MULTIPLE MATING AND MATE CHOICE IN *SITOPHILUS ORYZAE* (L.) (COLEOPTERA: CURCULIONIDAE)

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Abstract

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is a major pest of stored grains throughout the world. A recent study found a reduction in fecundity and fertility in females that are exposed to multiple males. However, the mechanism behind this is unknown. In the first experiment we examined female *S. oryzae* fitness decline and behaviour in response to male density (i.e. 1, 5 and 10 males) maintained with a single female. Results show that female fecundity and longevity significantly decreased with increased male density ($P < 0.01$). However there was no significant effect of male density on fertility. Mating time significantly increased with male density ($P < 0.01$) but feeding and foraging time decreased from male density 1 to male density 5 and 10 ($P < 0.03$). The decrease in fecundity under high male density is proposed to be caused by increased damage by male reproductive organs. The second experiment examined precopulatory sexual selection in *S. oryzae*. Results show that both sexes select for large genitals. In addition males select for the number of mature eggs in females. These traits may directly affect the reproductive fitness of these insects. In the final experiment we examined how multiple mating affected female reproductive fitness in the laboratory. Results indicate that over sixty days females permanently paired with males, mated twice with different males and four times with the same males did not suffer from significant fertility decline while females mated once, twice with the same males and four times with different males had significant fertility 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 fertility decline in females that mated with four different males may be caused by reproductive organ damage or other factors which decreases their fertility due to excessive polyandry.

KEY WORDS oviposition, multiple mating, rice weevil, *Sitophilus oryzae*, Curculionidae.

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Chapter 1

General Introduction

1.1 Introduction

Sitophilus species are among the most widespread and destructive primary pests of stored cereals in the world (Rees 1996). One of these species, the rice weevil *S. oryzae* (Linnaeus), is particularly destructive, attacking numerous crops including wheat, rice, maize and split peas (Longstaff 1981a; Grenier et al. 1997). This pest causes extensive damage to stored products reducing market value. Before this insect spread from the Oriental Region with traded grains, it was a quarantine insect, however due to its worldwide distribution these regulations have been relaxed (Kuschel 1961; Champ 1963). However, the lack of information on the mating behaviour of *S. oryzae* makes it difficult in understanding the general reproductive biology and thus the development of pest management programmes.

1.2 Economic importance of *S. oryzae*

Rice weevils characteristically infest whole cereal grains, but can breed in commodities other than cereal grains such as split peas and pasta. Cereal grains are the most consumed crop worldwide, providing humans more energy than any other type of crop (FAO 2008). In poorer nations, cereals make up almost the entire diet. In developed nations, cereal consumption also makes up a large part of the diet, but is more commonly used as animal feed (Kay 1976; Tilman et al. 2002; FAO 2008). The increase in demand from a growing population has resulted in the doubling of the global cereal production in the past 40 years (Tilman et al. 2002). The ability of *S. oryzae* to infest all cereal grains makes their control a priority when trying to feed a growing population and expanding stock feed industries.

1.3 Damage caused by *S. oryzae*

The major effect of an infestation by *S. oryzae* is the damage to grains caused by the feeding activities of adults and grubs (Longstaff 1981a). Males and females feed by chewing holes in grain. Females chew a deep hole with enlarged sides in which they place an egg (Longstaff 1981a). The resulting grub hollows out the grain before emerging as an adult to continue the cycle (Longstaff 1981a). The feeding process reduces the quality and weight of the grain and produces large amounts of grain dust (Hardman 1977). The grain dust attracts secondary invaders such as the sawtoothed grain beetle *Oryzaephilus surinamensis* (Linnaeus), the red flour beetle *Tribolium castaneum* (Herbst), the rusty grain beetle *Cryptolestes ferrugineus* (Stephens) and mites which consume grain dust and broken grains (Longstaff 1981a; Hill 2002). *S. oryzae* activity also increases the temperature and humidity of infested grain, increasing growth rate of secondary pests and making conditions optimum for pathogens and further infestation (Hardman 1977; Longstaff 1981a; Hill 2002).

1.4 Pest management

Conventionally, control of rice weevils relies on grain protectants such as malathion to prevent infestation in grains (Murray 1979). However, weevil populations quickly build up resistance to protectant chemicals (Murray 1979; Longstaff 1988). Fumigation is now prominently used to control *S. oryzae*, yet these fumigants also cause problems with resistance, toxicity and chemical residue (Longstaff 1988). Moreover, the leading fumigant methyl bromide was phased out in 2005 under the Montreal Protocol due to environmental toxicity (Maud 2009). Pest control using insecticides on grain crops for food is also undesirable, causing rejection of seeds containing residues (Allen & Desmarchelier 2000). Many alternative control techniques exist, including the use of botanicals (plant products) such as Neem, pea extract, and clove oil to control *S. oryzae* (Bloszyk et al. 1990; Dayal et al. 2003). Dusts such as diatomaceous earth are commonly used to protect grain (Athanassiou et al. 2005). Biological control with pathogens (Dal Bello et al. 2000) and parasitoids (Ryoo et al. 1996) are effective in maintaining low pest numbers, but they rarely kill enough insects to meet export requirements (Champ 1963; Longstaff 1988). The leading alternative control methods involve reducing the seed moisture content to below 10%, which prevents adults and larvae from gaining enough water while feeding (Longstaff 1981a). Other alternative methods employed include the use of heating or cooling grain (Evans 1982), oxygen depletion (Bailey 1956) and physical stress (Carde 1990) to manage the pest population. However, these methods are expensive to implement or maintain.

1.5 Aim and objectives

High male density has been shown to reduce the fertility and fecundity of the rice weevil (Campbell 2005). However, the mechanisms contributing to this reduction remain unclear.

The aim of this study is to investigate factors associated with multiple mating in *S. oryzae* with three objectives:

(1) To investigate the influence of male density on the reproductive fitness and behaviour of *S. oryzae* females;

(2) To study the effect of genitalia and external characteristics on mate choice in *S. oryzae*;

(3) To determine the effect of different mating treatments on fertility and fecundity of *S. oryzae* females.

Chapter 2

Literature Review

2.1 Introduction

This chapter reviews the current information on the behavioural and reproductive biology on *Sitophilus* species that are relevant to this study. Special references are given to facts about *S. oryzae*.

2.2 Taxonomy of *S. oryzae*

The rice weevil was initially described as *Curculio oryza* by Linnaeus (1763). Prior to the official name of *Sitophilus oryzae* (L.) by Floyd and Newstrom (1959), this weevil had several name changes including:

Calendra oryzae by De Clairville and Schellenberg (1798),

Sitophilus oryzae by SchŐnherr (1838),

Sitophilus zea-mais by Motschulsky (1855),

Calandra oryzae var. *platensis* by Zacher (1922).

The classification for this species:

Order: Coleoptera
  Superfamily: Curculionoidae
    Family: Curculionidae
      Subfamily: Dryophthorinae
        Genus: *Sitophilus*
          Species: *oryzae*

2.3 Identification of *S. oryzae*

The adult rice weevil is 2-3mm in length depending on the size of grain it feeds on, i.e. larger weevils develop from larger grains (Campbell 2002). It is reddish-brown to black in colour with a long rostrum and four orange/red spots on the corners of the elytra which fade inward to the middle point of the elytra (Figure 2.1a) (Rees 2004). The prothorax is strongly pitted and the elytra have rows of pits within longitudinal grooves (Figure 2.1a) (Rees 2004). The larvae are legless with white/cream coloured body and dark head capsule (Figure 2.3b) (Rees 2004). Males and females are similar in appearance but can be identified by the males having shorter, wider and irregular indentations on their rostra (Halstead 1963) (Figure 2.1b). Females have a longer, thinner rostrum with shallower indentations uniformly running down the rostrum (Figure 2.1b) (Halstead 1963).

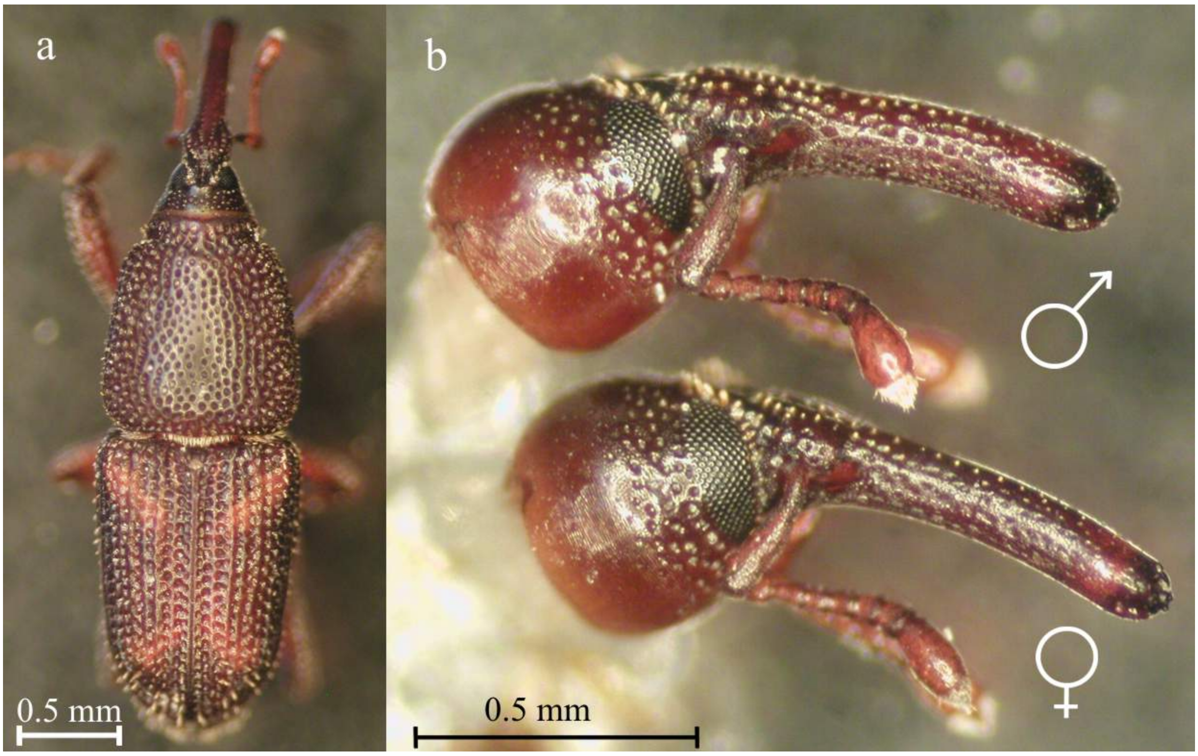

Figure 2.1. Identification of *S. oryzae*: (a) female, (b) male and female rostra. These pictures were taken in the present study.

The rice weevil is very similar in appearance to the granary weevil, *S. granarius* (L.) and externally identical to the maize weevil, *S. zeamais* (Motsch) (Rees 2004). The granary weevil is larger and darker, lacks colouration but has more prominent longitudinal rows on elytra. The rice weevil and maize weevil can be distinguished by examining the aedeagus, with the latter having three longitudinal ridges on the outer surface while the former bears a convex outer surface (Figure 2.2).

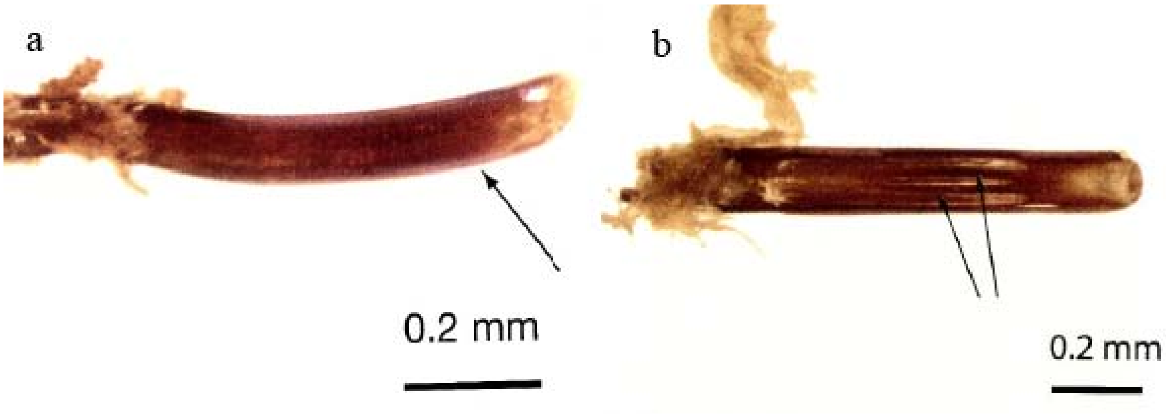

Figure 2.2. Aedeagus of: (a) *S. oryzae* and (b) *S. zeamais* (from Rees 2004).

2.4 Distribution and hosts

Sitophilus oryzae originally spread from the Oriental Region with traded grains and is now distributed worldwide (Kuschel 1961). This insect breeds on rice, wheat, maize, rye barley, sorghum, split peas, tamarind and macaroni (Longstaff 1981a). It attacks numerous other commodities but requires whole grains for breeding (Longstaff 1981a).

2.5 General biology

The life cycle of *S. oryzae* includes four stages: egg, larva, pupa and adult (Figure 2.3). The first three stages are completed inside the infested grain. Adults may survive for up to two years depending on the conditions (Ryoo & Cho 1988; Pittendrigh et al. 1997). Females lay 2-6 eggs per day and up to 300 eggs over their lifetime (Longstaff 1981a; Campbell 2005). The female uses strong mandibles to chew a small hole in a grain kernel which extends the full length of her rostrum, and then deposits a single egg within the hole and seals the hole with mucilaginous secretions from her ovipositor (Longstaff 1981a).

