

Sexual Receptivity and Mating Behavior of *Diaeretiella rapae* (Hymenoptera: Aphidiidae)

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Abstract

Sexual receptivity and mating behavior of the cabbage aphid parasitoid *Diaeretiella rapae* McIntosh were studied under laboratory conditions. When male and female *D. rapae* were paired females, males actively courted females, while females kept themselves away from males and displayed grooming behavior. Males became sexually active immediately after emergence and made mating attempts, whereas females took longer to become receptive to mating. Virgin males quickly detected female presence, resulting in a significant increase in the frequency of male courtship. Males encountered females within 5 min of pairing, and 90% of the males displayed courtship behavior by fanning their wings and chasing the female for mating. Before successful mating, males tended to approach females more often than females approached males. The time between pairing and mating in newly emerged females was longer than in 1-d-old females. The overall mating success in *D. rapae* was about 70%, and successful mating was largely dependent on females' decision to mate. Unlike females, males remained sexually receptive after mating.

Key words: mating behavior, sexual receptivity, courtship, multiple mating, *Diaeretiella rapae*

Mating is an integral part of reproduction in insects that directly affects their fitness and population dynamics. In haplodiploid insects, in which females can reproduce without mating, mating has a special significance, because to produce female offspring, a female needs to fertilize her eggs (Godfray 1994, Kant et al. 2012a). For mating success, the insects have to be receptive and able to locate their mates. Mate location and successful mating are generally mediated by female-derived sex pheromones (Quicke, 1997, Ruther 2013). Pheromone could be directly involved in long-distance mate finding, and also induce courtship behavior in males (Ruther et al. 2000).

While searching for mates, male and female insects often display precopulatory behaviors (courtship) to attract mates (McNeil and Brodeur 1995, Ruther et al. 2000, McClure et al. 2007). Diversity in insect mating system suggests that behaviors linked to reproduction, including mate location and courtship, evolve rapidly in insects (Gavrillets 2000, Ritchie 2007), and such signals vary considerably among insects (Emlen and Oring 1977, Choe and Crespi 1997). Male courtship behavior in parasitoids includes antennation, wing vibration (fluttering), and waving of forelegs (Reitz and Adler 1991, Abeeluck and Walter 1997, De Freitas et al. 2004). These courtship behaviors act to induce receptivity in females (Miller and Tsao 1974), which could display multiple signals to express receptivity (Ringo 1996). The primary receptivity may be stimulated by oviposition status or by the egg load of females.

Proovigenic females, which emerge with full complement of eggs, tend to be receptive sooner after emergence than synovigenic females (Jervis et al. 2001).

Sexual receptivity in female wasps can be usually induced only a limited number of times, and the opportunity for further matings tends to be low (Hardy et al. 2005). Females that mate multiple times undergo cyclic receptivity which fluctuates with frequency of mating, whereas monandrous females become unreceptive immediately after mating (Ringo 1996, Hardy et al. 2005). However, if a monandrous female mates with a sperm-depleted male, it will produce a limited number of female offspring. Thus, such female should be receptive after mating to maximize her fitness (Gordh and Debach 1976, Ridley 1993, Quicke 1997).

The study of mating behavior is useful in monitoring and manipulating host-parasitoid dynamics in biological control programs (Luck 1990, Suckling et al. 2002). Mating status of a parasitic wasp could directly affect the production of female offspring, and thus the efficiency of the wasp in suppressing pest population. Females are directly responsible for attacking the pest population; female-biased sex ratios could help to increase the efficiency of biological control (Godfray 1994, Ode and Hardy 2008). Thus, understanding mating systems could help to develop strategies for increasing female production in insectaries

In this study we examined the courtship and mating behavior of *Diaeretiella rapae* McIntosh (Hymenoptera: Aphidiidae). *D. rapae*

attacks several species of aphids (Pike et al. 1999), and has been used in biological control of the cabbage aphid *Brevicoryne brassicae* (Carver and Starý 1974, Vaughn et al. 1996). *D. rapae* is a solitary endoparasitoid, in which females emerge with the majority of their eggs matured (proovigenic condition; Jervis et al. 2001, Kant et al. 2012a). Our previous study suggested that the majority of adult *D. rapae* emerge during early- to mid-photophase; males emerge before females and mate throughout the photophase but not during the dark (Kant and Sandanayaka 2009). The study presented here further investigates sexual receptivity, courtship, and reproductive behavior that have not been previously examined in *D. rapae*.

