Costs and Benefits of Multiple Mating in the Beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae).

by

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Abstract

T. J. B. Lobley-Taylor, 2000: **Costs and Benefits of Multiple Mating in the Beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae).**

Two geographical strains of *Callosobruchus maculatus* beetles with contrasting life histories were compared. Female *Callosobruchus maculatus* beetles were manipulated to experience four different mating treatments. Multiple mating in a Brazil strain increased fecundity but decreased longevity of females. Multiple mating by Brazil-strain parents also reduced the longevity of their singly mated offspring. In contrast, multiple mating in a South India strain had no effect on longevity or fecundity of female parents or their singly mated offspring.

South-India-strain females mated more frequently than Brazil-strain females when mating opportunities were limited but not when females had constant access to males. Mating frequency decreased in Brazil-strain females when presented with virgin males. In contrast, South-India-strain females mated more frequently on the second and third days when presented with virgin South-India-strain males. Brazil-strain males mated for longer and males transferred proportionally larger spermatophores than South-India-strain males. Spermatophore mass was positively correlated with male emergence mass for the South-India-strain males only.

These differences are interpreted in relation to the contrasting life-history strategies of the two strains and trade-offs between the fitness components of longevity and fecundity.

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CHAPTER 1

CHAPTER 1: INTRODUCTION

1.1 GENERAL BIOLOGY OF *CALLOSOBRUCHUS MACULATUS*

1.1.1 Origins and distribution

Callosobruchus maculatus (Fabricus) (*C. maculatus.*) is a species of beetle (order Coleoptera) of the family Bruchidae. Bruchidae belong to the superfamily Chrysomeloidea, which also includes the Chrysomelidae (mostly plant leaf and stem feeders) and the Cerambycidae (mainly wood and root borers living mostly on dead tissue) (Haines, 1991). The family Bruchidae is made up of nearly 1400 species of bean and pea beetles that live on seeds of wild and cultivated leguminous plants (Taylor, 1981) and breed in every continent except Antarctica (Southgate, 1979). The species can be distinguished by examining the arrangement of teeth and ridges on the hind femur and identification can be confirmed by examining the male genitalia (Haines, 1991). *C. maculatus* is indigenous to Africa, where it is still the dominant species of *Callosobruchus* (Haines, 1991), and Asia and it is capable of inhabiting both tropical and relatively cooler sub-tropical regions. *C. maculatus* has been introduced to Europe, North and South America and Australasia. The spread of *Callosobruchus spp.* is believed to have occurred with the movements of harvested legumes within the continents and extended by shipments transported in the Indian Ocean. The establishment of *C. maculatus* in the West Indies and South America may have been facilitated by the advent of the slave trade across the Atlantic (Southgate, 1978). Some of the geographically isolated strains of *C. maculatus* show distinct differences in their life histories and behaviour (review in Mitchell, 1990).

The larvae of *C. maculatus* are efficient in the destruction of seeds of the Leguminosae and are serious pests of agricultural seed stock and stored consumable seeds, consuming protein that would otherwise be eaten by man (Southgate, 1979). *C. maculatus* is a pest species of great economic importance, and is the major primary pest of stored seeds of *Vigna unguiculata* (cowpea or black eyed bean), *Lens culinaris* (lentil) and *Vigna radiata* (mung bean) (Haines, 1991).

Infestation by *Callosobruchus spp.* in most parts of Africa typically starts in the field and generally continues in the seed storage environment. With certain species field

infestation soon terminates under storage conditions but in the majority of cases continuous infestation during storage leads to severe losses of seeds and has contributed to the spread and establishment of exotic species through commerce and trade (Taylor, 1981). High levels of infestation can be generated even when one or two generations are passed on the host.

1.1.2 Life cycle

Female *C. maculatus* lay their eggs directly onto the surface of a seed. The egg is a flattened ovoid in shape and is protected by a covering exuded at the time of laying, which also serves to fix the egg firmly to the seed surface (Southgate, 1979). Within a week the larva hatches and burrows into the seed through the seed coat. The larva consumes part of the cotyledons and creates a chamber within the seed. Frass is produced and fills the egg case changing its appearance from translucent to white. The larva remains in the seed throughout all stages of larval development and does not migrate between seeds (Wijeratne, 1991). Consequently the pupal stage is passed within the seed also and a larva will undergo four moults before becoming a pupa (Howe and Currie, 1964). In preparation for pupation, the larva eats as close to the surface of the seed as possible without breaking through (Southgate, 1979). This results in the construction of a so-called window where the seed coat is so thin that the developing larva can be seen. The larva pupates with its mandibles facing the window and following pupation the adult pushes out through the window using its head and legs and may also chew around the edge of the window to aid emergence (Howe and Currie, 1964). Developmental period can vary with temperature and adults may remain within the seed for several days before emerging. Howe and Currie (1964) reported the optimal conditions for rapid development to be 32.5°C and 90% relative humidity (RH) for *C. maculatus*. In addition, they possess a wide tolerance for temperature and will complete development within the range 20-37.5°C. Beetles emerge as fully developed sexually mature adults some 23 days after the egg was originally laid, at 30°C and 70% RH (Giga and Smith, 1983). Adults are capable of mating at emergence and begin laying eggs soon after. Qi and Burkholder (1982) described a calling behaviour exhibited by females that was synchronised with pheromone release, which generally lasted for three to five minutes. Pheromone release was initiated soon after emergence and this behaviour continued for up to one

week. Highly absorbent paper disks exposed to calling females were shown to excite 70% of males compared to 22.5% for those exposed to non-calling females. In addition, pheromone release decreased significantly after mating.

1.1.3 Female oviposition behaviour

Female *C. maculatus* are capable of laying approximately 70 - 100 eggs if unlimited pristine seed are available. Female oviducts contain approximately 8 eggs on emergence and an additional 15 eggs are matured the following day. Further maturation takes place in the presence of mates and oviposition sites (Wilson and Hill, 1989). The number of eggs laid by females peaks on the first day and declines steadily with age (Credland and Wright, 1989). Since larvae cannot move between seeds, the oviposition decisions of females will be critical to the survival of those larvae. In this way, natural selection will favour individual females that can discriminate suitability of oviposition sites in order to maximise survival for their larvae. It has been shown that females can discriminate between potential seed host species, between seeds that already have eggs laid on them from those that do not, seeds already containing emergence holes and seeds bearing different numbers of eggs (Mitchell, 1975; Messina and Renwick, 1985; Smith and Lessells, 1985; Wilson, 1988, Mitchell and Thanthianga, 1990; Ofuya and Agele, 1989 and 1990). Females have also been observed to delay or to suppress oviposition (Dick and Credland, 1984) or to retain eggs if densities of eggs per seed in an environment approach two per seed (Credland, 1986). Finally, females tend to hyper-disperse eggs over seeds, which reduces the incidence of competition between larvae and can increase offspring survival (Smith and Lessells, 1985).