The larva develops within the seed, hollowing it out while feeding, then pupates within the grain kernel and leaves the grain 2-4 days after eclosion (Longstaff 1981a). Male *S. oryzae* produce an aggregation pheromone to attract both sexes (Phillips & Burkholder 1981; Walgenbach & Burkholder 1986). Females also produce a pheromone which attracts males only (Trung et al. 1988). Adults can survive at 10.5-35°C, 60-100% RH and 10-16% seed moisture content with optimum conditions for adult growth and development being 25-30°C, 70-80% relative humidity (RH) and 12-14% seed moisture content (Longstaff 1981a). Eggs and pupae can tolerate harsher conditions than adults while larvae are more susceptible to environmental variation (Longstaff 1981a).

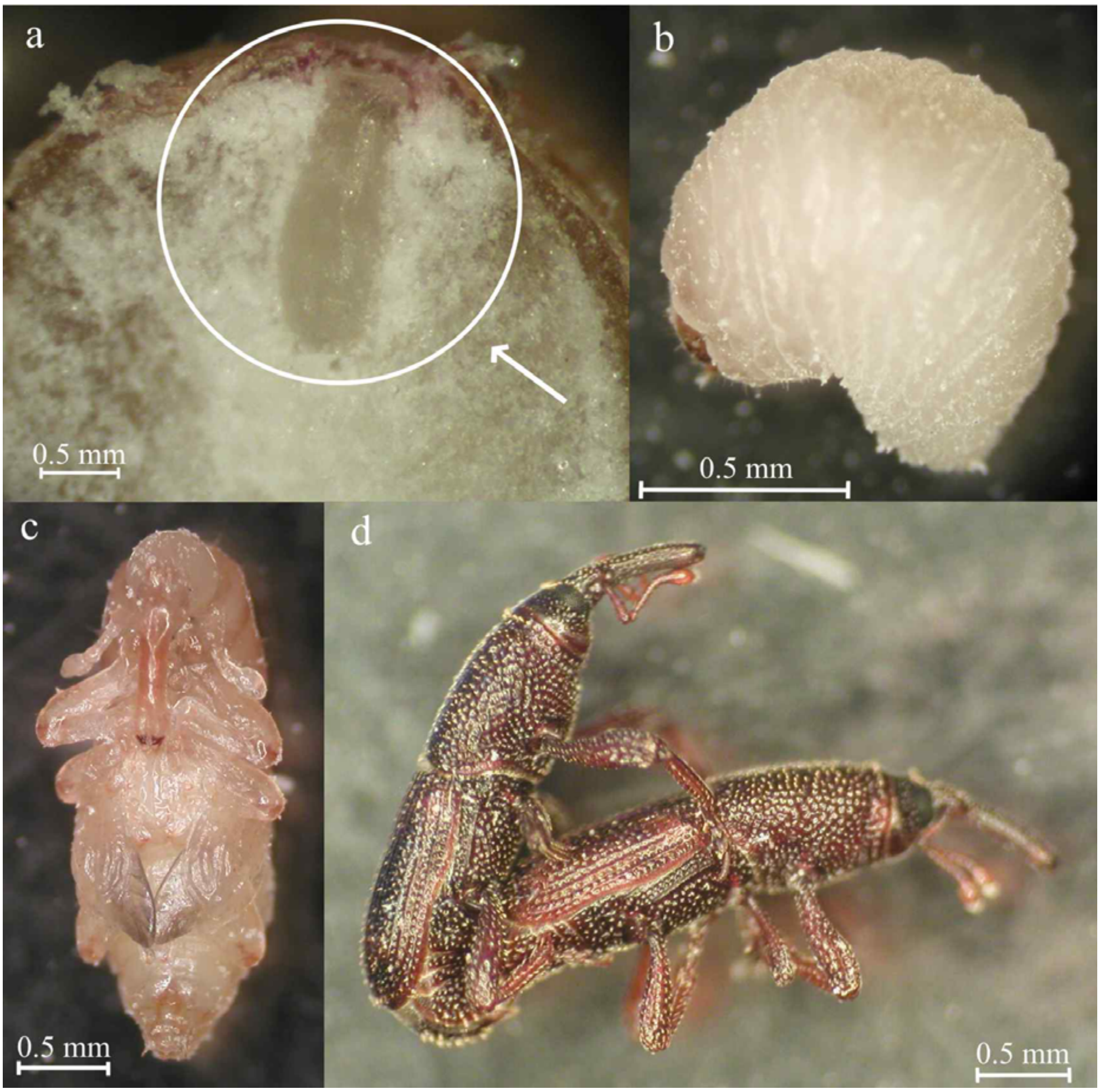

Figure 2.3. Life cycle of *S. oryzae*: (a) egg, (b) larva, (c) pupa and (d) adults. These pictures were taken in the present study.

2.6 Reproductive system of *S. oryzae*

2.6.1 Female reproductive system

Figure 2.4 shows the reproductive system of *S. oryzae*. The genitalia of female *S. oryzae* are located ventrally to the alimentary canal and consist of the spermatheca, bursa copulatrix, and common oviduct (Khan & Musgrave 1969). The spermathecal duct attaches the spermatheca to the dorsal side of the bursa copulatrix anterior to the attachment of the common oviduct (Khan & Musgrave 1969). The spermatheca is sclerotised and sausage shaped with compressor muscles attached to each end (Khan & Musgrave 1969). A sac-like spermathecal gland is attached to the spermatheca near the entrance of the spermathecal duct. The sperm enter the spermatheca from the duct through a number of small tubes at the end of a pinnacle. The tubes are so small that only one sperm can pass at a time (Khan & Musgrave 1969). The structure attached at the point where the common ovary duct splits into the two ovary branches has not been characterised.

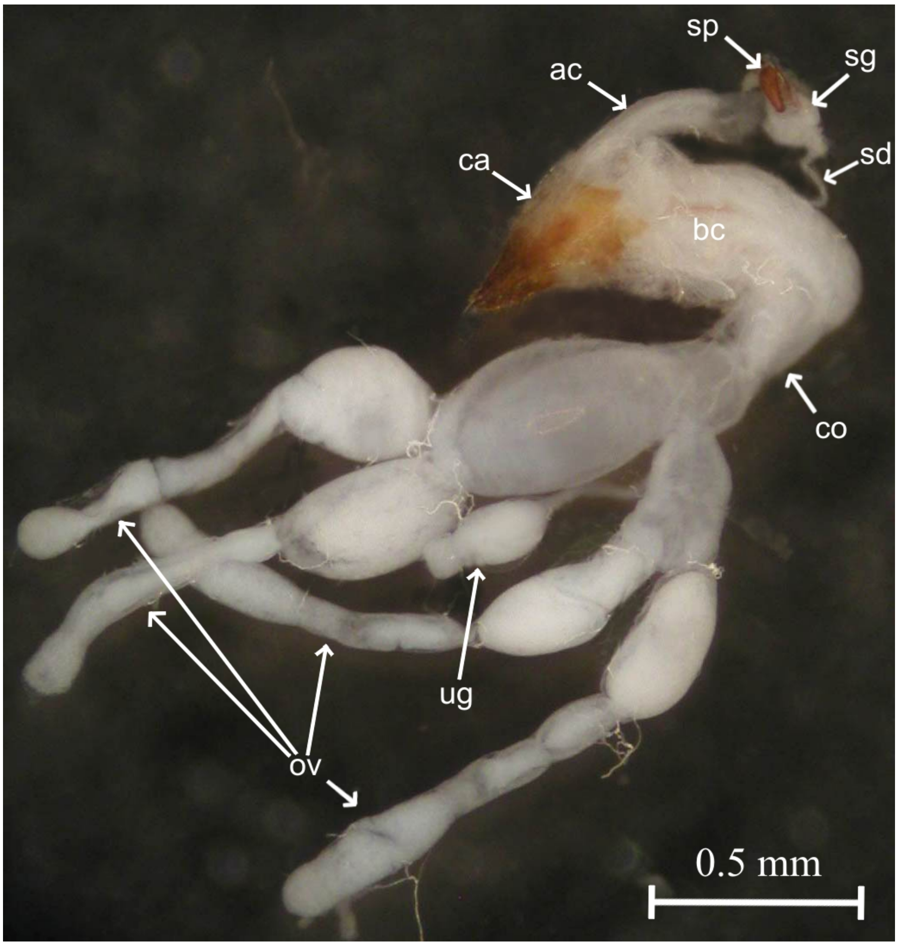

Figure 2.4. Reproductive system of *S. oryzae*: ca, capsule; ac, alimentary canal; sp, spermatheca; sg, spermatheca gland; sd, spermatheca duct; bc, bursa copulatrix; co, common oviduct; ug, unknown gland; ov, ovaries. This picture was taken in the present study.

2.6.2 Male reproductive system

As shown in Figure 2.5, the aedeagus of *S. oryzae* is surrounded by a tegmen and asymmetric sclerite, all of which are strongly chitinized (Marray & Tiegs 1935). When mating, the aedeagus extends into the female bursa copulatrix opening. The inflatable sac then extends from the end of the male aedeagus to facilitate sperm transport to the spermatheca (Marray & Tiegs 1935; Khan & Musgrave 1969). Sperm are produced in the testes and are activated when passing the prostate glands (Khan & Musgrave 1969). The structure attached at the point where the ejaculatory duct splits to meet the prostate glands has not been characterised.

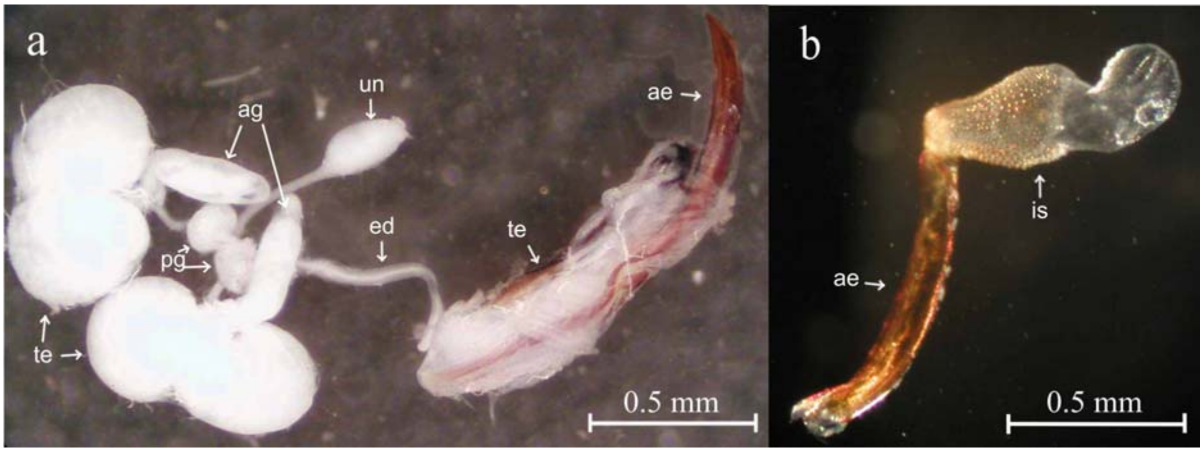

Figure 2.5. Reproductive system of male *S. oryzae*: (a) ae, aedeagus; te, tegmen; ed, ejaculatory duct; ug, unknown gland; pg, prostate glands; ag, accessory glands; te, testes. (b) ae, aedeagus; is, internal sac. These pictures were taken in the present study.

2.7 Mating behaviour

2.7.1 General mating behaviour

Upon contact with a conspecific, *S. oryzae* often hesitate and wave their outstretched antennae in recognition (Holloway & Smith 1987). After hesitating for a short period they either resume walking or the male orients himself in the same direction as the female and attempts to mount her (Holloway & Smith 1987). The female often keeps walking while a male attempts to mount, which may result in frequent dislodging of the male (Holloway & Smith 1987). Upon dislodgement the male either attempts to remount or walks away. The male which successfully mounts a female in the correct orientation will grip the outer edges of the female elytra with his legs. The male then periodically exhibits characteristic head wagging behaviour which consists of waving his rostrum laterally across the female thorax and then resting the rostrum on the mid thorax (Holloway & Smith 1987). Krautwig (1930) noted that male rice weevils inflate their aedeagus during copulation. This allows the inflated sac to reach the spermathecal duct opening where spermatozoa are discharged and subsequently sucked into the spermathecal duct through the action of spermathecal muscles. Male and female *S. oryzae* may mate repeatedly with many partners and all females can remove mating males by dislodging them on seed grains (Holloway & Smith 1987).

2.7.2 Female intrasexual competition

Most studies tend to focus on male intrasexual competition (MISC) while female intrasexual competition (FISC) is largely neglected. This may be due to the prominent expression of MISC compared to FISC in many species (Johnstone et al. 1996). This does not however indicate that MISC is more common than FISC because female competition may involve subtle and indirect forms of manipulation which do not require physical aggression (Jennions & Petrie 1997). This is observed in species like the grape root borer, *Vitacea polistiformis* (Harris) (Pearson et al. 2004). Females of this species move upwind and crosswind when in a competing pheromone plume is detected. Females exhibiting this movement mate significantly more often than those remaining stationary (Pearson et al. 2004). Aggressive intrasexual competition between females has been observed in brentid weevils, *Brentus anchorago* (Linnaeus). Females of this species disrupt the courtship and copulation of other females, thus, reducing the number of females ovipositing and increasing their offspring survival rates (Johnson 1982).

