly longer, than that of ovipositing females (unpublished data). However, in *F. arisanus*, female longevity is not prolonged by egg re-absorption (Wang & Messing 2003).

Synovigenic species may, or may not, mature their eggs without feeding; The former is known as autogenous and the latter as an anautogenous species (Jervis et al. 2005). In *M. flavus* and *M. Luteolus*, egg maturation occurs only after the adults are provided with carbohydrate food (Kapranas & Luck 2008). In *Venturia canescens* Gravenhorst, a larval parasitoid of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), egg production stops in the absence of food (Eliopoulos et al. 2003). In the present study, starved females were able to develop and mature some of their eggs, thus suggesting that *D. semiclausum* is an autogenesis species. However, when provided with a honey solution, females produced significantly more mature eggs, within the first 48 h after emergence. It is, therefore, suggested that food supply can stimulate egg maturation (Eliopoulos et al. 2003) and prevent eggs from being re-absorbed by females, for soma maintenance, which otherwise might occur during the time of starvation (Heimpel et al. 1997; Lee & Heimpel 2008).

Host stimuli (host and/or host-infested plant) are known to induce egg maturation in insect parasitoids. For example, *F. ceratitivorus* females are more attracted to and lay significantly more eggs, in fruit substrates containing odours of adults and eggs of the Mediterranean fruit fly (Bokonon-Ganta et al. 2007). However, in the present study the presence of host-infested plants, for 24 hours after emergence, did not enhance egg maturation in the *D. semiclausum* females. Winkler et al. (2006) reported that regardless of host and food availability, *D. semiclausum* females start to parasitise DBM larvae on day 2 or 3 following emergence. Results from the present study show that *D. semiclausum* females did not contain any mature eggs at emergence (Section 3.4.3.1) and the presence of host larvae did not enhance the initial egg maturation process (Section 3.4.3.5). Thus, the unavailability of mature eggs may be the reason of *D. semiclausum* females delayed their oviposition. It seems that ovarian development in *D. semiclausum* is influenced by inherent physiological mechanisms, if any, or stimulated by food resource, as discussed previously.

Although egg load is not related to female body size in some parasitoids (Ueno 1999b; Riddick 2005), the positive relationship between female egg load and body size

is common in many species (Shu-Sheng 1985; Mills & Kuhlmann 2000; He & Wang 2006b). In *D. semiclausum*, the number of developing oocytes at emergence, or the number of mature eggs in the ovaries of 8-d-old females, significantly increases with their increasing body size. Generally, large females are able to regenerate eggs faster when required (Cloutier et al. 2000) and the reproductive fitness of females (e.g. searching rate, longevity, fecundity, and the ability to parasitise hosts) is often positively correlated with their body size (van den Assem et al. 1989; Honék 1993; Godfray 1994; Visser 1994; Kazmer & Luck 1995; Cloutier et al. 2000; Arakawa et al. 2004; Sagarra et al. 2007). The positive relationship between female egg load and body size indicates that body size can be used as a straightforward indicator of the parasitoid quality, in laboratory-cultured or mass-rearing populations.

3.5   Factors Affecting Adult Longevity

3.5.1   Introduction

Adult longevity is an important factor in the population dynamics of parasitoids and it influences their effectiveness, when exploiting host populations (Jervis et al. 2005). The cost of reproduction in insect parasitoids is often considered as a trade-off between reproduction and survival (Ellers 1996). Males that live longer can inseminate more females and fertilise more eggs; Similarly, a long-living female can lay more eggs in her lifetime and thus increase the fitness (Jervis et al. 2005).

Parasitoid longevity is a highly variable characteristic and it may be affected by a number of factors, including sugar feeding (Fadamiro & Heimpel 2001; Lee et al. 2004; Winkler et al. 2006; Onagbola et al. 2007); oviposition activity (Ramadan et al. 1995); body size (Eliopoulos et al. 2005); and mating status (Onagbola et al. 2007). The ongoing interest in the potential utilisation of *D. semiclausum*, for the biological control of DBM, is however hindered by a lack of information on several aspects of its biology and life history strategy. For example, little is known about the effects of food provision and physiological factors, such as body size and mating, on survival and fitness of *D. semiclausum*.

The present study has investigated the effects of sugar feeding, body size, oviposition, and mating on the longevity of *D. semiclausum*. Knowledge relating to the

influence of these factors, on the fitness of *D. semiclausum*, should aid current efforts aimed at utilising this parasitoid for the biological control of DBM.

3.5.2   Materials and Methods

3.5.2.1 Effect of Food Supply and Body Size

In order to investigate the effects of food and body size on the longevity of *D. semiclausum* adults, four treatments were set up: Parasitoids were provided with (1) 10% honey solution (n = 86); (2) 10% honey solution during first 5 days after emergence (n = 108); (3) water only (n = 70); and (4) no food (n = 150). All parasitoids (males and females) used in the experiment were virgins, which were deprived of host access and individually maintained in transparent plastic containers (Figure 3.1H).

In order to determine whether body size affects longevity, the parasitoids used in the above experiment were categorised into two size groups, i.e. small and large. The pupal weight (PW) and body length (BL) of the parasitoids were positively correlated (n = 43; Linear regression: BL = 0.3838PW + 3.3; R^2 = 0.76; $F_{1,41}$ = 137.02, P < 0.001). The mean (± SE) pupal weight was 3.56 ± 0.02 and 3.97 ± 0.03 g for males and females, respectively. Male and female parasitoids, which emerged from the pupae having weighing less than the mean pupal weight, were considered to be small and those, which emerged from the pupae weighing more than the mean pupal weight, were considered to be large.

3.5.2.2 Effect of Mating

In order to investigate whether mating had any effect on the longevity of both sexes, mated and virgin wasps were individually maintained in transparent plastic containers (Figure 3.1H). Mated females were obtained by, pairing individual males in a glass vial for mating. Both males and females used in the experiment were 3 days old. All parasitoids were fed with a 10% honey solution until death and females were deprived of host oviposition. There were 36 mated and 43 virgin males and 29 mated and 43 virgin females, in this experiment.

3.5.2.3 Effect of Oviposition

In order to investigate whether oviposition had any effect on the longevity of *D. semiclausum* females, two experiments, each containing two treatments, were set up. In the first experiment, treatments included: (1) hosts + 10% honey solution and (2) 10% honey solution only (as a control), with 9 and 63 replicates, respectively. Mated females in treatment (1) were provided daily with 30 third instar DBM larvae, three days after emergence, until death. Similarly, the second treatments included: (1) host only, and (2) no hosts + no honey (as a control), with 36 and 51 replicates, respectively. Females in treatment (2) in this experiment were daily provided with 15 third instar DBM larvae after emergence, until death. Ovipositing females were maintained in the jars containing DBM larvae infested cabbage seedling. The longevity of each test parasitoid was recorded.

3.6 Statistical Analysis

A goodness-of-fit test was used, in order to test the distribution of data, before analysis. The data on longevity, which was affected by food and body size, were not normally distributed, and thus this was analysed using the nonparametric Kruskal-Wallis test, followed by Dunn's procedure for multiple comparison. Other data were normally distributed and hence, they were analysed by ANOVA, followed by a Tukey's studentised range test.

3.7 Results

3.7.1 Effect of Food Supply and Body Size

As shown in Table 3.2, the longevity of parasitoids was significantly longer when they were provided with a 10% honey solution, compared to that in other treatments. When fed on honey, evern during the first 5 days after emergence, parasitoids survived significantly longer, than those fed on water or those with no food supply (Table 3.2); However, if the duration of honey-fed was removed from the total longevity, the remaining lifespan was significantly shorter, than that of water-fed or starved individuals (ANOVA: $F_{3,410} = 765.28$, $P < 0.001$).

Regardless of food supply, body size did not affect the longevity of males. However, large and small females fed with a honey solution during their lifespan, lived significantly longer than males: In the absence of food or provided with water, larger females survived significantly longer, than small females and large and small males (Table 3.2). When a honey solution was provided, for only the first five days after emergence, no significant difference in longevity was found between males and females of different size (Table 3.2).

Table 3.2 Effect of food supply and body size on the longevity (days) of *D. semiclausum* adults.

Food	Male		Female		χ^2	P
	Small	Large	Small	Large		
Honey	36.71 ± 2.57 aαβ	28.16 ± 1.84 aβ	42.84 ± 2.44 aα	40.56 ± 3.90 aα	5.99	< 0.001
(n)	(17)	(26)	(25)	(18)		
Honey for 1st 5 days	6.32 ± 0.11 bα	6.36 ± 0.10 bα	6.29 ± 0.08 bα	6.53 ± 0.11 bα	2.84	> 0.05
(n)	(19)	(25)	(34)	(30)		
Water	2.00 ± 0.10 cβ	2.04 ± 0.10 cβ	2.00 ± 0.27 cβ	2.69 ± 0.12 cα	16.86	< 0.001
(n)	(20)	(26)	(8)	(16)		
No food	1.78 ± 0.10 cβ	2.08 ± 0.12 cβ	2.03 ± 0.09 cβ	2.40 ± 0.10 cα	17.10	< 0.001
(n)	(37)	(38)	(40)	(35)		
χ^2	76.08	97.03	95.24	85.00		
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001		

Means (± SE) followed by the same English letters in columns and Greek letters in rows are not significantly different (Kruskal-Wallis test: P > 0.05).

3.7.2 Effect of Mating

Virgin females lived significantly longer than males regardless of the latter's mating status (ANOVA: $F_{3,148}$ = 6.63, P < 0.001)); However no significant difference in longevity was detected between virgin and mated males (P > 0.05), or between virgin and mated females (P > 0.05) (Figure 3.10).
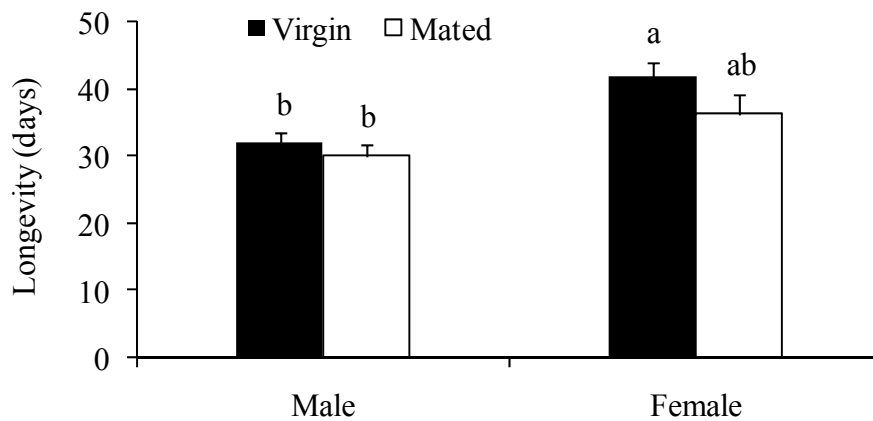
Figure 3.10 Effect of mating on the longevity (days) of *D. semiclausum* adults. Columns with the same letters are not significantly different (P > 0.05).

3.7.3 Effect of Oviposition

When provided with a honey solution, ovipositing females which accessed host larvae, survived significantly shorter, than females that were deprived of host larvae (ANOVA: $F_{1,70}$ = 6.10, P < 0.05) (Figure 3.11A). However, if no food was supplied, then no differences in longevity were observed between ovipositing and non-ovipositing females (ANOVA: $F_{1,49}$ = 0.00, P > 0.05) (Figure 3.11B).
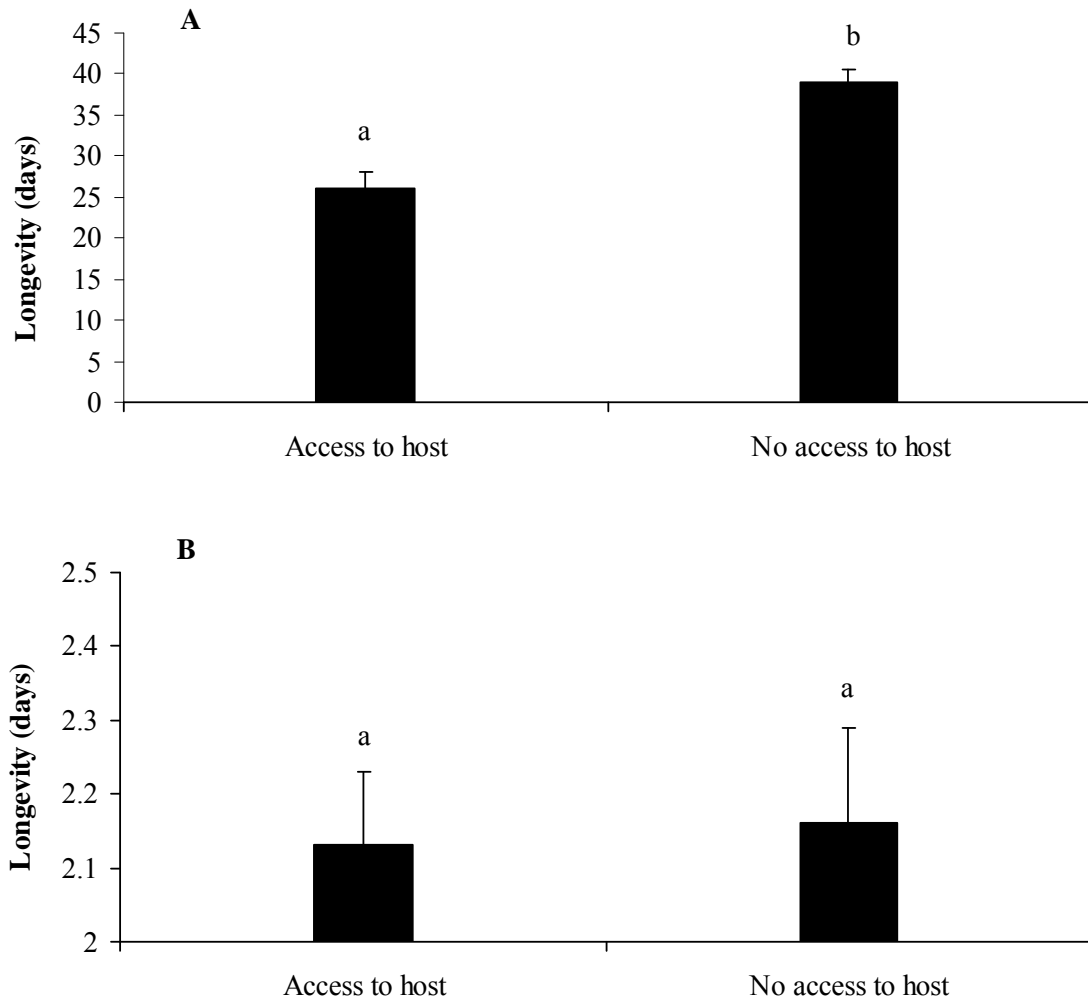
Figure 3.11 Effect of host access on the longevity of *D. semiclausum* females: **(A)** with food; **(B)** without food. Columns with the same letters are not significantly different (P > 0.05).

3.8   Discussion

Body size (Eliopoulos et al. 2005) and food supply (Heimpel et al. 1997; Baggen & Gurr 1998; Mitsunaga et al. 2004; Winkler et al. 2006) are two important factors that might affect parasitoid longevity. As reported in several other studies, for example, in *Trissolcus simoni* (Mayr), an egg parasitoid of *Eurygaster integriceps* Puton (Kivan & Kilic 2005) and *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae), an ectoparasitoid of several stored-product insects (Onagbola et al. 2007), males lived shorter than females in both honey-fed and starved conditions. In the present study, when parasitoids fed on honey during their lifespan, longevity amongst small and large

males or females did not differ. This contrasts with many findings where larger males (Sagarra et al. 2007) and larger females lived longer than the smaller ones (Irvin & Hoddle 2007). In the present study, when starved or provided with water only, body size did not affect the longevity of males; However, larger females survived longer than small females.

In the case of many wasp species, sugar consumption can increase their longevity (McDougall & Mills 1997; Fadamiro & Heimpel 2001; Lee et al. 2004; Winkler et al. 2006; Onagbola et al. 2007) and lifetime fecundity (Baggen & Gurr 1998; Tylianakis et al. 2004). Consequently, for these species, the availability of sugar sources in the field is important for their reproductive success. A semi-field study (Winkler et al. 2006) shows that sugar feeding is a major factor influencing the adult lifespan of female and male *D. semiclausum*. In this study, honey-fed adults survived nearly 15 to 20 fold longer than water fed or starved individuals. The reward from a single honey feeding can be critical for a forager's fitness. The risk of starving to death for sugar-fed wasps, *Cotesia rubecula* Marshall, was reduced by 0-73%, in comparison with unfed wasps, depending on the sugar concentration and the timing of feeding (Siekmann et al. 2001). In *Macrocentrus grandii* Goidanich, the lifespan of starved males and females is less than 3 days, but providing sugar, during the first day of life, increases the lifespan by 2 days for males and 4 days for females (Fadamiro & Heimpel 2001). Contrary to these studies, *D. semiclausum* adults fed with honey, for the first 5 days after emergence and then deprived thereafter, died significantly earlier than the adults provided with no food, or water only. It has been indicated that *Diadegma* adults, at emergence, have high levels of lipids and glycogen, which decline over time (Lee et al. 2004) and they can utilise dietary nutrients, in order to meet short-term energy demands but they are unable to convert excess carbohydrates to long-term storage in the form of lipids (Visser & Ellers 2008). Hence, food shortage, particularly when parasitoids become older, can put them at a high risk of death. The greater longevity in females, than that seen in males (and in larger females, than in smaller ones), in starvation could be due to a because of variation in lipid content. The lipid level of females of *D. insulars* (Cresson), at emergence, is higher than that of males (Lee et al. 2004).