The majority of species of Bruchidae attach their seeds singly, directly onto the seed surface (Southgate, 1979). Some species (e.g. *Caryedon fasicatum*, Prevett, 1966; *Mimosestes amicus*, Mitchell, 1977; *Sitophilus spp.*, Utida, 1967) are known to clump eggs, however, whereas *Acanthoscelides obtectus* scatters eggs among harvested seeds (Southgate, 1979). In *Callosobruchus* the tendency is to distribute eggs uniformly (Mitchell, 1975). In contrast, the eggs of *Caryedon fasciatum* are grouped and exposed eggs were often attacked by trichogrammatid parasites but covered eggs were not (Prevett, 1966). Similarly, *Mimosestes amicus* was observed to deposit eggs on each other although Mitchell (1977) argues that *Mimosestes* only does this when

stressed. Parasites and dessication were responsible for 80% of eggs lost from this species, however, covered eggs survived (Mitchell, 1977). In species where larval mobility between seeds is feasible (e.g. *Sennius morosus*, Johnson, 1967) oviposition decisions and larval competition are less important when considered against species whose larvae are constrained to a single seed. Problems soon arise from clumping when several larvae are present in the same seed and compete for that resource. Generally larval survival decreases with increasing larval density in a seed (Smith and Lessells, 1985). In this case decisions must be made by the female in order to maximise larval survival and hence the females own fitness. These decisions must take into account restraints such as time, egg number and seed resource availability. If seeds are the sole constraint and there are trade-offs between the number of offspring produced and their expected survival then the most productive clutch size observed will maximise the product of clutch size and offspring survival (Lack, 1947). It will not pay females to exceed this optimal clutch size, as fitness will then decrease (Smith and Lessells, 1985). If time is the constraint then in a species such as *C. maculatus* where adults typically do not feed energy must be partitioned between searching costs and egg laying costs. Smaller clutch sizes are favoured so the females can increase searching time (Smith and Lessells, 1985). Overall, when larvae are restricted to single seeds and larval survival is density dependent as in *C. maculatus* the optimal behaviour that results is to distribute eggs homogeneously with respect to the resource (Smith and Lessells, 1985).

1.1.4 Lifespan and mortality

Adults are short lived, living approximately 8-12 days while in a seed storage environment where they do not feed. In field conditions the adults do not feed on the host plants but may consume nectar and water (Bellows, 1990), which could increase their survival long enough to allow for dispersal. Resources in a seed are limiting. Since larvae do not migrate between seeds and are restricted to the same seed the egg was laid on, two or more larvae will compete for that resource (food and space). This is important because adults rely upon the reserves built up during the feeding (larval) stages for reproduction and maintenance of the body. The number of eggs that a female can lay will also be limited by these reserves (Mitchell, 1990). Consequently,

larvae exposed to high levels of competition or poor resources may emerge as smaller adults (Credland *et al.*, 1986).

Various other factors are reported as having an impact on the development and survival of *C. maculatus*. The Hymenopteran wasp, *Uscana lariophaga* Steffan (Trichogrammatidae), has been recognised to parasitise bruchid eggs both in the field and the seed-storage environment (Sagnia, 1994; van Huis *et al.*, 1994). Sagnia (1994) recorded that the highest mortality occurred in the egg stage in field conditions in Niamey, Niger. Mortality was largely due to parasitism by *U. lariophaga*, which was reported to be the most important cause of mortality in the egg followed by egg disappearance. Together, these two factors accounted for losses of over three quarters of eggs that were laid. Eggs may have disappeared because of the action of rain, mechanical removal via abrasion of the seeds or wind, predatory ants and other insects, or solar heating. Natural death in the larval stage was found to be the third most important mortality factor. Conversely, larval parasitism by the Hymenopteran wasp *Eupelmus vuilleti* (Cwf.) (Eupelmidae) and natural death at the pupal stage was found to be the least important of those mortality factors identified. Other mortality factors simply termed physiological were those where no obvious cause of death could be established. Overall mortality was high in the field with only 6.1% of initial eggs surviving to emerge as adults. Southgate, (1978) also reported the vulnerability of Bruchid egg, larval and pupal stages to parasitism in the field to a number of species of Hymenoptera, for example, *Anisopteromalus calandrae* (Howard) and *Dinarmus basilis* (Rond.) (Pteromalidae). These species are also capable of attacking a number of other stored product insects. In terms of their efficacy as a biological control agent, Southgate (1978) argued that there was little evidence that parasitoids affect natural control on field populations of the Bruchidae but must play a part among the restraints in the field ecosystem.

1.1.5 Appearance

The beetles are small, mostly less than 5mm in length, and are sexually dimorphic. Females are larger than males, weighing approximately 4-6mg at emergence in comparison to 3mg for males. The elytra of females are patterned and a white stripe is visible on the last abdominal segment (pygidium) whereas the elytra and pygidium of males are generally a uniform tan colour (see Southgate *et al.*, 1957).

1.2   STRAINS AND LARVAL COMPETITION STRATEGY

The different interfertile geographical strains of *C. maculatus* show extreme competitive behaviours (Thanthianga and Mitchell, 1987). Two such geographical strains of *C. maculatus* were used in this present study, referred to here as Brazil and South India. These strains were chosen as they display differing life-history traits (summarised in Table 1.1), contrasting especially in those related to larval competition.

1.2.1   South India strain

The South India strain displays the following characteristics (Mitchell, 1991):

1   **Larvae show strict contest competition with typically one adult surviving per seed.**

If more than one larva is present in a single seed they will actively compete for the seed resource. Vibrations caused by a dominant larva's chewing may slow or arrest the development of other larvae until the dominant larva has emerged (Thanthianga and Mitchell, 1987). Alternatively, one larva may seek out, attack and kill other larvae within the seed. Toquenaga believed that the ultimate cause of single larva emergence from a seed was due to biting behaviour (pers. comm. to Broadhurst, 1996).

2   **Females disperse eggs uniformly over the available seeds and oviposition is strongly inhibited if the available seeds carry eggs.**

Hyper-dispersion of seeds decreases competition between larvae (Smith and Lessells, 1985). Females will not benefit from laying eggs on seeds already containing older larvae, as their offspring are less likely to survive due to larval competition. Instead it may benefit females to withhold laying eggs until after dispersal to an area with unexploited seeds (Mitchell, 1991). Messina (1991) observed that females retained eggs if pristine seeds were not available.

3   **Larger seeds are selected for oviposition** (Thanthianga and Mitchell, 1990).

4   **Fecundity is variable, ranging from 40 to 90 eggs.**

The cultures in Leicester were derived from Mitchell's (1991) stock, supplied by Frank Messina of Utah State University.

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1.2.2 Brazil strain

The Brazil strain, commonly referred to by others as the Campinas strain (e.g. Credland, 1986), displays contrasting traits compared to the South India strain and is characterised by the following features (see Broadhurst, 1996):

1 Larvae show relatively weak exploitation or scramble competition.

Larvae passively compete for the seed resource and typically many larvae emerge from a single seed (e.g. Credland *et al.* (1986) observed more than twelve adults emerging from a single seed).

