Influence of multiple mating on female reproductive fitness in the rice weevil, *Sitophilus oryzae*

C.D. Flay, X.Z. He and Q. Wang

Institute of Natural Resources, Massey University, Palmerston North, Private Bag 11222, New Zealand

Corresponding author: q.wang@massey.ac.nz

Abstract The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is an important pest of stored grains throughout the world. This paper examined how multiple mating affected female reproductive fitness in the laboratory. Over 60 days, females that permanently paired with males, mated twice with different males or four times with the same males did not suffer from significant decline of offspring survival, while females mated once, twice with the same males and four times with different males had significant declines. It is suggested that mating once or twice with the same males is not enough for females to maximise their reproductive fitness. The significant decline of offspring survival in females that mated with four different males may be caused by reproductive organ damage or other factors that reduce their fertility due to excessive polyandry.

Keywords *Sitophilus oryzae*, rice weevil, multiple mating, fertility.

INTRODUCTION

Multiple mating of females has been found to enhance fecundity and fertility in many insect species (Arnqvist & Nilsson 2000). Multiple mating can occur repeatedly with one male (repeated mating) or with different males (polyandry). The benefits of repeated mating can be derived from sperm or chemical substances produced in male accessory glands during copulation (Thornhill & Alcock 1983; Schwartz & Peterson 2006). Male accessory glands can provide nutrition and protective substances, such as antibacterial agents, protease inhibitors and fungicides (Jennions & Petrie 2000; Chapman & Davies 2004). Repeated mating can also reduce the chance of infertility by restocking depleted sperm supplies (Campbell 2005), replacing degraded sperm (Siva-Jothy 2000; Reinhardt

& Siva-Jothy 2005; Wang & Davis 2006) or avoiding unsuccessful sperm transfer (Wedell et al. 2002; Pai et al. 2005; Hasson & Stone 2009). Genetic benefits of polyandry include offspring with increased genetic diversity (Baer & Schmid-Hempel 1999; Jennions & Petrie 2000; Calleri et al. 2006; Xu & Wang 2009), the opportunity to mate with higher quality males (Thornhill & Alcock 1983; Jennions & Petrie 2000), avoidance of inbreeding depression (Cornell & Tregenza 2007) and offspring parasite resistance (Baer & Schmid-Hempel 2001). These benefits, either separately or combined, increase female fitness, driving them to mate multiple times.

Excessive multiple mating can have a number of negative consequences. These include the time lost for feeding and oviposition (Keller &

Reeve 1995), energy lost while mating (Watson et al. 1998) and increasing risk of sexually transmitted diseases (Parker 1970; Hurst et al. 1995; Watson et al. 1998). Other negative consequences include risk of predation due to reduced mobility or increased visibility (Arnqvist 1989; Rowe 1994), and damage from deleterious chemicals produced in male sperm or accessory glands (Thornhill & Alcock 1983; Arnqvist & Nilsson 2000; Hardling & Kaitala 2005; Wigby & Chapman 2005). Furthermore, multiple mating may also increase the risk of external (Michiels & Newman 1998) and internal injury of female reproductive organs from male activity (Crudginton & Siva-Jothy 2000; Blanckenhorn et al. 2002). Female resistance to mating may also be costly to females (Rowe 1994; Watson et al. 1998). If males constantly harass females, resisting mating can be more costly than mating itself (Rowe 1994).

Many *Sitophilus* species, including *S. oryzae* (Linnaeus), *S. granarius* (L.) and *S. zeamais* (Motschulsky), are closely related and have similar biology and mating patterns (Longstaff 1981; Rees 2004). Thus, studying aspects influencing female fitness in *S. oryzae* may illuminate similar features in other *Sitophilus* species. Here, the effect of female partner diversity and mating frequency on reproductive fitness of *S. oryzae* was investigated. It was hypothesized that genetic and/or material benefits gained through partner diversity or mating frequency would increase fertility and fecundity of *S. oryzae* females.

MATERIALS AND METHODS

All experiments were carried out in Petri dishes (60 mm diameter $\times$ 10 mm height), each with about 100 grains of wheat, in a controlled environment room under red lights at $27 \pm 3^\circ\text{C}$ and $75 \pm 5\%$ relative humidity. Four-day-old virgin insects of average weight (1.61–2.10 mg and 1.57–1.96 mg, for females and males, respectively) were used for experiments.

