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Effects of male sterility on female remating in the Mediterranean fruitfly, *Ceratitis capitata*

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Mating-induced reductions in female receptivity are common in insects. These responses are of interest because of their utility in insect pest control. In addition, the control of receptivity is likely to be the subject of sexual conflict over remating frequency. We investigated the specific effect of male sterility on female receptivity in an important pest species, the Mediterranean fruitfly (medfly), in which sterile males are often used for population suppression. Sterile males performed less courtship, obtained significantly fewer first and second matings than fertile males, and reduced female receptivity significantly less effectively than did fertile males. We modelled the likelihood of fertile matings and show that the low mating success of sterile males represents a significant problem for medfly sterile insect technique (SIT) programmes.

Keywords: Mediterranean fruitfly; medfly; *Ceratitis capitata*; remating; sexual conflict; sterile insect technique

1. INTRODUCTION

Mating decreases female receptivity in many insects (reviewed by Chapman *et al.* 1998). Male control of female receptivity is expected to be a target of selection arising from sexual conflict over remating frequency. The post-mating receptivity of females of pest species is of interest because of its potential utility in insect control programmes (Partridge 1996) such as the sterile insect technique (SIT; Knipling 1955). In SIT, mass-reared males are sterilized and released into natural populations to mate with wild females (Knipling 1955). Success depends, critically, on the mating success of sterile males, a crucial feature of which is an unimpaired ability to induce female refractoriness.

We investigate experimentally the effect of male sterility on female mating and remating in the Mediterranean fruitfly, *Ceratitis capitata* ('medfly'), an agricultural pest of major economic importance. Female medflies mate multiply in the wild (Bonizzoni *et al.* 2002) and receptivity can subsequently be reduced for up to 10 days. However, despite the use of sterile males for control, the effect of male sterility on female receptivity has not previously been tested. Previous studies have reported increased remating by females after matings to sterile males. However, it is not possible to determine the contribution of male sterility to this effect because in a comparison between wild or wild-derived males and sterile mass-reared males (Mossinson & Yuval 2003) it is impossible to separate the effects of male sterility from genetic background. Three other studies that have claimed reduced refractoriness following sterile matings have no statistical support (Cavalloro & Delrio 1970; Katiyar & Ramirez 1970; Bloem *et al.* 1993). In addition, the effect of initial matings with sterile or non-sterile males on subsequent rematings by either sterile or non-sterile males has not previously been tested.

We determined the relative mating and remating success of fertile and sterile males from the same stock, and measured the effect of irradiation *per se* on a male's ability to induce refractoriness. We quantified the courtship delivered by fertile and sterile males. We used our observed mating and remating frequencies to model the likelihood of a female obtaining a fertile mating in a population containing fertile and sterile males in ratios characteristic of SIT programmes.

2. MATERIAL AND METHODS

(a) *Female mating and remating with fertile and sterile males*

Medflies were from a mass-reared strain maintained as described in Chapman *et al.* (1998). One to two days before emergence, half of a cohort of pupae were X-irradiated under partial anoxia at 10 krad, a dose used in SIT (Jang *et al.* 1998). All pupae were placed in plastic cages (22 cm × 15 cm × 8 cm) containing water and adult food (which was supplied to adults throughout). Virgins were collected within 24 h after eclosion, using light CO_2 anaesthesia followed by separation of the sexes on ice. One hundred and fifty females were placed individually in 100 ml plastic pots, each with two virgin fertile males, and 200 females each with two virgin sterile males. Each pot was supplied with water and food. The number of mating females was counted every hour for the first 8 h after lights on (i.e. the beginning of the 12 h light period). Nine hours after lights on, half of each once-mated female group was placed together with two sterile or fertile virgin males each. Females were checked for remating every hour for the first 8 h after lights on, over the subsequent 7 days. Every hour, we also recorded the number of males that were pheromone calling (by eversion of the rectal epithelium; Shelly (2000)). A replicate experiment was performed with 300 females using the above protocol, except that the females were given the opportunity to remate for 1 day only.