2 Females lay more than one egg per seed and show an oviposition strategy located somewhere between random and uniform (Messina and Dickinson, 1993).

Females can afford to be relatively less discriminatory over oviposition sites due to passive larval competition.

3 Observed to lay more eggs than South India strain females (Messina and Mitchell, 1989).

Females are constrained more by the number of eggs that can be laid rather than number of oviposition sites available.

The cultures in Leicester were derived from a starter culture collected in Campinas, Brazil by the late Ben Southgate in the 1970s and maintained by the National Resources Institute in Chatham, England.

1.2.3 Summary comparison of the two strains

Nicholson (1954) was the first to describe the terms contest and scramble. As larval density increased, the number of scramble competitors (share resource) surviving initially increased to the point where the amount of resource available per individual fell below that necessary for development to be completed. Then the number of survivors decreased catastrophically. In contrast, the number of surviving contest competitors (single larva monopolises resource) remained constant irrespective of the initial larval density. Later Smith and Lessells (1985) applied the terms Attack (contest) and Avoid (scramble). They also demonstrated that either Avoid or Attack could be an evolutionary stable strategy but there was no stable mixed strategy. Attack-strategist larvae (e.g. South India) characteristically tunnelled to the centre of

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the seed and would bite and kill any other larvae encountered. In contrast, Avoid-strategist larvae (e.g. Brazil) tunnelled below the seed surface and avoided contact with other larvae. Only paper thin walls separated the larvae within the seed. The larval competition strategy has influenced the adult life history and resulted in different optimal solutions to the trade-off between the fitness traits of longevity and fecundity. As a result, the South India strain is characterised by a relatively lower fecundity and higher longevity than the relatively shorter lived but more fecund Brazil strain. The life-history strategies of the two strains are discussed further in sections 3.1 and 6.1.

Table 1.1. Comparison of life-history strategies for the Brazil and South India strains of *C. maculatus*.

Brazil	South India
Relatively shorter lived than South India	Relatively longer lived than Brazil
Larvae share seeds and resources (Scramble / Avoid strategists) and many larvae emerge from one seed	Larvae compete for resources (Contest / Attack strategists) and one larva emerges from each seed
Eggs distributed randomly over seeds	Eggs distributed uniformly over seeds
Lay relatively more eggs than South India	Lay relatively fewer eggs than Brazil

1.3 MULTIPLE MATING

The term 'multiple mating' used in this study implies that a male or female has mated more than once with either the same or a different individual. Throughout this thesis, the abbreviations MM and SM will be used as a shorthand expression for individuals that mate multiply and mate singly respectively.

1.3.1 Costs and benefits of MM

The evolution of anisogamy has led to differential investment in offspring by the two sexes. In most species, the female typically makes the largest contribution as eggs are generally more costly in terms of nutrients and energy whereas male gametes or sperm are considered to be numerous and relatively cheap. Female reproductive success therefore is constrained primarily by the number of eggs that she can produce and male reproductive success is constrained primarily by the number of females that he can inseminate (Alcock, 1993). Females may need to mate only once to fertilise

all of their eggs and further matings may actually incur costs. In 1948, Bateman demonstrated in *Drosophila melanogaster* that male reproductive success increased with an increasing number of matings. Female reproductive success, however, did not increase past the first mating. More recently it has been shown that female *Drosophila* are actually poisoned by components of male semen (Chapman *et al.*, 1995). In the light of this, female *Drosophila* would be expected to reduce the number of matings, however, wild female *D. melanogaster* from a Viennese population have been shown to mate with up to four to six males (Imhof *et al.*, 1998). In reality, MM by females is widespread in most insect species (Ridley, 1988). Recent research has focused upon mechanisms that increase or decrease an individual's reproductive success, for example, sperm competition and cryptic female choice. From this research it has been shown that there are many potential costs and benefits to both the males and females in a species exhibiting MM (see reviews in Alcock, 1993).

1.3.2 Potential costs and benefits for MM females

The main potential costs and benefits encountered by females who MM are summarised in Table 1.2.

Benefits to females

Females may re-mate to ensure that they have sufficient sperm to fertilise all of their eggs (Sax *et al.*, 1998), to ensure fertilisation success or to avoid post-mating isolation or incompatibility where two species are sympatric (Dempster, 1996; Gallant and Fairbairn, 1997; Zeh, 1997). Similarly, females may re-mate to promote genetic variability and to dilute possibly deleterious effects of inbreeding. Stockley *et al.* (1993) showed that inbreeding resulted in reduced fitness of offspring in voles. They found that if females could not distinguish close kin they copulated with several different males and it was argued that this would reduce the risks of all the offspring being sired by a close relative. By MM, individuals ensured that at least some offspring would develop normally and survive. Ward (1998) also argued that female yellow dung flies, *Scathophaga stercoraria* (L) chose between the sperm of different males depending upon environmental conditions in order to give their offspring the best chance of survival.

Females may receive a nutrient contribution from the male either during copulation as a nuptial gift (e.g. the spider, *Pisaura mirabilis* presents females with a prey item wrapped in silk, Lang, 1996), as part of a spermatophore (Boucher and Huignard, 1987) or attached to a spermatophore (e.g. bushcrickets, Simmons *et al.*, 1999). These contributions by males may be used to provision eggs or female somatic condition (Fox, 1993a; Boucher and Huignard, 1987; Takakura, 1999). In some cases males transfer substances that will deter predators when incorporated into eggs rather than a nutrient donation that increases egg size (LaMunyon, 1997).

By encouraging or engaging in MM, females can also promote inter-male competition for copulations (Savalli and Fox, 1999a) or enhance competition among sperm for fertilisation (Eberhard, 1996). Similarly, females with sperm-storage organs may be able to exercise post-mating choice and selectively choose the sperm of the best males to fertilise their offspring (Eberhard, 1996; Ward, 1998).

Costs to females

MM can be costly to females as time may be lost that could be spent searching for suitable oviposition sites, searching for food or dispersing, particularly if courtship or copulation is long (e.g. as in milkweed leaf beetles, Dickinson, 1988) or if male quality is to be assessed. Continual harassment by males can result in physical damage to the female or her eggs during oviposition. Injury sustained during courtship or copulation may decrease fecundity or egg-laying ability. In a seed eating bug, male harassment is costly and females often abandon the host plant, which is the only source of oviposition sites, when male density is high (McLain and Pratt, 1999).

Copulating pairs may be more at risk of predation and females could be exposed to sexually transmitted disease (STD) or parasites (STP). One such example is the transmission of *Spiroplasma poulsonii sp.* in *D. willistoni*. This vertically transmitted pathogen is responsible for male lethality resulting in the production of female-only progeny (Williamson *et al.*, 1999). Frequent pheromone calling by females to attract males may incur energetic costs, manifested in reduced longevity or fecundity. By calling with pheromones, females may also alert predators or parasitoids to their location.

