

Effects of mating and oviposition delay on parasitism rate and sex allocation behaviour of *Diaeretiella rapae* (Hymenoptera: Aphidiidae)

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h i g h l i g h t s

- ▶ Female *Diaeretiella rapae* emerge with developed eggs.
- ▶ Oviposition delay resulted in decreased parasitism by *D. rapae*.
- ▶ Delay in mating did not affect courting but affected the reproductive capacity of male and female *D. rapae*.
- ▶ Mating delay caused overproduction of male *D. rapae*.
- ▶ One-two day old *D. rapae* adults could perform more efficiently than younger or older adults as biocontrol agents.

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Mating and oviposition complement each other in parasitoid reproduction; delay in either of these activities has disparate consequences for the fitness of the parasitoid. We investigated the effects of mating and oviposition delay on parasitism rate and progeny sex ratio in a parasitic wasp *Diaeretiella rapae*. Egg dynamics in the ovaries of females of different ages, and females which completed different numbers of oviposition bouts were also examined. Delay in mating and oviposition adversely affected reproductive performance of both male and female *D. rapae*. Delayed oviposition resulted in reduced parasitism rates in both mated and unmated females. The progeny sex ratio was female-biased, but the proportion of female offspring decreased towards the end of *D. rapae* life. Although females emerged with developed eggs, the egg load in the ovaries of unmated and naive females initially increased then continuously decreased with age, suggesting partial resorption of eggs towards the end of a females life. Oviposition

reproductive fitness ([Damiens et al., 2003](#); [Fauvergue et al., 2008](#); [Kant et al., 2012c](#)). Oviposition or mating could be delayed when hosts or mates, respectively, are limited.

When female parasitoids emerge, they have a choice between oviposition and mating ([Godfray, 1994](#); [Kant et al., 2012c](#)). Females can oviposit before or without mating, but pre-mating oviposition in arrhenotokous parasitoids will result in haploid male progeny and could imbalance the sex ratio of the population in the next generation ([King, 1962](#)). Mating is required for producing fertilised eggs which result in female offspring ([Godfray, 1994](#)). Mating is,

(Quicke, 1997). In several species female cannot use sperm immediately after copulation, and the period between mating and fertilization can vary considerably in parasitoids (Hardy et al., 2007). Our earlier study indicated that a female needs at least 6 h to fertilise her eggs. Therefore, females were held for 6 h to ensure that they had sufficient time to fertilise their eggs and produce female offspring. The number and sex of the offspring that emerged from parasitised aphids were recorded.

Similarly, the parasitism rate and sex ratio of offspring produced by the females mated with virgin males of different ages (1–2 days, 4–5 days and 7–8 days old) were investigated. Individual virgin males in each age-group were offered two 1-day old virgin females together for 24 h. This allowed each male to mate with one or both of the available females and it is known that sperm depletion occurs in *D. rapae* only after three or more matings (Kant et al., 2012d). Each of the mated females was then offered 15 unparasitised aphids per day for 3 consecutive days. The number of female offspring produced by the females mated with males of each age group was compared. The number of female offspring produced was used as an indirect estimate of sperm transfer (Godfray, 1994; Henter, 2004). More than ten males were tested in each male age group treatment.

The effects of different periods of mating delay (1–2 days, 4–5 days and 7–8 days) on parasitism rate and sex ratio of the female's offspring were tested. All females were offered 15 aphids for parasitism from the first day of emergence. Since haplodiploid females can reproduce before mating, offering hosts before mating likely to reflect natural conditions, and helps reveal the pre-mating and post-mating oviposition strategies of female *D. rapae*. When the females reached the desired mating age for the experiment, they were each paired with a 1-day old virgin male for 24 h. After mating, each female was again offered 15 aphids per day for three consecutive days, irrespective of the mating delay period. This means that in 1–2 day delay treatment, the females mated prior to first oviposition, while the group of females which were not exposed to males for the first four days of their emergence already oviposited for 3 days prior to mating; similarly, the group of females which were not exposed to males for the first 7–8 days of their emergence, oviposited for 6–7 days prior to mating. Each treatment was replicated 10 times. The total number of offspring and the proportion of female produced by each group of females were compared.

2.5. Statistical analysis

A goodness-of-fit test was used to assess the distribution of the data, and the non-normal data were transformed prior to analyses. The number of offspring produced by mated and unmated females in oviposition and mating delay experiments, and the number of eggs found in the ovaries of females of different age were analysed using analysis of variance (PROC GLM, SAS 9.2). The means of different treatments were compared using a Tukey's studentised range (HSD) test. Linear regression analysis (PROC REG) was used to examine the relationship between oviposition delay and the number of offspring produced by the females. Logistic regression with binary response variable (PROC LOGISTIC, SAS 9.2) was used to analyse the offspring sex ratio in different treatments. Values in results are untransformed means \pm SE. All analyses were carried out at $\alpha = 0.05$ level of significance.