2.7.3 Male intrasexual competition

Male intrasexual competition (MISC) may have evolved due to the ability of males to mate with many females and thus sire more offspring than females. Johnstone et al. (1996) proposed that if males sire more offspring than females, males will compete over females because most males wish to mate numerous females, making females a limited resource. Females will consequently be choosy due to the high competition for them and because their potential reproductive rate is not as readily affected by mating number as males (Johnstone et al. 1996). The competition between males for females varies considerably between species. Males can use a combination of contests, scramble tactics, endurance rivalry, coercion, mate choice, and sperm competition (Andersson & Iwasa 1996). In species such as *Librodor japonicas* (Motschulsky), the intrasexual competition is so intense that a dimorphism has evolved among males (Emlen 1997). In this species, males which receive ample nutrition grow large, using their size, strength, weaponry and agility as threat signals or for direct competition (Andersson & Iwasa 1996; Okada et al. 2008). Males receiving inadequate nutrition remain small and cannot compete directly with large males. Instead they compete by putting resources into large testes and exhibit sneaking behaviours when larger males are distracted by fighting (Okada et al. 2008). A third dimorphic group, receiving adequate nutrition, use scramble tactics where males put resources into wings to assist in dispersal from their initial location (Okada et al. 2008).

In some species male competition involves making themselves attractive to mates. This is seen in the dance fly, *Empis borealis* (Linneaus), whose fertilisation success is related to the size of their nuptial gift (Alcock et al. 1977; LeBas & Hockham 2005). Male cicadas, on the other hand, compete using sound, with females selecting males with the loudest song or in some species most intricate songs (Sueur & Aubin 2002). Pheromones are used for competition in *Drosophila grimshawi* (Oldenberg), and males of this species can adjust their pheromone output according to the competition in the lek (Widemo & Johansson 2006). Copulation time can also be used as a competition strategy. For example, male walking stick insects physically inhibit other male mating attempts by extending their mating duration (Thornhill & Alcock 1983).

Post-mating behaviour is also an important factor in sexual competition of insects (Thornhill & Alcock 1983). Males often guard over females they have mated with to ensure females do not obtain other mates (Thornhill & Alcock 1983). This is seen in the cricket, *Teleogryllus commodus* (Walker) which guards its mate with feeler contact. If the male is not present with feeler contact, females often leave the area in search of another mate (Thornhill & Alcock 1983).

Little is known about the intrasexual competition of male *S. oryzae*. To date, the only reported account states that larger males will pair with females faster than their smaller rivals (Holloway & Smith 1987). There is some indirect evidence for male intrasexual competition. For example, Holloway & Smith (1987) found that *S. oryzae* females remain mounted for much longer than required for successful sperm transfer, indicating that something other than fertilisation is occurring after insemination. In some species a longer mating allows the transfer of compounds which can delay female remating (Johnstone & Keller 2000). This occurs in bumble bees *Bombus terrestris* (L.), which secrete a sticky substance as a mating plug (Duvoisin et al. 1999). Males of other species transfer substances which harm females, entering them into a refractory period (Johnstone & Keller 2000).

2.8 Factors affecting reproductive fitness

2.8.1 Population density

MacLagan & Dunn (1935) were the first to demonstrate that high population densities reduce fecundity in *S. oryzae*. Longstaff (1981b) conducted similar experiments using a wider range of densities and came to the same conclusion. Later, Campbell (2005) carried out a related study which also found a reduction in female longevity and fecundity under high population densities. The reduction in fecundity and longevity is proposed to be caused by a high proportion of time spent in copula and male harassment (Campbell 2005). However, Campbell (2005) did not clarify how time spent in copula and male harassment are specifically linked to a reduction in fecundity and fertility.

2.8.2 Body size

Body size also affects the reproductive fitness of *S. oryzae* in numerous ways. It has been found that larger females are more fecund and produce more offspring to adulthood than small females (Richards 1947; Holloway & Smith 1987). Large males take less time to get a mate, mate for longer and sire larger progeny than small males (Holloway & Smith 1987). The genetic variability in body weight may be maintained by a size trade-off with survival or maturation time (Rose 1982).

2.8.3 Mate choice

2.8.3.1 Mate choice by females

Females of all sexually reproducing species are highly selective and male genital characteristics are especially important (Eberhard et al. 1998). The importance of male genitalia to fit female genitalia is exemplified in the relatively conserved sizes of male aedeagi in relation to body size and other sexually selected traits (Eberhard et al. 1998). Eberhard (1985) proposed that the conservation of aedeagus size functioned to allow males with intermediate size genitalia to copulate the largest proportion of females. Thus, males with aedeagi of average size will have a fitness advantage over males with either large or small aedeagi. Females may also indirectly alter sperm number, quality and morphology with the shape and size of their reproductive tracts (Rugman-Jones & Eady 2008; Gay et al. 2009).

The phenomenon of female restriction of the mating preference of competing females is not well studied (Jennions & Petrie 1997). However, when females select for direct benefits, the costs of sub-optimal choice may be significant (Jennions & Petrie 1997). Even if there are no direct benefits associated with mate choice, female mate selection may increase offspring viability (Petrie 1994). The failure to select high quality mates may consequently produce significant variation in the fitness of competitors offspring. This acts as an advantage to females who disadvantage others (Jennions & Petrie 1997).

2.8.3.2 Mate choice by males

Male mating strategies are expected to enhance a male's fertilization success throughout his life (Bateman 1948). This behaviour tends to select for female phenotypes associated with high fecundity or reduced sperm competition intensity (Bonduriansky 2001; Byrne & Rice 2006). In many species, the primary indicator of female fertility or fecundity is their body size (Honek 1993). Thus, males of *S. oryzae* (Holloway & Smith 1987)_and many other species tend to select for large females (Jimenez-Perez & Wang 2004; He & Wang 2006). Males of some species use display traits to select females of high quality. Male psylids, *Diaphorina citri* (Kuwayama) for instance, select females based on abdomen colours corresponding with high fecundity (Wenninger et al. 2009). Pheromones are used as display traits in insects such as *Drosophila serrata* (Malloch) (Chenoweth & Blows 2003). Male dance flies, *Rhamphomyia tarsata* (Zetterstetd), prefer to give nuptial gifts and inseminate females with large leg scales, which indicate high fecundity (LeBas et al. 2003; Simmons & Emlen 2008).

2.8.4 Mating frequency

2.8.4.1 Asymmetry in mating frequency

The asymmetry in mating interests between the sexes is thought to result from the cost of gamete production and parental care (Thornhill & Alcock 1983). The reproductive success of males is limited by the number of eggs they can fertilize, and consequently males often seek numerous mates to fertilize (Bateman 1948). The fitness of females on the other hand, is predominantly limited by their ability to produce eggs (Bateman 1948). This is influenced by the costs involved with mating, such as risk of parasitisation, predation, infection during copulation and time lost during mating (Moller 1990; Liersch & Schmid-Hempel 1998; Arnqvist & Nilsson 2000; Knell & Webberley 2004). As a result, females should mate infrequently in species which obtain enough sperm from one mating to fertilize all their eggs (Bateman 1948; Arnqvist & Nilsson 2000). However, this is not the case for many species as females often mate multiple times with many males (Arnqvist & Nilsson 2000).

2.8.4.2 Mating frequency of females

Female multiple mating is a key feature affecting the reproductive potential of many insects. However, the effects of multiple mating in many species are poorly understood (Arnqvist & Nilsson 2000; Campbell 2005). In a number of insects, multiple mating may have both positive and negative effects on female fertility, fecundity and longevity. Direct benefits from multiple mating can be produced in the form of nuptial gifts offered to the female (Thornhill & Alcock 1983). Nutritious sperm or protective substances such as antibacterial agents, protease inhibitors, and fungicides can also be passed from male accessory glands during copulation (Thornhill & Alcock 1983; Jennions & Petrie 2000; Chapman & Davies 2004; Schwartz & Peterson 2006). Genetic benefits can be gained by mating with higher quality males (Thornhill & Alcock 1983; Jennions & Petrie 2000), avoiding inbreeding depression (Cornell & Tregenza 2007), fertility assurance (Pai et al. 2005), parasite resistance (Baer & Schmid-Hempel 2001), and increasing genetic diversity or genetic bet hedging (Jennions & Petrie 2000; Calleri et al. 2006). Repeated mating can also reduce the chance of infertility by increasing the use of young sperm (Siva-Jothy 2000; Reinhardt & Siva-Jothy 2005)), avoiding unsuccessful sperm transfer (Hasson & Stone 2009), or by compensating for mating with sperm-depleted males (Wedell et al. 2002).

However, when mating frequency increases above which females are habituated to, multiple mating can have a number of consequences. These include: increasing time lost for feeding and oviposition (Keller & Reeve 1995), energy lost while mating (Watson et al. 1998a), an increased incidence of sexually transmitted diseases (Parker 1970; Hurst et al. 1995; Watson et al. 1998b), increased risk of predation due to reduced mobility or increased visibility (Arnqvist 1989; Rowe 1994), and increased damage from deleterious chemicals produced in male sperm or accessory glands (Thornhill & Alcock 1983; Arnqvist & Nilsson 2000; Moore et al. 2003; Campbell 2005; Hardling & Kaitala 2005). Furthermore, multiple mating may also increase the risk of external (Michiels & Newman 1998) and internal injury from male activity (Crudgington & Siva-Jothy 2000; Blanckenhorn et al. 2002).

Therefore, for optimum reproductive efficiency, the female mating frequency must limit the negative effects of mating and enhance their fertility and fecundity (Ridley 1988). The optimal mating frequency for females is not naturally supported by males. Males will instinctively mate at a higher frequency, creating sexual conflict (Chapman et al. 2003; Schwartz & Peterson 2006). Arnvist and Nilsson (2000) illustrated that when the optimal female mating rate was exceeded, mating became less effective as fewer eggs were produced. Campbell (2005) found a similar reduction in female longevity and fecundity in response to high male competition in *S. oryzae*. He found that the reduction in fecundity and longevity is likely caused by a high proportion of time spent in copula as well as male harassment (Campbell 2005). However, there is still no direct evidence supporting this notion.

2.8.4.3 Mating frequency of males

Male multiple mating is thought to have evolved in response to males' fitness increasing with the number of eggs they can fertilize (Bateman 1948). Consequently, the probability of fertilizing eggs increases with the number of females mated (Arnqvist & Nilsson 2000). A male may also initiate mating with many females to increase the genetic diversity of his offspring (Trontti et al. 2007). Although it seems that all males should mate with many females, there are many constraints on multiple mating. The primary constraints are the intrasexual competition among males for access to females and the intersexual competition between the sexes for fertilisation of eggs (Arnqvist & Nilsson 2000). However, there are other contributing factors to the number of females a male can mate. For example, male fitness in the parasitoid wasp *Anisopteromalus calandrae* (Howard) depends primarily on their sperm stock, which in turn is dependent on life history parameters, such as age and previous mating experience (Bressac et al. 2009). In species with a high investment in offspring, such as species with sex role reversal, multiple mating may be less pronounced due to the risks involved (Bressac et al. 2009). Conversely, species with little investment in offspring may have a high level of multiple mating, such as *S. oryzae* (Campbell 2005).

Chapter 3

Mechanisms of Female Fitness Decline Due to Increasing Male Density in *S. oryzae*

3.1 Introduction

Male density is a key feature affecting the reproductive potential of many insects (Lauer et al. 1996; Arnqvist & Nilsson 2000; Campbell 2005). It functions to increase mate competition, often through multiple mating (Arnqvist & Nilsson 2000). However, the effects of multiple mating on female reproductive fitness are still poorly understood in many species (Arnqvist & Nilsson 2000; Campbell 2005). In a number of insects, multiple mating may have both positive and negative effects on female fertility, fecundity and longevity. Direct benefits from multiple mating can be produced in the form of nuptial gifts offered to the female (Thornhill & Alcock 1983). Nutritious sperm or protective substances such as antibacterial agents, protease inhibitors, and fungicides can also be passed from male accessory glands during copulation (Thornhill & Alcock 1983; Jennions & Petrie 2000; Chapman & Davies 2004; Schwartz & Peterson 2006). Genetic benefits can be gained by mating with higher quality males (Thornhill & Alcock 1983; Jennions & Petrie 2000), avoiding inbreeding depression (Cornell & Tregenza 2007), fertility assurance (Pai et al. 2005), parasite resistance (Baer & Schmid-Hempel 2001), and increasing genetic diversity or genetic bet hedging (Jennions & Petrie 2000; Calleri et al. 2006). Multiple mating can also reduce the chance of infertility by increasing the use of young sperm (Siva-Jothy 2000; Reinhardt & Siva-Jothy 2005), avoiding unsuccessful sperm transfer (Hasson & Stone 2009), or by compensating for mating with sperm-depleted males (Wedell et al. 2002).

However, multiple mating can also have a number of consequences, including time lost for feeding and oviposition (Keller & Reeve 1995), energy lost while mating (Watson et al. 1998a), increased incidence of sexually transmitted diseases (Parker 1970; Hurst et al. 1995; Watson et al. 1998b), increased risk of predation due to reduced mobility or increased visibility (Arnqvist 1989; Rowe 1994), increased damage from deleterious chemicals produced in male sperm or accessory glands (Thornhill & Alcock 1983; Arnqvist & Nilsson 2000; Moore et al. 2003; Campbell

2005; Hardling & Kaitala 2005). Furthermore, multiple mating may also increase the risk of external (Michiels & Newman 1998) and internal injury during the mating process (Crudgington & Siva-Jothy 2000; Blanckenhorn et al. 2002). All of these mating costs, separately or in some combination, may affect fecundity, fertility and longevity of female insects.