Finally, males of some species have evolved ways of altering a female's physiology or behaviour resulting in a desired reproductive outcome for the male (see Eberhard, 1996). Ejaculates may contain components that stimulate oviposition and egg maturation, alter female refractory period and incapacitate rival males' sperm. However, in some cases these components also have the unfortunate side effect of being toxic to females resulting in reduced longevity (Chapman *et al.*, 1995; Partridge *et al.*, 1987) and reduced egg hatchability (Prout and Clark, 2000; Tregenza and Wedell, 1998). In the mite *Caloglyphus berlesei*, MM by females was found to reduce both longevity and fecundity (Radwan and Rysinska, 1999).

Table 1.2   Potential costs and benefits for females who MM.

Benefits to females	Costs to Females
1 An assurance of fertilisation	1 The need to assess male quality
2 Nutritional gain	2 Energetic cost
3 Avoidance of incompatibility	3 Time lost
4 Promote genetic variability	4 Physical damage
5 Promote inter-male competition at some level	5 STDs, STPs and increased predation risk
6 Disturbance and/or male harassment avoidance	6 Antiaphrodisiacs, egg production and maturation stimulants present in male seminal fluids, which may also have a toxic effect
7 Cryptic female mate-choice	

1.3.3   Potential costs and benefits for MM males

The main potential costs and benefits encountered by males that MM are summarised in table 1.3.

Benefits to males.

The most obvious benefit to males is from increased paternity as the number of offspring sired by a male is generally constrained only by the number of females that he can inseminate (Bateman, 1948). There are exceptions, however, for example in the pipefish *Syngnathus typhle* it is the male that cares for and provides nutrients for eggs in a specialised brood pouch. A single, large female can produce enough eggs to

fill the brood pouches of three males and can mature a fresh clutch of eggs faster than a male can raise one brood (Berglund *et al.*, 1986). Another potential benefit to males of MM is that it may increase the chances of successful fertilisations through sperm competition, diluting the effects of inbreeding and also avoiding incompatibility (as discussed for females above). Males may promote competition between females at some level such that females will compete to gain more matings (Bonduriansky and Brooks 1998; Simmons and Kvarnemo, 1997). Previous mating experience can also result in males being better able to secure additional matings when compared with virgin males (Teal *et al.*, 2000; Jouventin *et al.*, 1999).

Costs to Males.

Males risk injury during courtship or copulation, which may decrease the ability to re-copulate or transfer sperm and can even result in death (e.g. cannibalistic species such as mantids, Bukowski and Christenson, 2000; or spiders, Maxwell, 1999). In the sagebrush cricket females were observed to feed on the fleshy hind wings of males during copulation and to ingest haemolymph from the wounds created (Johnson *et al.*, 1999). The wounds were not fatal but reduced the chance of a male securing an additional mating as females may refuse to mate with males that cannot offer a chance to feed. Exposure to STDs and STPs is also increased through repeat matings. Energy will be expended on copulation, sperm and spermatophore production, courtship and searching for and assessing female quality (Schneider and Lubin, 1998). Energy budgets are particularly important for males who transfer large amounts of nutrients as part of a spermatophore (e.g. *Bruchidius dorsalis* males transfer ejaculates comprising 7% of their body weight to females during copulation, Takakura, 1999) or who present nuptial gifts to females (e.g. black tipped hanging flies, Thornhill, 1976).

Paternity may be lost after mating through processes such as sperm competition and males are forced to invest in other ways of securing more fertilisations and ensuring their paternity. These may include the production of compounds that increase female refractory periods or incapacitate rival males' sperm. Similarly, excess ejaculates may be produced such that males are more likely to pre-empt rival males and less likely to be pre-empted (Eady, 1995). Complex aedaegal structures may evolve enabling the removal of rival males sperm before males transfer their own (Waage, 1979). Pre- or post-copulatory guarding of females may also be necessary. Despite

all these methods, however, successful courtship, copulation and sperm transfer does not mean that a male will successfully sire the offspring if females exercise cryptic choice (Eberhard, 1996).

Table 1.3. Potential costs and benefits for males who MM.

Benefits to males		Costs to Males	
1	Increased paternity	1	The need to assess female quality
2	Avoidance of incompatibility	2	Cryptic female choice
3	Experience	3	Time lost
4	Promotion of inter-female competition at some level	4	Physical damage
5	Cryptic female choice	5	Energetic cost
		6	Sperm competition
		7	STDs, STPs and predation by female

1.3.4 Consequences of MM for *C. maculatus*

Both male and female *C. maculatus* beetles have been observed to MM. The obvious benefit to males is increasing the number of offspring that they sire. Eady (1991) demonstrated that when two male *C. maculatus* mate with the same female it is the last male to mate (last male sperm precedence, LMSP) that typically fathers the majority of offspring (approximately 82%). The cost or benefit of this process depends on whether the male is first or second to mate. Additionally, although LMSP still operates when female *C. maculatus* mate with up to three males (Lady and Tubman, 1996) it was observed to break down when female pseudoscorpions mated with more than three males (Zeh and Zeh, 1994). The result would be sperm mixing and a 'raffle' effect with each male who mated having an equal chance of fathering any offspring (Parker *et al.*, 1990). Mating with more than three males could result in a breakdown of LMSP for female *C. maculatus* with consequences for both male and female choice and intra- and inter-sexual competition.

Re-mating in order to replenish sperm stores is thought to be an unlikely reason for MM in *C. maculatus* (Fox, 1993a). Females may benefit nutritionally, however, by

metabolising spermatophore materials and excess sperm, which may allow females to invest more into egg production and maturation (Wilson *et al.*, 1999; Fox, 1993a). In *C. maculatus* fecundity has been observed either to increase (Savalli and Fox, 1999a; Wilson *et al.*, 1999; Ofuya, 1995; Fox, 1993a) or to be unaffected by female MM when compared with SM females (Fox, 1993a).

Given the life-history strategies of the two strains we could predict the responses of females to MM opportunities. Brazil-strain females might be expected to accept MM opportunities, as oviposition stimulation in these females would be favourable given the passively competitive nature of their larvae and their relatively low longevity. In contrast, oviposition stimulation in South-India-strain females could be detrimental to fitness given the highly competitive nature of their larvae and we might expect these females to reject MM opportunities if oviposition sites were limited.

1.4 RESEARCH EMPHASIS TO DATE

The majority of research to date on *C. maculatus* has been concerned with the pest status of the species, egg morphology and pest biology in relation to potential control methods. A large body of work has also been concerned with the variety of larval competition strategies within the *Callosobruchus* genus and also in the *C. maculatus* strains. More recently, the reproductive and behavioural aspects of mating behaviour, the mechanisms of sperm competition and the importance of MM have been studied (sections 3.1, 4.1 and 5.1).

