Mating behaviour of the European leafminer *Scaptomyza flava* (Diptera: Drosophilidae)

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Abstract European leafminer, *Scaptomyza flava* (Fallén), is an important pest of brassicas, peas and gypsophila in New Zealand. This study examined the mating behaviour of this species for the first time under laboratory conditions. Males were the active sex in the entire mating behavioural sequence involving approaching, courting and mounting females, and copulation. Copulation lasted for about 20 min. There was no significant difference in pre-courtship, courtship and mating durations between virgin and mated males. However, mated males were significantly more likely to perform courtship and to achieve mating than virgin ones. It is suggested that males learn from mating experience, which helps them achieve higher mating success. Both virgin and mated males tended to disrupt mating couples but could not separate them.

Keywords *Scaptomyza flava*, courtship, mate competition, mating success.

INTRODUCTION

Mating behaviour comprises the activities and events that take place as the animals seek, identify, win over and appraise, and finally accept partners in reproduction (Lloyd 1979). Many animal species have developed elaborate courtship rituals (Lazareva et al. 2007), and mating sequences contain valuable information needed to understand insects' mating systems (Jiménez-Perez 2003). The understanding of mating behaviour of a pest species is mandatory prior to establishment of any behaviour-based control technique (Butt 1991). For example, information on the mating behaviour of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), has led to the implementation of the sterile insect technique in pest management programmes (Hendrichs et al. 2002).

Mating behaviour of the Drosophilidae is complex, involving sequential steps of visual, chemical and auditory signals (Petfield et al. 2005), that require interactions between the sexes (Bacigalupe et al. 2008). Generally, after orienting and approaching a potential mate the male engages in courtship display before attempting to mount and copulate, and such courtships involve species-specific movements of various body parts such as wings, legs, mouthparts and abdomen, and these movements occur sequentially (Speith 1974). Male courtship behaviour in *Drosophila melanogaster* Meigen consists of a well-defined series of behaviours (Lazareva et al. 2007), while in *D. pagasa* Wasserman no courtship behaviour is displayed by the male before copulation occurs (Pruett-Jones et al. 2002).

So far, nothing is known about the mating behaviour of *S. flava*, making it difficult to develop strategies for behaviour-based management programmes for this pest. For example, to implement pest control programmes using sex pheromones and the sterile insect technique an understanding is needed of male-female interactions leading to mating and males' ability to find their mates. The objectives of this study were to observe the mating behavioural sequences and investigate the pre-courtship, courtship and mating duration of virgin and mated males in *S. flava*.

MATERIALS AND METHODS

Insect colony

A breeding colony of *S. flava* was maintained and experiments conducted at $20 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH under 16:8 h light:dark conditions. Pupae were collected and weighed on an electronic balance (Mettler AE 100-S, Switzerland) before being placed individually in clear glass vials (1.5 cm in diameter, 5.0 cm in height) with a 0.5 cm mesh covered hole in lids until adult eclosion. Males of 11.96 to 12.04 mg and females of 16.94 to 17.04 mg were considered as medium weight and used for experiments. As adult *S. flava* feed on cell sap of brassica leaves, a 3-cm long Chinese cabbage leaf was provided as food for each of the flies in clear glass vials. Flies were also provided with 4 ml of 10% honey solution.

Mating behavioural sequence

To observe the general mating behaviour, a 2-day-old virgin female was maintained with a 2-day-old virgin male in an above mentioned clear glass vial. Twenty pairs (replicates) were observed. The mating behaviours were observed and recorded using a Panasonic SVHS Camcorder (MS-4) (Panasonic, Japan) connected to a Samsung digital video cassette recorder (DVD-V530, Korea) and images viewed on a Panasonic colour monitor (TC-21T1Z, Japan). Percentage of behavioural events at each transition was calculated as the number of individuals that performed an observed behaviour divided by

the number of individuals that performed a previous behaviour.

Differential mating behaviour of virgin and mated males

To observe male-male interactions and mating success of virgin and once-mated males, a 2-day-old virgin female was maintained with a 2-day-old virgin male and a 2-day-old once-mated male in an above mentioned glass vial. The virgin and mated males were marked randomly on the thorax by different trace colour powders (Magruder Color Co., Elizabeth, NJ, USA) for identification. According to previous work, 80% of *S. flava* males can mate when they are 24 hours old (Shakeel et al. 2009) and males can remate 1 day after the first mating (M. Shakeel, Massey University, unpubl. data). Thus, to obtain mated males, 1-day-old virgin males were allowed to mate with 2-day-old virgin females.