In insect mating systems where females mate with multiple males, males will have a higher reproductive fitness if they maximise utilisation of their sperm for fertilisation (Simmons 2001). Males use numerous strategies to increase their reproductive fitness over that of other males. For example, male damselflies use aedeagal spines to remove sperm of previous males (Thornhill & Alcock 1983); male bean weevils wound the female with spines on the aedeagus to postpone her remating or increase her egg laying rate (Crudgington & Siva-Jothy 2000). These strategies can however divert female fitness away from their optimal levels, reducing their fertility, fecundity or longevity (Arnqvist & Rowe 2002; Arnqvist & Rowe 2005). The resulting conflict selects females which resist manipulation by males (Arnqvist & Rowe 2005). For example, the female water strider *Gerris buenoi* (Kirkaldy) carries a male to repel additional males who attempt to mate (Rowe 1992). Similarly, the female dung fly *Sepsis cynipsea* (Linnaeus) resists mating to reduce the risk of injury (Ward et al. 1992; Muhlhauser & Blanckenhorn 2002). The use of these strategies indicates that females increase their fitness by reducing damage caused by multiple mating (Blanckenhorn et al. 2000). Despite this damage mitigation, sexual conflict over mating is often costly to female fitness (Chapman et al. 2003; Arnqvist & Rowe 2005; Bonduriansky et al. 2008).

Sitophilus species including *S. oryzae*, *S. granarius* (L.) and *S. zeamais* (Motschulsky) rank as the most important pests of whole grains such as wheat, corn, rice, and sorghum worldwide (Longstaff 1981a; Rees 2004). These species share similar life history, and therefore, the factors affecting female fitness in *S. oryzae* may also influence the fitness of females in other *Sitophilus* species in a similar way. Previous research narrowly focuses on physical or behavioural aspects to understand the impact of multiple mating on female fitness in insects (Watson et al. 1998a; Chapman et al. 2003; Arnqvist & Rowe 2005; Campbell 2005; Bonduriansky et al. 2008; Flay et al. 2009). Few studies have looked at behavioural interactions, or the effects of those interactions on fertility and fecundity. These studies are essential to get a comprehensive and accurate picture of multiple mating and the costs of mating for female fitness in *S. oryzae*.

Campbell (2005) shows that the fecundity and longevity decrease under high male densities in *S. oryzae*, and attributes this phenomenon to the time cost in copulation (Campbell 2005). However, apart from the time cost, the underlying mechanisms involved in the reduction of fecundity and longevity are still not clear. Here, it was hypothesised that the decreased egg production and female longevity under high male density were caused by (1) increased damage to female reproductive organs by males due to longer mating duration and higher mating frequency and (2) decreased foraging and feeding time. To test these hypotheses the duration of female mating, feeding, foraging and walking under different male density, and then recorded female fertility, fecundity and longevity under different male density was recorded. Damage of female reproductive organs was examined by dissecting after the females had died.

3.2 Materials and methods

3.2.1 Insects and experimental conditions

A breeding colony of *S. oryzae* was initiated from a population provided by Plant and 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 a Sanyo versatile environmental chamber (model MLR-350HT, Japan). Around 200 *S. oryzae* adults were introduced into a 500 ml glass jar with a 0.5 mm wire-mesh lid where 250 ml of wheat grains were maintained (Figure 3.1). The adults were allowed to feed and lay eggs for 7 days and then sieved off the wheat grains. Grains with irregular colour patterns and without emergence holes (2-3 mm diameter) present were individually placed in ventilated Eppendorf tubes. Tubes containing infested wheat grains were maintained in the environmental chamber and examined daily to determine adult emergence.

Figure 3.1. Insect colonies on wheat in 500 ml glass containers.

Emerged individuals were sexed according to rostrum characteristics (Halstead 1963). All adults were individually maintained in Eppendorf tubes with five wheat grains each. Tubes were then put into the environmental chamber for 4 days to allow weevils to reach reproductive maturity (Longstaff 1981a) before initiating the experiment. Before experiments, all insects were individually weighed using an electronic balance (Mettler Toledo, AG135, Switzerland) with an accuracy of 0.01 mg.

All experiments in this chapter and others were conducted in a constant dark room under red lights at 27 ± 3°C and 75 ± 5% RH. Experiments were carried out in Petri dishes (60 mm diameter ×10 mm height) with ~100 grains of wheat. Fluon (an anti-grip substance) was applied to the inner wall of Petri dishes to prevent insect escape.

3.2.2 Effect of male density on mating behaviour

To observe the effect of male density on mating behaviour of *S. oryzae*, three treatments consisting of virgin females individually maintained with virgin males of different densities (i.e. ratios of 1, 5 and 10 males) were set up. Male density of 0 was applied as control. Male insects were marked with a silver permanent marker on the dorsal surface of abdomens for sex identification. Test insects were placed in Petri dishes. Eight Petri dishes (two for each treatment and control) were placed in random order on the bench and 20 grains of wheat evenly distributed in each dish. Twelve hours before the experiment, females in the control were placed individually in ventilated Eppendorf tubes, each with five grains of wheat and one male for mating. Upon initiation of the experiment, these females were removed from the males and added to Petri dishes.

In the three treatments, males were added into the Petri dishes according to the male densities marked on each Petri dish. With an exception of the control where no behaviour was observed, the behaviour of adults was recorded for one minute every six minutes for each dish in each density of 1, 5 and 10 males. Thus, a total of 75 one-minute-observations were carried out for each dish over 7.5 hours (Figure 3.2). After 7.5 hours, males from each dish were separated from the female and placed in ventilated Eppendorf tubes with one wheat grain. Females in dishes were maintained without males for 16.5 hours, after which time (day 2) the fed-wheat grains were replaced with fresh ones and males were reintroduced into the dishes they came from and maintained with their original mates. This procedure was repeated for three days. Ten replicates were achieved by repeating the entire experiment for five times.

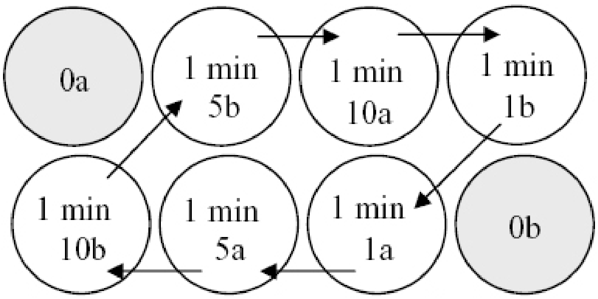

Figure 3.2. Treatment allocation pattern in a controlled environmental room. Each circle represents a randomly allocated Petri dish. Treatments are represented by the male density and replicates are indicated by a and b. Arrows represent observation movements from one dish to the next.

The duration of mating (the amount of time a female spent on copula), feeding (the amount of time a female spent on moving her mandibles on the surface of the grain and/or twisting her head while her rostrum was inserted within a grain), foraging (the amount of time a female spent on tapping or moving antennae <1 mm over the grain surface) and walking (the amount of time a female spent walking without any other behavioural patterns) under different male density were recorded. Insects were viewed with a dissecting microscope (Nikon, Japan) set on a trolley. This enabled the viewer to move the dissecting microscope over the Petri dishes to view the insects. A digital timer was used to time the one minute observations of the insects in each dish.

3.2.3 Effect of male density on female reproductive fitness

The effect of male density on reproductive fitness of *S. oryzae* females was investigated using the same 10 replicates as above. After the three-day long behavioural observations (Section 3.2.2) were complete, females were maintained with the same males until death to determine the life time fecundity and longevity of females. If a male died, it was replaced with another mated male of the same age. Petri dishes were checked every 24 hours for female death. Dead females were immediately dissected under a microscope to observe reproductive organ damage.

Wheat grains were replaced every three days with fresh ones and stained with acid fuschin to highlight egg plugs as described by Frankenfeld (1948). For each dish, the number of egg plugs highlighted by staining is assumed equal to the number of eggs laid (Frankenfeld 1948). Egg plugs were counted under the dissecting microscope. The stained wheat grains were kept in the controlled atmosphere room in ventilated Eppendorf tubes for 40 days to record adult emergence and obtain estimated fertility.

Reproductive organ damage consisted of darkened patches of tissue not present in virgin females on the bursa copulatrix wall or the eighth sternite. Similar damage is seen after mating in the bean weevil *Callosobruchus maculates* (Fabricius) (Crudgington & Siva-Jothy 2000). Both of these organs come in contact with the male aedeagus and are involved in female oviposition.

3.2.4 Statistics

Normally distributed data on mating, feeding, and walking, and mean fecundity, fertility and longevity were analysed using ANOVA followed by Tukey's Studentised Range test. Abnormally distributed data on foraging were analysed using the non-parametric Kruskal-Wallis (KWT) test followed by Dunn's procedure for multiple comparisons. The frequency of damage to bursa copulatrix and eighth sternite was analysed using a U test. All data were analysed using SAS (SAS 2006).

3.3 Results

3.3.1 Effect of male density on mating behaviour

Female *S. oryzae* spent significantly longer time on mating with increasing male density (ANOVA: $F_{2,\ 27} = 20.50$, $P < 0.01$) (Table 3.1). Female feeding and foraging duration at male density of 1 was significantly longer than that at male density of 5 and 10 (ANOVA: $F_{2,27} = 8.05$, $P = 0.0018$ for feeding; KWT: $\chi^2_{2,27} = 7.34$, $P = 0.0254$ for foraging) (Table 3.1). No significant difference was detected in walking duration between different male densities (ANOVA: $F_{2,27} = 1.28$, $P = 0.2948$) (Table 3.1).

Table 3.1: Mean duration (minutes) of mating, feeding, foraging and walking in *S. oryzae* females under different male densities

Male density	Mating	Feeding	Foraging	Walking
1	36.9 ± 3.5 c	39.8 ± 2.1 a	4.5 ± 0.4 a	9.2 ± 1.5 a
5	53.1 ± 3.1 b	25.0 ± 2.2 b	3.2 ± 0.4 b	7.5 ± 1.0 a
10	62.2 ± 1.7 a	26.1 ± 2.0 b	3.1 ± 0.4 b	6.8 ± 1.7 a

Means (± SEM) with the same letters in each column are not significantly different ($P > 0.05$).

3.3.2 Effect of male density on damage of female reproductive organs

The brown sclerotised spines on the inflated internal sac of the male aedeagus are orientated in the opposite direction to insertion (Figure 3.3).

The dark sclerotised scar on an excised section of the bursa copulatrix wall of females (Figure 3.4A) and a missing branch from the eighth sternite (Figure 3.4B) were observed after female death under male density of 1, 5 and 10 but not in the control density of 0.

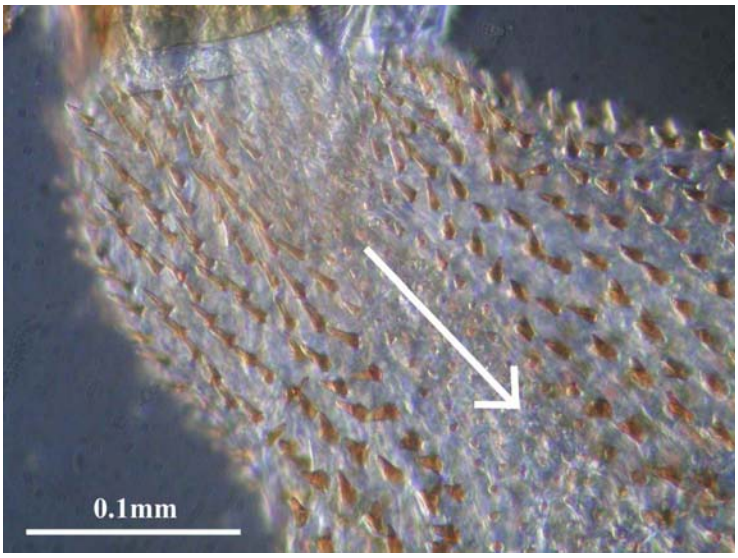

Figure 3.3. Spines on the everted internal sac of a *S. oryzae* male. Direction of insertion is indicated with the white arrow.

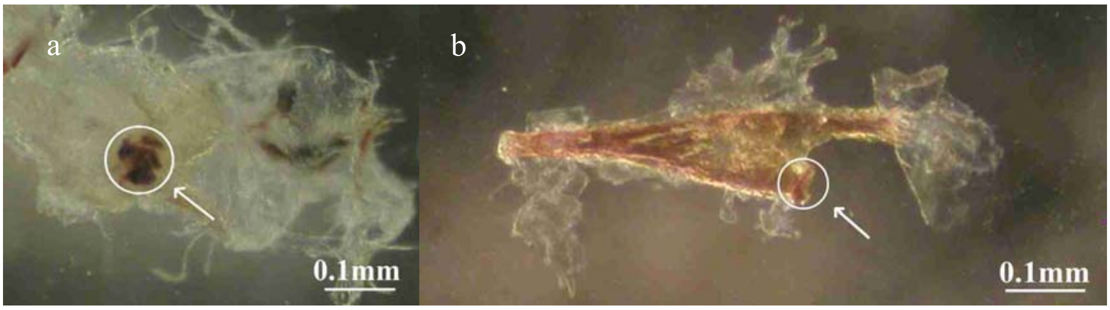

Figure 3.4. Damage shown on the bursa copulatrix (a) and eighth sternite (b) of multiply mated *S. oryzae* females.

With the increase of male density females had significantly higher chance to be damaged on their bursa copulatrix and eighth sternite ($U_0^{'}$ = 20.60 and 16.00 > $\chi^2_{3,0.05}$ = 7.82 for damage of bursa copulatrix and eighth sternite, respectively; $P < 0.001$) (Figure 3.5).