C. maculatus was chosen for this present study as females display a number of features that makes them ideal model organisms. They are relatively short lived, with egg to adult stage taking only four weeks and adults living only about two weeks, enabling short generation time and quick experiment turn round time. The grain-store environment is easily replicated under laboratory conditions and cultures are easy to maintain. *C. maculatus* reproduces readily with large population numbers generated from few individuals making them good for replicates. The sexes are easily distinguished and both males and females MM. In addition, reproductive output, longevity and mating opportunities can easily be manipulated. Finally, *C. maculatus* has different geographical biotypes that display differing larval competition strategies and behaviours allowing for intra-specific comparisons.

CHAPTER 2

CHAPTER 2: GENERAL MATERIALS AND METHODS

2.1   GENERAL CULTURE

2.1.1   Source of insects

Two strains of *C. maculatus* were used in experiments. These were the South India strain and Brazil strain. The South India strain in Leicester was derived from Mitchell's (1991) stock, supplied by Frank Messina of Utah State University. The Brazil strain in Leicester was derived from a starter culture collected in Campinas, Brazil by the late Ben Southgate in the 1970s and maintained by the National Resources Institute in Chatham, England. Both strains have been maintained separately on cowpea (*Vigna unguiculata*) and mung bean (*Vigna radiata*) seeds at 30°C and 70% RH in the controlled temperature and humidity (CTH) room in the Department of Biology in the University of Leicester.

2.1.2   Source of plant material used as insect hosts

Goodness Foods™ supplied cowpeas and mung beans in 50kg or 25kg sacks. Plant material was stored at –20°C for a minimum of two weeks then allowed to reach room temperature before being used in experiments or culturing. Only cultures maintained on cowpeas were used in experiments.

2.1.3   Maintenance of insect stocks

Beetle cultures were kept in 500ml culture bottles containing approximately 280 g of either cowpea or mung bean seeds. Fluon (Poly-Tetra-Fluoro-Ethylene (PTFE) suspension) was painted inside on the neck of the bottle and prevented insects from crawling up the sides of the bottle and escaping when a bottle was opened. Bottles were sealed with a 55 mm circle of metal gauze followed by a 55mm diameter Whatman® circular filter paper number 1. Both were held in place with a plastic screw lid with a 38 mm diameter circle cut out of the lid. All jars were placed in trays containing Risella oil to prevent the spread of any mite infestation or insect contamination. All cultures were kept in the CTH room set at 30°C and 70% RH. Conditions in the CTH room did fluctuate from time to time and a range of 28-31°C and 58-83% RH was recorded. The life cycle from egg to adult took approximately 23 days under these conditions.

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Insects used for routine culture were removed from the old culture medium by sieving through brass-framed Endecott sieves with stainless steel mesh. Emerged adult beetles were separated from the culture medium through two sieves. The first sieve, mesh size of 2.8mm, retained the seeds and the second sieve, mesh size 850μm, retained the beetles. The second sieve also allowed frass and dust to pass through into a third tray below. Sieve edges were painted with Fluon to prevent beetles from crawling up the sides. Approximately 200 unsexed adults were transferred to fresh culture media. New cultures were re-derived approximately every 4 weeks. Following re-culture the old jar was placed in the freezer for two weeks before discarding.

2.2 EXPERIMENTAL PROCEEDURES

2.2.1 Sexing of beetles

Beetle were sexed using descriptions of male and female characters described in detail by Southgate *et al.* (1957) using a Nikon SMZ-1 binocular microscope. For a brief description of differences see section 1.15.

2.2.2 Handling and weighing of beetles

Beetles were moved using BioQuip feather weight forceps. Fresh mass at emergence of individual beetles was determined using a Cahn C-31 microbalance. Individual beetles were placed inside a receptacle constructed from disposable Gilson P20 tips (see Figure 2.1). This allowed beetles to be weighed negating the need to expose them to decreased temperatures (which slowed activity) or carbon dioxide (CO_2) gas. It has been reported for *C. subinnotatus* that exposure to carbon dioxide, nitrogen (N_2) or cold temperatures has an effect on recovery period from anaesthesia, time to copulation following recovery, mating duration and numbers of eggs laid by adult females (Mbata *et al.*, 1998).

2.2.3 Isolation of virgin beetles

In order to obtain virgin beetles, seeds containing eggs were removed from the main culture jars and isolated individually in lidded plates measuring 10x10x2 cm and divided into 25 cells each measuring 2x2x2 cm. This is referred to throughout the thesis as a 5 x 5 plate. Cultures were sieved as described in section 2.3. Seeds with clearly visible emergence windows were collected from the top sieve. These were

held in the (CTH) room and virgin adult males and females were isolated on emergence.

2.2.4 Cauterisation of males

This technique was developed in order to produce males that would show a normal courtship behaviour but could not transfer any seminal material. Males were first anaesthetised with CO_2 and then placed on their backs on a CO_2 pad under a dissecting microscope. Pressure was gently applied to the abdomen with a pair of BioQuip feather weight forceps, which resulted in partial eversion of the aedeagus. The aedeagus could be fully everted by gently pulling with a pair of watchmaker forceps and then severed using a scalpel blade. The cut surface was then cauterised with an RB light and cautery unit (704FP73). Finally, males were allowed to recover and placed with non-experimental females for 10 minutes for observation to ensure that copulation and sperm transfer was not possible. Anaesthetisation with CO_2 was unavoidable in this case since the beetles could not be restrained otherwise.

2.2.5 Food source

Feeding was used to extend the life of adults (Møller *et al.*, 1989b); in the wild, beetles may feed on nectar and water collected in flower heads. A dilute solution of yeast extract (Marmite) and honey dissolved in water was used as a food source for experiments in chapters 3 and 4. 0.25g of Marmite and 4g of Sainbury's pure clear blended honey was dissolved in 20ml of water. This was presented to the beetles in a 1.5ml Eppendorf lid containing absorbent cotton wool.

2.3 EXPERIMENTAL CONDITIONS

Unless otherwise stated all experiments and behavioural observations were carried out in the CTH room.

2.4 STATISTICAL ANALYSES

All statistical analyses were performed in Minitab version 12.

2.5 SUPPLIERS ADDRESSES

BioQuip: 17803, La Salle Avenue, Gardena, CA 90248, USA.

Cahn Headquarters: 5225, Verona Road, Building #1, Madison, WI 53711, USA (WWW.CAHN.com).

Cahn UK Distributor: Scientific and Medical Products Ltd., Shirley Institute, 856 Wilmslow Road, Didsbury, Manchester, M20 8RX, UK.

Fluon GP1: Whitford Plastics Ltd., 10 Christleton Court, Manor Park, Runcorn, Cheshire, WA7 1SU, UK.

Goodness Foods™, South March, Daventry, Northants, NN11 4PH, UK (www.goodness.co.uk/order.shtml).

Humbrol water based acrylic hobby paint: blue, 5025; yellow, 5024; red, 5060. Humbrol, Hull, England, UK.

Whatman International Ltd., Maidstone, England, UK.

