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Mating or ovipositing? A crucial decision in the life history of the cabbage aphid parasitoid *Diaeretiella rapae* (M'Intosh)

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Abstract. 1. The reproductive fitness of a parasitoid depends on its mating and ovipositing success. Virgin haplodiploid females can reproduce, but produce only males, and may diminish fitness by producing more male offspring than required. Therefore, females must decide on whether to mate or oviposit first.

2. This study was conducted to assess the mating versus ovipositing decision and its impact on the reproductive fitness of *Diaeretiella rapae* (Hymenoptera: Aphididae), an endoparasitoid of the cabbage aphid *Brevicoryne brassicae* (Hemiptera: Aphididae).

3. When newly emerged females were given a choice between mating and ovipositing, about 62% of *D. rapae* females preferred to mate before ovipositing. Those females who oviposited before mating parasitised only 10% of the available aphids. After mating, females superparasitised their hosts with fertilised eggs, which resulted in a highly female-biased sex ratio in the offspring.

4. Mating success was very high (91%) in the presence of hosts (cabbage aphid nymphs) compared with that in the absence of aphids. However, mating success was not influenced by the quality (size) of the hosts present in the mating arena, despite a parasitoid preference for larger hosts during oviposition. The time between pairing and mating was also shorter in the presence of host aphids. The mean number of aphids parasitised and the parasitism rate were significantly greater after mating.

Key words. Mating, oviposition, reproductive fitness, sex ratio, superparasitism, trade-off.

Introduction

In sexual reproduction, females must mate to produce viable offspring. However, in haplodiploid Hymenoptera, in which males are haploid and females are diploid, virgin females can produce viable male offspring, but need to mate and fertilise their eggs to produce female offspring (Godfray, 1994). After mating, sperm transferred by the male is stored in the spermatheca; subsequently, throughout her life, the female fertilises the eggs facultatively with the stored sperm, adjusting the sex ratio of her offspring in response to the prevailing conditions (Charnov, 1982; Fauvergue *et al.*, 2008). Therefore, the sex ratio and ultimately the reproductive success of parasitic wasps are directly linked to the mating status of ovipositing females.

When a female parasitoid emerges from her host, she can choose between searching for a host and immediately producing male offspring, or searching for a mate and producing both male and female offspring later. Mate searching may differ between gregarious and solitary parasitoids. In gregarious parasitoids in which a number of males and females emerge from one host, parasitoids may immediately mate with one another (King & Copland, 1969; Greeff, 1996). However, solitary parasitoids may actively search for mates (Hardy *et al.*, 2005). For example, virgin females of the aphid parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) attract males for mating while foraging and males are attracted to host volatiles (Fauvergue *et al.*, 2008).

The decision by a virgin female on whether to mate or oviposit first can affect her fitness. If the female lays eggs before mating, she may produce more males than are required for inseminating all available females (Ode *et al.*, 1998). Females that emerge with a full or partial complement of developed eggs (pro-ovigenic or weakly synovigenic,

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respectively) are generally egg-limited and may therefore be cautious in choosing to oviposit before mating as they run the risk of reducing overall fitness by depleting their egg supplies and producing too many male offspring (Jervis & Ferns, 2004). However, females that can produce eggs throughout their lifespan (synovigenic parasitoids) are not constrained in this way. If they oviposit before mating, they may be able to compensate for pre-mating oviposition (production of only males) by increased oviposition after mating (production of female and males). Thus, they are capable of manipulating the overall sex ratio of their offspring by producing more fertilised eggs after mating later in life.