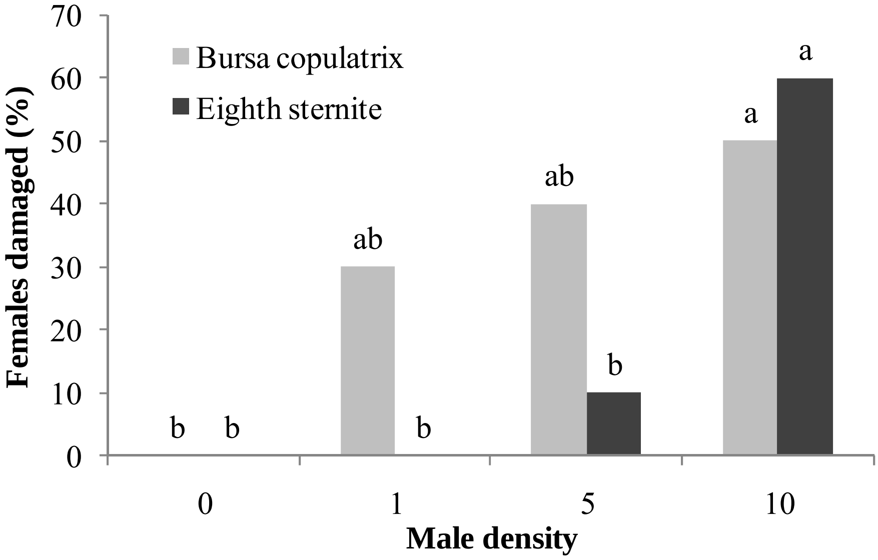

Figure 3.5. Percentage of *S. oryzae* females with damage to the bursa copulatrix and the eighth sternite. Within the same category (bursa copulatrix or eighth sternite) columns with the same letters are not significantly different ($P > 0.05$).

3.3.3 Effect of male density on female reproduction and longevity

The female fecundity significantly decreased with the increase of male density (ANVOA: $F_{3,36}$ = 13.34; $P < 0.0001$) (Table 3.2). However, male density had no effect on fertility rate (ANOVA: $F_{3,36}$ = 1.17; $P = 0.329$) (Table 3.2). Female longevity significantly decreased with increasing male density (ANOVA: $F_{3,36}$ = 12.67; $P < 0.0002$) (Table 3.2).

Table 3.2. Effect of male density on female fecundity, fertility and longevity in *S. oryzae*

Male density	Fecundity	Fertility rate (%)	Longevity (days)
0	213.3 ± 33.6 ab	67.3 ± 2.1 a	196.7 ± 30.1 a
1	269.1 ± 15.6 a	62.0 ± 2.1 a	107.9 ± 17.7 ab
5	118.7 ± 18.7 bc	70.0 ± 3.7 a	66.9 ± 9.6 bc
10	94.2 ± 12.3 c	63.6 ± 4.6 a	38.1 ± 5.8 c

Means (± SE) with the same letters in each column are not significantly different ($P > 0.05$).

3.4 Discussion

Like many other species (Parker 1970; Parker & Simmons 1994; Jablonski & Vepsalainen 1995; AlonsoPimentel & Papaj 1996; Andres & Rivera 2000; Simmons 2001; Wang et al. 2008), mating duration significantly increased with the increase of male density in *S. oryzae* (Table 3.1). This phenomenon may result from the fact that when male density increases, the probability that a male will find another mate decreases and the possibility that his sperm will be displaced increases (Wang et al. 2008). Therefore, mating duration appears to be mostly under male control (Arnqvist 1988; Carroll 1991; Arnqvist & Danielsson 1999b; Garcia-Gonzalez & Gomendio 2004; Wang et al. 2008). In the water strider *Gerris odontogaster* (Arnqvist 1992) and seed bug *Nysius huttoni* White (Wang et al. 2009), mating success rate increases under the increasing male-biased sex ratio. Although not tested, we assume that *S. oryzae* females mate more frequently when male density increases. Furthermore, previous studies suggest that the mating success is controlled by females in insects and other animals (Ortigosa & Rowe 2003; Gavrilets & Hayashi 2005; Wang et al. 2009). Mating duration controlled by males and mating success governed by females can result in sexual conflict between the sexes, i.e. male preference is to court more and mate longer when male density increases and female preference tends to resist prolonged mating and accept less mates for mating.

The present study confirms that female fecundity and longevity of *S. oryzae* decrease with the increase of male density (Table 3.2) as reported by Campbell (2005). These results also show that females were significantly more likely to be damaged on their bursa copulatrix (Figure 3.4A) and eighth sternite (Figure 3.4B) when male density increased (Figure 3.5). Further examination of male genitalia demonstrates that during copulation the reversed male internal sac penetrates through the vagina into the bursa copulatrix and deposits ejaculate. We propose that the backward pointing spines on the reversed internal sac (Figure 3.3) function as an anchor to keep the aedeagus and internal sac in place while transferring sperm may cause damage to the female reproductive system during the sexual conflict under the high male density due to longer mating duration and probably higher mating frequency in females. For example, females may attempt to resist long mating and have to accept more mates, where the spines on the reversed internal sac can grip and damage the female reproductive organs such as bursa copulatrix and eighth sternite. Similar genital damage by such spines has been reported in the bean weevil, *C. maculates* (Crudgington & Siva-Jothy 2000) and dung fly, *Sepsis synipsea* Linnaeus (Blanckenhorn et al. 2002). It is thus suggested that the decrease in female fecundity and longevity under higher male density may be caused by increased damage to female reproductive organs by males due to longer mating duration and higher mating frequency in *S. oryzae*.

These results indicate that female foraging and feeding time significantly decreased with the increase of male density in *S. oryzae* (Table 3.1). A similar reduction in feeding and foraging time in response to male density is also found in a solitary bee, *Anthophora plumipes* (Pallas) (Stone 1995). Consequently, females in the high male densities may have less energy for somatic maintenance and egg production, resulting in reduced female fecundity and longevity. For example, (Perez-Mendoza et al. 2004) suggest that if female *S. oryzae* become malnourished, they may reabsorb eggs for somatic maintenance, contributing to the reduction in female fecundity; Campbell (2005) demonstrates that exposure to multiple males reduces female survival.

During oviposition female *S. oryzae* drill a hole the depth of their rostrum in a grain and deposit a single egg in the hole (Longstaff 1981a). Therefore, under high male density the increased time and energy costs associated with increased mating duration and frequency may restrict female oviposition. This oviposition restriction is observed in treatments where a single female is present with a single male, females took an average of 6.4 hours to lay an egg. Conversely, females in treatments with 5 or 10 males present were not observed to lay an egg within the 7.5 hour duration (observed). Similar restrictions with the increasing male density are found in many insect species (Thornhill & Alcock 1983; Wilcox 1984; Stone 1995; Cordts & Partridge 1996; Clutton-Brock & Langley 1997; Bybee et al. 2005; Jennions et al. 2007). This suggests that the reduced oviposition time due to high male density may contribute to the decrease in female fecundity. Unlike the findings by (Arnqvist & Nilsson 2000), the fertility rate in *S. oryzae* females was not affected by male density in this study. The reason for this phenomenon is unknown.

In conclusion, the decrease in the fecundity and longevity of *S. oryzae* females under higher male density may be caused by increased damage to female reproductive organs by males due to longer mating duration and higher mating frequency, and by decreased feeding and foraging time leading to nutrition deficiency.

Chapter 4

Pre-copulatory Mate Choice in *S. oryzae*

4.1 Introduction

Sexual selection was proposed by Darwin (1871) as the competition existing within one sex for members of the opposite sex and the differential choice applied by members of one sex for members of the other sex. This selection acts to enhance traits that indicate high genetic quality in the opposite sex (Thornhill & Alcock 1983; Jennions & Petrie 2000), lack of parasites (Baer & Schmid-Hempel 2001) or indicate that a mate will supply more material benefits (Schwartz & Peterson 2006). This selection enhances the fitness of insects which mate with partners displaying these desirable traits (Thornhill & Alcock 1983). The study of sexual selection helps understand the direction of phenotypic trait evolution in relation to mate choice and reproductive fitness. It may also deliver vital information for the improvement of pest management measures such as sterilised insect techniques (Boake et al. 1996).

Phenotypic selection varies greatly between different insect species. For example, females of most species prefer large males due to their size indicating superior fitness attributes (Andersson 1994). Occasionally, sexual selection may also favour small males for greater agility (McLachlan & Allen 1987) or efficient searching for females (Fagerström & Wiklund 1982). In other species, sexual selection has been found to focus on genital traits rather than body size. For example, male water striders, *Aquarius remigis* (Say) with longer intermittent organs had an advantage under sexual conflict between the sexes (Sih et al. 2002). In a review of genitalia evolution and sexual selection, Eberhard (2004b) found that traditional sexual selection had more influence on genitalia evolution than intersexual conflict, further suggesting the influence of genital traits on sexual selection.

To date, attributes including genital and external characters have not been measured in relation to mate choice in *S. oryzae*. The present study recorded the sexual dimorphism to determine features which vary between the sexes and may thus be influenced by sexual selection. The morphological and genital characteristics of both sexes were then recorded in order to examine traits that might be selected in sexual selection.

4.2 Materials and methods

4.2.1 Mate choice by females

Female choice for male attributes was determined by placing one female with one heavy >1.96 mg and one light male <1.57 mg in a Petri dish (55 mm diameter × 13 mm height) until mating ceased. If mating did not occur within 30 minutes the replicate was discarded. Males and females used here were four day old virgins. The following male parameters were measured: body weight before mating, body length, prothorax length and width, antennal length, club length and width, width and length of rostrum from mandibles to the antennal cavity, abdomen width at 3rd and 6th sternites, left elytra length, and hind tibia length. The aedeagus length and width, length of aedeagal apodemes, internal sack length from aedeagus tip to end of tubercles, and tegmen length were measured after mating for males that mated and un-mated. Thirty replicates were performed.

4.2.2 Mate choice by males

Male choice for female attributes was similarly examined by placing one male with one heavy >2.10 mg and one light female <1.61 mg in an above-mentioned Petri dish until mating ceased. If mating did not occur within 30 minutes the replicate was discarded. Males and females used here were four day old virgins. The same external features measured in males were measured in females. Internal features measured after mating included: bursa copulatrix length, capsule (ovipositor) to spermatheca length, number of mature eggs, capsule length, length and width of the eighth sternite, length of spermithecal duct and length of spermitheca. Thirty replicates were performed.

4.2.3 Statistics

To estimate the net variation in external traits between the sexes and to analyse the external and internal traits under sexual selection by the choosing sex, standardised multivariate selection analysis was used (Lande & Arnold 1983). All independent variables were standardised by dividing the variable mean by its standard deviation before analysis. The standardising removes the effects of differential scaling and allows the comparison of each variable relative to others, e.g. mean = 0, variance

= 1 (Gibson 1987). Selection gradients were calculated as partial logistic regression coefficients (r_s) from regression of mating success to the standardised values of measured traits. Selection gradients quantify the strength of selection acting on a trait independently of variation in the other characters included on the regression model (Lande & Arnold 1983).

4.3 Results

4.3.1 Sexual dimorphism

Females were significantly heavier and had a significantly shorter but wider prothorax, wider antennal club, a longer but thinner rostrum and longer elytra than males (Table 4.1). Other features were not significantly different between the sexes (Table 4.1).

Table 4.1: Body measurements (mean ± SE) of *S. oryzae* females (n = 140) and males (n = 140)

Characteristics measured (mm)	**Female**	**Male**	$\mathbf{r_s}$	**P**
Weight (mg)	185.59 ± 3.92	172.43 ± 4.62	0.375	0.0337
Rhostrum length	0.70 ± 0.01	0.60 ± 0.01	2.286	<0.0001
Rhostrum width	0.18 ± 0.00	0.20 ± 0.00	-2.112	<0.0001
Prothorax length	1.07 ± 0.01	1.13 ± 0.01	-0.599	0.0016
Prothorax width	1.13 ± 0.01	1.06 ± 0.01	0.719	0.0003
Left elytra length	1.53 ± 0.01	1.49 ± 0.02	0.363	0.0454
Abdomen width at 3rd sternite	0.83 ± 0.01	0.81 ± 0.01	0.283	ns
Abdomen width at last sternite	0.71 ± 0.01	0.69 ± 0.01	0.385	0.0317
Club width	0.12 ± 0.00	0.11 ± 0.00	0.436	0.0171
Club length	0.20 ± 0.00	0.21 ± 0.00	0.257	ns
Antennal length	0.68 ± 0.01	0.66 ± 0.01	0.290	ns
Hind tibia length	0.58 ± 0.01	0.57 ± 0.01	0.341	ns
Body length	3.30 ± 0.02	3.21 ± 0.04	0.343	ns

ns = not significantly different ($P > 0.05$).

4.3.2 Mate choice by females

Females significantly preferred males with wider aedeagus for mating (Table 4.2). Other male features did not significantly affect mate choice by females (Table 4.2).

Table 4.2: Female selection of male *S. oryzae* traits (mean ± SE, n = 56)

Characteristics measured (mm)	Chosen male	Non Chosen male	r_s	P
Internal characteristics				
Aedeagus width	0.13 ± 0.00	0.12 ± 0.00	1.154	0.0010
Aedeagus length	0.79 ± 0.01	0.78 ± 0.01	0.251	ns
Length of basal apodemes	0.75 ± 0.01	0.74 ± 0.01	0.038	ns
Internal sac length	0.43 ± 0.01	0.44 ± 0.01	-0.333	ns
Tegmen length	0.91 ± 0.01	0.92 ± 0.01	-0.251	ns
External characteristics				
Body weight (mg)	183.50 ± 8.98	174.82 ± 7.98	0.199	ns
Body length	3.27 ± 0.07	3.18 ± 0.05	0.261	ns
Prothorax length	1.17 ± 0.03	1.13 ± 0.02	0.327	ns
Prothorax width	1.05 ± 0.02	1.03 ± 0.02	0.164	ns
Antennal length	0.65 ± 0.01	0.68 ± 0.01	0.446	ns
Club length	0.21 ± 0.00	0.21 ± 0.00	-0.219	ns
Club width	0.11 ± 0.00	0.11 ± 0.00	0.013	ns
Rostrum length	0.61 ± 0.01	0.59 ± 0.01	0.300	ns
Rostrum width	0.20 ± 0.00	0.20 ± 0.00	0.185	ns
Abdomen width at 3rd sternite	0.82 ± 0.01	0.80 ± 0.01	0.195	ns
Abdomen width at last sternite	0.68 ± 0.01	0.69 ± 0.01	-0.231	ns
Left elytra length	1.49 ± 0.03	1.47 ± 0.02	0.161	ns
Hind tibia length	0.57 ± 0.01	0.55 ± 0.01	0.333	ns

ns = not significantly different (P > 0.05).