Figure 2.1. The use of a 'beetle cage' made from P200 Gilson tips to allow the weighing of beetles without the need for exposure to carbon dioxide or chilling.
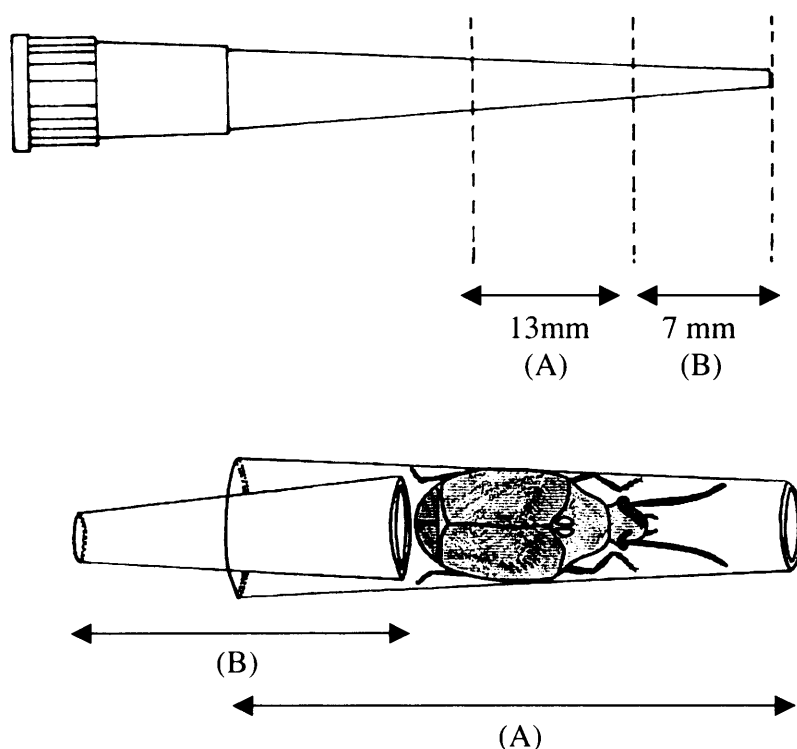
CHAPTER 3

CHAPTER 3: EFFECT OF MM ON LONGEVITY OF FEMALES AND THEIR OFFSPRING

3.1 INTRODUCTION

Adult longevity is an important component of fitness, even for relatively short-lived species such as *C. maculatus*. Living longer gives a female more opportunity to mature and lay eggs on seeds, and more time for selecting oviposition sites. The effect of MM by females on longevity is extremely variable between species. A decrease in longevity for females following multiple copulations compared to SM females has been observed in the moth *Heliocoverpa armigera* (Hou and Sheng, 1999), the mite *Caloglyphus berlesei* (Radwan and Rysinska, 1999), the Mexican fruit fly (Mangan, 1997), the European corn borer, *Ostrinia nubilalis*, Fadamiro and Baker, 1999) and *Drosophila melanogaster* (Chapman *et al.*, 1995 and Partridge *et al.*, 1987). In contrast, an increase in longevity after MM was observed in female dobsonflies, *Protohermes grandis* (Hayashi, 1998) and Mediterranean fruit flies, *Ceratitis capitata* (Whittier and Shelly, 1993). Finally, no effect on longevity from MM was observed in an arctiid moth, *Utetheisa ornatrix* (LaMunyon, 1997) or the green June beetle (Domek and Johnson, 1991).

The magnitude of the observed effect can also be affected by factors such as male status, (whether males are virgins or non-virgins), by the male's previous mating history (several previous matings or only one) and by the number of spermatophores received by females. For example, *C. maculatus* females mated to virgin males were found to live less long than those mated to non-virgins (Savalli and Fox, 1999a). The longevity of female Australian lycaenid butterflies decreased as the previous number of times mated by the male increased (Hughes *et al.*, 2000) and a negative relationship was observed between the number of spermatophores received and the longevity of female moths (Hou and Sheng, 1999).

To complicate matters further, the effect of MM on female longevity in *C. maculatus* has also yielded conflicting results. Savalli and Fox (1999a) observed that MM decreased female longevity, with the lowest longevity displayed by MM females mated with virgin males. In contrast, Fox (1993a; 1993b) found that MM increased female longevity when under starvation conditions. This effect disappeared, however,

when females were fed suggesting that the nutrient stressed females may have been re-mating in part to obtain nutritional benefit (Fox, 1993a). Male *C. maculatus* typically transfer oversized ejaculates such that the spermatheca, the site of sperm storage, is filled after one insemination as virgin males transfer approximately 46,000 (Eady, 1994b) to 56,000 (Eady, 1995) spermatozoa. This is 85% more than a female can hold in her spermatheca (Eady, 1994b), and this number decreases with each successive mating (Eady, 1995, Savalli and Fox, 1999a) although a male's ability to fertilise a female was not reported to decline until the male's fourth mating (Savalli and Fox, 1999a). Females may acquire a nutritional benefit from metabolising this excess sperm and rapid degradation of excess sperm in the bursa copulatrix has been observed for *C. maculatus* (Eady, 1994b). The incorporation of male-derived nutrients into both female tissue and eggs has been demonstrated in other species (*Caryedon serratus*, Boucher and Huignard, 1987; *Acanthoscelides obtectus*, Huignard, 1983; *Ellychnia corrusca* and *Photinus ignitus*, Rooney and Lewis, 1999).

It is relatively clear why females who receive a nutrient donation either as part of an ejaculate or as a nuptial gift could show increased longevity (e.g. Hayashi, 1998). In these cases additional nutrients received can be invested into eggs or into somatic maintenance or both, thus extending longevity over SM females who received less nutrient donation. When faced with a decrease in longevity, however, there are several possible explanations.

Repeat matings may further stimulate egg production and oviposition leading to a trade-off between the fitness traits of longevity and fecundity. Exposure to disease or toxic products or physical damage to females is also increased. Finally, repeat matings have even been shown to increase metabolism, which results in increased senescence. Metabolism increase can be measured via lipofuscin accumulation in the brain (Sohal, 1981) and lipofuscin accumulation is measurable in *C. maculatus* (Matt Sheehy, pers. comm.).

The trade-off hypothesis is central to the concept of life-history theory. Life-history traits, such as fecundity and longevity are components of fitness, which are the currency (decision variables) of life-history models. Møller *et al.* (1989a) argued that in an evolutionary context the trade-off concept implied that natural selection could not result in the unlimited increase of a particular fitness component. It is the idea

that you can never have something for nothing. One trait cannot be increased without losing out on another and trade-offs occur when the same resource (e.g. energy or time) limits two traits. Non-feeding *C. maculatus* adults have finite resources derived during the larval feeding stages to invest or allocate between reproduction and somatic maintenance. This could result in a trade-off between such fitness traits and the optimal life history is seen as the best compromise given a limited set of options (Sibly and Calow, 1986). Møller *et al.* (1989b) demonstrated in *C. maculatus* that fitness components of longevity and fecundity were traded-off, females displaying higher fecundity living less long than those with lower fecundity. Females were able to display adaptive phenotypic plasticity, however, and were able to oviposit maximally when fresh seeds were available, resulting in reduced longevity, but to refrain from ovipositing if oviposition sites were poor, therefore extending longevity (Møller *et al.*, 1990).

