

Effects of multiple matings on reproductive fitness of male and female *Diaeretiella rapae*

R. Kant^{1*}, S.A. Trewick¹, W.R.M. Sandanayaka², A.J.R. Godfrey³ & M.A. Minor¹

¹Ecology, Institute of Natural Resources, Massey University, Private Bag 11-222, Palmerston North 4474, New Zealand,

²Institute of Fundamental Sciences, Massey University, Private Bag 11-222, Palmerston North 4474, New Zealand, and

³The New Zealand Institute for Plant and Food Research, Private Bag 92-169, Auckland 1142, New Zealand

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Abstract

Mating frequency and the amount of sperm transferred during mating have important consequences on progeny sex ratio and fitness of haplodiploid insects. Production of female offspring may be limited by the availability of sperm for fertilizing eggs. This study examined multiple mating and its effect on fitness of the cabbage aphid parasitoid *Diaeretiella rapae* McIntosh (Hymenoptera: Aphidiidae). Female *D. rapae* mated once, whereas males mated with on average more than three females in a single day. The minimum time lag between two consecutive matings by a male was 3 min, and the maximum number of matings a male achieved in a day was eight. Sperm depletion occurred as a consequence of multiple mating in *D. rapae*. The number of daughters produced by females that mated with multiple-mated males was negatively correlated with the number of matings achieved by these males. Similarly, the proportion of female progeny decreased in females that mated with males that had already mated three times. Although the proportion of female progeny resulting from multiple mating decreased, the decrease was quicker when the mating occurred on the same day than when the matings occurred once per day over several days. Mating success of males initially increased after the first mating, but then males became 'exhausted' in later matings; their mating success decreased with the number of prior matings. The fertility of females was affected by mating with multiple-mated males. The study suggests that male mating history affects the fitness of male and female *D. rapae*.

Introduction

Mating is an integral part of reproduction in most insects, and it is essential for the production of viable offspring in sexually reproducing species. However, in parasitic wasps in which males are haploid and females are diploid, mating is needed only for producing female offspring (Godfray, 1994). Therefore, females of haplodiploid organism have a choice between ovipositing and mating, and the decision affects their fitness (Steiner & Ruther, 2009; Kant et al., 2012a). After mating, females store sperm in a specialized organ, the spermatheca, and use the sperm throughout their lives facultatively to fertilize their eggs for producing females (Thornhill & Alcock, 1983). Therefore, the

amount of sperm transferred during mating and stored in spermatheca is an important determinant of the population sex ratio and the population dynamics of the species (King, 2000; Chevrier & Bressac, 2002).

The reproductive fitness of a polyandrous female can depend on the number of mating and oviposition activities she undertakes during her life (Godfray, 1994; Bissoondath & Wiklund, 1996). For monandrous females, which mate only once in their lifetime, fitness depends on the number of ovipositions and on the mating history of the male she mates with (how much sperm transferred during copulation) (Marcotte et al., 2007). After mating with a sperm-depleted male, a polyandrous female might be able to replenish sperm stored in her spermatheca by remating, whereas a monandrous female will remain in shortage of sperm throughout her life. Thus, reproductive success in monandrous females is influenced both by the acquisition of mating and by the quality of the male (Smith, 1984). The number of female offspring produced by the mother

*Correspondence: R. Kant, Ecology, Institute of Natural Resources, Massey University, Palmerston North 4412, New Zealand.
E-mail: R.Kant@massey.ac.nz

provides an indirect estimation of the amount of sperm transferred during mating (Henter, 2004).

Reproductive fitness of males depends on the number of times they mate and/or the amount of sperm transferred during each mating (Bissoondath & Wiklund, 1996; King & Fischer, 2010). A male is capable of inseminating more than one female, because in most cases sperm stock of a high-quality virgin male is significantly greater than the number of eggs a single female fertilizes (Bressac et al., 2008). In some species, males emerge with already developed or fixed quantity of sperm (prospematogeny), whereas in other species males can replenish their sperm once it has been exhausted after mating (synspematogeny) (Boivin et al., 2005). The synspematogenic males should be able to increase their fitness by transferring a large amount of sperm, so that the mated female does not experience sperm shortage and does not need to re-mate. The prospematogenic males have a limited sperm stock and may enhance their fitness by transferring their sperm partially during each mating. By limiting the amount of sperm transferred during each mating, a male can avoid rapid depletion of sperm and eventually inseminate more females. However, this is only successful if females are monandrous; otherwise, a female can re-mate with a different male once she faces sperm shortage to replenish her spermatheca. The amount of sperm transferred to the female during each mating and the number of female offspring produced are used as measures of male fitness (Foster & Ayers, 1996; Bressac & Chevrier, 1998; Henter, 2004).