4.3.3 Mate choice by males

Males preferred females with longer bursa copulatrix, longer capsule (ovipositor) to spermathecal duct length and more mature eggs for mating (Table 4.3). Other features did not affect mate choice by males (Table 4.3).

Table 4.3: Male selection of female *S. oryzae* traits (mean ± SE, n = 42)

Characteristics measured (mm)	**Chosen Female**	**Non Chosen Female**	r_s	**P**
Internal characteristics				
Bursa copulatrix length	0.95 ± 0.01	0.91 ± 0.01	0.872	0.0225
Capsule to spermathecal length	0.83 ± 0.01	0.79 ± 0.01	1.127	0.0084
Number of mature eggs	1.33 ± 0.25	0.43 ± 0.13	0.053	0.0080
Capsule length	0.50 ± 0.01	0.50 ± 0.01	0.087	ns
Eighth sternum length (fork)	0.54 ± 0.01	0.52 ± 0.01	0.388	ns
Eighth sternum width	0.12 ± 0.00	0.11 ± 0.00	0.054	ns
Spermathecal duct length	0.43 ± 0.01	0.44 ± 0.01	-0.195	ns
Spermathecal length	0.29 ± 0.01	0.29 ± 0.00	0.062	ns
External characteristics				
Body weight (mg)	191.71 ± 8.83	179.33 ± 8.96	0.315	ns
Body length	3.47 ± 0.06	3.35 ± 0.05	0.494	ns
Prothorax width	1.08 ± 0.02	1.05 ± 0.02	0.448	ns
Prothorax length	1.20 ± 0.02	1.17 ± 0.02	0.377	ns
Antennae length	0.69 ± 0.01	0.67 ± 0.01	0.353	ns
Club length	0.22 ± 0.00	0.21 ± 0.00	0.245	ns
Club width	0.12 ± 0.00	0.12 ± 0.00	0.167	ns
Rostrum length	0.70 ± 0.01	0.68 ± 0.01	0.335	ns
Rostrum width	0.18 ± 0.00	0.18 ± 0.00	0.357	ns
Abdomen width at 3rd sternite	0.85 ± 0.01	0.83 ± 0.01	0.297	ns
Abdomen width at last sternite	0.73 ± 0.01	0.72 ± 0.01	0.222	ns
Left elytra length	1.56 ± 0.02	1.50 ± 0.02	0.630	ns
Hind tibia length	0.60 ± 0.01	0.57 ± 0.01	0.510	ns

ns = not significantly different ($P > 0.05$).

4.4 Discussion

Many insect species exhibit a body size difference between the sexes with females often being larger and heavier (Andersson 1994). This is thought to occur as a response to the sexual selection pressures acting on the sexes (Ferguson & Fairbairn 2000). Sexual selection by males favouring large females leads to larger females with higher fecundity (Andersson 1994). In addition, females favouring large males leads to larger males with higher fecundity and fertility (Blanckenhorn et al. 2000; Joyce et al. 2009).

Although the precopulatory sexual selection was reported to act on body weight and size in *S. oryzae* (Holloway & Smith 1987) and many other insects (Arnqvist et al. 1997), the present study shows that only genital characteristics in both sexes and number of mature eggs in females were directly selected while other traits including body size were not selected in pre-copulation sexual selection (Tables 4.2 and 4.3). These results support the notion that sexual selection is often targeted toward specific phenotypes rather than overall size (Andersson 1994; Eberhard 1996; Clutton-Brock 2009).

The present study suggests that males and females select mates based on immediate reproductive potential and indirect benefits. A female possessing more mature eggs is likely to lay eggs promptly. This increases the chance that sperm delivered by the immediate male will fertilise her eggs, increasing his immediate reproductive potential due to last male sperm precedence (Walker 1980). Conversely, a male mated to a female with few mature eggs may experience more sperm competition with other males that subsequently mate with the female before she lays eggs.

Males and females may sexually select mates' genital morphology based on direct and indirect genetic benefits (Jennions & Petrie 2000; Hosken & Stockley 2004; House & Simmons 2006). Although not measured, this study assumes that a wide aedeagus (inflated sac holding vessel) corresponds to a wide inflated sac. The wider inflated sac would enable better control over mating by allowing the male to inflate the sac further and anchor the spines on the inflated sac (Figure 3.2) deeper to the female bursa copulatrix which would prevent female removal of the male. Also, a long bursa copulatrix may allow better control over male mating by having a looser fit with the inflated sac of males, thus enabling females to remove unwanted males easier than those with a short bursa copulatrix. Males may gain direct genetic benefits by mating with virgin females with large genitals as these females have a superior genetics and possibly more control over paternity (Arnqvist & Danielsson 1999a; Hosken & Stockley 2004; House & Simmons 2006). Males may also gain indirect genetic benefits by selecting females with larger reproductive organs. Similarly, females could also gain indirect genetic benefits by passing the genes for a wide aedeagus to their sons (Jennions & Petrie 2000; Hosken & Stockley 2004; Eberhard et al. 2009).

This directional selection on genital size does not necessarily contradict the one-size-fits-all hypothesis which states that, intermediate sizes of reproductive organs are favoured regardless of body size, as large genitals can still be selected under certain circumstances (Eberhard et al. 2009). Phenotypic associations between genital size and lifetime reproductive success may be positive under favourable environmental conditions due to increased breeding success, and negative under poor environmental conditions due to reduced survival (Eberhard et al. 2009). Thus, in the natural environment, sexually selected phenotypes may change (Rose 1982; Hunt et al. 2004; Robinson et al. 2008).

A survey by Eberhard (2004b) has shown that 50% of all genital structures in 43 dipteran species function to achieve deeper penetration for transferring sperm, while 30% function to clasp the female in place while insemination occurs. Thus, female and male *S. oryzae* would gain a fitness advantage by having deeper insemination and having offspring that achieve deeper penetration by selecting partners with large genitals (Arnqvist & Danielsson 1999a; Eberhard 2004a; Wang et al. 2008). Sexual selection of large genital structures also occurs in many other insects (Arnqvist & Danielsson 1999a; Eberhard 2004b; Wang et al. 2008). These insects have been shown to exhibit a higher coupling rate and fertilisation efficiency when possessing large genitals (Arnqvist et al. 1997; Arnqvist & Danielsson 1999a; Hosken & Stockley 2004).

In conclusion, precopulatory sexual selection in *S. oryzae* directly selects for large genitals in both sexes and the number of mature eggs in females. These traits may directly affect the reproductive fitness of these insects. Genital evolution has been shown to be more divergent than any other traits in polygamous insects. Thus, *S. oryzae's* highly polygamous nature may explain the strong tendancy toward selection based on genital size.

Chapter 5

Influence of Multiple Mating on Reproductive Fitness in *S. oryzae*

5.1 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 (Hasson & Stone 2009). In addition to the above benefits from repeated mating polyandrous females may also gain genetic benefits by mating with multiple males, such as increasing genetic diversity (Baer & Schmid-Hempel 1999; Jennions & Petrie 2000; Calleri et al. 2006; Xu & Wang 2009), mating with higher quality males (Thornhill & Alcock 1983; Jennions & Petrie 2000), avoiding inbreeding depression (Cornell & Tregenza 2007), fertility assurance (Pai et al. 2005), parasite resistance (Baer & Schmid-Hempel 2001) or compensating for mating with sperm-depleted males (Wedell et al. 2002). These benefits, either separately or combined, increase female fitness, driving them to mate multiple times.

The rice weevil is not the only insect that should benefit from multiple matings. Other examples include the ground cricket, *Allonemobius socius* (Scudder) (Fedorka & Mousseau 2002), and the scorpionfly, *Panorpa cognata* (Rambur) (Engqvist 2006). In both of these examples, female fecundity increases with polyandry. This suggests that these insects are gaining material benefits. These polyandrous females may increase their fitness by risking the detrimental effects of mating with diverse partners (Thornhill & Alcock 1983; Hosken & Blanckenhorn 1999). Females which repeatedly mate with the same male may be less likely to experience negative consequences to their fitness but lack the genetic benefits gained through mate diversity (Hosken & Blanckenhorn 1999).

When mating frequency increases to an amount above which females are habituated to, 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. 1998a) or increasing risk of sexually transmitted diseases (Parker 1970; Hurst et al. 1995; Watson et al. 1998a). 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; Moore et al. 2003; Hardling & Kaitala 2005; Wigby & Chapman 2005). Furthermore, multiple mating may also increase the risk of external (Michiels & Newman 1998) and internal injury from male activity (Crudgington & Siva-Jothy 2000; Blanckenhorn et al. 2002). Female resistance to mating may also be costly to females (Rowe 1994; Watson et al. 1998a). If males constantly harass females, resisting mating can be more costly than mating itself (Rowe 1994).

Here, the effect of multiple mating on *S. oryzae* reproductive fitness was investigated. It was hypothesized that either genetic and/or material benefits gained through partner diversity or mating frequency would increase fertility and fecundity of *S. oryzae* females.

5.2 Materials and methods

5.2.1 Experimental design

Six treatments were set up with 4-day-old virgin insects of average weight (1.61 – 2.10 mg and 1.57 – 1.96 mg for females and males, respectively): a female was allowed to (1) mate once only (single mating), (2) mate twice with the same male (2SM), (3) mate four times with the same male (4SM), (4) mate twice with different males (2DM), (5) mate four times with different males (4DM), and (6) be permanently paired with the same male. For treatments (1)-(5), 3-day-old virgin individuals were paired until the end of mating, after which time the male was removed. Mating was performed once every 24 hours in treatments (2) to (5). After the mating regime, males were discarded.

Courtship period was recorded as the time between release into the Petri dish and the mounting of females by the male. Mating period was recorded as the period between the start and end of genitalia connection. After mating the female was held for 60 days in a ventilated Eppendorf tube with 50g (~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.

5.2.2 Statistics

A goodness-of-fit test was carried out before data analysis. Data on 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 (±SE).

5.3 Results

Courtship duration significantly increased with the number of matings when a female mated four times with the same male (Linear regression: $F_{1,118} = 5.54$, $P = 0.0202$) but did not significantly increase when mated to four different males (Linear regression: $F_{1,109} = 2.44$, $P = 0.1212$) (Figure 5.1A). When mated with the same male, mating duration significantly decreased with the number of matings (Linear regression: $F_{1,118} = 7.50$; $P = 0.0072$); however, mating frequency had no significant effect on mating duration when a female mated with four different males (Linear regression: $F_{1,108} = 9.38$; $P = 0.9923$) (Figure 5.1B).

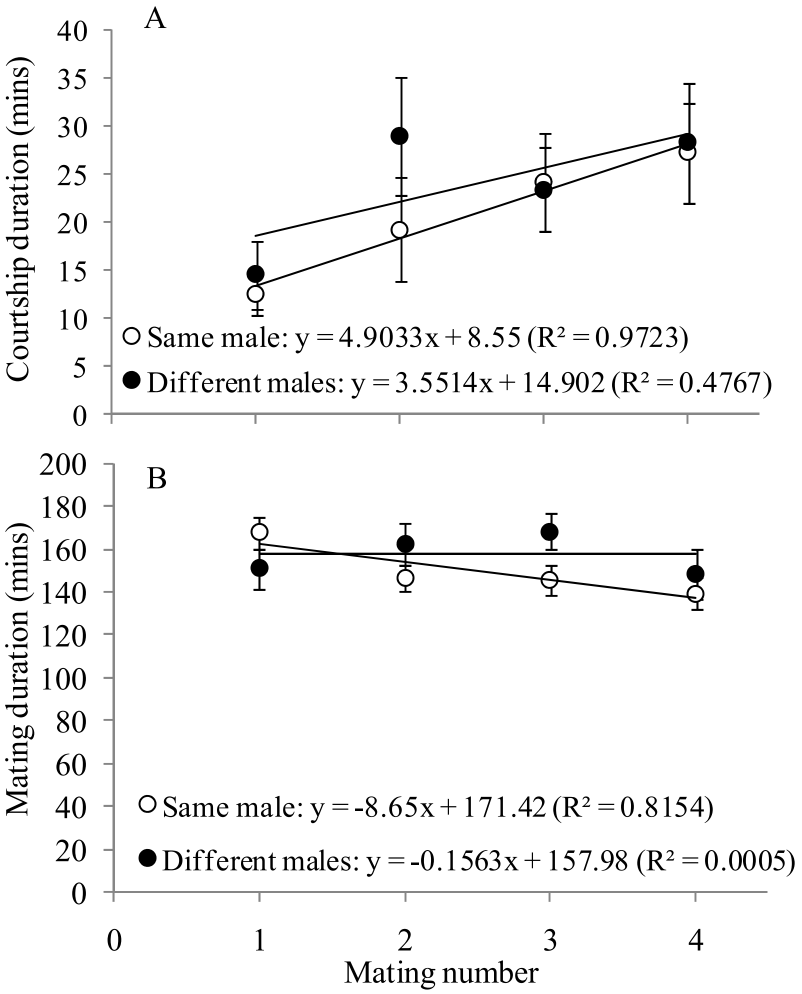

Figure 5.1. Mean (± SE) courtship (A) and mating (B) duration in four successive matings in *S. oryzae.*

The number of eggs laid by a female between 11 and 20 days after mating was significantly higher than that laid during her later life (i.e. 31-60 days after mating) (KWT: χ_5^2 = 78.62, 91.81, 101.00, 62.02, 78.09 and 31.86 for single mating, twice with the same male, twice with different males, four times with the same male, four times with different males and permanent pairing, respectively; $P < 0.0001$) in all treatments (Table 5.1).