(b) *Impact of sterile-male mating success on the likelihood of fertile matings*

A simple model was constructed to estimate the likelihood of a female obtaining a fertile mating when both sterile and fertile males are present in the population, as is the case in SIT programmes. Females were given two opportunities to mate, and encounter rates were determined by the ratio of sterile : wild males (the 'overflooding ratio'). We used the mating and remating success of fertile and sterile males from this study as parameters in the model. We then summed the probabilities of all of the outcomes involving matings with at least one fertile male.

(c) *Statistical analysis*

The proportion of males pheromone calling was arcsine transformed, checked for normality using Shapiro–Wilks tests and analysed using a general linear model in JMP v. 5 (SAS Institute 1989–2002). Patterns of mating and remating were analysed manually using *G*-tests, adjusted using William's correction.

3. RESULTS

In their initial matings, virgin females were significantly more likely to mate with a fertile male than with a sterile male (table 1; $G_{\mathrm{adj},1} = 63.77$, $p < 0.001$). The frequency of remating differed significantly between the four treatments (figure 1; table 1; $G_{\mathrm{adj},3} = 70.69$, $p < 0.001$). Females that mated first with a sterile male were significantly more likely to remate than females that initially

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Table 1. Total number of females mating and remating with fertile and sterile males.

initial number of females	first male	number of females mating	second male	number of females remating
150	fertile	48	fertile	23 (47.9%)
	fertile	47	sterile	0 (0%)
	total fertile	95 (63.3%)		
200	sterile	22	fertile	18 (81.8%)
	sterile	21	sterile	2 (9.5%)
	total sterile	43 (21.5%)		

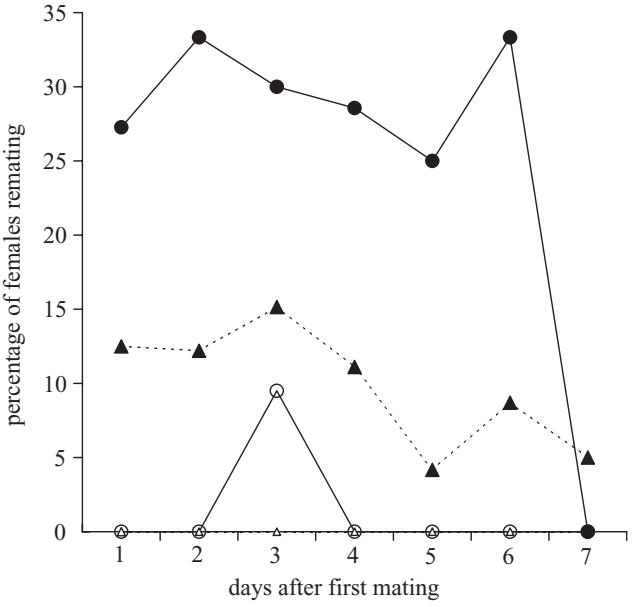

Figure 1. The percentage of females remating on each of the 7 days after their first mating. Further rematings (third, fourth etc.) were excluded. Fertile–fertile: filled triangles; fertile–sterile: open triangles; sterile–fertile: filled circles; sterile–sterile: open circles.

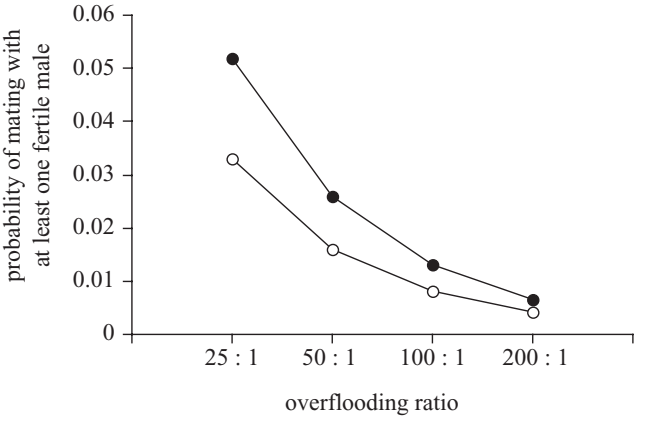

Figure 2. Model of the effect of sterile-male mating and remating ability on the likelihood of fertile matings. The probability of one or more matings with fertile males is plotted against the overflooding ratio (ratio of sterile to fertile males in the population). Sterile- and fertile-male mating successes were either set to the values observed in the experiments (filled circles), or were equalized by averaging the observed values (open circles).