Materials and Methods

Insect Colonies

Colonies of *D. rapae* and *B. brassicae* were collected from a commercial cauliflower field near Palmerston North, New Zealand. Insects were maintained in the laboratory at $20 \pm 2^\circ\text{C}$, 60–70% RH, and 16-h photophase on cabbage seedlings ('Autumn Pride') in Plexiglas cages (30 by 30 by 30 cm³). To get adults of uniform size, we used *D. rapae* emerged from cabbage aphid parasitized at 6–7 d old. Once parasitized aphids were mummified, each mummy was transferred to an individual glass vial (7.5 cm, 2.5 cm dia.). Upon emergence, *D. rapae* adults were offered 10% honey solution before being used in mating experiments. All bioassays were conducted during early photophase.

Sexual Receptivity in Male and Female Parasitoids

The time required for males and females to become receptive for mating after emergence was examined. Male and female *D. rapae* of four different ages (1, 2, 6, and 24 h) were individually paired with a 24-h-old adult of the opposite sex in a glass vial, and the behavior of individuals recorded until mating. Between 30 and 37 individuals were tested in each age group, and none were used in more than one experiment. *D. rapae* which did not mate within 60 min of pairing were considered unreceptive.

Courtship and Mating Behavior

Mating behavior of male and female *D. rapae* was examined using a microscope (Olympus SZX12) fitted with the video camera. Observations on the behavior of males before and after pairing with a female were recorded for 60 min in 1-d-old, honey-fed *D. rapae*. This time period was selected because our preliminary mating trials suggested that most pairs mate within 60 min after pairing. To study the behavior of males before pairing, virgin male *D. rapae* ($n=26$) were individually placed in glass vials, and activities including walking, standing, grooming, and antennal movements were recorded. To study the behavior of males during mating, virgin 1-d-old honey-fed male and female were placed together in a glass vial. The courtship and mating behavior of each mating pair ($n=37$) were observed and recorded, to develop ethograms for the mating behavior of *D. rapae*. The pairs that did not mate within the 60-min observation period were considered unreceptive and were not included in the data analysis. Post mating behaviors for males and females such as courtship, mounting, and re-mating were also observed in similar setups. The activities of mating adults were categorized following King et al. (2005):

- Walking: displacement of males and females per unit time after pairing (males and females)
- Standing: when displacement per unit time is zero (males and females)

- Grooming: rubbing of legs or antennae while standing (males and females)
- Approaching: male moving closer to the female and vice-versa (males and females)
- Encounter: male and female come close and touch each other (males and females)
- Male courtship behavior: male briefly moves his wings up and down, usually rapidly, and is often running toward the female at the same time (males)
- Mounting: more than half of male's body is on top of female's body (males)
- Copulation/mating: male inserts its aedeagus in female genitalia (males)
- Antennation (antenna tapping): vertical movement of male antenna during copulation

The pre-mating period (period between male–female pairing and copulation) and copulation time (period between start and termination of copulation) were recorded during experiment.

Multiple Mating in Male and Female *D. rapae*

The pairs which successfully mated in the experiments above were used for multiple mating experiments. The selected mated pairs ($n=18$) were given rest for 60 min and offered 10% honey solution. After the resting period, the males and females were transferred to individual glass vials and paired again with 1-d-old virgin females and males, respectively. The mating (second mating for the original male and female) was observed for 60 min. The mating for the original males and females was repeated eight times, and the number of times males and females mated during the entire process was recorded.