Diaeretiella rapae (M'Intosh) (Hymenoptera: Aphidiidae) is the only parasitoid of the cabbage aphid, *Brevicoryne brassicae* (Hemiptera: Aphididae), although it also parasitises several other aphids of cruciferous and non-cruciferous plants (Pike *et al.*, 1999). It is a solitary endoparasitoid, of which a single adult emerges per host, irrespective of the number of eggs laid in the host (Bernal & Gonzalez, 1997; Kant *et al.*, 2011). Previous studies of *D. rapae* have shown that males of this species mate several times, whereas females mate only once (R. Kant, unpublished data, 2012). In species in which this occurs, the production of additional females is considered as a fitness increment (Godfray, 1994). Multiple mating in males and single mating in females alters the operational sex ratio of a population [the proportion of males and females ready to mate at a given time (Emlen & Oring, 1977)] to one that is male-biased, despite the numerically female-biased population sex ratio. Females are weakly synovigenic (Bernal & Gonzalez, 1997) and can be considered egg-limited.

Male *D. rapae* emerge before females and mate at any time during daylight, but do not mate in the dark (Kant & Sandanayaka, 2009). Our research on the mating and oviposition of *D. rapae* in an open environment found a high proportion of male–male mating attempts in a female-biased population (Kant *et al.*, 2012a), suggesting that despite the high proportion of females in a population, the number of mating males at any time might exceed the number of females available for mating. Competition among males for mating with fewer females interferes with male–female mating success (Kant *et al.*, 2012a). Lack of or even a delay in mating may force virgin females to oviposit unfertilised eggs, exacerbating the overproduction of males and further affecting the gender imbalance in the subsequent generation.

The present study investigated the mating and ovipositing preferences of *D. rapae* under laboratory conditions. After emergence, females can choose between mating and ovipositing, and must respond to environmental conditions and related trade-offs. We hypothesised that the weakly synovigenic state of *D. rapae* females (Bernal & Gonzalez, 1997) would result in a preference for mating before ovipositing. The specific research questions in this study were: (i) Do females show a preference for mating or for ovipositing in the presence of males and of aphid hosts? (ii) How does the mating and ovipositing sequence affect fitness gain in *D. rapae*? (iii) Does the presence or absence of hosts affect mating success in *D. rapae* females?

Materials and methods

Insect culture and preparation

Cabbage aphids and the parasitoid *D. rapae* were collected from cabbage plants in Palmerston North, New Zealand. Aphids were reared on live cabbage plants (cv. Summer Globe Hybrid) grown in pots in cages. The parasitoids used in this research were reared on cabbage aphid nymphs aged 5–7 days in a controlled laboratory environment at 20 ± 2 °C, 60–70% RH under a photoperiod of LD 16:8 h. Parasitised aphid mummies were removed from the plants and kept individually in 2-ml microfuge tubes until adult emergence. Emerging *D. rapae* adults were sexed based upon the presence or absence of an ovipositor under a stereomicroscope (Olympus SZX12) and offered 10% honey solution for feeding. All bioassays were conducted under similar environmental conditions during daylight hours.

Experimental set-up

Two experiments were conducted to investigate the effects of host quality on the choice between mating and ovipositing. In the first experiment, individual females ($n = 15$) were offered larger (aged 6–7 days) aphid nymphs; in the second experiment, females ($n = 14$) were offered smaller (aged 1–2 days) aphid nymphs. In both experiments, a 1-day-old, honey-fed virgin female *D. rapae* was introduced into a closed Petri dish containing 10 cabbage aphid nymphs and a 1-day old honey-fed virgin male *D. rapae*. The number of aphid nymphs parasitised by the females was recorded and whether female parasitoids were standing or grooming (rubbing their legs) during foraging was noted. Pairs that copulated within 30 min of pairing were considered successful; unsuccessful pairs were discarded from the experiment. The time between pairing and mating (pre-mating time) was recorded for all successful matings. Females were further observed, and the time between mating and first oviposition (pre-oviposition time) was recorded. Females were allowed to continue oviposition until 60 min after the start of the experiment (i.e. from the beginning of male–female pairing), irrespective of how quickly they mated.