A trade-off between mating and longevity has been reported for several parasitoids (Li et al. 1993a; Carpenter 1995; Jacob & Evans 2000; Onagbola et al. 2007). Unlike these studies, mating did not affect the longevity of *D. semiclausum* adults, when fed on a honey solution with no hosts provided. Generally, a potential trade-off

between mating and longevity is more likely to occur, when energy resources are limited, or in the absence of high quality food such as honey, as demonstrated by Jacob & Evans (2000) in the *Bathyplectes cuculionis* Thomson (Hymenoptera: Ichneumonidae). However, in the present study, all the parasitoids were fed with a honey solution.

Oviposition may result in the early death of females. For example, the longevity of host-deprived and ovipositing female *Biosteres vandenboschi* (Fullaway) (Hymenoptera: Braconidae), a parasitoid of the early instar oriental fruit fly, was 28.6 and 23.7 days, respectively (Ramadan et al. 1995). Similar adverse effect of host availability, on the lifespan of female *D. semiclausum*, was found in the present study, when females were fed on a honey solution. It has been assumed that such adverse effects of oviposition, in the longevity of parasitoids, results from stimulated reproductive activities, such as oogenesis and egg maturation in females, that reduce resource available for other life processes (Rivero & Casas 1999; Jervis et al. 2007; Boggs 2009).

CHAPTER 4
REPRODUCTIVE BEHAVIOUR OF *DIADEGMA SEMICLAUSUM*

4.1 General Introduction

In hymenopteran parasitoids, such as *D. semiclausum*, reproductive behaviour, such as mating, oviposition and host-parasitoid relationships, may be directly related to progeny production and its fitness (Godfray 1994; Cloutier et al. 2000; Roitberg et al. 2001). Although, *D. semiclausum* has long been recognised as an efficient biocontrol agent of DBM, very little effort has been made to understand its aspects of reproductive behaviour, the knowledge of which is important for maintaining laboratory colonies for mass rearing and release programmes, in addition to understanding the population ecology in the field. This chapter, therefore, investigates the factors that affect mating, oviposition and host-parasitoid relationship, in response to host stages and densities in *D. semiclausum*.

4.2 Mating behaviour

4.2.1 Introduction

In haplodiploid parasitoids, virgin females can produce offspring: but only males (Quicke 1997). Therefore, copulation is essential for individual fitness and the long-term survival of this insect's population, and a study on their mating behaviour will help in an understanding of their their reproductive biology.

Parasitoid mating behaviour is also studied for applied purposes, for example, the development of efficient techniques for mass-rearing in biological control programmes (Sagarra et al. 2002; Hardy et al. 2005). In hymenopteran parasitoids, mating affects the outcome of various fitness parameters, such as longevity, lifetime fecundity and progeny production (Ridley 1988; Sagarra et al. 2002). An Investigation of the factors, which may potentially affect mating success, could provide necessary information for the development of biological control strategies. Moreover, such investigations are likely to be invaluable additions to the predictions, which habe been made by population models of host-parasitoid dynamics (Luck 1990).

The mating behaviour of parasitoids is affected by several factors, including age, body size and light. Age differences are an important source of variation in courtship behaviour (Tagawa et al. 1985). In many species of parasitoids, newly emerged females are unreceptive to mating attempts by males (Godfray 1994). Furthermore, unmated adult females become unreceptive after a certain period, in some species (Godfray 1994). Thus, it is important to understand how the age of individuals affects their mating behaviour. Furthermore, there is evidence that body size may affect male and female mating behaviour. van den Assem et al. (1989) demonstrated that small male and female *Lariophagus distinguendus* (Forster) were at a disadvantage, compared to larger conspecifics. Moreover, a daily activity pattern (including mating) can be rhythmic and affected by the presence of light (He 2008). Mating behaviour contains the fundamental information needed to understand parasitoids' reproductive biology. Furthermore, identification of potential factors, which might affect mating success, could be useful for the development of biological control strategies.

Apart from Abbas' (1988) work, where he suggested that *D. semiclausum* can mate immediately after emergence and that mating can occur during the day and night, the mating behaviour of this species is poorly understood. This section aims to investigate the general mating behaviour and factors, which affect the mating success of *D. semiclausum*.

4.2.2 Materials and Methods

4.2.2.1 General Methodology

The parasitoids used for the experiments and the experimental conditions are described in Section 3.2. In order to ensure the virginity of the parasitoids, before the experiments, pupae were maintained individually in glass vials (4 cm in height × 2 cm in diameter). All mating trials were conducted in glass vials (7.5 cm in height × 2.5 cm in diameter), by pairing one virgin male and one virgin female in each vial. Unless stated, mating behaviour was observed for 1 h during the photophase. Parasitoids produced from DBM, parasitised at the third instar larvae, were used in these experiments.

My preliminary study shows that mating usually lasted more than 5 minute and females, which mated for > 5 minutes, produced female progeny. Hence, mating was

considered successful, if the genitalia connection time exceeded 5 minutes. General mating behaviour was observed and described. The premating period and mating duration (defined in Chapter 3, Section 3.3.2.2) were recorded.

4.2.2.2 Effect of Mate Age on Mating Success, Premating and Mating Duration

In order to determine whether mate age affected mating success, premating period and mating duration, 455 pairs of adults (in seven different treatments) were set up as follows (n = number of replicates):

1. 12-h-old female × 12-h-old male, n = 22;
2. 12-h-old female × 72-h-old male, n = 24;
3. 24-h-old female × 24-h-old male, n = 226;
4. 24-h-old female × 72-h-old male, n = 55;
5. 72-h-old female × 12-h-old male, n = 28;
6. 72-h-old female × 24-h-old male, n = 51;
7. 72-h-old female × 72-h-old male, n = 49.

4.2.2.3 Effect of Body Size on Mating Success, Premating and Mating Duration

In order to examine whether body size affected the premating period, mating duration and mating success, 3-day-old virgin males and females of different body size were paired and their mating behaviour was observed. Experimental parasitoids were categorised into small and large, on the basis of their pupal weight measured (as described in Chapter 3, Section 3.5.2.1). Two hundred and four pairs of adults were used in this experiment with four treatments (n = number of replicates):

1. Small female × small male (SF × SM), n = 34;
2. Large female × large male (LF × LM), n = 89;
3. Small female × large male (SF × LM), n = 54;
4. Large female × small male (LF × SM), n = 27.

4.2.2.4 Effect of Light Regime on Mating Success, Premating and Mating Duration

In order to determine the effect of light condition on *D. semiclausum* mating behaviour, 1-day-old virgin males and females were paired for 1 h, during the photophase and scotophase. A red light was used for mating behaviour observation during the scotophase. There were 39 and 49 pairs (replicates) in the photophase and scotophase, respectively. Lights in the experimental room went on and off at 08:00 h and 24:00 h, respectively. Experiments were conducted between 08:00 and 10:00 h into the photophase and between 0:00 and 02:00 h into the scotophase.

4.2.2.5 Mating Pattern during the Photophase

Since mating generally occurred during the photophase, 73 pairs of 1-day-old virgin males and females were established, in order to determine the mating pattern during the entire 16-h photophase. A 10% percent honey solution was provided inside the glass vial, as food. Mating pairs were observed continuously during the photophase. After the first mating, the pair was maintained, in order to determine whether remating occurred, until the completion of the 16-h observation. The premating period, mating duration and subsequent number of matings, were recorded.

4.2.3   Statistical Analysis

The Marascuilo procedure of the nonparametric analysis (a multiple Chi-square test) (Daniel 1990) was used to assess the effect of mate age on mating success. A generalised linear regression (GLR) was used to analyse the relationship between mate age and mating success: $y = \exp(a + b \times Fage + c \times Fage^2 + d \times Mage + e \times Mage^2 + f \times Fage \times Mage)$, where a, b, c, d, e and f are parameters of the model, and Fage and Mage are female and male age, respectively. Only significant terms, after running the full regression model, were kept in the final model. A log likelihood ratio test was applied, in order to determine whether male age or female age had more influence on mating success. Data on premating period were not normally distributed (even after transformation) and thus it was analysed using the nonparametric Kruskal-Wallis test, followed by Dunn's procedure for multiple comparisons. Data on the mating period were normally distributed and analysed using ANOVA, followed by Tukey's studentised range test.

The relationships between the number of matings and mating period, in addition to that between the number of matings and the mating interval, were analysed by a nonlinear regression, given by the polynomial quadratic equation $y = y_0 + ax + bx^2$, where y_0, a and b are the parameters of the model.

4.2.4   Results

4.2.4.1   General Mating Behaviour

After a virgin male and a virgin female were released into a glass vial, the male always approached the female with fanning wings (Figure 4.1A) and attempted to mount the latter, but the female often ran or flew away. After the first antennation (Figure 4.1B), the male attempted to mount the female (Figure 4.1C). If the female was receptive after mounting, she lowered her antennae backwards, lowered her body posture and remained still and the male immediately moved backwards, curved his abdomen and inserted his aedeagus into her genitalia (Figure 4.1D). Once the genitalia were connected, both male and female remained completely immobile. However, when an unreceptive female was mounted by a male, she continued to move with her wings flapping and she pushed the male with her hind legs.

Mating was terminated when the male moved slightly and the female started walking forwards, at which time the male dismounted her. The average premating period was 10.42 ± 1.02, ranging from 0.5 to 59 mins (n = 193 pairs) and mating duration was 6.77 ± 0.66, ranging from 5.18 to 11.34 mins (n = 194 pairs).
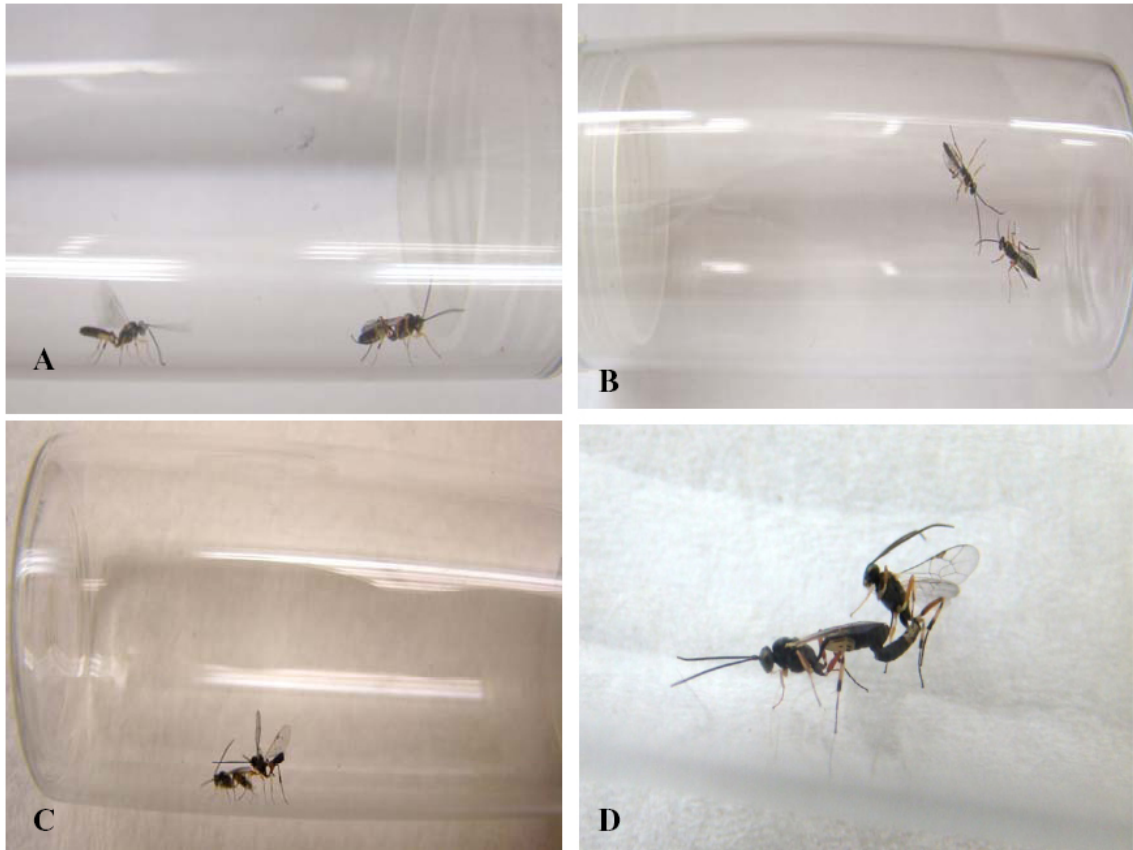
Figure 4.1 Mating behaviour of *D. semiclausum*: **(A)** a male approaching a female with fanning wings; **(B)** antennating; **(C)** mounting; and **(D)** mating. All the pictures were taken in the present study.

4.2.4.2   Effect of Mate Age on Mating Success, Premating and Mating Duration

Mating success significantly increased with an increasing in age of both sexes (GLR: $F_{3,3}$ = 15.23, P < 0.0001; R^2 = 0.94) (Figure 4.2). However, female age had significantly more impact on the mating success than the male's age (Likelihood ratio test: χ^2 = 700.81 > $\chi^2_{2,0.05}$ = 5.59, P < 0.001) (Figure 4.2).
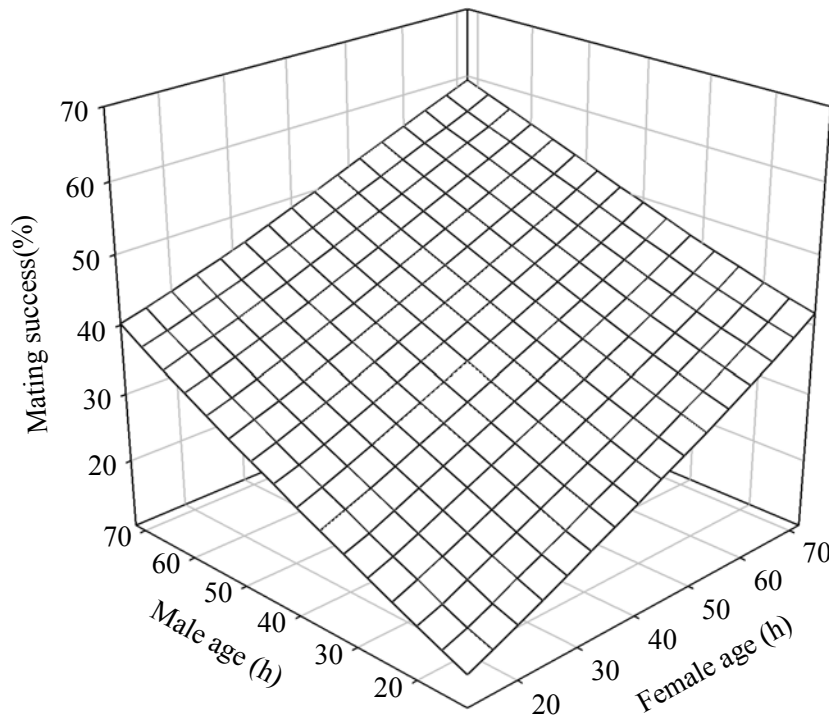
Figure 4.2 Effect of male (Mage) and female age (Fage) on mating success of *D. semiclausum*. Mating success (%) = Exp (- 2.2912 + 0.0196Fage + 0.0190Mage - 0.0002Fage×Mage).

However, mate age did not affect the premating period (Kruskal-Wallis Test: χ^2 = 4.05 < $\chi^2_{6,0.05}$ = 12.59; df = 6,148; P > 0.05) and mating duration (ANOVA: F = 1.39; df = 6,147; P > 0.05) (Table 4.1).

Table 4.1 Effect of mate age on premating period and mating duration.

Mate age	Premating period (min)	Mating duration (min)
12-h-old female × 12-h-old male	10.33 ± 8.84 a	6.68 ± 0.35 a
12-h-old female × 72-h-old male	12.11 ± 6.48 a	6.49 ± 0.13 a
24-h-old female × 24-h-old male	13.47 ± 2.12 a	6.91 ± 0.10 a
24-h-old female × 72-h-old male	7.07 ± 0.25 a	7.07 ± 0.25 a
72-h-old female × 12-h-old male	9.12 ± 3.03 a	6.49 ± 0.15 a
72-h-old female × 24-h-old male	9.05 ± 3.05 a	6.72 ± 0.11 a
72-h-old female × 72-h-old male	8.96 ± 2.66 a	7.66 ± 0.13 a

Means (± SE) followed by the same letters in the columns are not significantly different (P > 0.05).