Diaeretiella rapae McIntosh (Hymenoptera: Aphididae) is an important parasitoid of the cabbage aphid, *Brevicoryne brassicae* L. (Homoptera: Aphididae), and several other aphid species across the world (Pike et al., 1999). The parasitoid has been used in various biological control programmes globally, including in Australia for cabbage aphid (Carver & Stary, 1974) and in the USA for controlling Russian wheat aphid (Bernal & Gonzalez, 1993). Previous studies on this parasitoid suggested that mating occurs throughout the photophase, but not during the scotophase (Kant & Sandanayaka, 2009; Kant et al., 2012b). Although the sex ratio of *D. rapae* is female-biased (Kant et al., 2012b), multiple mating of males, females, or both, could alter the operational sex ratio of the population. Higher operational male/female sex ratio – specifically, more males being present in the population than the number required to inseminate all the females – can cause mating interference (Kant et al., 2012b) and leave some females unmated. As *D. rapae* is haplodiploid, oviposition by unmated females further contributes to overproduction of males in the population. Therefore, the study of mating in *D. rapae* is important for understand-

ing reproductive strategies of the parasitoid, which could help improve their manipulation for biological control (Godfray, 1994). The current study investigated (1) the capacity for multiple mating in *D. rapae* males and females, and behavioural display during multiple mating, and (2) the effects of multiple mating on reproductive fitness of *D. rapae*.

Materials and methods

Insect colonies

Colonies of the cabbage aphid *B. brassicae* and its parasitoid *D. rapae* were established from insects originally collected from a cauliflower field near Palmerston North, Manawatu, New Zealand. Insects were reared in the laboratory on cabbage seedlings (Summer Globe Hybrid) in Plexiglas cages (30 × 30 × 30 cm). The colonies were maintained at 22 ± 2 °C, 60–70% r.h., and 16L:8D photoperiod. Honey-fed adult *D. rapae* that emerged from 5- to 6-day-old cabbage aphid nymphs were used in the experiments. All mating experiments were done during the light period, because mating in *D. rapae* does not occur in the dark (Kant & Sandanayaka, 2009).

Multiple mating in female and male *Diaeretiella rapae*

Multiple mating in females was tested by pairing 1-day-old virgin females with 1-day-old virgin males in separate glass vials (7.5 × 2.5 cm) with a ventilated cap. The behaviours of each pair were observed and recorded up to 60 min or until copulation occurred if sooner. The male and female from each pairing were separated; after 1 h, the mated female was again paired with a 1-day-old virgin male and observed for a maximum of 60 min period or until copulation occurred. The mated females were then each offered 50 aphids per day. The mated and oviposited female *D. rapae* were paired again after 1 or 2 days with 1-day-old virgin males. Twenty-five females were examined for multiple mating.

Two experiments were conducted to examine multiple mating in males. First, multiple mating was examined every hour (hourly mating) where 1-day-old virgin males were paired with 1-day-old females for 60 min or until copulation occurred if sooner. Successful males were offered another virgin female after a gap of 1 h, and the process continued for the entire photoperiod. Behaviours of the males and the females, such as walking, standing still, grooming, and mating attempts were observed in each mating pair until copulation occurred. Mating attempt is defined as when a male started vibrating its wings in the presence of a female and chased the female for mating. The unsuccessful males were discarded. A total of 37 males were examined for hourly-multiple mating

success. In the second experiment, multiple mating was examined once every day (daily mating). Males and females were paired for 18–24 h in daily mating. On the following photophase, males were taken out of the mating arena and paired with another virgin female, and the process continued for the entire life of the males. Mating success in daily mating males was analysed. Fifteen males were examined in daily multiple mating.