Six treatments were set up: a female was allowed to (1) mate once (single mating), (2) mate twice with the same male, (3) mate four

times with the same male, (4) mate twice with different males, (5) mate four times with four different males and (6) be permanently paired with the same male. For treatments (1)–(5), 4-day-old virgin individuals were paired until the end of mating, after which time the male was removed. Mating was performed once every 24 h in treatments (2) to (5). After mating, males were discarded. Thirty replicates were performed for each treatment.

Courtship period was recorded as the time between release into the Petri dish and mounting a female by a male. Mating period was recorded as the period between the start and end of genitalia connection of sexes. After mating the female was held for 60 days in a ventilated Eppendorf tube with 50 g (about 100 grains) of wheat. Wheat grains were replaced every 10 days with fresh wheat acclimatised at 27°C and 75% RH. Twenty grains were sampled from each tube and stained with acid fuchsin to highlight egg plugs. Egg plug number was assumed equal to egg number (Frankenfeld 1948). Infested grains were maintained in ventilated Eppendorf tubes until adult emergence. Offspring survival rate was measured as the number of emerged adults divided by the number of eggs laid. Dead females were dissected soon after the experiment to determine damage to the female reproductive tract.

A goodness-of-fit test was carried out before data analysis. Data of total egg number were normally distributed and thus analysed using ANOVA followed by Tukey's procedure for multiple comparisons. Data on mean offspring survival rate were not normally distributed even after transformation, and thereby analysed using the non-parametric Kruskal-Wallis test (KWT) followed by Duncan's procedure for multiple comparisons. Linear regression was used to determine the relationship between offspring survival rate and time after the first mating as well as between courtship/mating duration and mating frequency. ANCOVA was used to analyse the slopes of regression. All data were analysed using the SAS statistical program (SAS 2006). All data reported here are means ($\pm$ SE).

RESULTS

Courtship duration significantly increased with the number of matings for females mated four times with the same male ($P < 0.05$) but did not significantly increase with the number of matings when mated to four different males ($P > 0.05$) (Figure 1a).

If a female repeatedly mated with the same male, mating duration significantly decreased with the number of matings ($P < 0.01$). However, mating frequency had no significant effect on mating duration when a female mated with different males ($P > 0.05$) (Figure 1b).

When all six treatments were compared, females that were permanently paired with the same males laid significantly more eggs than those mated to four different males ($P < 0.05$) (Figure 2a).

Offspring survival rate was significantly higher for females that mated with two different males than those that mated only once or four times with different males ($P < 0.01$) (Figure 2b).

Offspring survival rate significantly decreased over time when a female mated once, twice with the same male or four times with different males ($P < 0.01$) (Figure 3). Some females that mated four times with four different males showed damage to the bursa copulatrix and eighth sternite. However, the offspring survival rate did not significantly decrease when a female mated twice with different males, four times with the same male or was permanently paired with a male ($P > 0.05$) (Figure 3).

ANCOVA indicated that females mated to two different males had a significantly slower

Figure 1 (a) Courtship and (b) mating duration when *S. oryzae* females were mated up to four times with the same male or with different males. Values are the mean ($\pm$ SE).

Figure 2 (a) Number of eggs laid and (b) offspring survival rate by *S. oryzae* females over 60 days after mating once, twice or four times with the same or different males. In an additional treatment a male and a female were permanently paired. Values are the mean ($\pm$ SE). Columns with the same letters are not significantly different ($P > 0.05$).

decrease in offspring survival than those mated twice to the same male ($P < 0.05$). However, when females were mated four times to different males, the offspring survival rate decreased significantly faster than those mated four times to the same male ($P < 0.05$) (Figure 3).

DISCUSSION

Multiple mating appears to be a widespread mating strategy (Bateman 1948; Thornhill & Alcock 1983; Jennions & Petrie 2000; Ronkainen et al. 2010). However, only some studies differentiate between multiple mating with one male (repeated mating) or with different males (polyandry) (Tregenza & Wedell 1998; Arnqvist & Nilsson 2000; Ronkainen et al. 2010). Some research has shown that mating number is positively correlated with fecundity in females that repeatedly mate with the same males, suggesting that females acquire material benefits from males (Tregenza & Wedell 1998; Ronkainen

et al. 2010). However, the present study shows that multiple mating had no effect on fecundity in general (Figure 2a). It is thus suggested that in *S. oryzae* females do not receive more nutrition from males through multiple mating with a shorter interval (24 h) between matings. Because *S. oryzae* live for longer than 60 days and mate numerous times, prolonging the mating interval and increasing the mating number in future experiments may improve the understanding of the effect of multiple matings on egg production and offspring survival of *S. oryzae*.