4.2.4.3 Effect of Body Size on Mating Success, Premating and Mating Duration

In a no-choice situation, body size did not affect the mating success ($U_0^{'} = 1.76 <$ $\chi^2_{3,0.05} = 7.82$; df = 3,115; P > 0.05), premating period (Kruskal-Wallis Test: $\chi^2 = 1.11$ $< \chi^2_{3,0.05} = 7.82$; df = 3,115; P > 0.05) and mating duration (ANOVA: $F_{3,103} = 0.26$; df = 3,103; P > 0.05) of the parasitoid (Table 4.2).

Table 4.2 Effect of body size on premating period, mating duration and mating success.

Parasitoid	Premating period (min)	Mating duration (min)	Mating success (%)
SF × SM	5.84 ± 1.64 a	6.52 ± 0.13 a	64.70 a
LF × LM	6.08 ± 1.29 a	6.40 ± 0.10 a	58.42 a
SF × LM	8.55 ± 2.73 a	6.39 ± 0.15 a	51.00 a
LF × SM	4.76 ± 2.56 a	6.35 ± 0.13 a	62.96 a

Means (± SE) followed by the same letters in the columns are not significantly different (P > 0.05).

4.2.4.4 Effect of Light Regime on Mating Success, Premating and Mating Duration

Mating occurred both in the photophase and scotophase: However, significantly higher mating success occurred during the photophase, than during the scotophase (Chi-square: $\chi^2 = 8.62 > \chi^2_{1,0.05} = 3.84$; P < 0.05) (Figure 4.3). No differences were observed in the premating period (mean ± SE: 12.40 ± 3.61 and 24.00 ± 1.00 mins for photophase and scotophase, respectively) (ANOVA: $F_{1,11} = 1.74$; P > 0.05) and mating duration (7.06 ± 1.45 and 7.75 ± 1.45 mins for photophase and scotophase, respectively) (ANOVA: $F_{1,9} = 0.70$; P > 0.05) between photophase and scotophase.
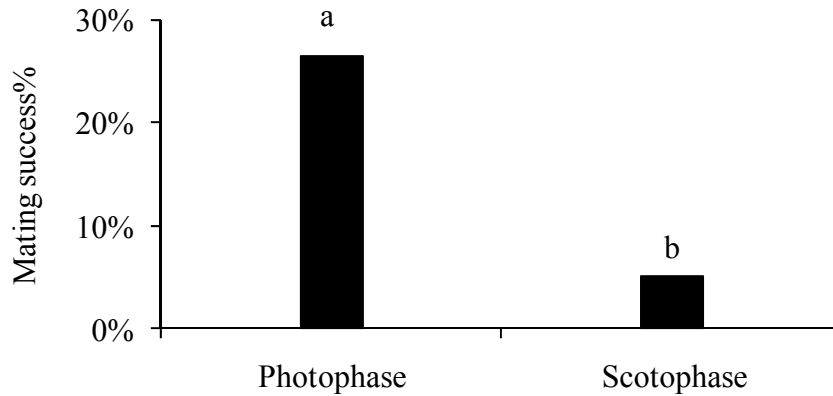
Figure 4.3 Effect of light on mating success of *D. semiclausum*. Columns with the same letters are not significantly different (P > 0.05).

4.2.4.5 Mating Pattern during the Photophase

Mating occurred throughout the 16-h observation period in the photophase but only 27 of 73 pairs (< 40%) mated. For the first mating, there was no significant difference in mating success between the first and second halves of the photophase (Chi-square: $\chi^2 = 0.04 < \chi^2_{1,0.05} = 3.8$; P > 0.05) (Figure 4.4).

30% of mated parasitoids remated (9 out of 27) and up to six rematings were observed. Significantly higher number of those rematings occurred during the second half of the photophase (Chi-square: $\chi^2 = 10.71 > \chi^2_{1,0.05} = 3.8$; P < 0.01) (Figure 4.4). Similarly, significantly higher number of total matings occurred during the second half of the photophase (Chi-square: $\chi^2 = 6.48 > \chi^2_{1,0.05} = 3.8$; P < 0.05) (Figure 4.4).
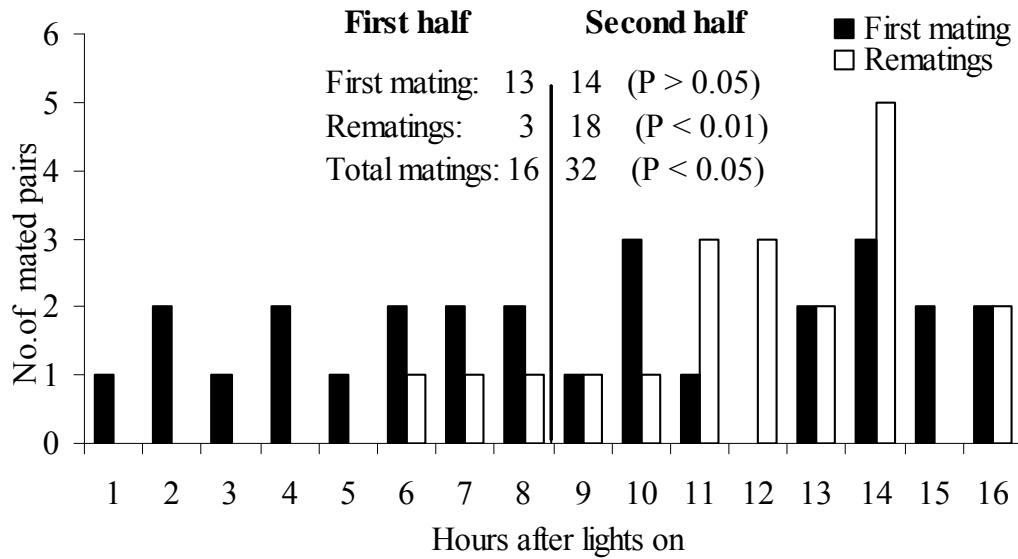
Figure 4.4 Mating pattern of *D. semiclausum* during the photophase.

The mating duration increased significantly with the increasing number of matings (Non-linear regression: $F_{2,42} = 22.40$, $P < 0.0001$) (Figure 4.5). The interval between two matings decreased with the increasing number of matings (Non linear regression: $F_{2,42} = 22.40$, $P < 0.0001$) (Figure 4.6).
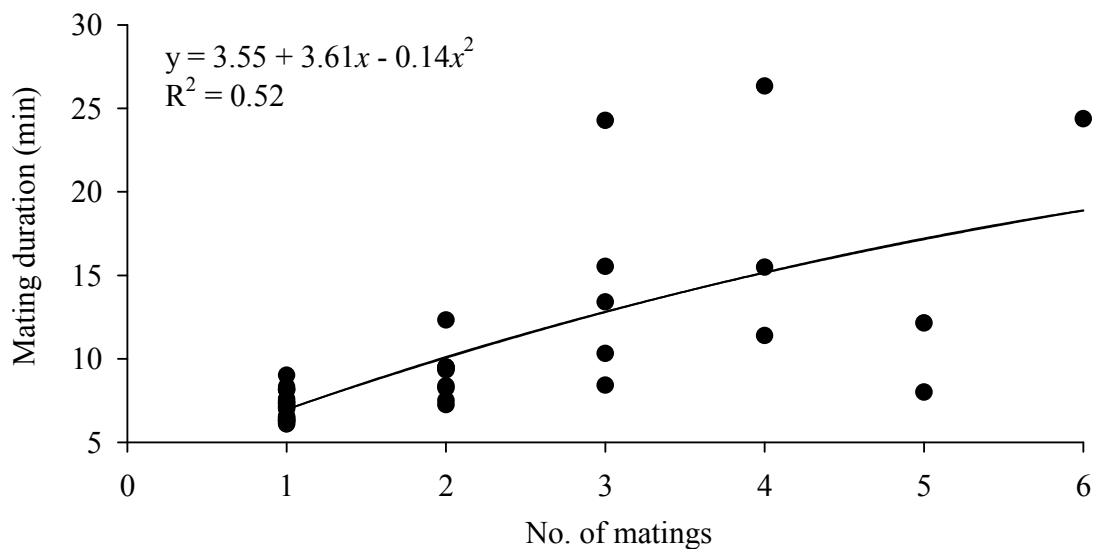
Figure 4.5 Relationship between the number of matings and mating duration of *D. semiclausum* during the 16-h photophase

$$y = 12.14 - 5.75x + 0.70x^2$$
$$R^2 = 0.40$$
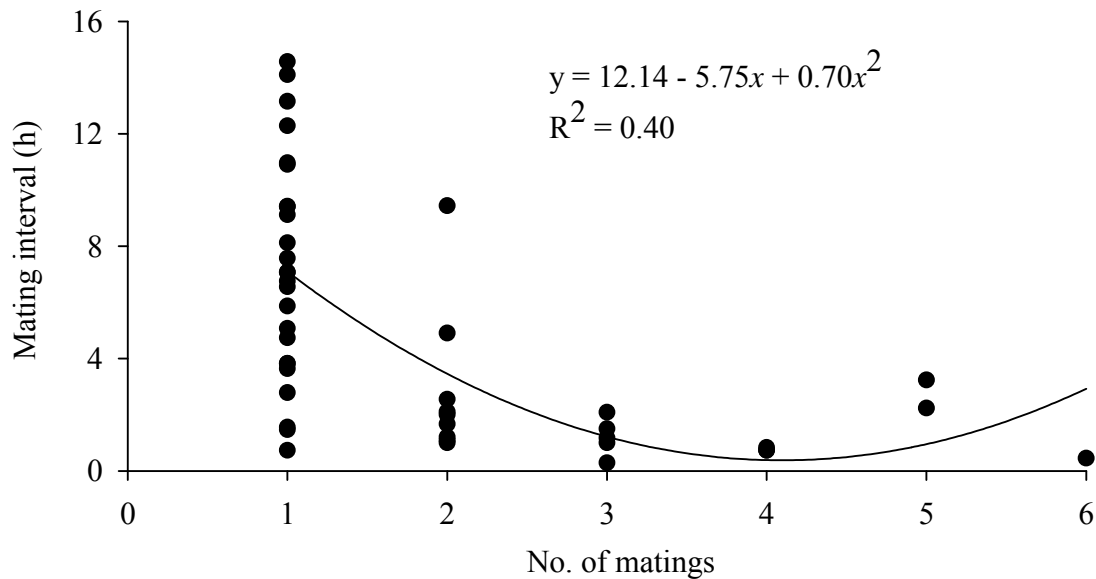
Figure 4.6 Mating interval (h) between two matings of *D. semiclausum* during the 16-h photophase

4.2.5   Discussion

As reported in several insect parasitoids (Collins & Grafius 1986; Antolin & Strand 1992; Liu et al. 2001; Cheng et al. 2004; Babendreier 2007), *D. semiclausum* males exhibit the active role in mating behaviour, executing the majority of observable behaviour and females are apparently more passive. Females of several ichneumonid species, such as *Eriborus terebrans* (Gravenhorst) (Shu & Jones 1993), *Campoletis sonorensis* (Cameron) (Vinson 1972) and *Syndipnus rubiginosus* Walley (Eller et al. 1984), are known to produce female sex pheromones that mediate the courtship and mating behaviour. Although the pheromone has not been identified for *D. semiclausum,* the active role, which males play in the mating system of this species, suggests that females may release a chemical cue that attracts males.

Parasitoid age is known to affect mating success (Schwörer et al. 1999), because the level of pheromone production may be associated with age (Tagawa et al. 1985; Cormier et al. 1998; Schwörer et al. 1999). Increasing egg load together with the age of the female (Section 3.4.3.1) may also have some stimulatory effect on the mating behaviour of males (Takemon 2000) or females (Carsten & Papaj 2005), thus leading to a higher mating success. In the present study, mating success increased significantly with the increasing age of both males and females. Female age-dependent mating success has been reported in *Apanteles glomeratus* Linnaeus (Tagawa et al. 1985),

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Dendrocerus carpenteri (Curtis) (Schwörer et al. 1999) and *A. ervi* (He & Wang 2008). Furthermore, female age was found to be more important in mating success, than male age. It could be possible that the increasing age of females may be related to a higher level of sex pheromone production, thus leading to higher mating success. The increased egg load together with the age of females (Section 3.4.3.1) may enable them to become more sexually receptive, thus resulting in higher mating success.

Size-assortative mating has been reported in *Eretmocerus eremicus* Rose & Zolnerowich, where body size incompatibility could prevent males from successfully copulating with receptive females (Hunter et al. 1996). In the present study, the body size of *D. semiclausum* adults did not affect mating success. However, my experimental design cannot test the size effect in a choice situation, i.e. each sex has a choice between mates of different size, where mating success may differ (van den Assem et al. 1989; Ueno 1998).

Abbas (1988) suggested that *D. semiclausum* adults mate during both day and night. The present study found that mating success was much lower during the scotophase, than during the photophase. During the photophase, males actively approached females and performed courtship displays and females responded to males, by running or flying away or accepting males, after several mounting attempts. However, during the scotophase, both sexes generally remained idle in the glass vial, with males occasionally attempting to mount females when they made contact with them. It is thus clearly indicated that, similar to emergence (Section 3.3.3.1) and oviposition (Section 4.3.4), light is probably the main factor that stimulates mating activity. As a result, visual cues may play an important role in the mating behaviour of *D. semiclausum* adults.

Ridley (1993) stated that, in parasitic Hymenoptera, solitary species tend to be monandrous, whereas gregarious species tend to be polyandrous. In the present study, *D. semiclausum* males showed a strong tendency to remate after the first mating and a single male remated with the same female up to six times, during the 16 h of pairing (Figure 4.5). Multiple mating has also been reported in some other solitary species, such as the *Cephalonomia tarsalis* (Ashmead) (Cheng et al. 2004). Since the *D. semiclausum* males and females were paired in a confined arena, during the present study, multiple mating may have resulted from the repeated courtship attempts performed by the males. However, it could be possible that multiple mating is a natural behaviour in this species. Burton-Chellew et al. (2007) suggested that prolonged exposure of males to females

may lead to an increase in courtship attempts, but it may not necessarily lead to mating success, unless females are ready for it.

Generally, the olfactory attractiveness of females diminishes after they are inseminated (Schwörer et al. 1999). However, in this study, mated males were observed to be more active and they attempted to remate with the females, even after first mating. It could be possible that the olfactory attractiveness of *D. semiclausum* females does not diminish immediately after mating. For example, in *L. distinguendus*, sex pheromones produced by females are still perceived by males, at least 5 days after mating (Ruther et al. 2000). Alternatively, mating could stimulate the mating behaviour of males, thus making them more inclined to mate. Evidence of *A. ervi* males learning from previous mating has been reported by He & Wang (2008). This could be the reason for the shorter mating interval between two matings, after the first mating (Figure 4.6).

From the present study, it is unclear whether remating is an adaptive strategy, which enhances the fitness of the parasitoids. Mated *D. semiclausum* females may benefit, by preventing males from mounting and courting and thus saving their time and energy, if they have received sufficient sperm from a single copulation: and mated males may gain more by waiting for their semen components to replenish, before mating again. However, in the present study, females of *D. semiclausum* repeatedly remated with the same males. It is possible that remating in *D. semiclausum* males may help them transfer more sperm, which the females need to fertilise their eggs throughout their life time, or it could be a sperm competition: strategy of males being to ensure their paternity by providing sufficient sperm, which otherwise females could acquire it from other males (Damiens & Boivin 2006). Remating leads to greater offspring production in some animals (Ridley 1988; Arnqvist & Nilsson 2000), while it could be costly in other species, shortening their longevity (Arnqvist & Nilsson 2000). However, the effect of multiple mating on the longevity of *D. semiclausum* is not clear.

Mating in many hymenopteran parasitoids is brief, usually lasting less than 1 min (Quicke 1997; He 2008). For example, in some ichneumonid species, mating lasts about 22 seconds in *Aptesis nigrocincta* Gravenhorst (Babendreier 2007) and 20–60 seconds in *Diadromus collaris* (Gravenhorst) (Liu et al. 2001). However, in this study, the mating duration of *D. semiclausum* is considerably longer, usually lasting 6 to 7 minutes. Although, to some extent, the mating duration is affected by several biotic and abiotic factors, such as age and body size (Field et al. 1999), such a large discrepancy in the mating duration of *D. semiclausum*, compared to other hymenopterans insects, may

be attributed to other reasons. Generally, the copulation process mainly consists of two stages, i.e. firstly, the males accomplish the task of intromission by inserting their aedeagus into the inner end of the female genitalia; and secondly, the males complete the sperm transfer (Eberhard & Pereira 1995). However, there could be a third stage, in which males complete sperm transfer but remain in copula, in order to deter females from remating with rivals (Alcock 1994). A variation in the length of any of these phases may result in longer copulation duration, which could be an adaptive reproductive behaviour to enhance the fitness. Although it has been less studied in hymenopterans wasps, a longer mating duration in many other insects has been found to be associated with an increase in sperm storage by females (Yamagishi & Tsubaki 1990; Cuatianquiz & Cordero 2006); greater female non-receptivity to subsequent males (Kuba & Ito 1993; Cuatianquiz & Cordero 2006); improved sperm precedence of first mates (Thornhill & Sauer 1991); and a prolonged ability to physically deter other males, through contact mate guarding (Alcock 1994). This present laboratory study shows that females could repeatedly mate. Thus, the longer mating duration in *D. semiclausum* could be associated with any of the aforementioned evolutionary reproductive strategies. On the other hand, a longer copulation duration in *D. semiclausum* may have resulted from some physical constraints of passing ejaculate (Rutowski & Gilchrist 1986). However, a concrete reason cannot be inferred from the present study.