Statistical Analyses

A goodness-of-fit test was used to test the distribution of the data, and nonnormal data sets were subjected to arcsine square root transformation. Differences in behavioral activities, including copulation, pre-mating time, and mating success, were tested using ANOVA Generalized Linear Models (PROC GLM, SAS 9.2). Pair-wise comparisons were made using Tukey's studentized range (HSD) test. Wilcoxon Signed-Rank Test was used to compare the activity of males before and after introduction of a female to the mating arena. To analyze the relationship between the pre-mating period and copulation time, data from all mating experiments of this study were pooled and analyzed using Ordinary Least Squares (OLS) regression. Mating success in the sexual receptivity experiment was analysed using logistic regression (PROC LOGISTIC, SAS 9.2). The relationship between male courtship display and the time between pairing and mating (pre-mating time) was analyzed using the S-function of PASW (SPSS) Statistics 18. Values in the results are untransformed means \pm SE. Analyses were carried out at $\alpha=0.05$ level of significance.

Results

Sexual Receptivity in Males and Females

Both male and female *D. rapae* were able to mate within 24 h of emergence. Younger males (1 h old) were more successful in mating than younger females (1 h old) (logistic regression: $\chi^2=4.03$; $P=0.041$). After pairing, mating attempts by males were made much sooner than mating attempts by females. When paired with 24-h-old females, younger male *D. rapae* took significantly less time to mate (19 ± 4 min) than younger females when paired with

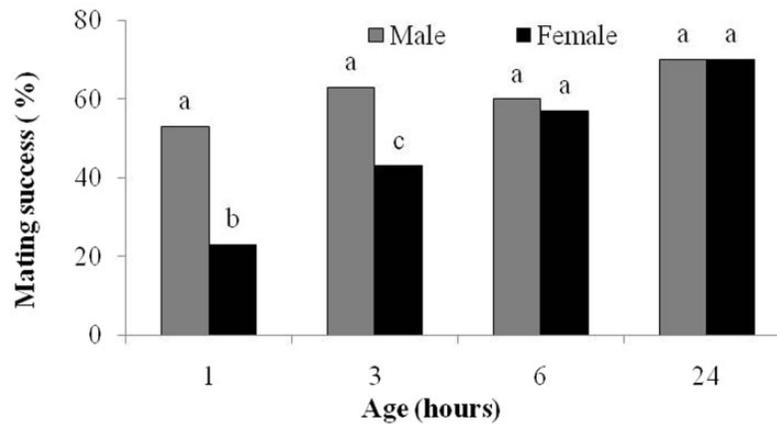


Fig. 1. Mating success (successful in copulation) of male and female *D. rapae* at different ages when paired with a 24-h-old mate. The pairs that failed to mate within 60 min after pairing were considered unsuccessful. Bars with the same letters in each sex are not significantly different (Tukey's HSD at $\alpha = 0.05$).

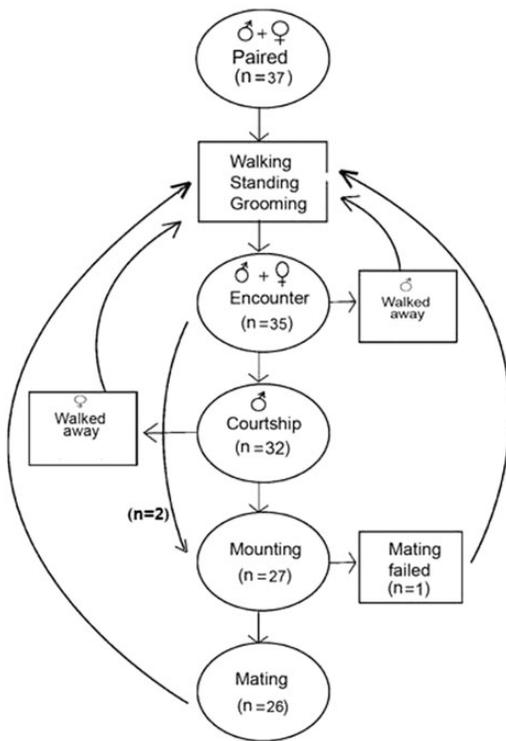


Fig. 2. The ethogram of behavioral transitions displayed by *D. rapae* after pairing ($n = 37$) until mating (copulation took place) or end of 60-min period. The number of males which displayed particular behavior or events is shown in parentheses.