Table 5.1: Mean (± SE) number of eggs laid by females in different mating situations

	Time period after mating					
Treatment	1-10	11-20	21-30	31-40	41-50	51-60
Single	13.60 ± 1.18a	14.13 ± 1.17a	11.76 ± 1.38a	6.89 ± 0.96b	3.37 ± 0.71bc	1.37 ± 0.32c
2SM	13.17 ± 0.68a	15.16 ± 1.34a	11.43 ± 1.30ab	7.59 ± 0.85b	3.59 ± 0.70c	0.92 ± 0.28c
2DM	13.28 ± 0.97a	15.28 ± 1.07a	11.43 ± 0.95a	5.93 ± 0.56b	3.75 ± 0.77bc	1.18 ± 0.46c
4SM	13.20 ± 1.19a	15.86 ± 1.38a	12.42 ± 1.35ab	8.36 ± 0.91bc	5.41 ± 0.90cd	2.73 ± 0.61d
4DM	11.96 ± 0.99ab	16.81 ± 1.33a	11.00 ± 1.78b	5.33 ± 0.88c	2.79 ± 0.68cd	1.38 ± 0.36d
Permanent	11.79 ± 0.73ab	14.04 ± 0.87a	13.89 ± 1.16ab	9.69 ± 0.67bc	7.67 ± 0.93c	10.27± 1.20bc

Means (± SEM) with the same letters in rows are not significantly different ($P > 0.05$).

Among different treatments, females permanently paired with males laid significantly more eggs than those mated to four different males (ANOVA: $F_{5,161} = 2.46$; $P = 0.0349$) (Figure 5.2A). Offspring survival rate was significantly higher for females that mated with two different males than that mated only once or four times with different males (ANOVA: $F_{5,170} = 5.24$; $P = 0.0002$) (Figure 5.2B).

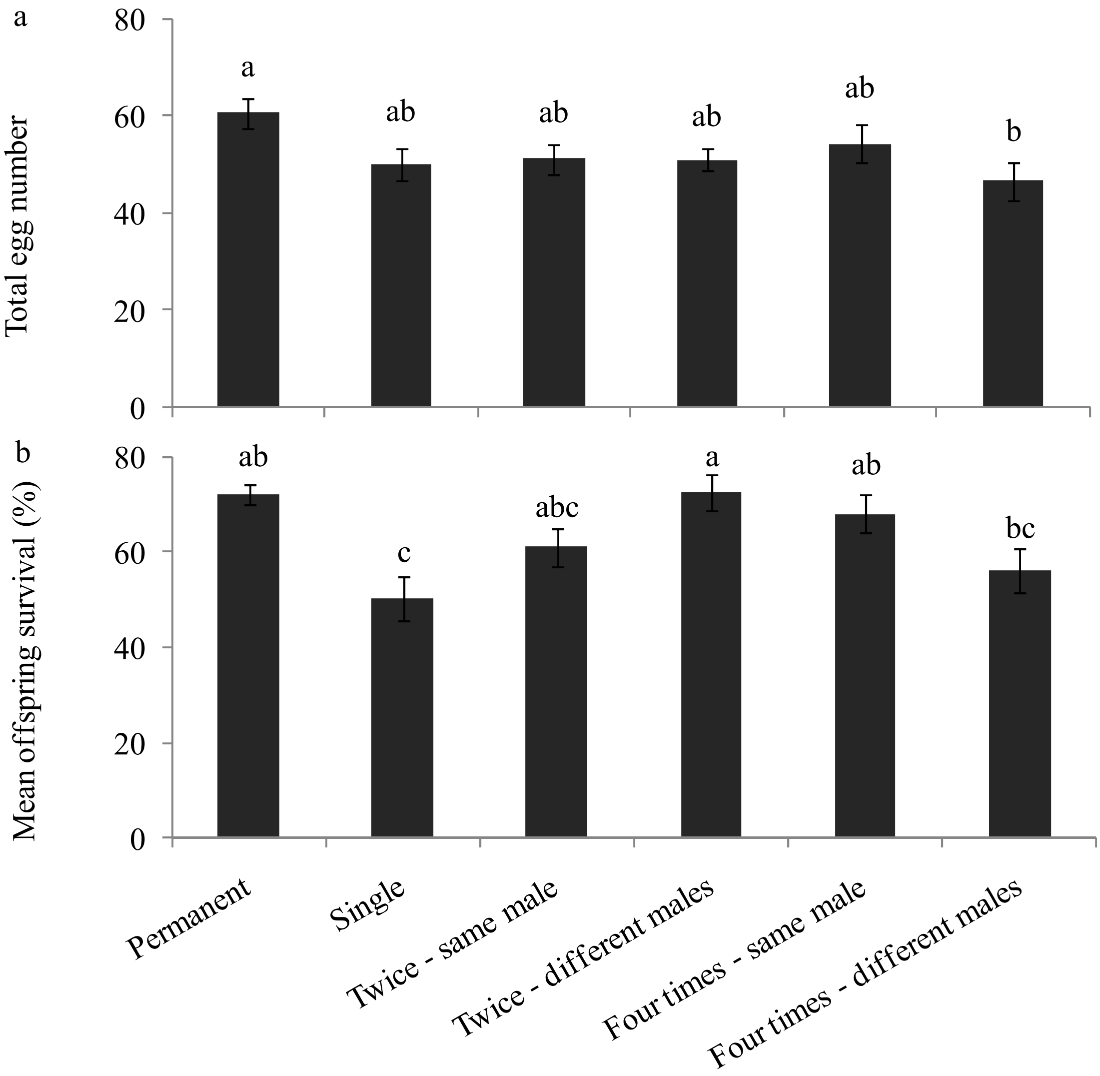

Figure 5.2. Mean (± SE) number of eggs laid (a) and offspring survival rate (b) by *S. oryzae* females over 60 days. Columns with the same letters are not significantly different ($P > 0.05$).

Offspring survival rate significantly decreased over time when a female mated once ($F_{1,141} = 11.62$, $P = 0.0009$), twice with the same male ($F_{1,141} = 11.62$, $P = 0.0009$) or four times with different males ($F_{1,128} = 7.05$, $P = 0.0090$). However, the survival rate did not significantly decrease when a female mated twice with different males ($F_{1,153} = 0.12$, $P = 0.7338$), four times with the same male ($F_{1,151} = 3.47$, $P = 0.0643$) or when permanently paired with a male ($F_{1,151} = 0.67$, $P = 0.7968$) (Figure 5.3).

ANCOVA indicated that a female that mated with two different males had a significantly slower decrease of offspring survival than that mated twice to the same male ($F_{1,10}$ = 6.60, P = 0.0293). However, when females mated four times with different males, offspring survival decreased significantly faster compared with females that mated four times with same male ($F_{1,10}$ = 8.08, P = 0.0193) (Figure 5.3).

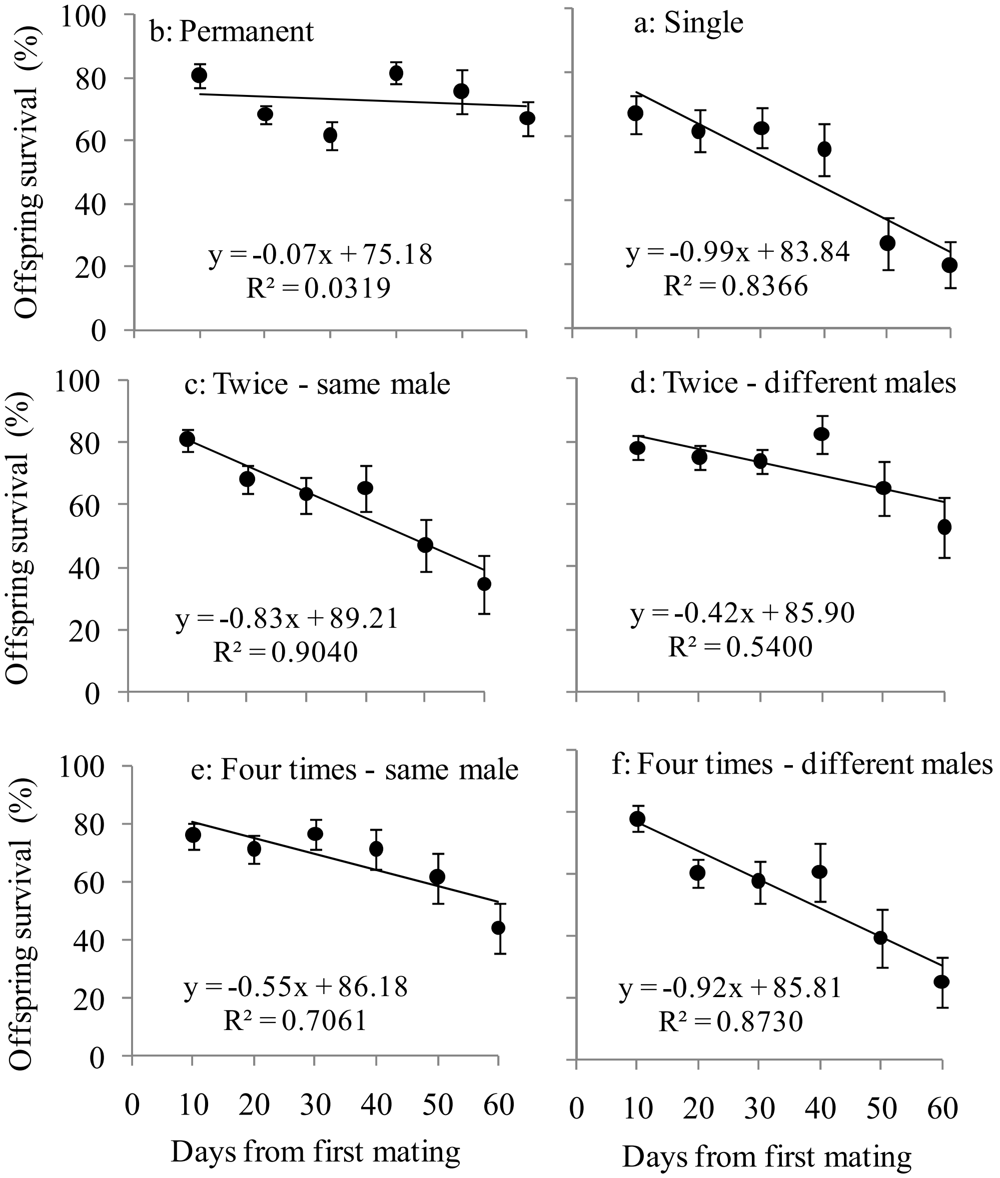

Figure 5.3. Offspring survival rate (± SE) of *S. oryzae* females over 60 days with different mating treatments.

5.4 Discussion

Multiple mating appears to be a widespread mating strategy (Bateman 1948; Thornhill & Alcock 1983; Eberhard 1996; 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 (Svärd & McNeil 1994; Tregenza & Wedell 1998; Ronkainen et al. 2010).

However, the present study shows that multiple mating had no effect on fecundity in general (Figure 5.2A). 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 our 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 5.2B), and the offspring survival rate of once-mated females declined significantly faster over time than that of females mated four times to the same males (Figure 5.3). 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). 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). 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 5.3).

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 5.3). 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 (Crudgington & 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 multiply mate with the same males (Figure 5.1B). This can exacerbate the damage caused by mating with different males that force the female to mate for longer than her optimum mating duration (Arnqvist & Nilsson 2000). Furthermore, spines are found on the male aedeagus (Figure 3.3) which may damage female reproductive system during the extended mating.

Finally, it was found that the number of eggs laid peaked 11-20 days after mating and then declined over time in all treatments (Table 5.1). Previous studies also show that in *S. oryzae* a single mating cannot fertilize the full egg load of a female throughout her life but females may increase fecundity when remating later in life (Campbell 2005). Consistent with this, multiple mating with the same or different males increases offspring survival rate when compared with a single mating (Figure 5.2A). Many species are found to become sperm depleted faster if not allowed to remate but females will increase their fertility over time if allowed to remate (Ridley 1988; Wang & Davis 2006).

Chapter 6

General Discussion and Conclusion

6.1 Introduction

According to Longstaff and Rees (1981a, 2004), *Sitophilus* species including *S. oryzae* (Motschulsky) are among the most destructive pests of whole grains worldwide. In order to better understand these pests and to find ways of managing them, numerous studies have been undertaken (Longstaff 1981a,b; Rees 2004; Campbell 2005). In this thesis, factors contributing to the reduction in fertility and fecundity of *S. oryzae* with high male density, the dimorphism between the sexes, genital and external traits in relation to mate choice in *S. oryzae*, and the effect of female partner diversity and mating frequency on *S. oryzae* offspring survival rates have all been studied. This knowledge is essential for a better understanding of *S. oryzae* reproduction.

This chapter summarises and discusses the main findings and their relevance to reproduction in *S. oryzae* in order to provide background information for further study.

6.2 The influence of high male density on female *S. oryzae* reproductive fitness

High male density was found to have numerous effects on the behaviour and reproductive fitness of *S. oryzae*. In agreement with Campbell (2005), it was found that the female fecundity and longevity of *S. oryzae* decrease with the increase of male density (Table 3.2). The results suggest that the decrease in fecundity and longevity was caused by a combination of factors including damage to the female reproductive organs, a reduced feeding time, reduced foraging time and increased restrictions on oviposition.