Similarly to *A. kamali*, a parasitoid of hibiscus mealybug (Sagarra et al. 2007), the premating period and mating duration of the first mating appeared to be pre-programmed in *D. semiclausum*, regardless of mate body size and age. A similar observation was made by Teder (2005) on several other ichneumonid wasps. However, the mating duration increased with the number of successive matings. Thus, a variation in mating behaviour can occur, depending on the mating status. In insects, ejaculates transferred by males contain sperm, in addition to accessory glands secretion (Leopold 1976) and multiple mating may deplete a male's reserve of ejaculate constituents (Rutowski et al. 1987). Ejaculates are physiologically costly for males. Increasing the mating period after the first mating may have been the result of a depletion in the ejaculates' reserve and thus males necessarily need a longer time to rejuvenate their ejaculates (Bissoondath & Wiklund 1996). A significant increase in the duration of copulation, together with an increase in the number of successive matings by a male, has been reported in an egg parasitoid *Trichogramma evanescens* Westwood (Damiens & Boivin 2005).

4.3   Oviposition Rhythms of *D. semiclausum*

4.3.1   Introduction

Insect oviposition is often rhythmic (Saunders 1982). Quicke (1997) suggested that in many parasitoid species, oviposition generally occurs in the morning: For example, in *Coccophagus bartletti* Annecke & Insley, an parasitoid of scale insects, the majority of eggs (81%) were laid early in the morning (Walter 1988). However, in some other species, such as *Uscana lariophaga* Steffan, an egg parasitoid of the cowpea bruchid beetle *Callosobruchus maculats* (Fabricius), eggs were laid throughout the 24-h period with about 70% taking place during the first 12 h, regardless of it being in the photophase or the scotophase (van Huis & Appiah 1995).

A knowledge of circadian oviposition patterns can be useful, in order to optimise a mass-rearing system, in the context of a biological control programme (Idoine & Ferro 1990). Frequently, such patterns are associated with the production of females (van Huis & Appiah 1995; Sagarra et al. 2000): For example, *C. bartletti* females lay most of their female eggs in the early morning (Walter 1988) and thus the parasitised larvae, during the first few hours of the photophase, are ideal for the production of more females in the mass-rearing programme. In *Anagyrus kamali* Moursi, a parasitoid of the hibiscus mealybug *Maconellicoccus hirsutus* (Green), lifetime fecundity and reproductive period are optimum, when it is reared in conditions of no light and constant temperature. A knowledge of this behaviour could help save tremendous energy costs, in the mass production of this parasitoid in an insectary (Sagarra et al. 2000). Moreover, a determination of the approximate rate of egg deposition, during the foraging period, could provide useful information for estimating the appropriate host density, which could be offered to parasitoids in laboratory rearing. This would help in the optimisation of resources, without constraining the reproductive capacity of the parasitoids.

To date, the oviposition behaviour of *D. semiclausum* is still poorly understood. The aim of the following section is to describe and discuss the circadian oviposition pattern of *D. semiclausum*.

4.3.2 Materials and Methods

In order to investigate whether parasitoids could oviposit during the photophase or scotophase, nine mated 3-day-old females were individually (and daily) provided with 10 third instar DBM larvae a jar (Figure 3.1E), for two days prior to the experiment to allow them to obtain oviposition experience. Soon afterwards, these females (5 days old) were individually introduced into a jar, which maintained a cabbage seedling infested by 30 third instar DBM larvae, immediately after lights on. Sixteen hours later, the seedling (together with the DBM larvae) was removed (prior to lights off) and replaced immediately with another seedling infested by 30 healthy larvae. Cabbage seedlings infested with DBM larvae parasitised by parasitoids, during the photophase and scotophase, were maintained individually in plastic containers, until pupation (Figure 3.1C). Parasitoid pupae were counted, to determine the number of parasitised larvae.

In order to determine the oviposition patterns of *D. semiclausum* during the 16-h photophase, 19 mated 5-day-old females, which were treated as above, were individually offered 20 third instar DBM larvae feeding, on a cabbage seedling in a jar (Figure 3.1E), immediately after lights on. Two hours later, the seedling (together with the DBM larvae) was removed and replaced with another seedling infested by 20 healthy DBM larvae. This process was repeated, until 8 oviposition bouts were completed during the photophase. Cabbage seedlings infested with DBM larvae, parasitised by parasitoids in each oviposition bout, were maintained individually in a perplex container (Figure 3.1C), until pupation. 12 of 19 experimental female parasitoids were dissected, in order to count the matured eggs left in the ovaries, at the end of the experiment. In order to determine how long parasitoids take to start the oviposition activity, after lights on, females were monitored soon after the establishment of the first oviposition bout, until they performed the first sting to a DBM larva. Adults emerging from parasitised larvae in different oviposition bouts were counted and sexed. DBM larvae, which were dead during the experiment, were dissected to detect whether they were parasitised.

4.3.3 Statistical Analysis

Data on the number of larvae parasitized, in different oviposition bouts in the photophase were normally distributed and thus they were analysed using ANOVA,

followed by a Tukey's studentised range test. Data on the number of female offspring, which emerged from different oviposition bouts, were not normally distributed and hence they were analysed using the nonparametric Kruskal-Wallis test, followed by Dunn's procedure for multiple comparison.

4.3.4 Results

Oviposition did not occur during the scotophase. The mean total number of DBM larvae, which were parasitized by a female in a 16-h photophase, was 24.4 ± 1.0.

Females were observed to oviposit throughout the photophase. Before landing on the plants, these females cleaned their legs and antennae and fanned their wings on the container's walls. After they landed on the plants, they started searching and stinging the larvae. The time that females required, to perform the first stinging of DBM larvae, was 71.18 ± 14.05 minutes, after they were released into the jar. The number of larvae parasitised gradually increased during the first two oviposition bouts and it significantly decreased (ANOVA: $F_{7,44} = 7.54$, $P < 0.001$) (Figure 4.7). The total number of larvae parasitised during the 16 h of photophase ranged from 7 to 57, with a mean of 31.42 ± 3.16.
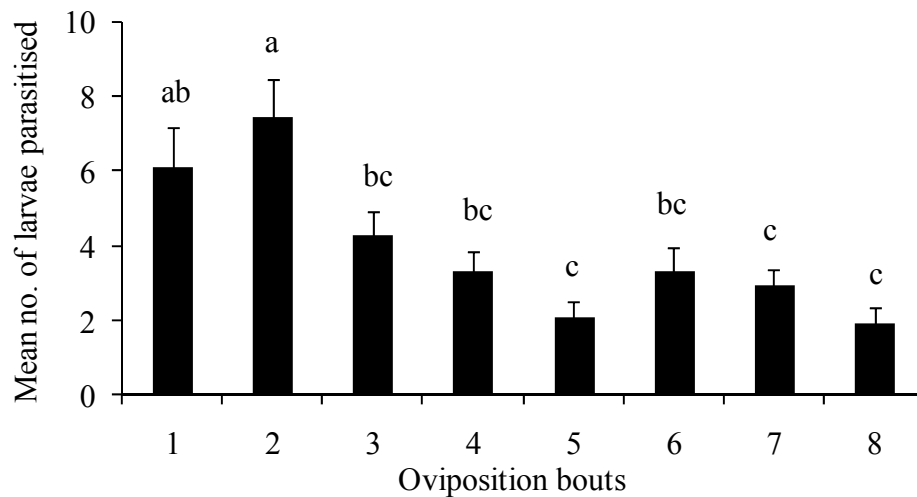
Figure 4.7 Mean ($\pm$ SE) number of DBM larvae parasitised during the 16 h photophase. Columns with the same letters are not significantly different ($P > 0.05$).

Significantly more female offspring emerged from the larvae parasitised during the first two oviposition bouts (Kruskal-Wallis test: $\chi^2 = 30.28 > \chi^2_{7,0.05} = 18.48$; $P < 0.001$) (Figure 4.8). No females emerged from the last oviposition bouts.
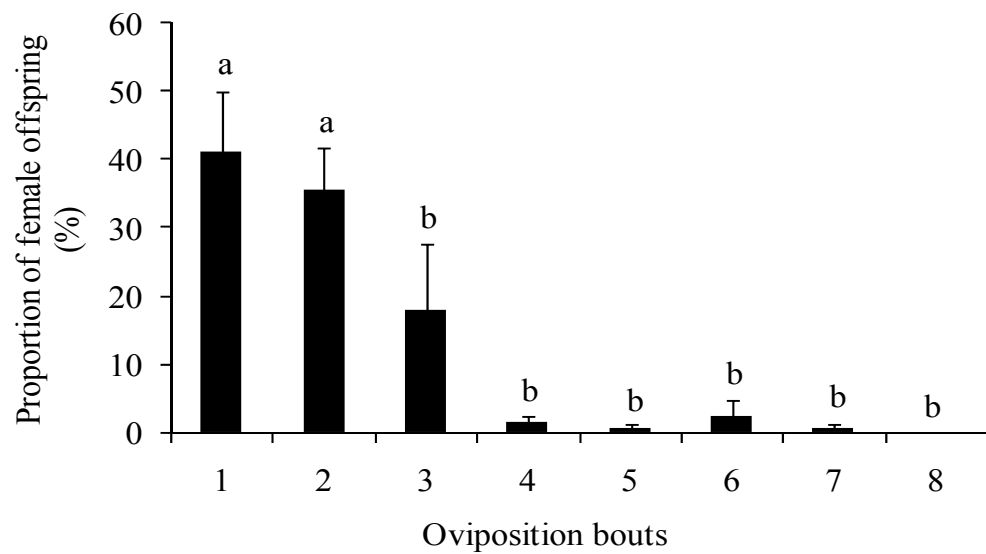
Figure 4.8 Proportion of female progeny emerged in different oviposition bouts. Columns with the same letters are not significantly different (P > 0.05).

old) (Figure 4.9A), the ovipositing females did not have any mature eggs in their oviduct and calyx (Figure 4.9B).
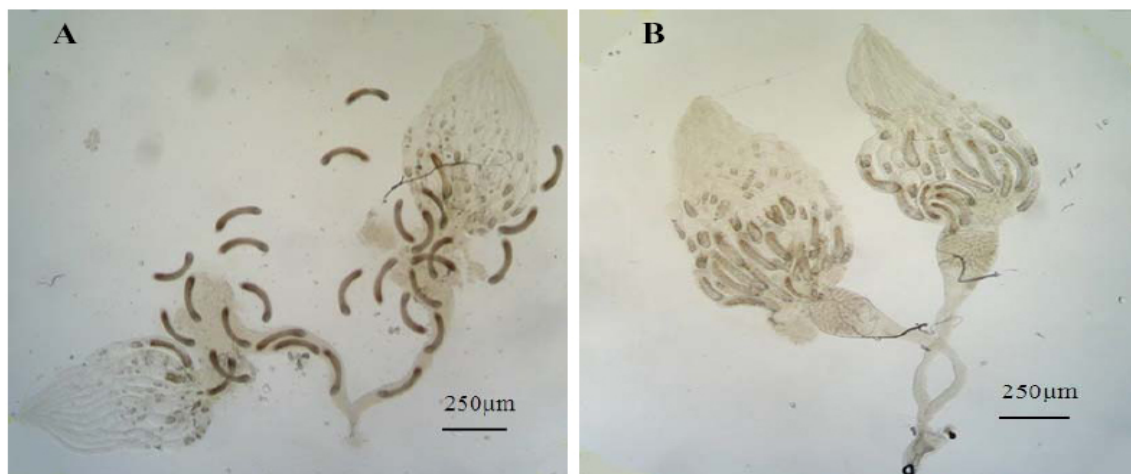
Figure 4.9 Mature and/or developing eggs in the ovaries of 6-day-old females: (**A**) non-ovipositing females; and (**B**) ovipositing females.

4.3.5   Discussion

Daily insect activities are often determined by an endogenous or exogenous rhythm, or a combination of both (Beck 1980). The present and previous study (Talekar & Yang 1991) confirm that *D. semiclausum* females only oviposit during the day time.

This suggests that oviposition in *D. semiclausum* is determined by an exogenous factor, the light.

Females parasitised more larvae during the early oviposition bouts. The dissection of females, at the end of oviposition, clearly shows the lack of mature eggs in the oviduct and calyx regions of the ovipositing females, when compared to those completely deprived of hosts (Figure 4.9B). Thus, the decreasing parasitism, during the photophase, is the result of a decreasing load of mature eggs. Egg load mediating oviposition behaviour is well known in insect parasitoids, as seen in *V. canescens* (Hughes et al. 1994) and *Coccophagus atratus* Compere (Donaldson & Walter 1988).

Ovipositing females, with depleted mature eggs, were found to possess some developed or nearly mature eggs in their ovaries, when compared to the non-ovipositing ones (Figure 4.9A, B), thus suggesting that oviposition may stimulate egg maturation. Furthermore, females were found to deposit more fertilised eggs during the first two oviposition bouts, as evidenced from the higher number of female progeny emerging from parasitised larvae, during first 4 h of photophase. Walter (1988) reported that females of *C. bartetti* produced more female progeny in the morning. The early deposition of fertilised eggs during oviposition sequence has also been reported in the parasitic wasp *Bracon hebeter* Say (Antolin et al. 1995). It appears that a sex allocation decision by parasitoids is also affected by their egg load, or it could be the reproductive strategy of a female to enhance the fitness, by ensuring the continuity of her generation.

As females parasitise more larvae and lay more fertilised eggs, during the early oviposition bouts, the provision of host larvae, for 4-6 hours after light on, may help to produce more female progeny.

4.4   Host Stage Preference and Its Effect on Reproductive Fitness of *D. semiclausum*

4.4.1   Introduction

Parasitoid fitness is usually measured by life-history traits, such as developmental time, survival, fecundity, sex ratio and body size (Godfray 1994; Roitberg et al. 2001). Oviposition decisions (e.g. host selection and sex allocation), by female parasitoids, are expected to correspond with host quality, since fitness for their progeny depends on the quantity and quality of the resources provided by a single host

(Nicol & Mackauer 1999; Chau & Mackauer 2001). Charnov (1979) first demonstrated that host size-dependant sex allocation can be adaptive, if there is a differential effect of host size on the reproductive success of males and females. The host size model assumes that host quality is a linear function of host size, with larger hosts containing a greater quantity of resources, and thus thay are more profitable for parasitoid progeny, than smaller ones. Several studies have shown such adaptive behaviour in parasitoids (Fox et al. 1990; Nishimura 1993; Sequeira & Mackauer 1993; Urano & Hijii 1995; Bernal et al. 1999; Ueno 1999b). In koinbiont parasitoids, such as *D. semiclausum*, which allow the hosts to feed and grow after parasitisation, there is a higher degree of uncertainty about the resource for their progeny, since host quality varies during the course of the parasitoid's development (Mackauer 1986; Mackauer 1996; Harvey & Strand 2002). Many studies have suggested that the host's stage, at the time of parasitism, affects the reproductive fitness of the resulting progeny, for example, altering developmental time (Petitt & Wietlisbach 1993; Jenner & Kuhlmann 2006); progeny sex ratio and body size (King 1988; Ueno 1998; Jenner & Kuhlmann 2006); and mortality and emergence rate (Jenner & Kuhlmann 2006).

An understanding of the role of host quality, within such fitness parameters, is important for studies of evolutionary ecology and applied biological control. For example, in an applied sense, body size (particularly in females) is an important component of fitness, since it is usually positively correlated with longevity (van den Assem et al. 1989; Visser 1994; Ueno 1999b), pheromone production (Cloutier et al. 2000); egg load (Visser 1994); egg size (Visser 1994); fecundity (Bai et al. 1992; Visser 1994); dispersal and host searching ability (Visser 1994; Ellers et al. 1998); oviposition success (Ueno 1999b); and an innate capacity for increase (Cloutier et al. 2000).

Sex ratio is another important consideration in biological control programmes, where the production of more female progeny is required, in laboratory mass-rearing programmes (Bernal et al. 1999). Knowledge, relating to host quality induced behaviour on sex allocation, helps to develop efficient rearing techniques. For example, Chow & Heinz (2006) developed a modified technique using a mixed proportion of large and small hosts for the rearing of the *Diglyphus isaea* (Walker), a parasitoid of agromyzid leafminers *Liriomyza langei* Frick on chrysanthemum. Their technique increased the female progeny production by 13%.

The nutritional physiology in DBM larvae is known to be affected by parasitisation of *D. semiclausum* (Huang et al. 2008a). Parasitisation results in a

significant decrease in growth, feeding, excretion, assimilation and respiration of the DBM larvae (Cai et al. 2005; Huang et al. 2008a). Cai et al. (2005) suggested that parasitised second instar DBM larvae consumed less food, compared to unparasitised larvae, with no difference found between third and fourth instar larvae. Thus, it is expected that this differential nutritional contribution of host stages may have a significant impact on the growth and development of developing parasitoid larvae. However, the role of host size, in life history traits, such as growth, development, survival and sex ratio in *D. semiclausum*, is still poorly understood and further study will help in the consideration of proper host stage, in laboratory mass-rearing. This section aims to study the effect of host stage on the reproductive fitness of *D. semiclausum*.