24-h-old males (41 ± 5 min) ($F_{1, 23} = 12.3$; $P < 0.002$). Variations in mating success in different aged males were not significant ($P = 0.56$), but mating success was significantly lower in younger females (1 h and 3 h old) compared to older ones (6 h and 24 h old; $P < 0.001$; Fig. 1).

Courtship and Mating Behavior of *D. rapae*

Male *D. rapae* were less active before they were paired with females. Activities such as wing vibration and upward and downward movements of the antennae in males were observed after females were introduced in the arena. About 55% of males spread their wings, and

75% showed antennal movement within 1 min of a female introduction. The proportion of time spent walking and grooming by males increased significantly after introduction of females from 47% in males without females to 85% after pairing (Wilcoxon Stats = 252; $n = 22$; $P < 0.001$).

When placed in a test arena together, both male and female *D. rapae* started waving their antennae searching for mates. The first male–female encounter took place 4.8 ± 0.5 min after pairing. A large percentage of males (91%) displayed courtship behavior upon encountering the female or just before the encounter event. Males followed females and attempted to mount them for copulation. However, females appeared to initially avoid mating by walking away from the encounter site. The detailed behavioral sequences and transition between the behaviors of *D. rapae* during courtship are illustrated in Figure 2. The number of times males approached females for mating (4.3 ± 0.6) was significantly larger than the number of times females approached males (0.8 ± 0.2 ; $F_{1, 47} = 20.61$, $P < 0.001$). In 75% of the cases, male courtship behavior upon encountering a female resulted in mating. The mean premating time (time period after pairing and mating) for successful mating was 17 ± 3 min (min 1, max 56 min). Males were persistent in their mating attempts. The courtship did not stop until mating occurred. The relationship between premating time and number of courtship displays ($R^2 = 0.51$, $P < 0.001$) is presented in Fig. 3a. The intensity or frequency (per minute) of male courtship decreased when the females rejected male mating attempts. Relationship between rate of courtship displays with time ($R^2 = 0.46$, $P < 0.001$) is presented in Fig. 3b.

The number of *D. rapae* pairs that successfully mated within the 60-min period was larger than the number of unsuccessful pairs (logistic regression: $\chi^2 = 5.72$; $P = 0.016$). Only two males mounted and mated the female without showing courtship behavior. Males generally followed and chased the female and attempted to mount it from behind or from the side. About two thirds of mounting occurred from the side. Females rejected initial mating attempts by a male, but 13% of females mated with the same male after initial rejections. Male *D. rapae* made 4.5 ± 0.6 mating attempts before successfully mating. Unsuccessful males made 10.3 ± 0.9 attempts during the allocated 60 min ($F_{1, 32} = 31.07$, $P < 0.001$; Fig. 4).

After mounting the female, males used their legs to grasp the female, aligned their body with the dorsum of the female, and slowly slid behind the female until the tip of the male's abdomen reached the female's ovipositor. The males then extended their genitalia to