The number of aphid nymphs parasitised between the first host encounter after parasitoid pairing and the start of mating was recorded as representing pre-mating oviposition. Similarly, the number of aphids parasitised between the first host encounter after mating and the end of the experiment (60 min from pairing) was recorded as representing post-mating oviposition. After the experiments, aphids were transferred to a cabbage seedling leaf for feeding and development. Subsequently, all mummified aphids were transferred to individual 2-ml microfuge tubes until the emergence of the parasitoids. The number of emerged adults was recorded and their sexes determined under the stereomicroscope.

In order to compare the respective effects of the presence and absence of hosts on mating success, a separate experiment was conducted. In this experiment, a male and a female ($n = 37$) were paired in the absence of host aphid nymphs for

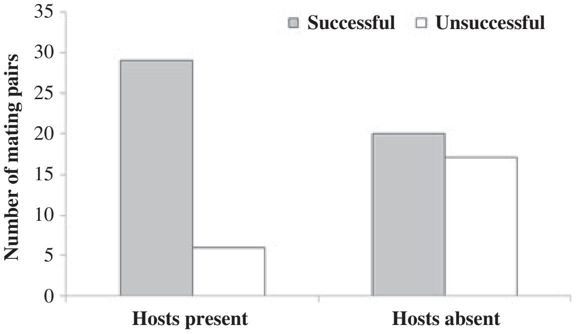

Fig. 1. Numbers of male–female *Diaeretiella rapae* pairs that successfully copulated within 30 min of pairing in the presence and absence of host cabbage aphid nymphs (*Brevicoryne brassicae*).

30 min; the configuration of the experiment was identical to that of the first experiment in all other respects. Pairs that copulated within 30 min were considered successful. Pre-mating time was recorded for each successful pair.

Statistical analyses

Logistic regression was used to analyse mating success in *D. rapae* adults in the presence and absence of the aphid host. Pre-mating times in the absence and presence of hosts and parasitism in large and small hosts were compared using analysis of variance (ANOVA). Paired *t*-tests were used to compare parasitism rates before and after mating. Regression analyses were used to elucidate the relationship between the length of the oviposition period and the number of aphids parasitised during pre-mating and post-mating oviposition. The sex ratio of the offspring resulting from each experimental exposure was compared using the Chi-square tests. All statistical analyses were performed using SAS Version 9.2 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Mating in Diaeretiella rapae *in the presence and absence of host aphids*

More *D. rapae* mating pairs were successful when paired in the presence of host cabbage aphids than in the absence of hosts ($P < 0.001$) (Fig. 1). There was no significant difference in mating success between *D. rapae* adults paired in the presence of small and large hosts ($P = 0.58$). Therefore, data on mating success in the presence of large and small hosts were pooled in order to compare mating success in the presence and absence of aphid hosts (Fig. 1).

Mating in *D. rapae* occurred throughout the allocated 30-min period. Pre-mating time was significantly shorter for pairs that mated in the presence of host aphids (6.9 ± 1.2 min) compared with those that mated in the absence of aphids (11.9 ± 1.5 min) ($P = 0.01$) (Fig. 2). The minimum time between pairing and mating was observed to be 1 min in a pair that mated in the presence of hosts. How quickly the pairs mated was independent of the size of the aphid host ($P = 0.78$) (Fig. 2).

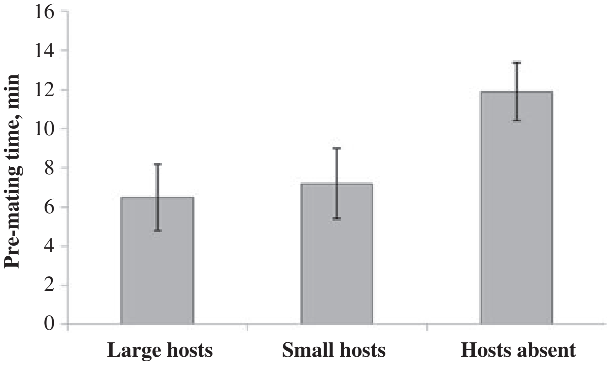

Fig. 2. Mean (± standard error) pre-mating time (duration between pairing and copulation) of mated pairs of *Diaeretiella rapae* in the presence of large, small or no cabbage aphid nymphs (*Brevicoryne brassicae*).