4.4.2   Materials and Methods

4.4.2.1 Identification of Host Stages

The first instar DBM larvae mine into the leaves, whilst the second, third and fourth instar larvae feed outside the leaves (Shi et al. 2002). Therefore, the first instar was not tested for parasitism. The head width of DBM larvae is the most reliable measure, in order to identify host stages (Poelking 1992). The present preliminary study found that, immediately after moulting, the head width increases by 0.4, 0.6 and 1.0 mm, for the second, third and fourth instar larvae, respectively: Newly moulted, second instar larvae have a distinct dark head and newly moulted fourth instar ones have light green last four abdominal segments (Figure 4.10). In the present study, the developmental period of DBM larvae, since oviposition, was recorded as 9~10, 12~13 and 15~16 days for the second, third and fourth instars, respectively. Therefore, in the present study, I identified the host stages, using the above three parameters.

Figure 4.10 Different stages of DBM larvae. This picture was taken in the present study.

4.4.2.2 Host Stage Preference and its Effect on Development, Body Size, Emergence and Sex Allocation of *D. semiclausum*

In order to determine the host preference by *D. semiclausum*, in relation to host stage and its effect on *D. semiclausum* reproduction, 15 DBM larvae (5 from each of the second, third and fourth instar larvae), which were feeding on a cabbage seedling, were offered to a 1-day-old mated female for oviposition in a plastic chamber (Figure 3.1E). The female was allowed to stay in the chamber for 24 h, and then moved to another chamber, together with 15 healthy DBM larvae at three different stages as above, etc. until she died. After each trial, the DBM larvae were separated according to stage and maintained on a cabbage seedling until pupation. Ten replicates were conducted in this experiment. Parasitoid cocoons were collected from the plant, then weighed and kept individually in glass vials, until emergence (Figure 3.1G). Cocoons were checked every day and emerged adults were sexed. The developmental time from oviposition to emergence was recorded for each parasitoid. The parasitism rate was estimated (i.e. number of parasitoid cocoons /total number of hosts).

4.4.3 Statistical Analysis

A goodness-of-fit test was used to test whether the data were normally distributed. The data on the host stage and developmental time were not normally

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distributed, and thus they were analysed using the nonparametric Kruskal-Wallis test, followed by Dunn's procedure for multiple comparison. The remaining data were normally distributed and analysed using ANOVA, followed by a Tukey's studentised range test. Proportional data were arcsine transformed, before ANOVA.

4.4.4 Results

4.4.4.1 Host Stage Preference

The parasitism rate ranged from 78.03 ± 3.33 to $78.18 \pm 3.53\%$ which was not significantly different between the three DBM instars (ANOVA: $F_{2,27} = 0.01$, $P > 0.05$), indicating that *D. semiclausum* females do not have any preference for host stages, for their oviposition.

4.4.4.2 Effect of Host Stage on Parasitoid Development

Diadegma semiclausum were able to parasitise and develop in all three host stages. Parasitoids developed significantly faster when the third and fourth instar larvae were parasitised than when the second instar ones parasitised (Kruskal-Wallis test: $\chi^2 = 41.63$, 22.69 and 35.62 for male, female and average, respectively, $P < 0.001$) (Table 4.3). Developmental time was significantly shorter for males than for females, in all host stages (Kruskal-Wallis test: $\chi^2 = 20.21$, 17.51 and 13.47 for the first, second and third instars, respectively; $P < 0.001$) (Table 4.3).

Table 4.3 Effect of host stage at parasitisation on developmental duration (days) of *D. semiclausum*

Host stage parasitised	Male	Female	Average
2nd instar	16.93 ± 0.09 aβ	17.59 ± 0.13 aα	17.08081 ± 0.08 a
3rd instar	16.30 ± 0.06 bβ	16.75 ± 0.09 bα	16.43345 ± 0.05 b
4th instar	16.24 ± 0.06 bβ	16.67 ± 0.09 bα	16.35974 ± 0.05 b

Means ($\pm$ SE) followed by the same English letters in columns and Greek letters in rows were not significantly different ($P<0.05$).

4.4.4.3 Effect of Host Stage on Parasitoid Body Size

The mean pupal weight was significantly greater, when parasitoids developed from DBM parasitised at the third and fourth instars (ANOVA: $F_{2,1154} = 32.04$, $P < 0.0001$) (Table 4.4). In each host stage, females were significantly heavier than males (ANOVA: $F_{1,372} = 78.82$, $P < 0.0001$ for second instar; $F_{1,385} = 46.25$, $P < 0.0001$ for third instar; $F_{1,394} = 9.58$, $P < 0.05$ for fourth instar) (Table 4.4).

Table 4.4 Effect of host stage on pupal weight (mg) of *D. semiclausum*

Host stage	Male	Female	Average
2nd instar	3.33 ± 0.03 bβ	3.85 ± 0.05 bα	3.47 ± 0.03 b
3rd instar	3.68 ± 0.04 aβ	4.13 ± 0.06 aα	3.83 ± 0.03 a
4th instar	3.66 ± 0.04 aβ	3.90 ± 0.06 bα	3.73 ± 0.03 a

Means (± SE) followed by the same English letters in columns and Greek letters in rows are not significantly different ($P < 0.05$).

4.4.4.4 Effect of Host Stage on Emergence Rate and Sexual Allocation

Host stage did not have significant effect on emergence (ANOVA: $F_{2,27} = 0.12$, $P > 0.05$) and sexual allocation (ANOVA: $F_{2,27} = 0.24$, $P > 0.05$) (Table 4.5). The sex ratio of *D. semiclausum* was highly male-biased (> 70%), from all host stages (Table 4.5).

Table 4.5 Effect of host stage on emergence and sex ratio of *D. semiclausum*

Host stage	Emergence (%)	Male progeny (%)
2nd instar	93.42 ± 1.59 a	73.28 ± 6.05 a
3rd instar	94.13 ± 0.06 a	67.67 ± 5.74 a
4th instar	92.83 ± 1.68 a	71.83 ± 5.03 a

Means (± SE) followed by the same letters in columns are not significantly different ($P > 0.05$).

4.4.5   Discussion

Optimal models of host–parasitoid relationships have assumed that host quality increases, as a function of host size or age at parasitisation (Charnov 1979; Charnov et al. 1981). Results of the current study contribute to the growing literature that suggests that this generalisation is not necessarily valid for koinobiont parasitoid species and therefore it has to be looked at as being species specific.

Many studies show that host stage selection affectes, parasitoid fitness by restricting the proper survival of progeny, for example, smaller hosts may impose a higher reproductive cost, through higher host mortality after parasitisation (Jenner & Kuhlmann 2006), or a lower parasitoid emergence rate (Elzinga et al. 2003). However, in the present study, such effects were not evidenced, i.e. host instars did not affect the survival and emergence of *D. semiclausum*. On the other hand, *D. semiclausum* females showed no preference to a particular host size, since parasitism rate was similar at all stages. Thus, this suggests that all host stages are equally suitable for the development of *D. semiclausum* offspring.

Host size, which affects the developmental time of parasitoid progeny, has been reported in many species, for example: *Opius dissitus* Muesebeck (Hymenoptera, Braconidae) (Petitt & Wietlisbach 1993); *Compoplex dubitator* Horstmann (Hymenoptera: Ichneumonidae) (Jenner & Kuhlmann 2006); and *Microplitis tristis* Nees (Hymenoptera: Braconidae) (Elzinga et al. 2003). In this study, when the third and fourth instar larvae were parasitised, *D. semiclausum* developed faster, indicating that it has the ability to adjust its development and growth rate, according to the host stage. Happe et al. (1988) also reported that the developmental time of *D. semiclausum* is shorter, when it is reared from larger host larvae. Irrespective of host stage, the developmental time of female progeny was longer, than that of male progeny. A longer developmental time (of females over males) has been reported in other ichneumonid parasitoids, for example, *Aptesis nigrocincta* Gravenhorst (Babendreier 2007) and *Diadromus collaris* (Gravenhorst) (Liu et al. 2001). King (1988) suggested that the developmental time of males being shorter than females, is a typical characteristics in most parasitic wasps, which has resulted from selection pressure for small males, leading to earlier emergence. Results of the previous (i.e. emergence pattern in Section 3.3.3.1) and present studies support this hypothesis.

Charnov et al. (1981) assumed that large hosts, which contain more resources than small ones will result in larger parasitoid progeny. It has been generally accepted that, in insect parasitoids, males are smaller than females (van den Assem et al. 1989; Mackauer 1996) and if female body size is more important in reproductive fitness than male body size, then sexual dimorphism is expected to occur (Mackauer 1996). The asymmetrical developmental period and body size for male and female progeny, from a given size of hosts, suggest that females of *D. semiclausum* may utilise more of the host resource and they need a longer time to become physiologically more mature than males.

Developmental time and body size are considered to be two important and often conflicting components of fitness (Godfray 1994; Jenner & Kuhlmann 2006). Harvey & Strand (2002) suggested that parasitoids attacking foliar-feeding hosts may favour rapid development, over a larger size, whilst those parasitising concealed hosts favour size over developmental duration. However, contrary to this general belief, both the development and body size of *D. semiclausum* were favoured in larger hosts. Similar findings have been reported in some other parasitoids, for example, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) developing in *Plodia interpunctella* (Hiubner) (Lepidoptera: Pyralidae) (Harvey et al. 1994).

Several studies have suggested that the mothers control the sex of their offspring according to the host size (King 1988), since it has greater effect on the ultimate reproductive success of emerging females. In the present study, sex allocation decision by *D. semiclausum* females was not affected by host stage. This study does not support the parasitoid sex allocation strategy suggested by Charnov et al. (1981). It may probably be that host size does not play any, or even a small role, in the reproductive success of developing parasitoids and thus it fails to alter females' sex allocation strategy. However, it may affect some other aspects of reproductive success such as egg load (Section 3.4.3.2) and longevity of progeny (Section 3.7.1), through its influence on body size and/or developmental time of both male and female progeny. Furthermore, the progeny sex ratio was highly male biased ($\approx$ 70%), irrespective of host stages. An extremely high male biased sex ratio has also been reported, in both laboratory (78~83%) (Honma et al. 2005; Takashino et al. 2005; Rossbach et al. 2006) and field studies (80%) (Chua & Ooi 1986).

There is widespread variation in the sex ratio of this species, as reported in several studies. Natural enemies used for biological control frequently go through genetic bottlenecks during collection and rearing and also following estabilishment in

the field, which decreases genetic variability and leads to the poor adaptability of exotic parasitoids to the new environment (Unruh et al. 1983). In Hymenoptera, a reduced genetic variability causes an additional problem: the production of homozygous diploid males, as a consequence of sex-determination mechanism (Stouthamer et al. 1992). As in many hymenopteran parasitoids, inbreeding depression may result in the production of more males in *D. semiclausum* (Butcher et al. 2000). The highly male biased sex ratio, in the present study, may have been a result of the breeding colony, which may have been estabilished from a highly male-biased field population. Since *D. semiclausum* adults were introduced into New Zealand a long time ago (Beck & Cameron 1992), there is possibility that the existing field population may have lost its genetic variability. In an attempt to improve the sex ratio of *D. semiclausum*, by reintroducing the New Zealand strain, Takashino et al (2005) found a highly male biased (≈ 84%) sex ratio in the New Zealand *D. semiclausum* strain. Takashino et al. (2005) also reported that the Japanese strain of *D. semiclausum* also produced a highly male-biased sex ratio. Thus, the insufficient control of DBM population in summer, by this species in New Zealand, may have been a result of highly male-biased sex ratio of *D. semiclausum*.

Diploid males are considered as reproductively dead, since they have low viability (Petters & Mettus 1980); they are sterile (Krieger et al. 1999); or they produce unreduced diploid sperm and sterile triploid offspring (Smith & Wallace 1971; Krieger et al. 1999). In such cases, females mated with diploid males should produce all males. However, in the present study, the production of at least some females' offspring, by these parasitoids, suggest that either the diploid males in the species are reproductively viable, as suggested in some other species (Elias et al. 2009), or there are other mechanisms that may result in a highly male biased sex ratio. The reproductive ability of diploid males, in this species, needs further investigation. Highly male-biased laboratory cultures can be attributed to several other reasons. One common reason could be the existence of *Wolbachia pipientis* Hertig, an endosymbiont bacterium found in many insects (Werren 1997). *Wolbachia* often results in cytoplasmatic incompatibility. *Wolbachia*-induced cytoplasmic incompatibility has been reported in *Nasonia* species, which can result in all male progeny, when an infected male mates with an uninfected female and mixed progeny, when both parents are infected (Breeuwer & Werren 1990; Reed & Werren 1995). Research on whether such a sex-distorter exists is vital, in order to explain the reason for highly-male biased ratios in *D. semiclausum*.

4.5   Effect of Host Density on Reproduction of *D. semiclausum*

4.5.1   Introduction

The impact of a parasitoid on its host population depends upon its ability to find and parasitise hosts and to increase the number of its offspring, when needed (Waage & Hassell 1982; Mackauer 1983). The spatial patterns of parasitism by insect parasitoids, and the importance of these to the population dynamics of host-parasitoid interactions, have been topics of widespread interests amongst pure and applied ecologists. Many models have been developed, in which heterogeneity in the distribution of parasitism has played an important part in population regulation (Hassell & May 1973; Hassell & May 1974; Murdoch & Oaten 1975). However, the potential effects of host density, on other aspects of reproductive behaviour, have been neglected. Apart from parasitism, few studies have noted the effect of host density on the reproductive rate (Liu 1985), sex allocation (Sandlan 1979; Asante & Danthanarayana 1993), progeny emergence rate (Sagarra et al. 2000) and longevity (Ellers et al. 2000) of the ovipositing parasitoids.

Life history models suggest that trade-offs exist between major components of fitness in organisms, due to constraints on the rate at which resources can be acquired and used for vital functions (Bell 1980; Reznick 1985; Bell & Koufopanou 1986). Trade-offs represent the costs paid in the currency of fitness, when a beneficial change in one trait links to a detrimental change in another (Stearns 1989). One of the most common ways of a expressing trade-off is the negative correlation between early and late reproduction (Richner & Tripet 1999), or between reproduction and survival (Roitberg 1989; Ellers et al. 2000). Insect parasitoids have a limited amount of energy resources accumulated during the larval stage and this is supplemented by non-host food, such as nectar and honeydew (in non-host feeding species) and the hosts' fluids or tissues (in host feeding species), during the adult stage (Rivero & Casas 1999; Jervis et al. 2007). The energy acquired is used for somatic maintenance, locomotion and future egg production (Rivero & Casas 1999; Jervis et al. 2007; Visser & Ellers 2008). More investment in reproduction means less energy available for somatic maintenance and a lower probability of survival. Host density may affect the fecundity and reproductive rate of the parasitoids (Liu 1985; Tripathi & Singh 1991). As a result, parasitoids may adopt different oviposition strategies, at different host density (He et al. 2006).

The sex ratio in hymenoptera parasitoids has long been an interest amongst researchers. Although many factors affect the parasitoid sex ratio (King 1987; Godfray 1994), the effect of host density has received less attention. Few studies have provided evidence that sex allocation of parasitoids is also influenced by host density (Andersen 1961; Chong & Oetting 2006a; He et al. 2006). In analytical host-parasitoid models, changes in the density-dependent sex ratio of parasitoids influence the level of host population equilibrium and the stability of the host-parasitoid relationships, thus affecting the success of biological control (Waage & Hassell 1982; Hassell & Waage 1984).

An understanding of the influence of host density, on the reproductive fitness of parasitoids may have a direct application in biological control programmes. Useful behaviours can be manipulated to optimise mass rearing procedures. For example, Montoya et al. (2000) studied the functional response of tephritid fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead) in the larvae of *Anastrepha ludens* (Loew) and they suggested that lower parasitoid/host ratios would be better way to optimise mass rearing, since higher ratios did not produce a greater host mortality. Host distribution in the field may vary, both spatially and temporally. Therefore, when a large number of parasitoid females are released, for biological control, they may have variable opportunities to encounter their hosts, depending on host density and distribution pattern. An understanding on how these conditions affect the important fitness components of parasitoids will help in the decision making process for inundative releases.

Host density-dependent aggregation behaviour has been reported in *D. semiclausum* (Wang et al. 2004; Legaspi & Legaspi 2005). However, whether host density affects life history traits, in particular longevity, sex ratio, reproductive period and parasitism rate of the parasitoid, is unknown. The present section aims to investigate the effect of host density in the aforementioned life history traits of *D. semiclausum.*

4.5.2   Materials and Methods

Two host densities (5 and 30 third instar DBM larvae) were set up in this experiment. Mated 3-day-old females were used for the experiment. As mentioned in section 3.2.1, parasitoids were individually released into a plastic chamber containing

the host larvae, which had infested a cabbage seedling. The seedling, together with the larvae, was removed 24 h after exposure to the parasitoid. A new seedling infested by the same number of larvae was provided for the parasitoid, for 24 h. This process continued until the parasitoid female died. The number of cocoons and adults produced by each female parasitoid was recorded, on a daily basis. Emerged adults were sexed. The parasitism rate was calculated, as mentioned in section 4.4.2.2. The longevity of the experimental females was recorded and dead females were dissected, in order to count the number of mature eggs remaining in their ovaries. There were 10 and 9 female parasitoids tested for a host density of 5 and 30 larvae, respectively.