Progeny production and sex ratio from multiple-mated males

The effects of hourly- and daily-multiple mating of males on progeny production and progeny sex ratio was examined using the insects from multiple mating experiments described above. The mated females from hourly-mating experiments were each offered 15 fresh aphids for 2 h in a Petri dish per day for three consecutive days. The parasitized aphids were transferred to a cabbage seedling to feed and develop. Once the aphids were mummified, they were transferred to individual 2-ml microfuge tubes. The mummies were observed daily for adult emergence. The total number of adults and the proportion of female resulting from each mating were examined.

Similarly, progeny production and sex ratio of the progeny from daily mating was tested. Mated females were each offered 30 aphids on cabbage seedlings in a 2-l transparent and ventilated plastic container for 24 h. Fresh 30 unparasitized aphids were offered to the mated females for three consecutive days. The parasitized aphids were allowed to feed and develop on cabbage seedlings and the process was followed as above. The total number and sex ratio of adults emerged from hourly- and daily-mating experiments were compared. Ten males that mated at least five times were used in each hourly- and daily-mating experiment.

Statistical analysis

Binomial logistic regression was used to analyse mating success during daily- and hourly-multiple mating. When modelling the proportion of male wasps that were successful during mating, we use the model:

$$y_{ij} = m_i + f_j + (mf)_{ij} + \delta_{ij},$$

where y_{ij} is proportion of mating success, m_i is the mating number, f_j is the indicator for the hourly/daily mating frequency, $(mf)_{ij}$ is the interaction of these effects, and δ_{ij} is the random error term for each observation. A general linear model (ANOVA) was used to compare the number of mating attempts made by successful and unsuccessful males during first and fifth matings. The sex ratio, among the *D. rapae* offspring (proportion of female offspring) resulting from different matings was analysed using logistic regression (Wilson & Hardy, 2002). The log linear

model was used to understand the relationship between the mating history of males and the number of female offspring produced (Crawley, 1993). The model was fitted as a generalized linear mixed model using penalized quasi-likelihood (glmmPQL) (Venables & Ripley, 2002):

$$y_{ijk} = m_i + f_j + (mf)_{ij} + w_k + \delta_{ijk},$$

where m_i is the mating number, f_j is the indicator for the daily/weekly mating frequency, $(mf)_{ij}$ is the interaction of these effects, w_k is the random effect for each male wasp, and δ_{ijk} is the random error term for each observation. The response y_{ijk} and the error δ_{ijk} change for the last three models. When modelling the proportion of females as the response, a logistic regression was used. For modelling the counts of females and then the total offspring, the response, and the error term were assumed to be Poisson distributed. All analyses were done at $\alpha = 0.05$ level of significance by SAS (v9.2) (SAS Institute Inc., Cary, NC, USA) and R (v2.11.1) R Foundation for Statistical Computing, Vienna, Austria (<http://www.R-project.org>).

Results

Multiple mating in female and male *Diaeretiella rapae*

Females mated only once during their lifetime. Females did not accept a male for re-mating on the same day or after 1–2 days after first mating. Furthermore, female *D. rapae* did not re-mate even after oviposition. In contrast, male *D. rapae* remained sexually receptive after the first mating and showed courtship to previously mated as well as virgin females.

Mating success of males depended on their mating history (sequential analysis of deviance: $G = 7.42$, d.f. = 15, $P = 0.006$) (Figure 1). The proportion of mating success also depended on mating approaches, whether mating

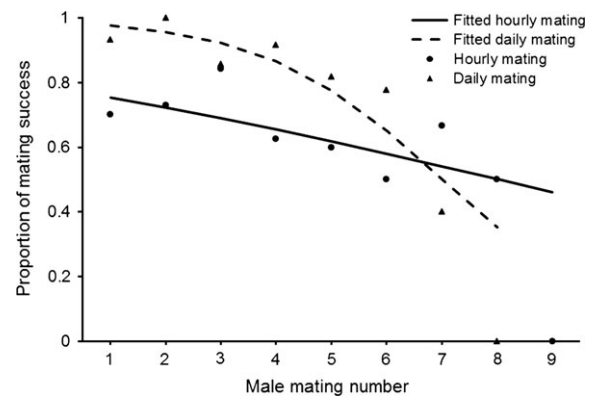


Figure 1 The effects of hourly and daily multiple matings on mating success of *Diaeretiella rapae*. Logistic regression model was used to analyse the mating success of multiple mated males.

