Multiple mating in the traumatically inseminating Warehouse pirate bug, *Xylocoris flavipes*: effects on fecundity and longevity

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1. INTRODUCTION

Generally, mating carries costs for both males and females [1]. However, theory predicts that males should have higher optimum mating rates than females, as the cost of each mating is lower than for the females they fertilizes [2,3]. Consequently, males should seek higher mating frequencies, and this can lead to sexual conflict [4,5]. Some invertebrate mating systems display a sexual conflict known as traumatic insemination (hereafter TI; [6]), where males use external genitalia to pierce and penetrate the female abdominal wall and ejaculate into the abdominal cavity. Here, males and females are unlikely to share an optimal reproductive outcome, but instead show antagonistic strategies [7].

In a TI mating system, the reproductive tract of the female is fully functional, but during mating, the male uses a specialized hypodermic penis (a coupling of the aedeagus and left paramere as in the Cimicidae; figure 1a) to penetrate the female’s abdomen into which the sperm is deposited. This behaviour is observed across many invertebrate orders, but is widely practiced among the Heteroptera (true bugs) and the infraorder Cimicomorpha, whereas it has evolved at least three times [8] and is most well-known in bed bugs (*Cimicidae*) [9].

The significant work on the costs of TI has considered only those species of blood-sucking (parasitic) systems of the Cimicomorpha [10–12], whereas most species that display TI are generalist predators. Costs associated with a TI mating system for such species are more likely to be affected by factors such as adult densities and prey availability. Here, we study another Cimicomorphan, the Warehouse pirate bug *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) that performs the extragenital form of TI. Warehouse pirate bug males use a scythe-like penis to penetrate the female on the surface of her abdomen ([13]; figure 1a and electronic supplementary material, appendix). Female *X. flavipes* are known to have a spermalege [6], which is thought to be a counter-adaptation to the risks of abdominal TI mating [11]. The function of the spermalege is to reduce the likelihood of infection being introduced to females via the piercing of their body wall with a potentially unhygienic male penis. Scanning electron microscopy reveals fluid seeping from the surface of the abdomen plate where we observed mating scars (figure 1b). Unlike in bed bugs, there is no single fixed position of entry and penetration can occur along much of the length of the join between the ‘TI-II’ and T-III segment on the upper right dorsal surface of the abdomen [13].

This study determined the costs incurred during single versus multiple mating as a representation of varying mating frequencies between the sexes of the Warehouse pirate bug. We measured the effects of TI mating on longevity, fecundity, fecundity schedules, offspring viability and prey consumption, an assessment of post-copulatory resource requirements, on mated females in comparison with virgin females and males.

2. MATERIAL AND METHODS

Specimens of *X. flavipes* (provided by Biologische Beratung Ltd.; [14]) were kept with their prey *Ephestia kuehniella* in a 1.5 cm deep bran mix in 25 × 25 × 16 cm plastic boxes and incubated at 28 ± 1°C/60 per cent relative humidity (RH). Individuals of known age and virginity were obtained by mass-rearing from eggs. Juvenile bugs were kept together, and fed *E. kuehniella* eggs until third or fourth instar when individuals were separated to prevent cannibalism and ensure virginity [15]. Once mature, adults were sexed, and virgin females were kept with ad libitum food for 48 h before mating treatments. Females were randomly assigned to one of three treatments: unmated (U, control), mated once (M1) or mated three times (M3). These mating rates were designed to represent natural low- and high-daily mating rates in *X. flavipes*, determined from 6 h video recordings of eight pairs of adult virgins where most mating attempts were prevented by the female. Control females were placed with a dead male. M1 and M3 females were placed with a
3. RESULTS

(a) Fecundity

M3 females started laying eggs earlier (average 3.0 days after mating) and peaked at a higher total number of eggs laid (3.7 on day 7), whereas M1 females took on average 5.14 days to start laying, with a peak output of 2.62 eggs on day 9. We found a significant interaction between mating frequency and time, because female age influenced the number of eggs laid per day (repeated-measured generalized linear model (GLM); $F_{2,18}(\text{mating} \times \text{time}) = 4.112, p < 0.001$). This interaction reflects that female age influenced the number of eggs laid per day, such that mated females in M1 and M3 differed in their lifetime fecundity schedule. In addition, lifetime fecundity was not significantly different between M1 and M3 females (mean ± 2 s.e. 16.47 ± 1.87 versus 18.05 ± 2.34, Welch two-sample $t$-test; $t_{38.15} = -1.0492, p > 0.3$; figure 2).

(b) Post-copulatory costs

We found no significant difference in the number of viable or unviable eggs laid by M1 and M3 females (unviable eggs laid per female; Welch two-sample $t$-test, $t_{35.48} = -0.6557, p = 0.5162$). All viable eggs hatched.