4.5.3 Statistic Analysis

A goodness-of-fit test was used to test whether the data were normally distributed. Data on adult emergence was not normally distributed and thus analysed, using the nonparametric Kruskal-Wallis test, followed by Dunn's procedure for multiple comparison. All other data in Table 4.6 were normally distributed and thus analysed by using ANOVA, followed by a Tukey's studentised range test. Proportional data were arcsine transformed before ANOVA.

The variation in the number of male progeny produced by *D. semiclausum* females, during the oviposition period, at a host density of 30 DBM larvae, was analysed using a cubic-linear regression and that at a host density of 5 DBM larvae was analysed, using a linear regression. The number of larvae parasitised by *D. semiclausum* females, during their oviposition period, at both density conditions, were analysed using a quadratic-linear regression.

4.5.4 Results

The total number of host larvae parasitised was significantly higher in the density of 30 DBM larvae (ANOVA: $F_{1,17} = 216.61$, $P < 0.001$), but sex ratio and parasitism rate were significantly higher in a host density of 5 larvae (ANOVA: $F_{1,17} = 4.81$, $P < 0.05$ for sex ratio; $F_{1,17} = 17.26$, $P < 0.001$ for parasitism rate) (Table 4.6). However, mother longevity (ANOVA: $F_{1,17} = 0.01$, $P > 0.05$) and egg load at death (ANOVA: $F_{1,17} = 2.46$, $P > 0.05$) and offspring adult emergence (Kruskal-Wallis test:

$\chi^2 = 2.67 < \chi^2_{1,0.05} = 3.84$; P > 0.05) were not significantly different between these two host densities (Table 4.6).

Table 4.6 Effect of host density on reproductive fitness of *D. semiclausum*.

	Host density	
	5	30
Total no. of parasitism	112.7 ± 15.28 b	602.33 ± 31.13 a
Parasitism (%)	95.61 ± 0.82 a	78.31 ± 4.31 b
Male progeny (%)	87.15 ± 2.66 a	80.35 ± 1.37 b
Mother longevity (days)	25.70 ± 3.12 a	26.11 ± 1.44 a
Adult emergence (%)	92.21 ± 2.56 a	92.35 ± 0.93 a
No. of eggs left in ovaries	30.11 ± 3.93 a	28.90 ± 3.93 a

Means ($\pm$ SE) followed by the same letters in rows are not significantly different (P > 0.05).

The number of larvae parasitised was lower in both host density conditions, during the first few days and then it increased to maximum level and finally decreased, at the end of the females' life time. (Non-linear regression: $F_{2,32} = 22.74$, P < 0.0001 for density of 30; $F_{2,35} = 5.92$, P < 0.01 for density of 5) (Figures 4.11 and 4.12). At both host density conditions, females were able to parasitise higher number of DBM larvae, with an oviposition period of $\sim$ 25 to 26 days.
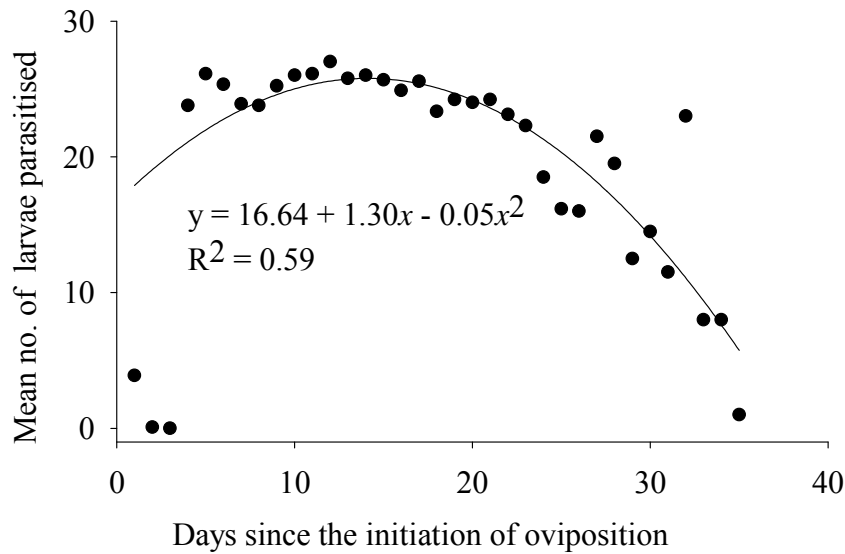
Figure 4.11 Mean number of larvae parasitised during the oviposition period of females at a host density of 30 DBM larvae.
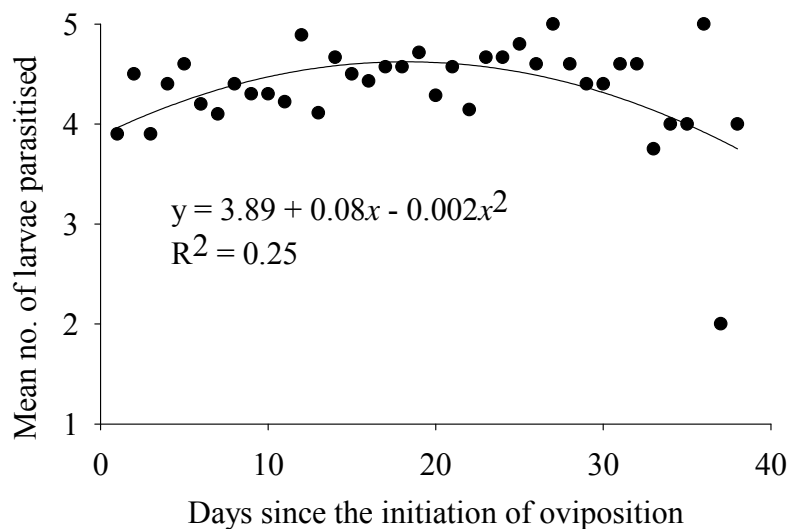
Figure 4.12 Mean number of larvae parasitised during the oviposition period of females at a host density of 5 DBM larvae.

When viewed over the entire oviposition period, the parasitoid offspring sex ratio followed different patterns in different host density treatments. In the density of 30 DBM larvae, the proportion of male progeny significantly decreased, during the first 10 days of oviposition and it reached a minimum between 10 to 20 days after the initial oviposition and then significantly increased (Non-linear regression: $F_{3,30} = 36.68$, $P < 0.0001$) (Figure 4.13). However, at a host density of 5 DBM larvae, sex allocation by

female parasitoids did not appear to have a clear pattern (Linear regression: $F_{1,36}$ = 3.20, P > 0.05) (Figure 4.14).

$$y = 98.64 - 2.42x + 0.13x^2 - 0.002x^3$$
$$R^2 = 0.78$$
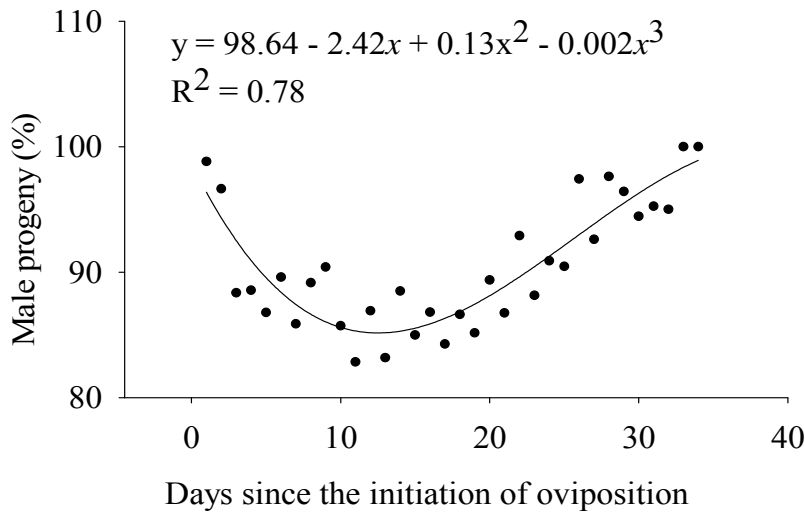
Figure 4.13 Proportion of male progeny produced during the oviposition period of females at a host density of 30 DBM larvae.

$$y = 86.61 + 0.18x$$
$$R^2 = 0.08$$
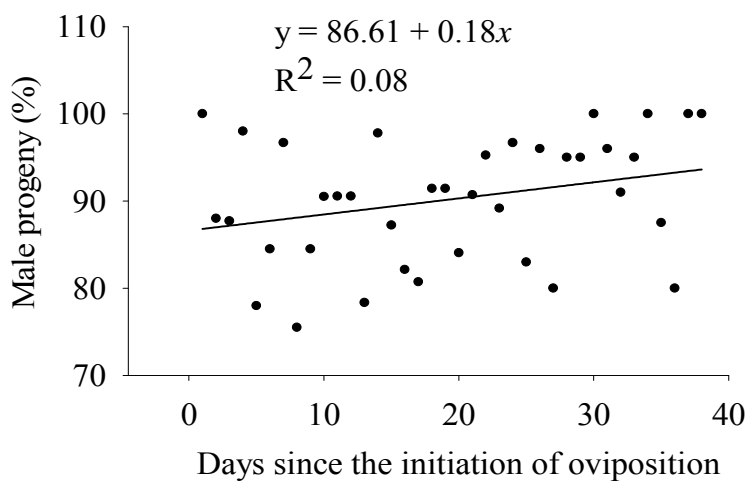
Figure 4.14 Proportion of male progeny produced during the oviposition period of females at a host density 5 DBM larvae.

4.5.5 Discussion

Host density influences the parasitoid's behaviour in various ways. A change in the progeny sex ratio, in respect to host density, has been reported in many parasitoid species (Sandlan 1979; Asante & Danthanarayana 1993; Ferreira De Almeida et al. 2002; He et al. 2006). In *A. ervi*, the proportion of female progeny increases with the

increase of host density, from 15 to 50 aphids (He et al. 2006). In the present study, *D. semiclausum* also produced more female progeny, at a higher host density. In insect parasitoids, host encounter may stimulate the females' ovipositional behaviour, by activating a synchronised released of sperm and eggs (Flanders 1946). Therefore, it could be possible that at a higher host density, *D. semiclausum* has a higher chance to encounter hosts and such conditions may have influenced the oviposition behaviour, thus producing more females.

Many parasitic wasps produce progeny with different sex ratios during their reproductive period. In some cases, mated females produce significantly more males soon after mating and the proportion of female progeny increases gradually, thereafter (Donaldson & Walter 1984). However, in the majority of species, females lay a high proportion of fertilised eggs immediately after mating (Tillman 1994; Bressac & Chevrier 1998; Cheng et al. 2004). In *D. semiclausum*, mated females produced a higher proportion of female progeny during the earlier days of oviposition and then the progeny gradually became even more male biased. Fecundity and sex allocation in insects are known to be maternal age dependent. Egg supply and sperm reserves are depleted and the mother's investment, in terms of clutch size, egg size and nutrient contents, decreases with time (Giron & Casas 2003). It is possible that, with increasing age, ovipositing female may receive depleted sperm and thus produce more male progeny towards the end of her reproductive period (King 2000; Santolamazza-Carbone et al. 2007), or it may be a result of physiological constraints due to ageing (Bressac & Chevrier 1998; Santolamazza-Carbone et al. 2007).

Stiling (1987) examined 171 cases of parasitoid oviposition and found that density-dependent parasitism occurs in only 25% cases. As reported by Legaspi & Legaspi (2005), density-dependent parasitism was not detected for *D. semiclausum*, in this study. One of the common reasons cited for density-independent parasitism, despite parasitoid aggregation, may be simply due to egg limitation in the searching parasitoid (Smith & Maelzer 1986). In the present study, foraging *D. semiclausum* adults, which were dissected at the end of their reproductive life, were found to contain a similar number of eggs in their ovaries, at both densities (Table 4.6). The high number of eggs remaining in the ovaries indicates that females did not suffer egg limitation: Rather, lower parasitism at a higher host density by *D. semiclausum*, may have been a result of more time spent to handling and overcoming the host's defences (Wang & Keller 2004). Waage (1983), for example, has suggested that aggregation by *Diadegma* sp.

(Hymenoptera: Ichneumonidae) is countered by an increase in handling time, at high host densities.

As reported in other studies (Tillman 1994; Pérez-Lachaud & Hardy 1999), the number of larvae parasitised by *D. semiclausum* varied with the age of ovipositing females. The number of larvae parasitised increased with the increase in the female's age, during the first 10 days of oviposition, reached a maximum and then decreasing afterwards. The decrease in parasitisation of host larvae may be due to decreasing physiological activity related to ageing (Uckan & Gulel 2002; Santolamazza-Carbone et al. 2007). The reduction of egg supply has been thought to be another reason it occurs in weakly synovigenic species (Santolamazza-Carbone et al. 2007). However, *D. semiclausum* females are strongly synovigenic and they are able to produce eggs, until death (Section 3.4.3.1). At low host density, the number of larvae being parasitised declined with parasitoid age, although females still contained a sufficient number of eggs in their ovaries, at death (Table 4.6). Hence, physiological constraint, due to ageing rather egg limitation, could be the reason for such a diminishing parasitisation rate.

It has been generally assumed that reproduction costs energy and that host density affects the survival of parasitic wasps (Roff 2002). A decrease in mean lifespan, as a result of increasing egg production, has been found in *Asobara tabida* (Nees) (Ellers et al. 2000). As reported in section 3.7.3, ovipositing *D. semiclausum* females survived a shorter time than non-ovipositing females, thus suggesting that reproduction costs energy: However, this is independent of host density. In the present study, host density did not significantly affect the parasitoids' longevity. The survival rate of female parasitoids *Lysiphlebia mirzai* Shuja-Uddin parasitising cereal aphid *Rhopalosiphum maidis*, at six different host densities were unaffected when measured under constant laboratory conditions (Tripathi & Singh 1991).

The lifetime fecundity of *D. semiclausum*, which was about 600, at a host density of 30 DBM larvae, was much higher than that in other studies (from 117 to 390; Chua & Ooi (1986); Abbas (1988); Rivera & Salazar (1993); Winkler et al. (2006). The lower lifetime fecundity in those studies could have resulted from the limited number of hosts supplied, for example, Chua & Ooi (1986) and Abbas (1988) provided ~20 DBM larvae to their parasitoids. The present study and those by Chua & Ooi (1986) and Winkler et al. (2006) found that *D. semiclausum* females were able to parasitise larvae until death, with an average reproductive period of >25 days. However, a shorter reproductive period of only 12~15 days, in this species, has been reported in several

other studies (Abbas 1988; Rivera & Salazar 1993; Honma et al. 2005). Such variations in the fecundity and reproductive periods may be expected to occur, due to differences in the experimental procedures, environmental conditions and strains of the insects (Chong & Oetting 2006b; Bokonon-Ganta et al. 2007; Huang et al. 2009; Potting et al. 2009).

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

5.1 Introduction

In this thesis, I have studied the general reproductive biology of the *D. semiclausum.* Such knowledge is important for an understanding of its reproductive behaviour, which has implications for laboratory mass-rearing and field releases of these parasitoids.

5.2 Emergence and Sexual Maturation

My study shows that adult *D. semiclausum* emerge only in the photophase, with males emerging mainly in the early hours of the photophase and females emerging during the latter hours of the photophase (Section 3.3.3.1). This indicates that *D. semiclausum* is a protandrous species, as reported in several other studies (Hirose & Vinson 1988; Doyon & Boivin 2006).

Although both sexes become sexually mature, within 12 h after emergence, males need more time than females (after emergence) to be able to mate. The protandrous emergence pattern allows newly emerged females to mate immediately with sexually mature males. In some parasitoids, females emerge earlier than males and therefore, they have time to develop their eggs and lay eggs immediately after mating (He et al. 2004). *D. semiclausum* females do not lay eggs within a 24 h period of emergence (Section 3.4.2.1). This naturally allows *D. semiclausum* females to mate before oviposition, so that they can produce both male and female offspring.

5.3 Egg Maturation Dynamics

Although Jervis et al. (2007) suggested that *D. semiclausum* is a weakly synovigenic species, my study shows that it is in fact a strongly synovigenic species. Similar to *A. nigrocincta* (Babendreier 2007) and *D. collaris* (Liu et al. 2001), *D. semiclausum* females do not contain mature eggs at emergence. Forty-eight hours after emergence, all females have mature eggs in their ovaries and the number of mature eggs

continues to increase, until they are 8 ~ 10 days old. The number of eggs decreases in the ovaries of older females (> 20 d old), which are provided with a 10% honey solution and deprived of host access, suggesting that egg re-absorption occurs in this species. Furthermore, the longevity of such females is longer, than that of the females which have laid eggs (Section 3.7.3). This may be attributed to the egg re-absorption, which increases life expectancy, as found in many species (Bell & Bohm 1975; Collier 1995; Tatar & Carey 1995; Heimpel et al. 1997).