The present results indicate that multiple mating significantly increased offspring survival rate as compared to single mating (Figure 2a), and the fertility rate of once-mated females declined significantly faster over time than that of females mated four times to the same males (Figure 3). This is consistent with a previous study that a single mating cannot fertilise all of a female's eggs throughout her life in *S. oryzae* (Campbell 2005).

Figure 3 Offspring survival of *S. oryzae* females over 60 days after mating (a) once, (b) permanent pairing, (c) twice with the same male, (d) twice with different males, (e) four times with the same male and (f) four times with different males. All data were pooled for regression but means ($\pm$ SE) are presented only.

These findings suggest that *S. oryzae* females can mate repeatedly to gain sufficient sperm to maximise their reproductive fitness.

Corresponding with the benefits gained by sperm loading is the potential genetic benefits associated with polyandry. There is an increasing amount of evidence supporting the theory that

polyandry is predominantly for genetic benefits (Zeh et al. 1998; Jennions & Petrie 2000; Fedorka & Mousseau 2002; Xu & Wang 2009). Possible genetic benefits to be gained through polyandry include female fertility or offspring hatching success (Tregenza & Wedell 1998; Jennions & Petrie 2000; Pai et al. 2005). The present results

show that females mated twice with the same male experienced a significant decrease of offspring survival over time, while the offspring survival of females that mated twice with different males did not significantly reduce over time (Figure 3). Conversely, the offspring survival of females that mated four times with the same male did not significantly decrease over time while that of females that mated four times with a different male significantly decreased over time (Figure 3). It is thus suggested that females obtain some genetic benefits in terms of offspring viability when mating twice with different males compared to mating twice with the same male.

However, like the water strider, *Aquarius paludum* (Fabricius) (Ronkainen et al. 2010), the costs of mating four times with different males outweighed the slight genetic benefits to offspring gained in *S. oryzae*. Exceeding the optimal mating rate may lead to internal damage to the female reproductive system from male activity (Crudginton & Siva-Jothy 2000; Blanckenhorn et al. 2002). The fastest decrease in offspring survival rate over time in females that mated four times, each with a different male, may be caused by damage to the bursa copulatrix and eighth sternite of the female reproductive tract during mating. This is supported by behavioural data showing that mating duration does not significantly decrease when females mate multiple times with different males compared to that when females mate multiple times with the same males (Figure 1b). This can exacerbate the damage caused by mating with different males that force females to mate for longer than her optimum mating duration (Arnqvist & Nilsson 2000). Furthermore, spines are found on the male aedeagus (C.D. Flay, unpublished data), which may damage the female reproductive system during extended mating.

ACKNOWLEDGEMENTS

We would like to acknowledge Kees (C.W.) van Epenhuijsen from The New Zealand Institute for Plant & Food Research Limited, Palmerston North, for providing the insect stock.

REFERENCES

- Arnqvist G 1989. Multiple mating in a water strider – mutual benefits or intersexual conflict. *Animal Behaviour* 38: 749-756.
- Arnqvist G, Nilsson T 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour* 60: 145-164.
- Baer B, Schmid-Hempel P 1999. Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. *Nature* 397: 151-154.
- Bateman AJ 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2: 349-368.
- Blanckenhorn WU, Hosken DJ, Martin OY, Reim C, Teuschl Y, Ward PI 2002. The costs of copulating in the dung fly *Sepsis cynipsea*. *Behavioural Ecology* 13: 353-358.
- Calleri DV, Reid EM, Rosengaus RB, Vargo EL, Traniello JFA 2006. Inbreeding and disease resistance in a social insect: effects of heterozygosity on immunocompetence in the termite *Zootermopsis angusticollis*. *Proceedings of the Royal Society B-Biological Sciences* 273: 2633-2640.
- Campbell JF 2005. Fitness consequences of multiple mating on female *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Environmental Entomology* 34: 833-843.
- Chapman T, Davies SJ 2004. Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. *Peptides* 25: 1477-1490.
- Cornell SJ, Tregenza T 2007. A new theory for the evolution of polyandry as a means of inbreeding avoidance. *Proceedings of the Royal Society B-Biological Sciences* 274: 2873-2879.
- Crudginton HS, Siva-Jothy MT 2000. Genital damage, kicking and early death – The battle of the sexes takes a sinister turn in the bean weevil. *Nature* 407: 855-856.
- Fedorka K, Mousseau T 2002. Material and genetic benefits of female multiple mating and polyandry. *Animal Behaviour* 64: 361-367.
- Frankenfeld JC 1948. Staining methods for detecting weevil infestation in grain. *USDA Bureau of Entomology and Plant Quarantine* 256: 1-4.