Exposure to toxic components of male spermatophores, physical damage to females from repeat matings and sexually transmitted pathogens/disease (STP/STD) can also reduce longevity. Spermatophores are produced from substances secreted by the male accessory glands along with additional peptide secretions that are passed along with the sperm (Davies, 1988). Females also possess accessory glands and these are responsible for secreting an adhesive substance used to cement eggs either to each other or to an oviposition site or substrate (Davies, 1988). These additional secretions passed by males along with the spermatophore can often induce favourable responses in female physiology and behaviour such as stimulating or increasing the rate of egg maturation, ovulation, oviposition or reducing receptivity to further matings (see reviews in Eberhard, 1996). In addition to stimulating such responses in the female some accessory-gland product or products may also be toxic or may have toxic by-products when broken down and metabolised, which may result in a decrease in longevity. Accessory-gland products in the beetle *Acanthoscelides obtectus* stimulated oviposition and egg maturation (Huignard, 1970), however, one component of the active fraction of the male spermatophore also had toxic effects (Huignard *et al.*, 1977). A decrease in longevity for MM *Drosophila melanogaster* females compared with SM females was observed as a result of exposure to the seminal products from the main cells in the male accessory glands and not by the presence of sperm itself (Chapman *et al.*, 1995). Seminal products can also have an

invasive nature inside the female and may cause localised damage to female physiology in order to reach their site of principal action. In the housefly *Musca domestica,* seminal product components break down cells lining the walls of the vaginal pouches and enter the female's haemolymph. These substances inhibit female sexual receptivity and may act directly on the brain (Leopold *et al.,* 1971). In the blowfly *Lucilia sericata,* males have saw-like structures on the male genitalic paraphallus, which may inject accessory gland-material into the haemolymph by piercing the walls of the bursa resulting in the development of granules that may be scar tissue (Lewis and Pollack, 1975). Repeated exposure to these invasive products may adversely affect female fitness, however any affect on longevity in the last two examples was not reported.

Female *C. maculatus* receive sperm during copulation via a spermatophore that forms inside the bursa copulatrix (Ouedrago, 1978 described in Eady, 1994a). The oversized ejaculate has consequences not only for sperm competition but, if it contains components that have toxic effects, may have additional consequences for female fitness. This assumes that any accessory gland products present vary proportionally with spermatophore size.

STPs or STDs can also be transferred to females as part of the ejaculate and have detrimental effects on female fitness (e.g. bacterial contamination in Red-winged Blackbird ejaculate, Westneat and Rambo, 2000; transmission of parasitic mites between individuals during copulation in a coccinellid beetle, Hurst *et al.,* 1995; *Spiroplasma poulsonii sp.* transmitted in *Drosophila willistoni* and resulting in unisexual female progeny, Williamson *et al.,* 1999).

The first aim of this chapter is to quantify whether there is a direct/indirect cost or benefit to female *C. maculatus* in terms of longevity through MM. SM versus MM was contrasted and also the effects of male exposure and copulation (exposure to seminal products) were contrasted using cauterised males. Similarly, the effect of MM with one male or several males was contrasted thus highlighting any effects of having multiple partners. The second aim is to examine any direct/indirect costs or benefits to the F_1. In other words, do the parent females mating experiences affect the F_1 offspring longevity? A third, very important aim is to compare the effects of MM between two strains of *C. maculatus* with differing life histories (see section 1.2.3 for

a description of life-histories). As in the rest of the thesis the abbreviations MM and SM will be used as a shorthand expression for individuals that mate multiply and mate singly respectively.

3.2   AIMS

This chapter aims to test the following null hypotheses.

- MM has no effect on female longevity.
- MM by female parent has no effect on the longevity of either F_1 or F_2 offspring.
- The effect of MM on female longevity is not affected by strain.

3.3   MATERIALS AND METHODS

3.3.1   The effect of MM on the longevity of females

Source of insects

Beetles were reared on Cowpeas and kept in culture jars in the CTH room. Virgin males and females were obtained using the isolation procedure outlined in section 2.2.3 and were used no more than 12 hours after emergence. Two strains were used. These are the South India strain and the Brazil strain. As discussed in section 1.2, Brazil is a scramble strain and South India is a contest strain.

Apparatus

Beetles were held in 120ml Beatson jars containing a food source and sealed with filter paper, gauze and a plastic screw lid as described for culture jars in section 2.1.3. Treatments were replicated 20 times. Where oviposition substrates were required each female had access to 100 fresh cowpea seeds. These were changed daily for the first four days and replaced with 100 pristine seeds. From days five until the females' death, 50 fresh seeds were provided and changed daily.

Food source

A food source was provided in the form of a honey and Marmite solution (section 2.2.5), which had been used successfully by Møller *et al.* (1989b). The food source was provided to ensure that females were not energy limited thus enhancing any other physiological effects of the mating treatment upon longevity.

Experimental design

Males and females were paired together at random, numbered and observed until mating took place. Pairs that did not mate within 20 minutes were discarded. Following mating, males and females were separated to prevent re-mating before being transferred at random to one of eight possible treatments. These are summarised in Table 3.1. The experiment had a factorial design with three main design elements or factors: mating treatment, continuation through the F_1 generation to the F_2 and access to seeds.

Mating treatment: Females were observed to mate once as described previously and then transferred to one of four possible mating treatments (Figure 3.1). Cauterised males (see section 2.2.4) were used in treatment B and provided the behavioural responses of a male without mating and sperm transfer taking place. Copulation was impossible since these males had their aedeagus removed. This would allow separation of any effects caused by the presence of the male and from effects caused by the seminal products transferred or mechanical stimulation. Cauterised males appeared to behave as intact males and attempted to mate as normal (pers. obs.).

Access to seeds: Females were either provided with unlimited seeds (more seeds than they could lay eggs on in one day) or no seeds at all. Since females that lay eggs generally live less long due to trade-offs, not allowing females to lay would extend their lifespan (Møller *et al.*, 1989b). This may highlight the effects of a mating treatment that might otherwise be masked by the strong longevity/fecundity trade-off. It also represented one aspect of the ancestral environment where oviposition substrates might have been limited.

Table 3.1. Summary of mating treatment received by female and whether access to seeds was granted.

Mating treatment	Access to seeds	Number of males females were exposed to	Number of potential matings
A	Y	1	single
B	Y	7	single
C	Y	7	multiple
D	Y	1	multiple
A	N	1	single
B	N	7	single
C	N	7	multiple
D	N	1	multiple

Figure 3.1. Mating treatments imposed upon females where exposure was to one or seven different males with SM and MM.

A: Female mated once and then kept alone until death.

B: Female mated once. Male cauterised and returned to female. The male was replaced with a new cauterised male daily for a further six days after which females were kept alone until death.