Larger females, at emergence, have more developing oocytes and a larger number of mature eggs at 8 days old (Section 3.4.3.2). A positive relationship between body size and egg load has been reported in several other parasitoids, such as *Campoplex dubitator* Horstmann (Jenner & Kuhlmann 2006) and *V. Canescens* (Eliopoulos et al. 2003). Access to food, after emergence, enhances egg maturation in *D. semiclausum* females (Section 3.4.3.4). In several parasitic species, sugar feeding is known to affect the egg load directly, either by reducing the rate of egg re-absorption (Bell & Bohm 1975; Jervis & Kidd 1986; Antolin & Williams 1989; Heimpel & Rosenheim 1995; Heimpel et al. 1997; Quicke 1997; Lee & Heimpel 2008), or by facilitating egg maturation (England & Evans 1997; Burger et al. 2004; Tylianakis et al. 2004).

5.4 Longevity

As reported in many other hymenopterean parasitoids (Babendreier 2007; Onagbola et al. 2007), *D. semiclausum* females live longer than males (Section 3.7.1). The provision of food is very important for the longevity of *D. semiclausum* adults. Adults that have access to a honey solution live 15 to 20 fold longer, than those that are not provided with food or water only (Section 3.7.1). The provision of sugar is vital in meeting their daily energy requirements and prolonging their lifespan (Heimpel et al. 1997; Baggen & Gurr 1998; Mitsunaga et al. 2004; Winkler et al. 2006). Food is more important, as the parasitoids get older. Parasitoids fed with honey for 5 days (and deprived thereafter) die significantly sooner than those deprived of all food at emergence. The shorter longevity of older adults may be due to the declining level of reserve materials, such as lipid and glycogen, in the body (Lee et al. 2004).

5.5 Mating Behaviour

As reported in *B. hebetor* (Antolin & Strand 1992), *D. semiclausum* males, rather than females, show active courtship behaviour. Mating usually lasts 6-7 minutes. Mating success increases with the increasing age of both sexes (Section 4.2.4.2). Female age appears to have more affect (than male age) on mating success. According to Tagawa et al. (1985), *A. glomeratus* females increase pheromone production, with an increase of age. Therefore, the increased mating success, together with an increase in female age, may have resulted from increased female sex pheromone production. Furthermore, older females carry more mature eggs (Section 3.4.3.1), which may also increase female receptivity, resulting in a higher mating success.

In a no-choice test in this study, body size had no effect on mating success in *D. semiclausum*, as reported in other studies (Sagarra et al. 2001).

Most hymenopteran female parasitoids mate only once (Ridley 1993). However, a single *D. semiclausum* male mates repeatedly with the same female, during the entire photophase (Section 4.2.4.5), suggesting that multiple mating occurs in this species. After the first mating, mating duration increases in subsequent matings (Section 4.2.4.5). A longer mating period, after the first mating, may have resulted from a depletion of ejaculated reserves and males necessarily need a longer time in order to rejuvenate required ejaculates in subsequent matings (Bissoondath & Wiklund 1996). However, the mating interval between two matings decreases after the first mating (Section 4.2.4.5). These shorter mating intervals (in the subsequent matings) could be due to males learning from the previous mating, which may then affect their subsequent behaviour, thus making them more successful in further mating, as reported in *A. ervi* (He & Wang 2008).

5.6 Oviposition Behaviour

Females parasitise larvae only in the photophase, suggesting that visual cues are essential for such activity (Hofstetter & Raffa 1998). As reported by (Donaldson & Walter 1988), oviposition activity is associated with the number of mature eggs in the ovaries of *D. semiclausum*. Therefore, with the increasing number of oviposition bouts during the photophase, the parasitoids run out of mature eggs, resulting in fewer parasitisms in the later stage of the photophase. Dissection shows that, at the end of the

photophase, ovipositing parasitoids have fewer mature eggs in their oviduct and calyx region (Section 4.3.4).

D. semiclausum females lay more fertilised eggs during the first two oviposition bouts than subsequent bouts, suggesting that the sex allocation decision is also affected by egg load, as reported in an aphelinid parasitoid, C. bartetti (Walter 1988). Furthermore, D. semiclausum females do not parasitise larvae in the scotophase, during which time they can produce more eggs for the next photophase.

5.7   Host Stage and Reproductive Behaviour

The host stage affects body size and the developmental time for growing parasitoid offspring. Irrespective of host stages, female offspring are heavier than male offspring (Section 4.4.4.3). Such variation in body size is expected to occur, if body size is more important for the fitness of females (Mackauer 1996). In the present study, female body size is positively correlated to egg load (Section3.4.3.2) and longevity (3.7.1).

Offspring body size is positively correlated with the host stage, when parasitised. This may be attributed to more nutritional resources in large hosts (Charnov et al. 1981). The developmental time for female offspring is significantly longer than that for male offspring, at all host stages. King (1988) suggested that a shorter developmental time for males may have evolved, due to selection pressure for small males, leading to earlier emergence. My results, in the present study, (Section 3.3.3.1) support this hypothesis.

Parasitoid larvae grow and develop faster, if their mothers parasitise the third and fourth instar DBM larvae (Section 4.4.4.2). Host size dependent growth (King 1988; Ueno 1998) and developmental time (Petitt & Wietlisbach 1993) have been reported in other studies. However, the host stage has no effect on sex ratio, parasitism rate, host larval mortality and parasitoid pupal mortality, suggesting that all host stages are equally suitable for parasitoid development.

5.8   Host Density and Reproductive Behaviour

Host density significantly affects the sex ratio of D. semiclausum offspring, as reported in other studies (Chong & Oetting 2007) (Section 4.5.4). The parasitoid produces more female offspring with an increase of host density. This could be due to

higher host encounter rate at high host density, which may stimulate the release of sperm and eggs (Flanders 1946).

Parasitism rate is inversely related to host density in this parasitoid (Section 4.5.4). Smith & Maelzer (1986) suggested that egg limitation could be the prominent reason for such a relationship. In the present study, the dissection of parasitoids (which were reared in two different host densities, at the end of their reproductive period) shows no difference in the egg load (Section 4.5.4). Therefore, such inverse relationship, in response to host density, may be attributed to reasons other than egg limitation. One potential reason for lower parasitism, at higher host density, may be because *D. semiclausum* females spend more time handling and overcoming the host's defences (Waage 1983; Wang & Keller 2004).

5.9   Conclusion

In this section, I summarise the conclusions and implications of the findings in my thesis as follows:

- Most activities of these parasitoids, such as emergence (Section 3.3.3.1), oviposition (Section 4.3.4), and mating (Section 4.2.4.4) occur during the light period. Hence, all studies on behaviour should be made during the light period.

- Newly emerged females do not contain mature eggs in their ovaries (Section 3.4.3.1). Females, aged 48 h old or older, have significantly more mature eggs (Section 3.4.3.1). Mating success is higher in older females (Section 4.2.4.2). As a result, 48-h-old or older parasitoids are recommended to be used, in order to achieve a higher mating success and parasitism in the laboratory, or in augmentative releases.

- Females parasitise more larvae and lay more fertilised eggs in the early oviposition bouts (Section 4.3.4). Providing host larvae for 4-6 hours (after lights on) helps parasitoids parasitise larger numbers of larvae and more female progeny produced.

- Providing honey immediately after emergence increases the reproductive potential of the parasitoids, by enhancing their longevity (Section 3.7.1) and promoting egg maturation (Section 3.4.3.4). Thus, food supply is important in the improvement of laboratory mass rearing.

- More female offspring can be produced, when parasitoids are reared on a higher host density (Section 4.5.4).

- Rearing *D. semiclausum*, in the third instar DBM larvae, results in the production of large sized female offspring (Section 4.4.4.3), which have a higher egg load (Section 3.4.3.2) and greater longevity (Section 3.7.1).

- Ovipositing adults parasitise most larvae and produce more female offspring during the first 3 weeks of the oviposition period. Hence, using females up to that stage will help optimise the reproductive potential of these parasitoids

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MATING BEHAVIOUR AND EGG MATURATION IN *DIADEGMA SEMICLAUSUM* HELLEN (HYMENOPTERA: ICHNEUMONIDAE)

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ABSTRACT

Diadegma semiclausum is an important larval parasitoid of diamondback moth, *Plutella xylostella*. Little was known about the reproductive biology of this parasitoid. The present study investigated mating behaviour and egg maturation dynamics of *D. semiclausum* in the laboratory at 21±1°C, 16:8 h (light:dark) and 50-60% RH. Both males and females became sexually mature <12 h after emergence. When paired with 3-day-old virgin mates, significantly more newly emerged females (<12 h old) mated compared to newly emerged males (P<0.001). Both sexes could mate more than once. Females immediately after eclosion (<1 h old) did not contain mature eggs in the ovaries, suggesting that it is a synovigenic species. Maternal age affected the egg load, which was greatest in 72-h-old females. Egg maturation occurred without food supply, suggesting that *D. semiclausum* is an autogenous and non host feeding species. The implication of this study in mass-rearing in the laboratory is discussed.
Keywords: *Diadegma semiclausum*, sexual maturation, egg maturation, synovigeny, autogeny.

INTRODUCTION

Diadegma semiclausum (Hellen) (Hymenoptera: Ichneumonidae) is an important, solitary larval parasitoid of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Talekar & Shelton 1993). *Diadegma semiclausum* mainly attacks DBM larvae and has been introduced in several countries for the control of DBM (Poelking 1992; Talekar 1996; Amend & Basedow 1997). In Papua New Guinea highlands, the release and successful establishment of *D. semiclausum* since 1995 have substantially reduced crop losses by DBM (Saucke et al. 2000). The parasitoid was introduced to New Zealand from England in 1936, and is currently a prominent species in the field (Beck & Cameron 1992; Talekar & Shelton 1993). However, this parasitoid fails to prevent economic losses to crops in New Zealand due to the asynchronisation of parasitoid and DBM populations in the field.

The biology and ecology of *D. semiclausum* have been studied to some extent (Abbas 1988; Yang et al. 1993; Kwon et al. 2003; Winkler et al. 2006). Knowledge of the reproductive biology of parasitoids is crucial if biological programmes based on augmentative releases are to be developed. Surprisingly, little information is available about the reproductive biology of *D. semiclausum*. Huang et al. (2008) reported that *D. semiclausum* females need several days after eclosion to fill the ovaries with mature egg. However, these authors did not fully discuss the mating behaviour and egg maturation dynamics in *D. semiclausum*. The research here therefore aims to elucidate in detail the reproductive biology of *D. semiclausum*.

MATERIALS AND METHODS

Breeding colony and experimental conditions

The breeding colonies of DBM and *D. semiclausum* were started from DBM larvae collected from a commercial farm in Palmerston North in January 2008. The insects were maintained at the Entomology and IPM Laboratory, Massey University at $21 \pm 1°C$, 16:8 h (light:dark) (0800 h lights on, 2400 h lights off) (high-frequency, broad-spectrum Biolux tubes, Osram, Germany) and $50 \pm 10\%$ RH. All experiments were carried out under these environmental conditions.

The insects were reared on potted cabbage plants (Summerglobe hybrid, *Brassica oleracea* var *capitata*) (Terranova seeds Pty Limited, Australia) in plexiglass cages ($30 \times 30 \times 30$ cm). Two holes (13 cm in diameter) were made on the opposite sides of the cage and covered with a metal mesh (aperture size of the mesh=0.25 mm) for ventilation. One side of the cage was fitted with a circular opening (16 cm in diameter) for handling plants and insects in the cage. Twenty newly emerged DBM adults (ten males and ten females) were released into a plexiglass cage containing a potted cabbage plant (6-8 weeks old). After 24 h, the cabbage plant together with the DBM eggs were removed and maintained in a transparent plastic jar (25 cm in height × 17 cm in diameter). A circular metal frame covered with a nylon net (aperture size of the mesh=0.25 mm) was kept on top of the jar in order to prevent the newly emerged larvae from moving away from the plant.

To maintain the parasitoid colony, a mated parasitoid female was released into a plastic chamber (25 cm height × 17 cm diameter) with two circular openings (5 cm in diameter) covered with fine metal mesh for ventilation. This contained a cabbage plant along with 10 DBM larvae (3rd and 4th instars). After 24 h, the parasitised larvae were removed and reared in the same infested cabbage plant until pupation in a plastic container (10 cm in height × 8.5 cm in diameter) with two circular holes (5 cm in diameter) covered with fine metal mesh for ventilation. Pupae were maintained in individual glass vials (7.5 cm in height × 2.5 cm in diameter) until emergence. Virgin males and females of *D. semiclausum* were mated and used for further parasitisation of the DBM larvae. Both DBM and parasitoid adults were fed with 10% honey solution soaked in cotton balls (0.5 cm in diameter).

Mating behaviour

To study the mating behaviour of *D. semiclausum*, two experiments were set up: (1) 3-day-old virgin females were paired individually with newly emerged males (<12 h), and (2) 3-day-old virgin males were paired with newly emerged females (<12 h). Pairing was maintained for 12 h continuously. Mating duration was recorded using a stop watch. Subsequent mountings and remating time of the parasitoid were also recorded. All the mating experiments and behaviour observations were undertaken by pairing one virgin male and one virgin female of *D. semiclausum* in an above mentioned glass vial. The premating period (time period between pairing and copulation) and duration of copulation (time period from genitalia connection to disconnection) were recorded. There were 26 and 34 replicates for newly emerged females and males, respectively.

Egg maturation

To determine egg maturation period, females (0, 12, 24, 48 and 72 h old) were killed by freezing at -20°C and dissected in a droplet of Ringer's solution (15 g NaCl, 0.7 g KCl, 0.4 g $CaCl_2$ in 1 litre sterile water) on a slide under the stereomicroscope (Leica MZ12, German) equipped with micrometer eye piece. The specimen was then covered gently with a cover slip and observed under compound microscope (Olympus, GH, Japan) equipped with transmitted light and a micrometer eyepiece. Mature (fully chlorinated) eggs present in the ovaries were counted. There were 13, 16, 15, 14 and 15 replicates for above age groups, respectively. As mating and oviposition may affect the egg maturation rate, parasitoids used in the experiment were virgin and not provided with DBM larvae for oviposition.

Effect of food on egg maturation

To determine the effect of food on egg maturation of *D. semiclausum*, two treatments were set up: (1) 10% honey solution was provided for females immediately after emergence, and (2) no food was provided for females. The parasitoids were killed by

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freezing at -20°C 48 h after emergence and dissected for egg count as described above. There were 14 and 15 replicates for honey-fed and starved parasitoids, respectively. As mentioned in the last experiment, parasitoids used in this experiment were also virgin and not provided with DBM larvae for oviposition.

Data analysis

Data on sex maturation were analysed using a Chi-square test. The relationship between the number of matings and mating duration was analysed using regression analysis. Data on egg maturation and effect of food supply on egg production were analysed using ANOVA followed by Tukey's studentized range test.

RESULTS

Mating behaviour

Both males and females (<12 h old) after eclosion were able to mate. However, mating success was significantly higher in newly emerged females than in newly emerged males when paired with 3-day-old mates ($P<0.001$) (Table 1). After perceiving the presence of a female, the male started chasing and grasping her in order to mount her and achieve the genital contact. Once the male was able to connect his genitalia with hers, mating was successful.

TABLE 1: Mean (±SE) premating (h) and mating (min) periods, and mating success (%) of newly emerged adults (<12 h) of *D. semiclausum.*

	Premating period	Mating period	Mating success
<12-h-old female×3-day-old male	5.84 ± 0.69	7.00 ± 0.22	57.70 a
<12-h-old male×3-day-old female	5.09 ± 3.99	6.70 ± 0.40	6.25 b

When newly emerged females were allowed to remain continuously with the same males after the first mating, 45% (5 out of 11) of females remated. On average, *D. semiclausum* mated 2.6±0.24 times when left with the same mate. The mating duration increased significantly with increasing number of matings (Linear regression: $F=12.40$, $P=0.0048$) (Fig. 1).

Within the figure: $y = 5.1862x + 1.1224$, $R^2 = 0.5299$
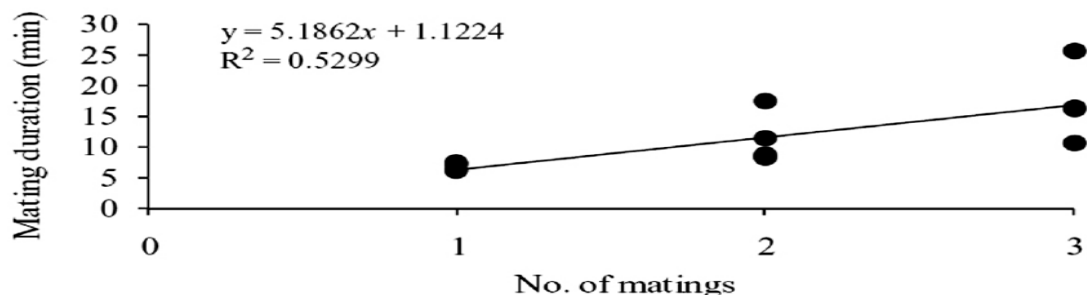
FIGURE 1: Relationship between number of matings and mating duration (min) in *D. semiclausum.*

Egg maturation

Newly emerged females did not contain mature eggs. Egg maturation started when the females were 12 h old. Mature eggs were semicircular, sausage shaped and 0.32 mm long and 0.05 mm wide. A significantly greater number of mature eggs was found in the ovaries at 48 and 72 h after emergence than at 12 or 24 h ($P<0.001$) (Fig. 2).
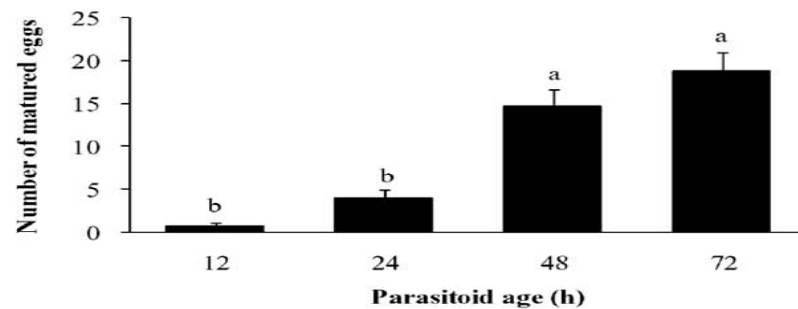
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FIGURE 2: Mean (±SE) number of mature eggs in parasitoids of different ages. Columns with the same letters are not significantly different (P>0.05).

