INFLUENCE OF MALE DENSITY ON THE COURTSHIP AND MATING DURATION OF MALE RICE WEEVILS, *SITOPHILUS ORYZAE*

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ABSTRACT

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is a major pest of stored grains throughout the world. This experiment examined the capacity of male *S. oryzae* to adjust their mating behaviour in response to male density (i.e. 1, 5 and 10 males) maintained with a single female. Results show that males spent less time in courting females with antennal contact at density 1 when compared to densities 5 and 10. Copulation duration increased in response to the increase in rival densities. The mechanism behind this phenomenon may be (1) a mate guarding strategy to enhance reproductive success by reducing the chance of sperm displacement by competitors and (2) a strategy to avoid damage to the reproductive organs of their mates by multiple mating.

Keywords: *Sitophilus oryzae*, male density, mating behaviour.

INTRODUCTION

Both theoretical and empirical studies suggest that, under different population densities, male and female behaviour should alter to maximise fitness (Eshel 1979; Crowley et al. 1991; Jirotkul 1999). Thus, males that can adjust their mating behaviour in response to a change in density may gain a mating advantage (Crowley et al. 1991). For example, at low male densities, a male may increase his mating success by courting and staying with one female; conversely, at high male densities, he may gain a mating advantage by courting many females, each for less time in order to increase his probability of finding a receptive female (Crowley et al. 1991). It is expected that competitive behaviour, such as mate guarding, should decrease at low male densities because of less competition by rivals; whereas, at high male densities, competition may be high due to the increasing number of rivals (Parker 2006).

The rice weevil, *Sitophilus oryzae* (L.), is an important pest of stored cereal crops including rice, wheat and maize, and is found throughout the world (Longstaff 1981). *Sitophilus oryzae* is an excellent subject for the study of the effects of population density on male–male competition because it is polygamous and its population density in grain stores is spatially and temporally dynamic (Armitage et al. 1983). However, mating behaviour of *S. oryzae* is still poorly understood (Campbell 2005). This study investigated whether *S. oryzae* alters its mating behaviour in response to male density.

MATERIALS AND METHODS

Insect rearing

A breeding colony of *S. oryzae* was started from a population provided by Plant & Food Research, Palmerston North, New Zealand. Insects were reared on wheat (*Triticum aestivum* (L.)) grains in constant dark at 27±1°C and 75±3% RH in an environmental incubator (MLR-350HT, Sanyo). Around 500 *S. oryzae* adults were introduced into a 500 ml transparent glass jar with a 0.25 mm wire-mesh lid where 200 ml of wheat grains had been placed. The adults were allowed to feed and lay eggs

for 25 days and then sieved off from the wheat grains. Grains with irregular colour patterns but without emergence holes (2-3 mm diameter) were considered infested and individually isolated in ventilated Eppendorf tubes. Tubes containing infested wheat grains were placed in the environmental incubator and checked daily to determine adult emergence. Emerged adults were sexed according to rostrum characteristics and the dorsal surface of the abdomen of each female was marked with a silver permanent marker (Halstead 1963). All adults were individually introduced to a tube with five uninfested wheat grains. Tubes were then put into the incubator for 3 days so that weevils could achieve reproductive maturity before initiating the experiment.

Experiment

The experiment was carried out in Petri dishes (60 mm diameter × 10 mm height) in a controlled environmental room under red lights at the same temperature and RH as in the incubator. Three treatments were set up, i.e. a virgin female was maintained with males of three densities: 1, 5 and 10. Fluon (anti-grip substance) was added to the inner wall of the dish base to prevent the escape of insects. Six Petri dishes with two dishes for each treatment were randomly placed on the bench, and 20 grains of wheat evenly distributed in each dish. The mating behaviour of adults in each dish was observed for 1 min every 6 min under a microscope (Olympus, Japan) during the 7.5 h observation period on the first day, i.e. each dish was observed for 75 min during the 7.5 h observation period. After 7.5 h, males from each dish were separated from the female and placed individually in a ventilated Eppendorf tube maintained with one wheat grain. Wheat grains in the Petri dish were replaced with fresh ones and left with the female overnight. After 16.5 h (day 2) the males were released into the dishes and maintained with their original mate, and observations continued for a further 7.5 h. This procedure was repeated for a third day. This 3-day sequence was carried out on different insects a further four times to give ten replicates for each treatment.

Courtship duration was recorded as the duration males waved their antennae when <5 mm away or in contact with the female, and mating duration was recorded as the duration of copulation during the 7.5 h observation period.

Statistics

Data on the mean courtship and mating duration over 3 days, and mating duration on different days at different male densities were normally distributed and analysed using ANOVA followed by Tukey’s Studentised Range test. All other data were not normally distributed even after transformation and therefore analysed using non-parametric Kruskal-wallis (KWT) test followed by Dunn’s procedure for multiple comparisons.