was hourly or daily ($G = 9.54$, $d.f. = 14$, $P = 0.002$) (Figure 1). As the number of male mating sessions increased, the mating success decreased. This was true for both hourly- and daily-mating sessions; however, the rate of decline was greater in hourly mating ($G = 5.01$, $d.f. = 13$, $P = 0.025$) (Figure 1). All males died by the ninth day in the daily multiple mating. The mean (\pm SE) number of times males mated in the single day was 3.4 ± 0.5 . The minimum time gap between two consecutive matings was 3 min, and the maximum number of successful matings a male achieved in a day was eight.

When activities of males (walking and mating attempts) during mating were compared in early (first) and late (fifth) matings, males were less active in the late mating bouts. Before copulation in the fifth mating, males spent 35% of their time walking around the arena and the rest of the time standing still or grooming, whereas in the first mating they spent 85% of the time walking. The mean number of mating attempts by males that were unsuccessful during their first mating was significantly higher than the number of mating attempts by males that were unsuccessful during their fifth mating (10.9 ± 0.9 vs. 4.6 ± 1.3) ($F_{1,15} = 13.65$, $P < 0.002$). The mean number of mating attempts by successful males during the first mating (4.0 ± 0.6) and fifth mating (4.8 ± 0.8) were similar ($F_{1,23} = 0.60$, $P = 0.45$).

Progeny sex ratio from multiple-mated males

The proportion of female offspring from multiple-mated males decreased irrespective of whether the multiple mating occurred hourly in a single day (glmmPQL: $t = 2.66$, $d.f. = 85$, $P = 0.009$) or daily over a period of several days

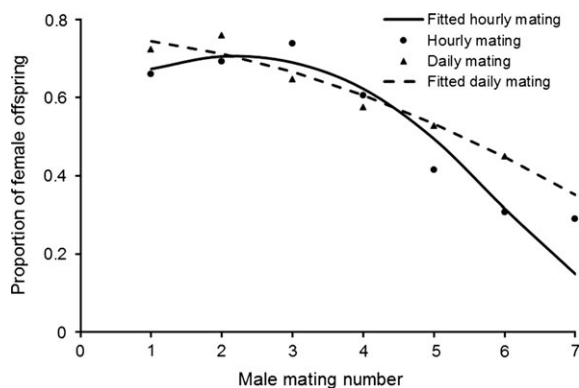


Figure 2 Sex ratio (proportion of female offspring) of the offspring produced by female *Diaeretiella rapae* that mated with multiple-mated males. Logistic regression model was used to analyse the decrease in the proportion of female offspring. The model was fitted as a generalized linear mixed model using penalized quaslikelihood (glmmPQL).

($t = 2.51$, $d.f. = 85$, $P = 0.014$) (Figure 2). However, the decrease in the proportion of female offspring was significantly quicker in hourly mated compared to daily mated males ($t = 2.35$, $d.f. = 18$, $P = 0.03$) (Figure 2).

Effect of male-mating history on progeny production of mated females

The fertility of females was affected by the mating history of their mates (glmmPQL: $t = 2.02$, $d.f. = 18$, $P = 0.057$) (Figure 3). Females that were mated with multiple-mated males produced fewer progeny. The reduction in the total number of offspring produced by females also depended on whether the females were mated in hourly or daily mating sessions ($t = 2.49$, $d.f. = 86$, $P = 0.014$) (Figure 3). The total offspring resulted from hourly mating was much less than daily mating.

Females also produced significantly fewer female offspring when they mated with multiple-mated males (glmmPQL: $t = 3.11$, $d.f. = 18$, $P = 0.006$) (Figure 4). The number of female offspring produced also depended on whether the females were mated in hourly or daily mating sessions ($t = 2.53$, $d.f. = 85$, $P = 0.013$) (Figure 4).