Effect of food in egg maturation

Egg maturation occurred without food supply. However, food supply for females significantly stimulated egg maturation, with fed females producing 14.64±1.90 mature eggs compared to 5.40±1.30 mature eggs from unfed females (P<0.001).

DISCUSSION

Results of the present study show that very few newly emerged males were able to mate compared to newly emerged females, suggesting *D. semiclausum* males require a longer time than females to become sexually mature. In *D. semiclausum,* developmental duration of females is longer than that of males, and thus females emerge later than males (D. Khatri, personal observation). Therefore, it would seem that the longer developmental duration in females and longer sex maturation period in males are adaptive strategies to synchronize with the availability of sexually mature mates. Protandry in the mating system has been reported in grasshopper, *Sphenarium purpurascens* (Charpentier), (del Castillo & Nunez-Farfan 2002) and in the parasitoid *Cardiochiles nigriceps* Viereck (Hirose & Vinson 1988).

Newly emerged *D. semiclausum* females did not contain mature eggs, suggesting that this is a synovigenic species. The length of time to attain the maximum egg load following eclosion varies depending upon species, ranging from 4-12 days (Ramadan et al. 1995; Wang & Messing 2003). *Diadegma semiclausum* adults require at least 48 h to achieve maximum mature egg load.

In the present study, starved *D. semiclausum* females were able to develop and mature some of the eggs, suggesting that it as an autogenesis species (Jervis et al. 2005). However, when provided with honey solution, females produced significantly more mature eggs within 48 h. It has been suggested that food supply can prevent eggs from being reabsorbed by females for soma maintenance, which otherwise may occur during a time of starvation (Lee 2008), and food supply can stimulate egg maturation.

Results from this study have implications for mass-production and release of parasitiods for biocontrol in the field. For example, the probability of mating success could be increased by pairing newly emerged females with older males rather than pairing newly emerged males with older females. Females aged 48 h old or older have significantly more mature eggs and thus should be used for augmentative releases. Supply of honey solution to the parasitoid adults is highly recommended to maximise reproductive potential in *D. semiclausum*.

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DEVELOPMENT AND REPRODUCTION OF *DIADEGMA SEMICLAUSUM* (HYMENOPTERA: ICHNEUMONIDAE) ON DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (LEPIDOTERA: PLUTELLIDAE)

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ABSTRACT

This study investigated the development, emergence and mating of *Diadegma semiclausum* Hellen, an important parasitoid of diamondback moth in the laboratory at $21 \pm 1°C$ and 50-60% RH, with a photoperiod of 12:12 h (light:dark). The parasitoids' developmental period was significantly shorter and pupal weight significantly higher if their mothers parasitised the fourth instar larvae of DBM ($P<0.01$). The offspring sex ratio was strongly male-biased (<20%) but no significant difference was detected between host instars. Most adults emerged during the first few hours of the photophase and none emerged during the scotophase. Mating had no effect on the longevity of both sexes, but small females lived significantly longer than the larger ones ($P<0.05$). Male age and size had no effect on mating success, whereas small females achieved higher mating success than large ones ($P<0.05$). The implication of this study in mass-rearing in the laboratory is discussed.

Keywords: *Diadegma semicalusum*, *Plutella xylostella*, emergence, body size, mating.

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.), is the most destructive pest of cruciferous crops worldwide including New Zealand (Talekar & Shelton 1993; Cameron et al. 1997). *Diadegma semiclausum* Hellen is one of the most important, solitary larval endoparasitoids of DBM (Talekar & Shelton 1993), and was introduced into New Zealand from England in 1936 to manage the DBM problems (Beck & Cameron 1992; Talekar & Shelton 1993). *Diadegma semiclausum* has great prospects for the biological control of DBM because of its high specificity and searching ability (Shi et al. 2004). The parasitism rate of DBM by *D. semiclausum* can be as high as 70% (Iga 1997; Walker et al. 2002; Chen et al. 2003; Kwon et al. 2003), but the rate is only 12-27% in commercial crops in spring (Cameron et al. 1998). The high resistance of DBM to the major classes of insecticides (Cameron et al. 1997) and biopesticides (Shelton et al. 1993) has led to increasing interest in the role of parasitoids in the biologically based integrated management of DBM aiming to reduce reliance on insecticides (Talekar & Shelton 1993; Liu & Sun 1998).

The biology and ecology of *D. semiclausum* have been studied to some extent (Abbas 1988; Yang et al. 1993; Kwon et al. 2003; Winklera et al. 2006). The male-biased sex ratio for this species (Yang et al. 1993; Kwon et al. 2003) may contribute to the failure in suppressing the spring population and restrict the quality of mass-rearing in the laboratory. However, the reasons for the male-biased sex ratio are poorly understood. The aim of this research was to investigate the development, emergence and mating behaviour of *D. semiclausum*, information of which is vital to the understanding of its basic biology and improvement of mass-rearing programmes.

MATERIALS AND METHODS

Breeding colony

The breeding colonies of DBM and *D. semiclausum* started from DBM larvae collected from a commercial farm in Palmerston North in January 2008 and reared at the Entomology and IPM Laboratory, Massey University, at 21 ± 1°C and 50-60% RH, with a photoperiod of 12:12 h (light:dark). Ten DBM larvae were transferred to each potted cabbage seedling (4-5 leaves), and the plants were individually maintained in transparent plastic jars (25 cm in height × 17 cm in diameter) with two holes (5 cm in diameter) covered with metal mesh (aperture size of the mesh <0.5 mm) on opposite walls for ventilation. The parasitoid colony was maintained in a similar way where a mated parasitoid female was released into a jar with DBM larvae each day. DBM and parasitoid adults were provided with 10% honey solution soaked in cotton balls (0.5 cm in diameter) as food.

Development and emergence

To determine whether hosts of different instar stages affected the development and body weight of *D. semiclausum*, a mated parasitoid female was released into the jar where 10 second, third or fourth instar larvae of DBM were feeding on a cabbage seedling. The parasitoid was allowed to stay for 24 h for oviposition and then removed. There were 6, 6 and 10 mated female parasitoids used to parasitise the second, third and fourth instar larvae of DBM, respectively. Parasitised DBM larvae were allowed to feed on cabbage seedlings until pupation. Once the parasitoids pupated, they were removed from the silken web and weighed using an electric balance (Mettler Toledo, AG135, Switzerland) with a readability of 0.01 mg. Parasitoid pupae were individually maintained in glass vials (4 cm in height × 2 cm in diameter) for emergence. Emergence was recorded hourly during the photophase, and the sexes of adults and the developmental period of parasitoids from eggs to emergence were also calculated. Emergence observation was also performed immediately after light-on to determine whether parasitoids emerged during the scotophase.

Longevity

To investigate whether mating and body size had any effect on the longevity of both sexes, mated and virgin adults were individually maintained in transparent plastic containers (10 cm in height × 7.5 cm in diameter) with two 3 cm mesh-covered ventilation holes. The parasitoids were fed with honey solution as above. Longevity of adults was recorded. The parasitoids used were those that emerged from DBM pupae parasitised at fourth instar larvae.

Mating behaviour

To investigate the effect of age and body size on mating success of both sexes, one male and one female *D. semiclausum* were paired in eight treatment combinations (n = number of replicates):

(1) 12-h-old small female + 3-day-old small male, n = 8;
(2) 12-h-old large female + 3-day-old large male, n = 19;
(3) 24-h-old small female + 3-day-old small male, n = 16;
(4) 24-h-old large female + 3-day-old large male, n = 34;
(5) 12-h-old small male + 3-day-old small female, n = 10;
(6) 12-h-old large male + 3-day-old large female, n = 17;
(7) 24-h-old small male + 3-day-old small female, n = 10;
(8) 24-h-old large male + 3-day-old large female, n = 15.

The body size of parasitoids was defined as small when pupal weight was less than 3.5 mg and large when pupal weight was greater than 3.5 mg. The mating behaviour of each pair was observed in a glass vial (7.5 cm in height × 2.5 cm in diameter) for 1 hour during the photophase. The number of mating successes, premating and mating periods were recorded.

Data analysis

A goodness-of-fit test was used to test the distribution of data before analysis. Developmental period data were not normally distributed even after transformation and

thus analysed using the nonparametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons. Data on pupal weight, adult longevity, and premating and mating periods were normally distributed and thus analysed using ANOVA followed by Tukey's studentized range test. Because mating had no effect on adult longevity, longevity of mated and virgin individuals was pooled for analysis of the effect of body size on longevity. Parasitoid emergence data from second, third and fourth instar larvae were pooled and the emergence pattern during the photophase was analysed using non-linear regression. Chi-square tests were applied to analyse the mating success.

RESULTS
Development and emergence

Parasitoids developed significantly faster ($P<0.0001$) and resulting pupae were significantly heavier ($P=0.006$) when developing on DBM fourth instar larvae (Table 1). No significant difference was found in developmental time and pupal weight between males and females ($P>0.05$) (Table 1). The proportion of female progeny (13.9, 7.8 and 20.0% for second, third and fourth instar, respectively) did not vary significantly between the host larval stages at parasitisation ($P>0.05$).

TABLE 1: Developmental period from eggs to emergence (days) and pupal weight (mg) of *D. semiclausum* that parasitised DBM larvae of different instars. Means (±SE) followed by the same letters in columns are not significantly different ($P>0.05$).

DBM	Developmental period			Pupal weight		
instar	Male	Female	Mean	Male	Female	Mean
2nd	17.0 ± 0.1	17.4 ± 0.4	17.1 ± 0.1 b	3.4 ± 0.1	3.6 ± 0.2	3.5 ± 0.1 b
3rd	16.8 ± 0.1	16.7 ± 0.3	16.7 ± 0.3 b	3.5 ± 0.1	3.5 ± 0.2	3.5 ± 0.2 b
4th	16.2 ± 0.1	16.1 ± 0.1	16.2 ± 0.1 a	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1 a

No individuals were observed to emerge during the scotophase. The emergence of parasitoids was confined to the first 2 hours of photophase and then significantly decreased with time ($P=0.03$; Fig. 1).
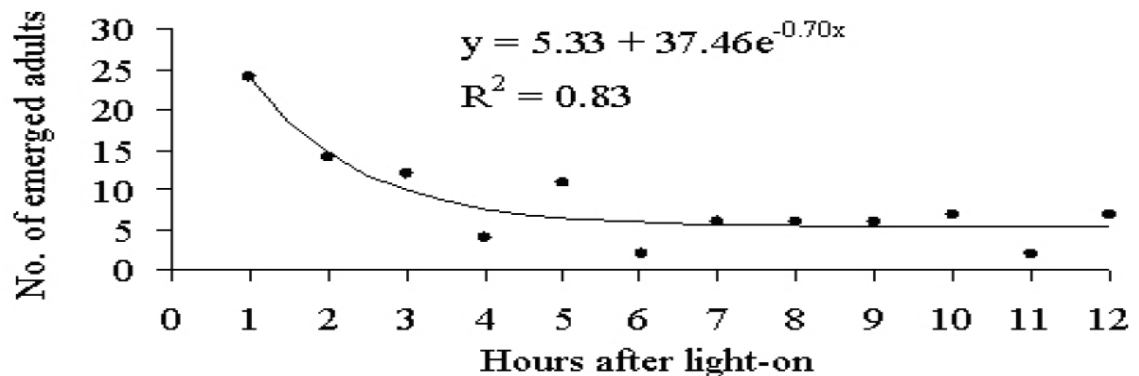

$$y = 5.33 + 37.46e^{-0.70x}$$
$$R^2 = 0.83$$

FIGURE 1: Emergence of *D. semiclausum* (number of adults/h) during the photophase.

Longevity

Mating did not significantly affect the longevity of either sex (P=0.41). However, the longevity of small females (34.6 ± 6.1 days) was significantly greater than that of large females (22.4 ± 3.5 days) and small males had greater longevity than large males (26.6 ± 2.78 and 24.6 ± 1.2 days) (P=0.0129).

Mating behaviour

When both sexes were released together, males always approached females with their wings vibrating. Upon female reception, they mated in tail to tail position. Mating success was significantly higher in small females than in larger ones when they paired with 3-day-old males (P<0.05) (Table 2). However, no significant differences were detected in mating success between ages or sizes of males when they were paired with 3-day-old females (P>0.05) and *vice versa* (Table 2). Parasitoid age and body size had no effect on the premating and mating periods (Table 2).

TABLE 2: Premating period (PMP, min), mating period (MP, min) and mating success (MS, %) of *D. semiclausum* at 12 or 24 h of age and with small or large body size.

	12- or 24-h-old females mated with 3-day-old males					
Female age	Small female + small male			Large female + large male		
	PMP	MP	MS	PMP	MP	MS
12 h	21.0 ± 7.5	7.9 ± 0.6	75.0	13.3 ± 4.5	6.5 ± 0.2	36.8
24 h	12.0 ± 8.3	7.1 ± 0.5	75.0	16.5 ± 7.0	8.3 ± 0.3	38.2
	12- or 24-h-old males mated with 3-day-old females					
Male age	Small female + small male			Large female + large male		
	PMP	MP	MS	PMP	MP	MS
12 h	16.4 ± 11.1	5.9 ± 1.5	50.0	20.3 ± 8.1	8.6 ± 1.2	41.2
24 h	13.3 ± 6.7	7.3 ± 0.6	42.9	14.2 ± 6.4	8.4 ± 0.8	47.1

DISCUSSION

Harvey & Strand (2002) suggested that parasitoids attacking foliar-feeding hosts may favour rapid development time over size while parasitoids that attack concealed hosts favour size over development time. In this study, when *D. semiclausum* attacked fourth instar larvae of DBM, it developed faster and produced larger progeny, indicating that *D. semicalusum* has the ability to adjust its development and growth rate according to host stage as reported in some other parasitoids, e.g. *Aphidius ervi* Haliday (Sequeira & Mackauer 1992). This may be because for the solitary parasitoids, large hosts contain more resources than small hosts (Charnov et al. 1981). The similar developmental period and body size for both male and female progeny from a given size of hosts suggests that sexes of *D. semiclausum* utilise similar amounts of host nutrients.

Diadegma semiclausum is a haplodiploid species where fertilised eggs develop to females and unfertilised eggs develop to males. Charnov et al. (1981) suggested that parasitoids can make efficient use of the size variation in the hosts by depositing fertilised eggs to large hosts to produce females and unfertilised eggs to small ones to produce males. However, in the present study, no significant difference in proportion of female progeny was found when *D. semiclausum* attacked hosts of different size, which does not support the parasitoid sex allocation strategy suggested by Charnov et al. (1981). The male-biased sex ratio of this species has been reported in both the laboratory (Yang et al. 1993) and field (Kwon et al. 2003). The fact that fertilised eggs of *D. semiclausum* may develop to either females or males (Noda 2000) may be attributed to the male-biased sex ratio in the laboratory and field populations.

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The emergence pattern of parasitoids is usually linked to behavioural adaptations and reproduction strategy such as feeding, mating and oviposition (He et al. 2004). Results from this study and that of Yang et al. (1993) show that *D. semiclausum* mainly emerged in the first few hours in the photophase. Talekar & Yang (1991) reported that oviposition of *D. semiclausum* also occurs during the photopase. Furthermore, nectar is crucially important for *D. semiclausum* survival and reproduction in the field, and without nectar supply, females delay oviposition for 2-3 days after emergence in the laboratory (Winklera et al. 2006). Results from the present study show that *D. semiclausum* became sexually mature within 12 hours after emergence. Thus, morning emergence of *D. semiclausum* is in their favour in that it permits the search for food and mates.

In this study, smaller individuals, especially females, lived longer than the larger ones, which contrasts with the findings in other parasitoids where large individuals live longer than the smaller ones (Ueno 1998). The reason is unknown. Furthermore, small male and female parasitoids were more likely than large ones to achieve mating (Table 2). The lower mating success in larger males is also reported in *Itoplectis naranyae* Ashmead (Ueno 1998).

Due to the failure of *D. semiclausum* in suppressing the spring populations of DBM, augmentation of parasitoids in the spring must be done with caution. Results from this study imply that the production of small individuals of both sexes may improve the mating success. However, production of male-biased progeny is the major issue in laboratory mass-rearing and thus further investigation on how to produce female-biased progeny in mass-rearing programmes is warranted.

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