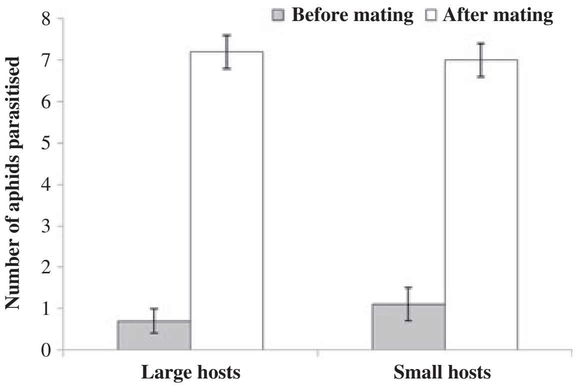

Fig. 3. Mean (± standard error) number of cabbage aphid nymphs (*Brevicoryne brassicae*) parasitised per *Diaeretiella rapae* female before and after mating when provided with small and large hosts.

Mating choice and oviposition before and after mating

When female *D. rapae* were given a choice between mating and ovipositing, 62% of them chose to mate first. The mean number of hosts parasitised before mating was significantly lower than that parasitised after mating ($P < 0.0001$) (Fig. 3). Female *D. rapae* that oviposited before mating parasitised only 10% of the aphids present in the arena during pre-mating oviposition. The number of aphids parasitised before mating was not affected by the size of the aphids offered ($P = 0.73$) (Fig. 3).

Time between mating and ovipositing

Female *D. rapae* did not parasitise aphids immediately after mating. Instead, after mating, the females spent time grooming before starting to oviposit. The mean [± standard error

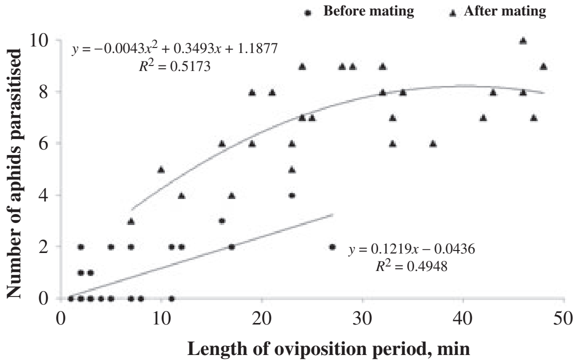

Fig. 4. The relationship between oviposition duration and the number of cabbage aphid nymphs parasitised by *Diaeretiella rapae* during pre-mating and post-mating oviposition. Length of oviposition period for 'pre-mating' is the time between the first host encounter after pairing and mating; for 'post-mating' it is the time between the first host encounter after mating and the end of the experiment (60 min from pairing).

(SE)] time between mating and ovipositing (pre-oviposition time) was significantly longer (24.4 ± 1.8 min) than the mean time between pairing and mating (6.9 ± 1.2 min) ($P < 0.001$). However, there was no correlation between pre-mating time and pre-oviposition time (Pearson correlation $= -0.12$, $P = 0.50$).

Parasitism rate

The mean number of aphids parasitised per unit time before mating (0.12 ± 0.04 hosts/min) was significantly lower than the number of aphids parasitised per unit time after mating (0.33 ± 0.03 hosts/min) ($P < 0.0001$). The relationship between oviposition period and the number of aphids parasitised was positive and linear during pre-mating oviposition (number of aphids $= 0.1219x - 0.0436$, where $x =$ length of pre-mating oviposition period), but positive and curvilinear during post-mating oviposition (number of aphids $= -0.0043x^2 + 0.3493x + 1.1877$, where $x =$ length of post-mating oviposition period) (Fig. 4). Virgin females were found to stand and groom for longer periods during pre-mating oviposition than mated females during post-mating oviposition.