The number of female offspring produced by sperm-constrained females (mated with males in their fifth and sixth mating) was greater in their first oviposition bouts than in their second and third oviposition bouts ($F_{2,84} = 21.59$, $P < 0.001$) (Figure 5). A similar number of female offspring were produced by females mated with sperm-depleted males during their second and third oviposition bout ($t = 1.28$, $d.f. = 67$, $P = 0.41$) (Figure 5).

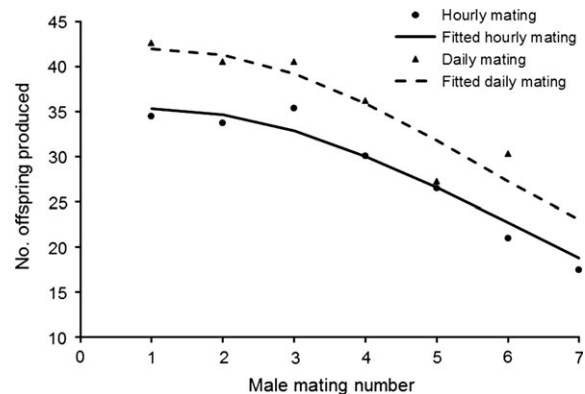


Figure 3 Total number of offspring produced by *Diaeretiella rapae* females that mated with multiple-mated males. The log linear model was used to understand the relationship between the mating history of males and the number of offspring produced. The model was fitted as a generalized linear mixed model using penalized quaslikelihood (glmmPQL).

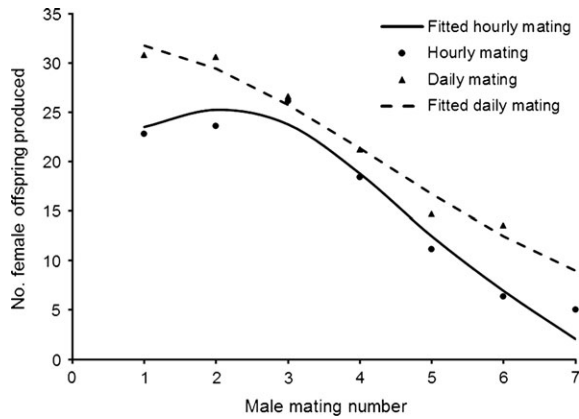


Figure 4 Mean number of female offspring produced by female *Diaeretiella rapae* that mated with multiple-mated males. The log linear model was used to understand the relationship between the mating history of males and the number of female offspring produced. The model was fitted as a generalized linear mixed model using penalized quasikelihood (glmmPQL).

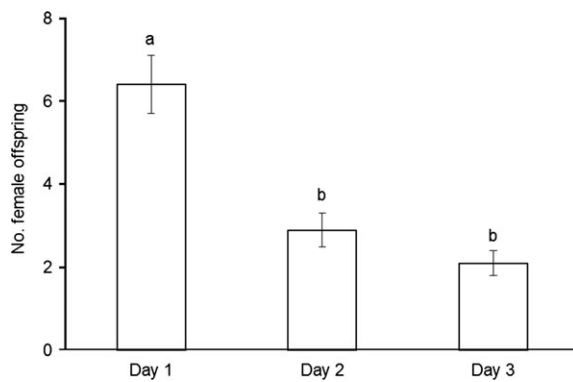


Figure 5 Mean (\pm SE) number of female offspring produced from three consecutive ovipositions (Day 1–3) by female *Diaeretiella rapae* that mated with four or five times already-mated males. Means with same letters are not significantly different (Tukey's HSD: $P > 0.05$).

Discussion

Multiple mating occurred in male but not in female *D. rapae*. Multiple mating in males affected the reproductive performance of females. Sperm depletion as a consequence of multiple mating by male *D. rapae* probably caused the low proportion of female offspring. Multiple mating was more exhaustive in a single-day (hourly) mating than daily mating. Males that mate frequently or in quick succession deplete their sperm stock more rapidly than males mating after longer intervals (Hardy et al., 2005a). The number of female progeny resulting from matings involving a virgin male was higher than the number of females produced

from matings with multiple-mated males. This suggests that multiple-mated males become sperm depleted and transfer a reduced amount of sperm during copulation. Reduction in sperm transfer with successive mating has been documented in other species of Hymenoptera (Nadel & Luck, 1985; King, 2000; Bressac et al., 2008).