(c) Longevity

Female longevity was found to vary significantly between groups (ANOVA; $F_{2,60} = 4.579, p < 0.01$), where M3 females died earlier than M1 or control females (mean ± 2 s.e. M3: 11.90 ± 1.36; M1: 15.57 ± 0.89 and U: 18.71 ± 1.12 days).

(d) Predation rates

We found significant differences in the number of prey items consumed following mating across female mating treatments and males versus females (GLM log prey with quasi-Poisson error; $F_{3,80} = 100.48, p < 0.001$). All female groups consumed more prey than males. In comparison with controls and M3 females, which did not differ, M1 females had a reduced predation rate (mean ± 2 s.e. prey items consumed per treatment groups: U = 22.61 ± 1.28, M1 = 16.61 ± 1.56, M3 = 21.09 ± 1.13, males = 8.47 ± 1.01).

4. DISCUSSION

Increased TI mating frequency led to a reduced adult female lifespan, earlier onset and higher daily rates of egg laying, but there was no overall difference in lifetime fecundity in female Warehouse pirate bugs.

In the bed bug, Cimex lectularius, females with high TI mating frequency died earlier, but daily fecundity schedules between females of different mating frequencies were identical, resulting in reduced lifetime fecundity [12]. Increased mating rates also reduced female lifespan in C. lectularius, but increased daily fecundity, such that mating frequency had no effect on lifetime fecundity [10]. Here, we found that an increase in TI mating frequency led to an earlier death, but multiply mated females began to produce offspring approximately 40 per cent earlier following mating and daily fecundity rates peaked approximately 40 per cent higher than females mated once.

Differences in the methodology between the studies mentioned above are important to consider. In our experiment, females were mated 48 h after eclosion and then separated from males. In the bed bug studies,
females and males were paired throughout the experiments. We chose two mating treatments based on our observations of natural frequencies, but it is possible that one versus three matings do not adequately represent the range, and it is also possible that the length of time our experiments ran was insufficient to uncover long-term effects on lifetime fecundity. However, as we found that increased TI mating increased daily fecundity earlier in the adult lifespan, which stands as a contrast to studies on *C. lectularius* [10,12], our results can indeed be interpreted in the light of selection on lifetime reproductive strategies.

There are a number of potential mechanisms to explain why multiply mated females oviposit earlier and at a higher rate in our study. Generation and maintenance of high-daily oviposition may require multiple mating. This would explain the early onset and post-peak sharp decline in daily oviposition rates in multiply mated females. It is known that males can manipulate female oogenesis and oviposition behaviour to their advantage [4,5,16]. Alternatively, the costs of multiple mating to adult longevity could be detected by females and early oviposition could be adaptive female behaviour. These hypotheses are not mutually exclusive. It has been suggested that females from TI mating systems are significantly counter-adapted to the costs of reproduction, which are mediated by the female spermalege [10,11]. We suggest that our results support this hypothesis and we now witness the proximate responses to TI mating frequency.

Prey consumption was recorded to try to understand the role of resource needs in recovery from TI. Furthermore, as this species is a biocontrol agent, any factor that could reduce kill rates of target species following release should be minimized. Females mated once showed consistently lower prey consumption in the first 24 h after mating. The difference in prey consumption as a result of variation in TI mating frequency in female *X. flavipes* suggests that the onset of oviposition and adult longevity is not independent; reduced prey consumption could influence subsequent survival or fecundity. We consider that the reduction in prey consumption during these first 24 h would be compensated for later and is therefore unlikely to directly affect adult longevity. We propose two mechanisms that could explain our results that require further research. Reduced feeding by low mating frequency females is caused when consumption is reduced by mating in general, but exposure to multiple matings requires more resources to recover and/or the increased seminal fluid exposure increases both foraging and egg-laying behaviours [17]. A second mechanism could be our methods have created this result. By continuing our mating treatments throughout the experiment, the increased exposure to seminal fluids may have increased daily fecundity and prey consumption rates for both M1 and M3 females.

In conclusion, we have shown that increased TI can reduce adult female lifespan. By our methods, where females were mated over 24 h and monitored until death without any further mating, this did not result in over-compensatory increases in lifetime fecundity. Multiply mated females lived fast and died young, but we cannot determine whether the cause of this mating responsive life history is male manipulation of female behaviour or perfect compensation via the female counteradaptation to antagonistic sperm competition avoidance behaviour of male pirate bugs.

Figure 2. Mean ± 1 s.e. of total eggs laid per female per day for mated once and multiply mated females. Each female was monitored until death (*n* = 21 per group). Day 1 = day 1 following mating/day 3 following eclosion. Solid grey line with diamonds, M1; Solid black line with squares, M3.

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