Adult emergence and sex ratio

The number of aphids from which adult *D. rapae* emerged was greater when oviposition occurred both before and after mating (9.0 ± 0.3), compared with when oviposition occurred only after mating (7.3 ± 0.5) ($P < 0.01$).

More than half of the females that oviposited before mating subsequently superparasitised the host with fertilised eggs after mating, causing a highly female-biased sex ratio among emergent offspring. The mean number of females that emerged from the parasitism of 10 cabbage aphids was significantly greater than the number of males ($P < 0.001$) (Fig. 5). There was no significant difference in the respective numbers of adult parasitoids that emerged from small and large host aphids ($P > 0.45$), but there was a significant interaction between host size and the sex of emerging offspring in that a greater proportion of females emerged from large aphids ($P < 0.004$).

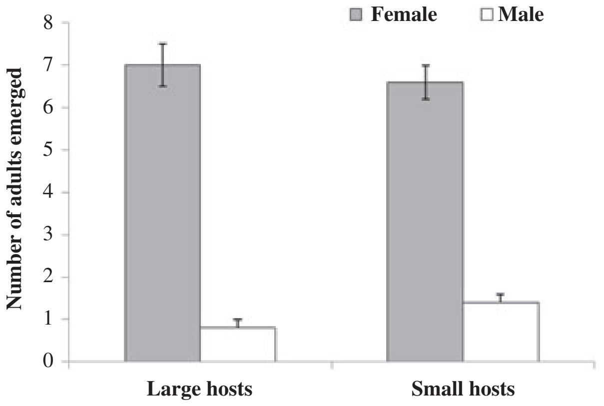

Fig. 5. Mean (± standard error) number of male and female *Diaeretiella rapae* offspring that emerged from large and small cabbage aphid nymphs (*Brevicoryne brassicae*).

Discussion

Mating and ovipositing are the two components of reproduction in insects. Female *D. rapae* emerge after males (Kant & Sandanayaka, 2009) and thus generally can choose between mating and ovipositing. When a choice between aphids for ovipositing and a male for mating was available, virgin *D. rapae* females preferred to mate before ovipositing. Females that did not mate probed (inserted the ovipositor into the host) few aphids before mating. However, *D. rapae* and some other parasitoids are known to sometimes probe their hosts not only for the purpose of ovipositing, but also to assess the quality of the host and to temporarily paralyse the host appendages to reduce the host defence (Phillips, 2002; Kant *et al.*, 2008, 2011).

In the present experiment, females were 10 times more likely to encounter an aphid than they were to encounter the male parasitoid, because one male parasitoid and 10 aphids were present in the arena (assuming that the male was not actively searching for the female). Despite the high probability of encountering an aphid, females probed very few hosts. Given a choice among differently sized hosts, mated *D. rapae* females preferred to attack and allocate fertilised eggs to larger hosts (Kant *et al.*, 2008, 2012b). By mating first, females gain the option of choosing the sex of their offspring and are able to allocate fertilised eggs to higher-quality hosts in order to produce female offspring (Godfray, 1994; Bernal & Gonzalez, 1997).

The mating success of *D. rapae* was significantly greater in the presence than in the absence of aphids. This suggests that mating and ovipositing are interdependent in *D. rapae*. The presence of and ready access to ovipositional resources, such as hosts, are known to induce sexual receptivity and mating in some insects (Ringo, 1996; Carsten & Papaj, 2005; Harano *et al.*, 2006). The longer pre-mating time in the absence of aphid hosts suggests that female *D. rapae* choose when they are receptive to males. Females rejected males more often when mating occurred in the absence of aphid hosts. Sexual receptivity in female wasps is usually induced only a limited number of times and opportunities for further mating tend to be few (Hardy *et al.*, 2005). The presence of a host also increases male efficiency in locating virgin females (Metzger *et al.*, 2010). The present results suggest that, in *D. rapae*, a female's decision to mate first when she has access to both a host and a mate might help in optimising her reproductive fitness. However, it is possible that females do not choose between mating and ovipositing, but rather between ovipositing and not ovipositing depending on whether or not they have mated.