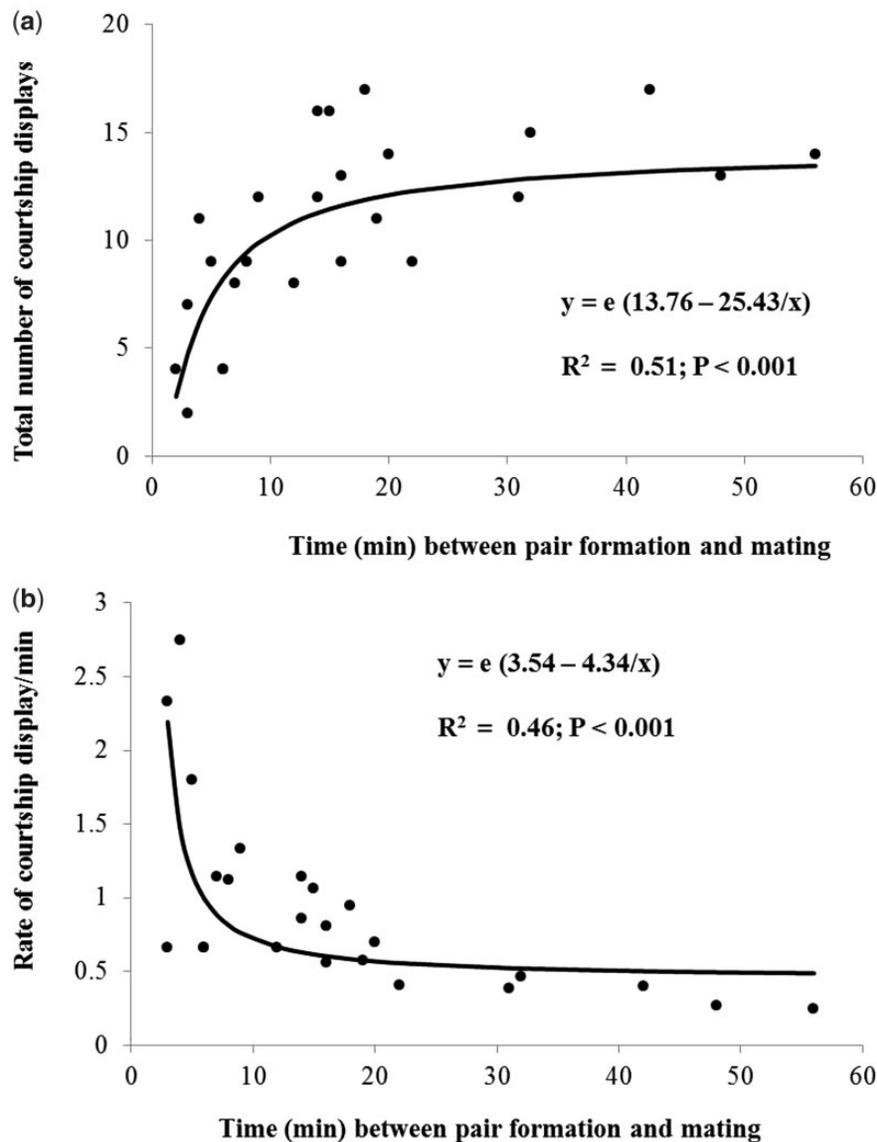


Fig. 3. (a) Relationship between the total number of courtship displays, such as wing fanning, by male *D. rapae* and pre-mating time (time between pairing and mating); (b) relationship between rate (frequency) of courtship displays (per unit time) by male *D. rapae* and the pre-mating time. Regression analysis was done using S-function of PASW Statistics.

copulate with the female. During copulation, females raised their antennae backward and were stationary, while the mating males continuously stroked the female body with their antennae. Copulation in *D. rapae* lasted for 58 ± 5 s; the copulation time was not affected by pre-mating time ($R^2 = 0.0047$, $P < 0.57$) shown in Fig. 5. Once copulation was completed, the mating pairs separated; the males started walking away, while the females were observed standing and grooming.

Multiple Mating in Male and Female *D. rapae*

Female *D. rapae* became unreceptive after copulation, and multiple mating did not occur in females with either previously mated males or with virgin males. However, about 56% of males displayed courtship to females that had already mated, and 20% of those who displayed courtship to mated females also mounted and made copulation attempt. Unlike females, males remained sexually active after their first mating and mated on average 1.2 ± 0.2 times during the 60-min period.