RESULTS

Males spent more time in courtship when other males were present (KWT: $\chi^2=15.38, 17.90, \text{ and } 12.49$ for day 1, 2 and 3, respectively, $P<0.001$; $P<0.0001$ for mean) (Table 1). Courtship duration increased from day 1 to day 3 at male densities of 1 and 10 (KWT: $\chi^2=6.72, \text{ and } 7.74$ for male density of 1 and 10, respectively; $P<0.05$). Courtship duration did not differ between days 1-3 when male density was 5 (KWT: $\chi^2=2.96$; $P>0.05$) (Table 1).

TABLE 1: Courtship duration (min) of *S. oryzae* at three male densities measured for 7.5 h periods over 3 consecutive days.

No. males	Day 1	Day 2	Day 3	Mean
1	1.9 ± 0.6 bβ ¹	2.7 ± 0.4 abβ	4.8 ± 1.2 aβ	3.1 ± 0.5 β
5	8.1 ± 1.0 αα	12.3 ± 2.4 αα	20.2 ± 6.5 αα	13.5 ± 2.7 α
10	7.7 ± 1.5 βα	9.4 ± 1.7 abα	15.9 ± 3.5 αα	11.0 ± 1.7 α

¹Means (±SE) followed by the same English letters in rows and Greek letters in columns are not significantly different ($P>0.05$).

Mating duration increased with increasing male density (KWT: $\chi_{2,27}=12.65$, 15.31 and 14.48 for male density of 1, 5 and 10 males, respectively, $P<0.001$; $P<0.0001$ for mean) (Table 2). Mating duration decreased from day 1 to day 3 at male density of 1 ($P=0.04$). No significant difference was detected in mating duration over days when male density was 5 or 10 ($P>0.05$) (Table 2).

TABLE 2: Mating duration (min) of *S. oryzae* at three male densities measured for 7.5 h periods over 3 consecutive days.

No. males	Day 1	Day 2	Day 3	Mean
1	44.3 $\pm$ 3.4 a β ¹	34.3 $\pm$ 4.5 ab β	31.2 $\pm$ 3.2 b β	36.6 $\pm$ 3.5 γ
5	54.7 $\pm$ 3.4 a $\alpha\beta$	57.3 $\pm$ 3.0 a β	47.4 $\pm$ 5.4 a β	53.1 $\pm$ 3.1 β
10	63.5 $\pm$ 1.5 a α	63.5 $\pm$ 2.3 a α	59.5 $\pm$ 2.7 a α	62.2 $\pm$ 1.7 α

¹Means ($\pm$ SE) followed by the same English letters in rows and Greek letters in columns are not significantly different ($P>0.05$).

DISCUSSION

In insects, males may regulate their mating strategies in response to changes in population density or sex ratio (Wang et al. 2008). It is expected that at higher male density, males will adjust their courtship strategy and attempt to mount and mate as soon as a female is detected; this may be advantageous to males since mounting quickly may allow them to guard their mates from rivals (Sato & Kohama 2007). However, results from the present study do not support this hypothesis, because the time *S. oryzae* males spent in courtship significantly increased from a male density of 1 to 5 and 10. The increased courtship duration over 3 days may result from females avoiding multiple mating due to the risk of damage and a reduction in fecundity and longevity when mated repeatedly (Campbell 2005; Flay et al. 2008). The avoidance of multiple mating also occurs in bean weevils *Acanthoscelides obtectus* (Say), whose males may damage the reproductive organs of their mates (Crudginton & Siva-Jothy 2000). An alternative explanation may be that courtship duration is a physiological response governed by the female condition, i.e. recently mated females being less receptive to courtship than virgins or sperm-depleted females (Campbell 2005).

The increased mating duration of *S. oryzae* with increasing male density supports the prediction that males should prolong their copulation duration in response to a male biased sex ratio or high population density, as reported in many other insects (Arnqvist 1988; Arnqvist & Danielsson 1999; Wang et al. 2008). In the present study, because only one female was exposed to the males of different density, males may increase their mating duration due to the low probability they will find another mate, and the high probability of their sperm being displaced (Wang et al. 2008). Thus, in an attempt to reduce the chance of sperm displacement by competitors, males may prolong copulation time (Wang et al. 2008).

According to Simmons (2001) and Wang et al. (2008), males prolonging copulation time over that required for insemination could be assumed as mate guarding. In this study, no evidence of mate guarding was observed for *S. oryzae* males after their genitalia were disconnected. Therefore, mate guarding, if any, should occur during copulation in this species. Mate guarding by prolonging copulation is found in many insects where sperm displacement risk and competition intensity are high (Andres & Rivera 2000; Elgar et al. 2003; Harari et al. 2003).

In conclusion, *S. oryzae* males increase copulation duration in response to higher rival density, and this behavior may increase reproductive success. The mechanism behind the relationship between copulation duration and male density may be in order to reduce mate competition and thus sperm competition.

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