The sequence of mating and ovipositing may be further influenced by the egg load of females, which may also affect the sex ratio of the parasitoid population. Species in which females emerge with developed eggs, including *D. rapae*, are generally egg-limited (Bernal & Gonzalez, 1997); therefore, it is advantageous for females to mate before ovipositing. Given a choice between mating and ovipositing, about a third of females in this study chose to oviposit first. However, these females attacked aphids at a lower rate during pre-mating oviposition than after mating, and parasitised only 10% of hosts offered before mating. The presence of more males in a population increases competition among a female's male offspring to find mates (Ode *et al.*, 1998), which has been reported to interfere with mating in *D. rapae* (Kant *et al.*, 2012a). The increase in parasitism of up to 90% after mating suggests the adaptive nature of ovipositing practice in *D. rapae* and indicates that mating preferentially precedes oviposition in this species.

Female *D. rapae* did not parasitise aphids immediately after mating but spent time grooming before starting to oviposit. The time lag between mating and oviposition decreased the time available to females for oviposition in the present experiment. The time gap may reflect the physiological process in which females must engage in order to store sperm in the spermatheca and to fertilise eggs. The length of this period between mating and oviposition has been shown to vary substantially across insect species (Hardy *et al.*, 2005). Previous studies suggested that females were not able to fertilise their eggs immediately after mating (R. Kant, unpublished data, 2012); therefore, by delaying oviposition after mating, females may be able to produce more female offspring.

The number of *D. rapae* adults that emerged was slightly greater when females oviposited both before and after mating. The sex ratio of *D. rapae* adults that emerged from parasitised hosts was highly female-biased, irrespective of whether oviposition occurred only after mating or both before and after mating. The proportions of females to emerge from larger and smaller hosts were similar, which is contrary to previous findings in which females preferred to allocate fertilised eggs to larger hosts in a host-size choice test (Kant *et al.*, 2012b). This also suggests that female parasitoids adjust their oviposition and vary their sex allocation strategies in conditions in which they do and do not have a choice among hosts (Godfray, 1994; Murray *et al.*, 2010). Another reason for the finding of a uniform sex allocation in small and large hosts may be that the low density of aphids encourages parasitoids to deposit more fertilised eggs in hosts regardless of their quality.

Parasitoids regulate clutch size and progeny sex ratio to maximise their overall fitness for a given host density (Godfray, 1994; Yu *et al.*, 2003). In the present experiments, females that oviposited in their hosts before mating later superparasitised some of their hosts with fertilised eggs to produce female offspring. Superparasitism in *D. rapae* may be considered an adaptive strategy on the part of females to maximise their fitness (and balance the sex ratio) in a low-density host population (such as in the present experiment), in which *D. rapae* females have the opportunity to attack the same hosts again (Kant *et al.*, 2011).

This research suggests that mating and ovipositing choices are not mutually exclusive, but can complement one another to maximise reproductive fitness. A trade-off exists between mating and ovipositing, and the choice made by females probably depends on the immediate availability and density of either hosts for oviposition or males for mating. In addition, the sequence of the two activities plays an important role in fitness gain and eventually in population dynamics. Mating first enables the female to utilise host resources more efficiently to produce a female-biased sex ratio in her offspring. The efficiency of parasitoids can be increased by releasing mated females during augmentative field or glasshouse release. As the increase in the proportion of females is a primary requirement for *D. rapae* to suppress the host population (Kant *et al.*, 2012a), releasing mated females would probably enhance the proportion of females in the next generation and offer better suppression of aphids in a biological control programme.

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