Discussion

For a successful mating, male and female need to be sexually mature and receptive. Our study showed that male *D. rapae* are more active than females when they are paired together, but mating success is largely limited by female's receptivity. Increased courtship activities of males (antennal movement and wing spreading) were observed in female presence; this suggests that olfaction plays an important role in mating in this species. Evidence of pheromone-mediated mating in *D. rapae* has been suggested in previous studies (Askari and Alishah 1979, Vaughn et al. 1996), which is supported by existence of long and short distance pheromones in other parasitoids (McNeil and Brodeur 1995, Ruther et al. 2000, Steiner et al. 2006, Ruther 2013). The frequency of male courtship displays increased after pairing, and the courtship did not stop until mating occurred.

Wing vibration also produces acoustic signals (Michelsen 1983), which can help to induce sexual receptivity in parasitic wasps (Miller and Tsao 1974). Wing vibration by males upon detection of

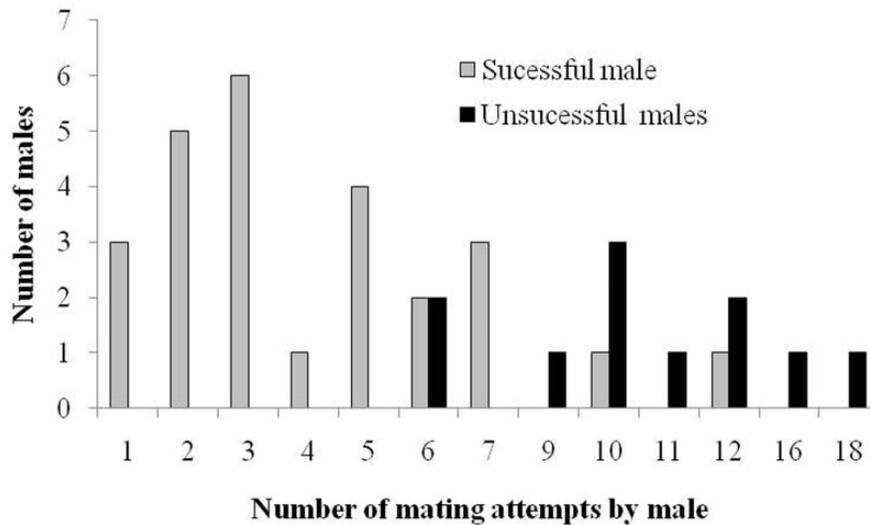


Fig. 4. The number of mating (copulation) attempts by successful and unsuccessful male *D. rapae*. Males were regarded as successful if they mated within 60 min of pairing. Mating attempts of males were recorded until they mated successfully or until the end of 60-min period.

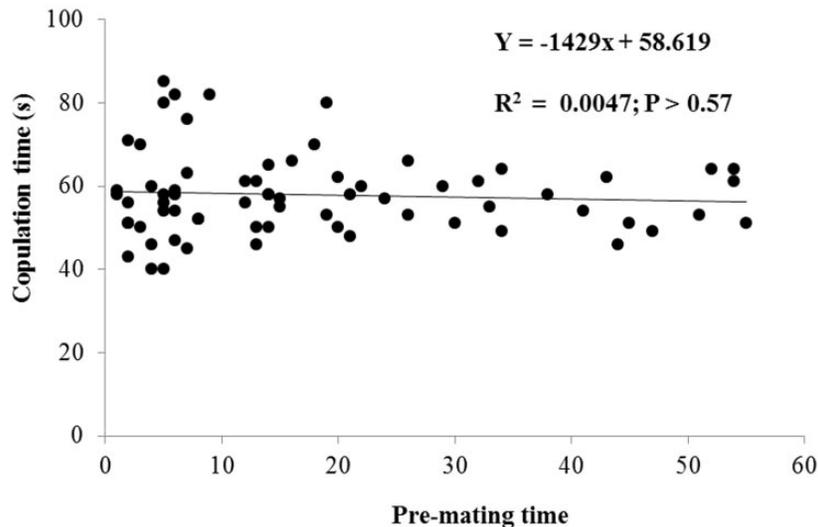


Fig. 5. Relationship between pre-mating time (time between pairing and copulation) and copulation time in *D. rapae* was analyzed using Ordinary Least Squares regression.

