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 $21 \pm 1^\circ\text{C}$, 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 $21 \pm 1^\circ\text{C}$, 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 $\times$ 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 $\times$ 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 $\times$ 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 $\times$ 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

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 ($\pm$ 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 $\times$ 3-day-old male	5.84 $\pm$ 0.69	7.00 $\pm$ 0.22	57.70 a
<12-h-old male $\times$ 3-day-old female	5.09 $\pm$ 3.99	6.70 $\pm$ 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).

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).

FIGURE 2: Mean ($\pm$ 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 parasitoids 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*.

ACKNOWLEDGEMENTS

This study was funded by New Zealand's International Aid and Development Agency (NZAID). We acknowledge the support.

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