Pre-courtship (period between release of a male into a vial and orientation of the male to a female), courtship (period between initiation of orientation and mating) and mating (period from genital connection to disconnection) periods were recorded for both virgin and mated males. Twenty replicates (vials) were observed.

Statistic analysis

Data on pre-courtship, courtship and mating periods were subjected to ANOVA, while data on the difference in number of courtships, mating and mating disruption between virgin and mated males were analysed using Chi-square test ($\chi^2 > 3.81$).

RESULTS

Mating behavioural sequence

The general courtship and mating behaviours are shown in Figure 1. Sexual behaviour started 2-3 min after release into the vial when the male became excited, walked towards the female and started courting. Courtship display by the male included flapping his wings around the female and tapping her body with his fore tarsi. After courtship started, 60% of females and 25% of males moved away. Then all males and females

Figure 1 Flow chart of the mating behavioural sequences of *S. flava*. Probabilities of a particular transition between stages are given.

moved back to their mates when courtship was resumed. At this stage most females remained stationary and males walked around stationary females, and then 95% of courting males mounted females. Upon mounting, 16% of mounted females rejected males by kicking them. If mating failed to occur, the male again started courting the female. Ninety-five percent of mounted females finally mated. Mating lasted for about 20 min. After mating *S. flava* males vibrated their wings, and made zigzag movements for about 15 s. Then males did not show any interest in females.

Differential mating behaviour of virgin and mated males

In triad trials, both virgin and mated males displayed similar mating behaviour, and had similar pre-courtship (2.93 ± 0.34 and 2.19 ± 0.32 min for virgin and mated males, respectively), courtship (2.14 ± 0.27 and 2.55 ± 0.26 min for virgin and mated males, respectively) and mating periods (20.11 ± 0.65 and 18.61 ± 0.73 min for virgin and mated males, respectively) ($P > 0.05$). However, mated males performed significantly more courtship displays ($P < 0.05$) and were significantly more likely to achieve mating than virgin males ($P < 0.05$) (Figure 2). Both mated and virgin males tried to

disrupt the mating couples by moving towards stationary mating couples, chasing the moving mating couples, striking their heads with fore legs, and mounting them. However, there was no significant difference in mating disruption between mated and virgin males ($P > 0.05$) (Figure 2). Mating disruption by males could not separate the mating couples.

DISCUSSION

The male *S. flava* perform certain pre-mating behaviour, which is composed of a succession of behavioural sequences typical of the Drosophilidae that can break off and resume from the beginning. The male approaches the female and then performs courtship display, after which he mounts and mates with her. Similar mating behaviour is reported in male *D. melanogaster* (Cobb & Ferveur 1995). The present study has also shown that as the courtship proceeds, *S. flava* females become stationary. Cobb & Ferveur (1995) demonstrate that in *D. sechellia* Tsacas and Bachli, one of the main signs of imminent copulation is the reduction of female movement. Like *D. pseudoobscura* Frolova & Astaurov (Noor 1997), courtship display is critical to mating success in *S. flava*.

In many drosophilid species, females release sex pheromones to attract males and elicit

Figure 2 The percentage of mated and virgin *S. flava* males engaging in courtship display, achieving mating success and showing mating disruption behaviour. Columns with the same letters in each category are not significantly different ($P > 0.05$).

their courtship displays (Antony & Jallon 1982; Tompkins et al. 1983; Oguma et al. 1992; Wicker-Thomas 2007; Dickson 2008). Although the sex pheromone of *S. flava* has not been identified, male's approaching and courting females in this species may be stimulated by a female sex pheromone.

As reported in some *Drosophila* species (Hoikkala et al. 2000), *S. flava* males perform post-copulation courtship behaviour by vibrating wings and making zigzag movements around females after copulation. However, whether such post-copulation display in *S. flava* serves as post-insemination mate guarding reported in other insects (Allen et al. 1994; King & Fischer 2005) remains unknown.

No significant differences were found in pre-courtship, courtship and mating periods between virgin and once-mated males of *S. flava*, supporting the same type of observation in *D. melanogaster* (Markow et al. 1978). However, the present results show that compared to virgin males, mated males are significantly more likely to display courtship behaviour and achieve mating success. This suggests that *S. flava* males are able to learn from their previous mating experience. Tompkins et al. (1983) and Dukas (2005) also report that previous courting experience permits fruit fly males to refine their courtship behaviour in a way that could increase their mating success.

The findings reported here can be an important starting point for further investigation into this leafminer and future development of behaviour-based control strategies for the pest. For example, to develop pest control programmes using sex pheromones and the sterile insect technique an understanding is needed of male-female interactions leading to mating and males' ability to find their mates.

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