females has been observed in other aphidines, including *Aphidius ervi* (McClure et al. 2007). In contrast, presence of a male never results in overt female receptivity in parasitoids; males need to display courtship to entice female(s) to mate, which is common in sexually reproducing organisms, and is an important investment of time and energy by the male (Hardy et al. 2005). A female *D. rapae*, in turn, responds to courtship behavior by becoming still and allowing mating to occur, or by walking away which could be a sign of rejecting the male (Abeeluck and Walter 1997). However, male *D. rapae* followed females for mating even after the female walked away, and made persistent mating attempts. Our results suggest that initial refusal to mate by female *D. rapae* might not be permanent, and could be due to either unreceptiveness of the female itself, or unsuitability of the available male partner.

Most mating occurred within 30 min after pairing, and the pairs that did not mate quickly after pairing were less likely to mate successfully. The males always made their mating attempt from back or

side of females, and seldom encountered females from the front. Some males that encountered empty aphid mummies during the mating (from which the female parasitoid emerged) attempted to copulate with the mummies (R. Kant, personal observation), which may indicate the use of chemical cue in locating females in this species (Van den Assem 1996). Copulation of *D. rapae* adults lasts for about 1 min. The copulation time was not affected by the amount of time male and female spent together before mating. This suggests that the energy spent on walking, grooming, and courtship display did not affect the duration of copulation or sperm transfer. During copulation, male stroking female body with their antennae is consistent with findings from other studies (Van den Assem 1996, Romani et al. 2005, Ruther 2013).

Sexual receptivity in female wasps is usually induced for a limited time, and the opportunity for further mating tends to be low (Hardy et al. 2005). In this study, male *D. rapae* were found receptive immediately upon emergence, but females took comparatively

longer time to become sexually receptive. Immediate receptivity in pro-ovigenic species would help both a male and a female to maximize their fitness by mating multiple times. However, our study showed that males mated multiple times while females mated just once. Thus, quick receptivity or aggressive nature of mating would maximize the fitness of males (Emlen and Oring 1977, Choe and Crespi 1997, Maklakov et al. 2005, Kant et al. 2013). Females became unreceptive after mating despite mating attempts made by the males (Ruther 2000).

In synovigenic species (e.g., *Bracon hebetor*), neither males nor females are receptive to mating immediately after emergence (Ode et al. 1996). The unreceptiveness in female *D. rapae* could be directly linked to shutting off of the short-range pheromone after mating, as pheromone production would cost both energy and time (McClure et al. 2007, Ruther 2013). Female *D. rapae* start host searching and oviposition immediately after mating (Kant et al. 2012a), and further energy expenditure on pheromone release and mating could negatively affect their oviposition or reproductive success. Also, a short delay in receptiveness of a monandrous female allows that female to assess the suitability of male before mating. Because fitness of a female parasitoid is not generally decided by the number of times she mates, but by the number of offspring she produces in her lifetime (Hardy et al. 2005, King 2008, Kant et al. 2011), this can be maximized by mating with a better male.

The results showed that male *D. rapae* display active courtship and repeated mating attempts to maximize their fitness. The females have evolved to be choosy to maximize their fitness by mating once but with a good quality male. The courtship and mating system of *D. rapae* could have direct implications on its use in the biological control. Females must mate before oviposition and this could be achieved by offering newly emerged males, which are most receptive, in mass production in insectaries. The problem of over production of males (unsuccessful parasitoid mating) during mass production could be minimized by manipulating the male age and emergence to achieve female-biased sex ratio (Ode and Hardy 2008, Kant et al. 2012b). Releasing mated *D. rapae* adults in the field or in a glasshouse could help to improve performance of biological control program.

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