From the female's perspective, mating with a sperm-depleted male might result in sperm shortage in her spermatheca, which could reduce the number of fertilized eggs and hence the number of female offspring. Because female *D. rapae* mate once and do not replenish their sperm supply by re-mating, they may ultimately produce more sons than are required for inseminating all the females of the subsequent generation, and enhance imbalance in the sex ratio in the parasitoid population (Damiens & Boivin, 2005). Thus, monandrous females are expected to evolve mechanisms acting against the male multiple-mating strategy (Chapman et al., 2003). If a female can recognize male-mating history, it would allow her to avoid mating with sperm-depleted males (Harris & Moore, 2005). In our experiments, less than 20% of males were able to reach the fifth mating and the remaining males were rejected by females. This suggests that female *D. rapae* might be able to recognize this aspect of male quality. The strategy of the female to initially avoid mating might explain the large number of unsuccessful matings and longer pre-mating period for the pairs who mated successfully. It should be noted that in our experiments females had no option to choose between a virgin and a multiple-mated male, but could only accept or reject the male provided. Furthermore, in this study, opportunity and time for females to make mating decisions was limited.

Although the sex ratio of New Zealand populations of *D. rapae* has been found to be female-biased in laboratory as well as in open systems (Kant et al., 2012b,c), the monandrous and polygynous nature of *D. rapae* (R Kant, unpubl.) biases the operational sex ratio in favour of males. Thus, to inseminate all the females in a population, a male would on average mate three to four times in its life. Large numbers of males could also lead to an increase in male-male interactions, reducing mating success in females, and further decreasing the efficiency of *D. rapae* to suppress aphid populations (Kant et al., 2012b). In this study, we found that sperm depletion in male *D. rapae* (as reflected by production of male offspring) did not occur until the third mating in hourly matings. However, sperm depletion in daily multiple matings is obvious in third matings, which is probably because of the age factor. Age of male *D. rapae* negatively affects the sperm production ability (R Kant, unpubl.). Furthermore, under natural conditions, where females are abundant in the population, it is less likely that a male would mate with only one female

per day. Therefore, under natural/field conditions the chances of females encountering sperm-depleted males should be low (Godfray, 1994; Hardy et al., 2005b).

We found that female *D. rapae* mate only once in their life. Mating with a sperm-depleted male did not increase the tendency of females to mate again. This could be due to some physiological constraint on re-mating in female *D. rapae*. During copulation, males might transfer certain chemicals with the spermatozoa that might prohibit females from mating again (Davey, 1985; Neubaum & Wolfner, 1999; Avila et al., 2011). By doing so, sperm-depleted males would increase their reproductive success by inseminating more females and preventing the females from mating with other males (Gardner & West, 2004; Damiens & Boivin, 2006). Absence of re-mating in females experiencing sperm shortage has also been reported in other species (Gardner & West, 2004; Damiens & Boivin, 2006).

In some parasitoid species, the longevity of adult females is relatively longer than the offspring developmental time which provides the opportunity for females mate with their own sons and produce female offspring (King & Copland, 1969; Greeff, 1996). However, in *D. rapae*, development takes longer than the lifespan of adult females (Kant et al., 2012c), thus, there is no opportunity for inbreeding (females to mate with their own sons) in this species. Therefore, the strategy for females that live for a shorter period of time is probably to mate once and spend more time on oviposition. In contrast, females of longer lived species have more options; they can mate several times if they become sperm deprived in later life and/or choose best sperm stored in their spermatheca (Jacob & Boivin, 2005). As *D. rapae* is a monandrous species, there is no opportunity for sperm competition.

The results of this study suggest that male *D. rapae* continue to mate even after sperm-depletion. They probably increase their reproductive fitness by optimizing the amount of sperm transferred per female to maximize the number of matings. Ageing negatively affected the insemination capacity of males. Female *D. rapae* rejected multiple-mated males, and the females mated with multiple-mated males received less sperm and produced fewer offspring.

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