- Hardling R, Kaitala A 2005. The evolution of repeated mating under sexual conflict. *Journal of Evolutionary Biology* 18: 106-115.
- Hasson O, Stone L 2009. Male infertility, female fertility and extrapair copulations. *Biological Reviews* 84: 225-244.
- Hurst GDD, Sharpe RG, Broomfield AH, Walker LE, Majerus TMO, Zakharov IA, Majerus MEN 1995. Sexually-transmitted disease in a promiscuous insect, *Adalia-bipunctata*. *Ecological Entomology* 20: 230-236.
- Jennions MD, Petrie M 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews* 75: 21-64.
- Keller L, Reeve HK 1995. Why do females mate with multiple males? The sexually selected sperm hypothesis. *Advances in the Study of Behavior* 24: 291-315.
- Longstaff BC 1981. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): a critical review. *Protection Ecology* 3: 83-130.
- Michiels N, Newman L 1998. Sex and violence in hermaphrodites. *Nature* 391: 647.
- Pai A, Bennett L, Yan GY 2005. Female multiple mating for fertility assurance in red flour beetles (*Tribolium castaneum*). *Canadian Journal of Zoology* 83: 913-919.
- Parker GA 1970. Sperm competition and its evolutionary consequences in insects. *Biological Reviews of the Cambridge Philosophical Society* 45: 525-530.
- Rees D 2004. *Insects of stored products*. CSIRO Publishing, Collingwood, Australia. 192 p.
- Reinhardt K, Siva-Jothy M 2005. An advantage for young sperm in the house cricket *Acheta domesticus*. *The American Naturalist* 165: 718-723.
- Ronkainen K, Kaitala A, Kivela SM 2010. Polyandry, multiple mating, and female fitness in a water strider *Aquarius paludum*. *Behavioral Ecology and Sociobiology* 64: 657-664.
- Rowe L 1994. The costs of mating and mate choice in water striders. *Animal Behaviour* 48: 1049-1056.
- Schwartz SK, Peterson MA 2006. Strong material benefits and no longevity costs of multiple mating in an extremely polyandrous leaf beetle, *Chrysochus cobaltinus* (Coleoptera: Chrysomelidae). *Behavioral Ecology* 17: 1004-1010.
- Siva-Jothy MT 2000. The young sperm gambit. *Ecology Letters* 3: 172-174.
- Thornhill R, Alcock J 1983. *The evolution of insect mating systems*. Harvard University Press, Cambridge, MA, USA. 547 p.
- Tregenza T, Wedell N 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution* 52: 1726-1730.
- Wang Q, Davis LK 2006. Females remate for sperm replenishment in a seed bug: Evidence from offspring viability. *Journal of Insect Behavior* 19: 337-346.
- Watson P, Stallmann R, Arnqvist G 1998. Sexual conflict and the energetic costs of mating and mate choice in water striders. *The American Naturalist* 151: 46-58.
- Wedell N, Gage M, Parker G 2002. Sperm competition, male prudence and sperm-limited females. *Trends in Ecology & Evolution* 17: 313-320.
- Wigby S, Chapman T 2005. Sex peptide causes mating costs in female *Drosophila melanogaster*. *Current Biology* 15: 316-321.
- Xu J, Wang Q 2009. A polyandrous female moth discriminates against previous mates to gain genetic diversity. *Animal Behaviour* 78: 1309-1315.
- Zeh J, Newcomer S, Zeh D 1998. Polyandrous females discriminate against previous mates. *Proceedings of the National Academy of Sciences* 95: 13732-13736.