The increase in mating duration with increasing male density (Table 3.2) may result in increased sexual conflict between the sexes. As a result of this conflict females were significantly more likely to be damaged on their bursa copulatrix (Figure 3.3A) and eighth sternite (Figure 3.3B) when male density increased (Figure 3.4). Further examination of male genitalia demonstrates that during copulation the aedeagus penetrates through the vagina into the bursa copulatrix. After this, the male internal sac is reversed from within the aedeagus and inflated to deposit sperm. Here it is proposed that the backward pointing spines on the inflated sac (Figure 3.2) function as an anchor to keep the aedeagus and internal sac in place while transferring sperm. However, they may cause damage to the female reproductive system during sexual conflict under high male density due to longer mating duration and possible higher mating frequency in females. It is thus suggested that the decrease in female fecundity and longevity under high male density may be caused by increased damage to female reproductive organs by males due to longer mating duration and higher mating frequency in *S. oryzae*.

Female foraging and feeding time also significantly decreased with the increase of male density in *S. oryzae* (Table 3.1). Consequently, females in the high male densities may lose energy required for somatic maintenance and egg production, resulting in reduced female fecundity and longevity (Perez-Mendoza et al. 2004). Furthermore, females spent increasingly less time on oviposition with the increase of male density Thus, the mechanical disturbance and physical restriction while mating may contribute to the reduction in fecundity of *S. oryzae*.

6.3 Pre-copulatory mate choice in *S. oryzae*

Although precopulatory sexual selection was reported to act on body weight and size in *S. oryzae* (Holloway & Smith 1987) and many other insects (Arnqvist et al. 1997), the present study shows that only genital characteristics in both sexes and number of mature eggs in females significantly affected mating success while other traits including body size were not selected in pre-copulation sexual selection (Tables 4.2 and 4.3). These results support the notion that sexual selection is often targeted toward specific phenotypes rather than overall size (Andersson 1994; Eberhard 1996; Clutton-Brock 2009).

This study suggests that males and females select mates based on immediate reproductive potential and indirect benefits. A female possessing more mature eggs is likely to lay eggs promptly. This increases the chance that sperm delivered by the immediate male will fertilise her eggs, increasing his immediate reproductive potential due to last male sperm precedence (Walker 1980). Conversely, a male mated to a female with few mature eggs may experience more sperm competition with other males that subsequently mate with the female before she lays eggs. Males and females may also sexually select mates' genital morphology based on direct and indirect genetic benefits (Jennions & Petrie 2000; Hosken & Stockley 2004; House & Simmons 2006). Although not measured, it is assumed that a wider aedeagus (inverted inflated sac holding vessel) indicates a correspondingly wider inflated sac. The wider inflated sac would enable better control over mating by allowing the male to anchor the inflated sac (Figure 3.2) more firmly in the female bursa copulatrix, which would prevent the female from removing the male.

A long bursa copulatrix may allow better control over male mating by having a looser fit with the inflated sac of males, thus enabling females to remove unwanted males more easily than those with a short bursa copulatrix. Males may gain direct genetic benefits by mating with females with large genitals as these females have superior genetics and possibly more control over paternity (Arnqvist & Danielsson 1999a; Hosken & Stockley 2004; House & Simmons 2006). By mating with females with superior genetics males have a chance of siring offspring to these females and thus enhancing their offspring's genes (Jennions & Petrie 2000). Moreover, the selectivity of these females may reduce the competition that the sperm of superior males endure. This is because genetically inferior males may be removed before mating or their sperm may be cryptically selected against by the female (Walker 1980; Eberhard 1991). Males may also gain indirect genetic benefits by selecting females with larger reproductive organs, such as enabling their female offspring to have more selective choice over males. Similarly, females could also gain indirect genetic benefits by passing the genes for wider aedeagus to her progeny which would subsequently enhance the fertilisation ability of her male offspring (Jennions & Petrie 2000; Hosken & Stockley 2004; Eberhard et al. 2009).

6.4 Influence of multiple mating on reproductive fitness in *S. oryzae*

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 general benefits of repeated mating were found in this experiment to be gained through increased offspring survival rate and a slower decline in offspring survival rate over time (Figures 5.2B, 5.3). Even though polyandry and repeated mating had similar effects, the strength of these effects was found to be different. These results show that the females that mated with two different males have a higher offspring survival rate than those that mated twice with the same males. However, polyandrous females experienced a decline in offspring hatch rate over time when mated 4 times to different males (Figure 5.3). Conversely, females that repeatedly mated with the same male four times experienced increased fertility over time (Figure 5.3). 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). These insects thus require multiple mating, to the same or different males, to increase their egg hatch rate. Since egg hatch rate is an indicator of fertility rate (Wang & Davis 2006), these findings suggest that *S. oryzae* females mate repeatedly to gain sufficient sperm to maximise their reproductive fitness. This behaviour is similar to the ground cricket, *Allonemobius socius* (Scudder) (Fedorka & Mousseau 2002), and the scorpionfly, *Panorpa cognata* (Rambur) (Engqvist 2006).

This study also indicates that females which repeatedly mate four times with the same male experience less negative consequence to their fitness than those that mate four times to different males. This may be attributed to exceeded mating rate, which leads to internal damage to the female bursa copulatrix and eighth sternite from male activity (Figure 3.3) (Crudgington & Siva-Jothy 2000; Blanckenhorn et al. 2002) or from deleterious chemicals produced in male accessory glands (Thornhill & Alcock 1983; Arnqvist & Nilsson 2000; Moore et al. 2003; Hardling & Kaitala 2005; Wigby & Chapman 2005). This is supported by behavioural data which show that mating duration does not decrease when females mate with different males as compared to when those that mate with the same males (Figure 5.1B). This could intensify the damage caused by mating with different males by forcing females to mate for longer than her optimum mating duration (Arnqvist & Nilsson 2000). Furthermore, the spines found on the male aedeagus (Figure 3.2) may damage female reproductive system during the extended mating. Females which repeatedly mate with the same male also lack the genetic benefits of high egg hatching rate gained through mate diversity (Hosken & Blanckenhorn 1999).

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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: χ^2 =15.38, 17.90, 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: χ^2 =6.72, 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: χ^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 aα	12.3 ± 2.4 aα	20.2 ± 6.5 aα	13.5 ± 2.7 α
10	7.7 ± 1.5 bα	9.4 ± 1.7 abα	15.9 ± 3.5 aα	11.0 ± 1.7 α

[1] Means (±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 ± 3.4 aβ[1]	34.3 ± 4.5 abβ	31.2 ± 3.2 bβ	36.6 ± 3.5 γ
5	54.7 ± 3.4 aαβ	57.3 ± 3.0 aβ	47.4 ± 5.4 aβ	53.1 ± 3.1 β
10	63.5 ± 1.5 aα	63.5 ± 2.3 aα	59.5 ± 2.7 aα	62.2 ± 1.7 α

[1] Means (±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 (Crudgington & 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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Influence of multiple mating on female reproductive fitness in the rice weevil, *Sitophilus oryzae*

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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 (Crudgington & 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 × 10 mm height), each with about 100 grains of wheat, in a controlled environment room under red lights at 27±3°C and 75±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 (±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).

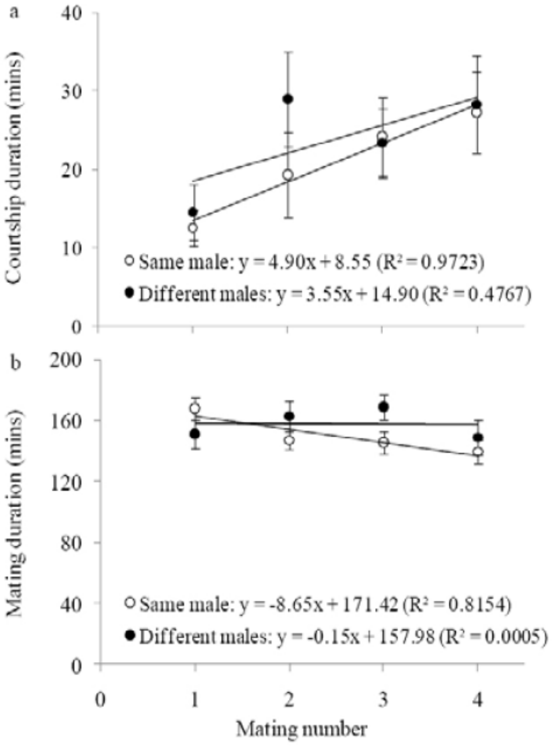

Figure 1 (a) Courtship and (b) mating duration when *S. oryzae* females were mated up to four times with the same male or with different males. Values are the mean (±SE).

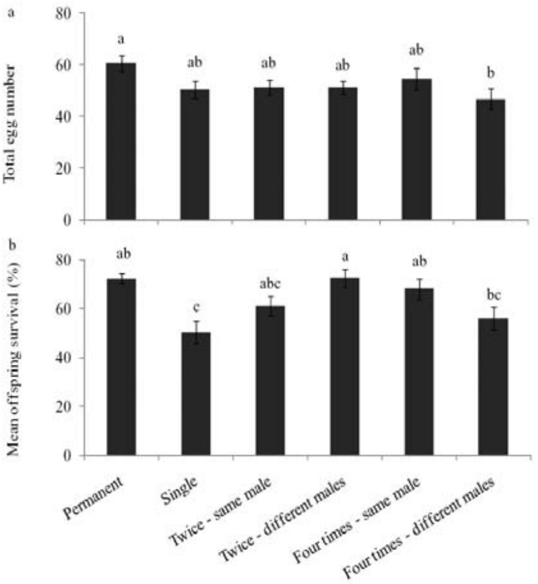

Figure 2 (a) Number of eggs laid and (b) offspring survival rate by *S. oryzae* females over 60 days after mating once, twice or four times with the same or different males. In an additional treatment a male and a female were permanently paired. Values are the mean (±SE). Columns with the same letters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

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 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).

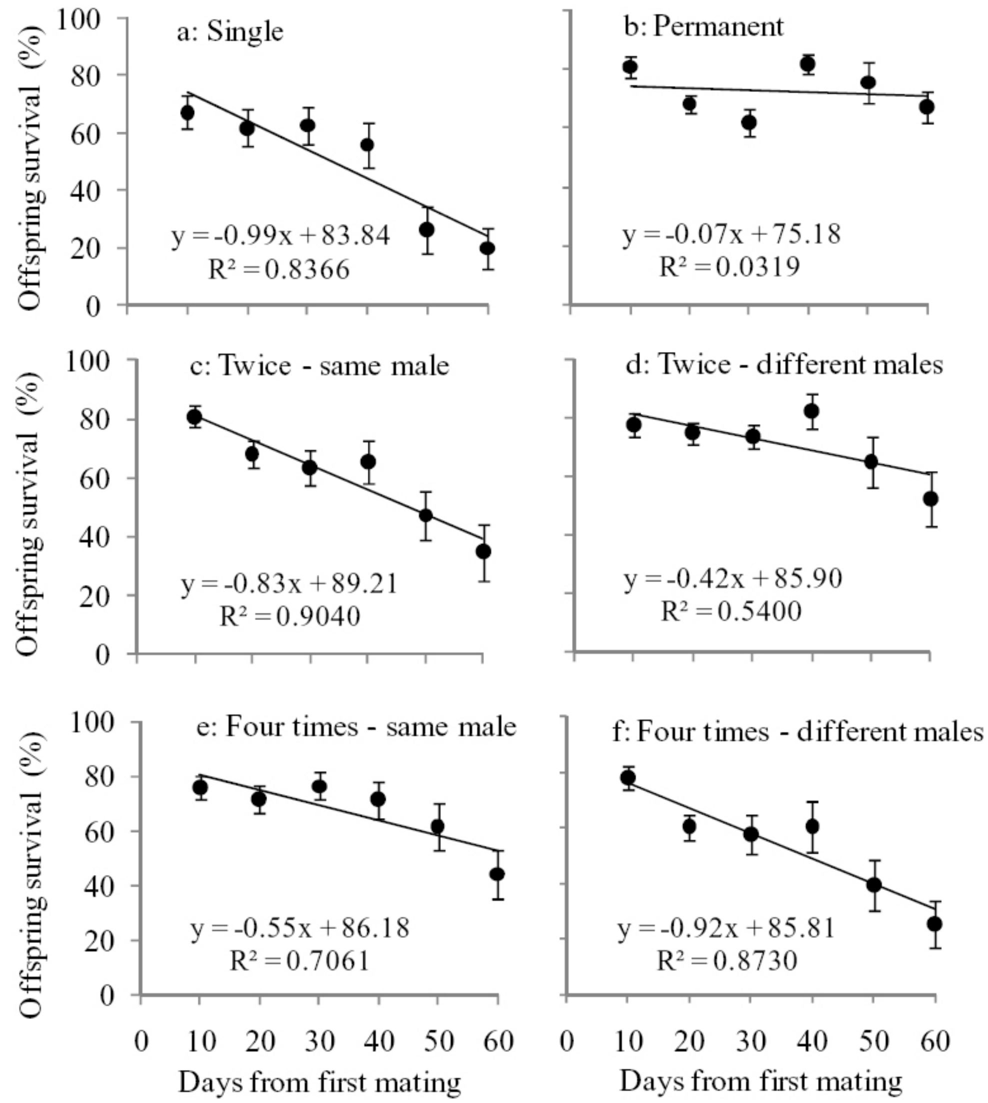

FIGURE 3: Fertility of *S. oryzae* females over 60 days in different mating treatments. All data are pooled for regression but means (±SE) are presented only.

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). 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 (Crudgington & 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.

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