mated with a fertile male ($G_{adj,1} = 6.66$, $p < 0.01$). Once-mated females were significantly less likely to remate when the second male was sterile ($G_{adj,1} = 58.20$, $p < 0.001$). However, the magnitude of the response to second males was influenced by whether the first male was sterile or fertile ($G_{int,adj,1} = 4.89$, $p < 0.05$). Pheromone-calling activity for all males dropped significantly over the course of each day ($F = 806.09$, $p < 0.0001$), but was significantly lower at all times in sterile than in fertile males ($F = 307.59$, $p < 0.0001$). The replicate experiment produced similar results. Significantly fewer virgin females mated with sterile males (20%) than with fertile males (53%, $G_{adj,1} = 36.68$, $p < 0.0001$). Significantly more females initially mated to sterile males remated (30%) than females initially mated to fertile males (10%; $G_{adj,1} = 7.54$, $p < 0.01$). In females first mated to fertile males, significantly more remated with fertile (20%) than with sterile males (0%, $G_{adj,1} = 11.27$, $p < 0.001$).

The model showed that the probability of fertile matings decreased as the overflooding ratio (number of sterile males) increased (figure 2). When the fertile male advantage was removed (by averaging the observed mating and remating success values), the probability of fertile matings decreased across all overflooding ratios (figure 2). The predicted remating frequency for females in the model was 2.3%.

4. DISCUSSION

The most important findings were that virgin and once-mated females were less likely to mate with sterile than with fertile males, and that females were significantly more likely to remate following initial matings to sterile males. Unlike previous studies, which report reduced mating success in sterile mass-reared males compared with wild males (reviewed in Hendrichs *et al.* 2002), we show a direct effect of sterilization *per se*. Sterile males had reduced pheromone-calling activity compared with fertile males, which may explain why sterile males were less successful in attracting mates. This result is consistent with the observation that sterilization negatively affected male courtship (Papadopoulos *et al.* 1998; Lux *et al.* 2002; but see Liimatainen *et al.* 1997) and pheromone composition (Heath *et al.* 1994).

Sterile males reduced receptivity in their mates less effectively than fertile males. More than 90% of females mated to either sterile or fertile males stored sperm (data not shown). However, sterile males transfer fewer sperm than fertile males (Seo *et al.* 1990; Taylor *et al.* 2001), which could explain the impaired post-mating success of sterile males, if stored sperm number is related to female receptivity (Mossinson & Yuval 2003). Alternatively,

receptivity-inhibiting seminal fluid molecules could be involved. Seminal fluid-mediated functions appear unimpaired by sterilization (Jang *et al.* 1998). However, in *D. melanogaster*, seminal fluid molecules that affect receptivity adhere to sperm (Saudan *et al.* 2002). Thus, sterile males may have impaired ability to reduce refractoriness because they have fewer sperm to act as 'carriers'. Alternatively, irradiated sperm may be less able to act as carriers. These possibilities remain to be tested.

Our model shows that the low mating success of sterile males increases the probability of fertile matings and is therefore a significant problem for SIT programmes. In fact, our estimates of this problem are extremely conservative. First, females in the model had only two opportunities to mate, and remating rates were low (the 2.3% predicted by the model is below the lowest estimate for wild females; Bonizzoni *et al.* (2002)). Higher remating rates exacerbate the problem of low sterile-male mating success (model results not shown). Second, sterile males were competing against fertile males from the same mass-reared strain. Mass-reared strains score lower than wild males in a range of fitness-related traits (Hendrichs *et al.* 2002). Third, the radiation dose used in SIT programmes varies between 10 and 15 krad (e.g. Lux *et al.* 2002), and higher doses than used in our study could reduce the mating competitiveness of the sterile males even further. Therefore, the poor mating success of sterile males in real SIT programmes represents a more significant problem than shown in our model.

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