3. Results

3.1. Oviposition delay in unmated females

The number of offspring produced by female *D. rapae* was negatively affected by the duration of oviposition delay (number of

offspring produced = $41.03 - 4.49x$, where x = delay in number of days, $R^2 = 0.48$, $P < 0.001$). Unmated females that were not allowed to oviposit for 6 days, produced less than a third of the total number of offspring they would have produced if their oviposition started soon after emergence ($F_{3,36} = 11.31$, $P < 0.001$) (Fig. 1). There was no significant reduction in the number of offspring produced by females whose oviposition was delayed for a single day.

The egg count in the ovaries of unmated and naïve female *D. rapae* changed with age (Fig. 2). Although females emerged with developed eggs, their egg count initially increased with age, reaching a maximum 3–4 days after emergence, before decreasing ($F_{4,63} = 62.23$, $P < 0.001$) (Fig. 2).

3.2. Oviposition delay in mated females

Mated female *D. rapae* that were prevented from ovipositing for the longest period (6 days) produced the least offspring ($F_{3,36} = 30.99$, $P < 0.001$) (Fig. 3). Mated females whose oviposition was delayed for 6 days also produced the least female offspring (7.7 ± 0.63) compared to other mated females ($F_{3,72} = 20.83$, $P < 0.001$) (

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Mating delay adversely affected the reproductive potential of male *D. rapae*. Females mated with older males (7–8 days old) produced significantly fewer offspring than females mated with younger males ($F_{2,31} = 4.31$; $P = 0.022$) (Fig. 4). The mean number of female offspring produced by females was also affected by the age of the males they mated ($F_{2,31} = 7.99$; $P = 0.002$) (Fig. 4). However, the number of male offspring produced by females was not significantly affected by the age of their mating partner ($F_{2,31} = 1.14$; $P = 0.33$) (Fig. 4).

The reproductive capacity of female *D. rapae* was negatively affected when their mating was delayed. The number of offspring produced per day reduced significantly in females whose mating was delayed for a longer period (6 days) compared to when delay was short (1 day) ($P < 0.01$) (Fig. 5). The sex ratio (female:male) of their offspring decreased significantly when their mating was delayed ($F_{2,27} = 24.89$; $P < 0.001$) (Fig. 5).

4. Discussion

Delay in mating and oviposition affected the reproductive fitness of *D. rapae*. The number of offspring produced by female *D. rapae* decreased when their mating was delayed. Older male *D. rapae* transferred less or lower quality sperm, which was reflected by the decrease in the resulting female offspring. Female *D. rapae* produced viable male offspring through ovipositing unfertilised eggs immediately after emergence. However, the number of offspring produced by female *D. rapae* prior to mating was less than the number of offspring produced after mating. Pre-mating oviposition can cause overproduction of males in the following generation, so the reduced oviposition rate by unmated female *D. rapae* could be a strategy to minimise the overproduction of males. Results also revealed that the number of subsequent ovipositions was also reduced when the females were deprived of hosts for a period of time, and the number of ovipositions decreased with increasing length of delay, irrespective of the mating status of the female.

Although females emerged with developed eggs, their egg load increased during their early life, suggesting that *D. rapae* is a weakly-synovigenic species (Jervis et al., 2001). The reduced oviposition and decreasing egg count in females whose oviposition was delayed suggests either deterioration or partial resorption of eggs by the females towards the end of their life. Resorption of eggs occurs in various parasitoid species, and females usually have full control over the resorption process (Guo et al., 2011; Hegazi et al., 2007; Richard and Casas, 2009). The number of eggs in the ovaries of females whose oviposition was delayed for 1 or 2 days remained unchanged, suggesting that egg resorption at a later stage of female life could be due to nutritive stress at old age (Bell

3.3. Mating delay in males and females

All females that were mated with newly emerged males produced female offspring. This confirms that male *D. rapae* are able to successfully mate and transfer viable sperm immediately upon emergence. Delay in mating did not affect the courtship capacity of males. When 7–8 day old males were paired with females, they displayed courtship immediately after pairing in a way similar to the newly emerged males, and 80% of them mated with both females. However, the duration of copulation was longer in males whose mating was delayed for 7–8 days (72.1 ± 1.8 s) compared to newly emerged males (58.8 ± 1.9 s) ($F_{1,40} = 24.89$; $P < 0.001$).

and Bohm, 1975; Rosenheim et al., 2000). Resorption of eggs reduces fecundity and is a last resort that could help a female to live longer (Jervis and Kidd, 1986). The increase in egg count early in the life of *D. rapae* females followed by later decrease, suggests that females can mature eggs but cannot produce new eggs.

Sex ratio of the progeny of mated *D. rapae* female was female-biased irrespective of the length of oviposition delay. However, the number and proportion of female progeny decreased with increasing oviposition delay. Since female *D. rapae* mate once in their life times while males mate multiple times (Kant et al., 2012d), the operational sex ratio of *D. rapae* is male-biased even when numerically more females than males are present in the population (Kant et al., 2012b). In ideal conditions (without any delay), a female will produce more female progeny in order to have only enough males to ensure that all her daughters will be mated

Kant, R., Minor, M.A., Trewick, S.A., 2012c. Mating or ovipositing? A crucial decision in the life history of the cabbage aphid parasitoid *Diaeretiella rapae* (M'Intosh).