C: Female kept with the original male that she mated with. A new male replaced the old male for a further six days after which the female was kept alone until death.

D: Female kept with the same intact male that she originally mated with for seven days. After seven days the male was removed and the female was kept alone until death.

3.3.2 The effect of MM by parent females on the longevity of the F_1 offspring

Source of insects

Seeds containing eggs laid on days three and four of the parental experiment were isolated in 5 x 5 plates (described in section 2.2.3) to be used in the experiment. Individuals from day three and four were chosen since parent females would have been exposed to the mating treatment for several days. Offspring isolated from seeds bearing one egg were chosen at random from a pool of offspring collected from the 20 females in the same treatment. Offspring from seeds containing more than one egg were discarded in order to avoid any effects of larval competition on an individual's fitness.

Apparatus

As described in section 3.3.1 except that no food source was provided.

Experimental design

Non-sibling males and females were weighed at emergence, paired at random and allowed to mate. Beetles were separated after mating. Females were transferred to their experimental jar alone and males were held individually in 1.5 ml Eppendorf tubes. All females experienced mating treatment A only and were labelled according the treatment experienced by their parent Female parent. Seeds were changed daily as described in section 3.3.1 and the day of death was recorded.

3.3.3 The effect of MM by the grandmother on the longevity of the F_2 offspring

Source of insects

Seeds containing single eggs laid on days three and four by the F_1 generation were isolated into 5 x 5 plates and held in the CTH room until the beetles emerged. On emergence virgin beetles were sexed and isolated. As before, beetles were isolated with respect to the original parent treatment such that individuals from treatment A were paired with others from A and so on.

Experimental design

Non-sibling males and females were paired at random and allowed to mate. After mating beetles were separated and held individually in 1.5 ml Eppendorf tubes. As there was no effect of the parent female treatment on the fecundity of the F_1 and the greatest effect of longevity was observed when females did not oviposit (ancestral environment) F_2 offspring were not provided with seeds. Beetles were checked daily and the day of death was recorded. No food source was provided.

3.4   RESULTS

3.4.1   The effect of MM on the longevity of females

First, the effect of seed availability and hence oviposition was examined with respect to the longevity of females. Second, the effect of mating treatment on longevity in concurrence with oviposition was examined. Since all females were mated at least once and would readily lay eggs if provided with seeds those females with access to seeds are referred to as ovipositing females and those females denied access to seeds are referred to as non-ovipositing females. Non-ovipositing females were not observed to lay eggs on the sides of the glass jars in which they were kept. The

strains will be considered separately with the Brazil strain being examined first then the South India strain. All data were log transformed and analysed using either ANOVA (GLM) or ANCOVA (analysis of covariance) with beetle emergence mass as a covariate in Minitab version 12. The data were also analysed using survival analysis in SPlus, however, in comparison to the method used, the results obtained were unaffected by the analysis method.

Seed availability: Brazil-strain females

Seed availability and hence the opportunity for a female to oviposit had a significant effect on longevity. Non-ovipositing females lived significantly longer than ovipositing females with non-ovipositing females living an average of 10 days longer. This was also found to be true for females within the four mating treatments with non-ovipositing females living significantly longer than ovipositing females (Table 3.2). Note the difference in longevity between ovipositing and non-ovipositing females within the treatments. In A, B and D the difference is an average of 11 to 12 days whereas in treatment C the average difference is only 5 days. Thus a greater reductive effect on longevity is seen in treatment C. Although non-ovipositing females still lived significantly longer than ovipositing females the difference of 5 days is only half that seen in the other three treatments.

Seed availability: South-India-strain females

The longevity of non-ovipositing females was compared to that of ovipositing females and the results were similar to those of the Brazil strain such that the presence of an oviposition substrate had a significant effect on longevity. Non-ovipositing females lived significantly longer than ovipositing females irrespective of treatment (table 3.3).

Table 3.2. Mean longevity in days for Brazil-strain females experiencing one of four different mating treatments when seeds were present or absent.

(a)   Means for log transformed data and SED values. SED values are standard errors of the difference between the means.

Treatment	Significance level	Seeds present	Seeds absent	SED
SM alone (A)	$F_{(1,37)} = 67.89$ p<0.001	2.652	3.282	0.0539
SM 7 males (B)	$F_{(1,37)} = 52.15$ p<0.001	2.524	3.192	0.0654
MM 7 males (C)	$F_{(1,37)} = 8.20$ p<0.05	2.478	2.842	0.0895
MM 1 male (D)	$F_{(1,37)} = 77.05$ p<0.001	2.387	3.095	0.0567
Average*	$F_{(1,151)} = 156.37$ p<0.001	2.510	3.103	0.0336

*see Table 1, appendix 1 for full ANOVA table.

(b)   Back transformed means.

Treatment	Seeds present	Seeds absent	Difference in longevity
SM alone (A)	14.182	26.629	12.447
SM 7 males (B)	12.478	24.337	11.859
MM 7 males (C)	11.917	17.150	5.532
MM 1 male (D)	10.881	22.087	11.206
Average*	12.305	22.265	9.96

Table 3.3. Mean longevity in days for South-India-strain females experiencing one of four different mating treatments when seeds were present or absent.

(a)   Means for log transformed data and SED values. SED values are standard errors of the difference between the means.

Treatment	Significance level	Seeds present	Seeds absent	SED
SM alone (A)	$F_{(1,37)} = 27.04$ p<0.001	2.159	2.489	1.044
SM 7 males (B)	$F_{(1,37)} = 21.26$ p<0.001	2.196	2.598	1.064
MM 7 males (C)	$F_{(1,37)} = 23.98$ p<0.001	2.179	2.644	1.069
MM 1 male (D)	$F_{(1,37)} = 19.43$ p<0.001	2.179	2.669	1.082
Average*	$F_{(1,151)} = 84.29$ p<0.001	2.179	2.599	0.0322

*See Table 2, appendix 1 for full ANOVA table.

(b)   Back transformed means.

Treatment	Seeds present	Seeds absent	Difference in longevity
SM alone (A)	8.662	12.049	3.387
SM 7 males (B)	8.989	13.437	4.448
MM 7 males (C)	8.837	14.069	5.232
MM 1 male (D)	8.837	14.426	5.588
Average*	8.837	13.450	4.613

Mating treatment

For the analysis it was important to separate any effect on longevity caused by the number of matings that a female had from the effect of the number of males that she encountered. Thus the effect of SM or MM opportunities on longevity was analysed by combining the results from treatments A and B and contrasting them against the combined results from treatments C and D. Similarly to see the effect on longevity of being exposed to one or seven males the results from treatments A and D were combined and contrasted against the combined results from treatments B and C. The data were also analysed according to whether seeds were provided or not.

Number of matings: Brazil-strain females

SM females lived significantly longer than MM females irrespective of whether seeds were either present or absent (Table 3.4). In all three analyses mean longevity was higher for non-ovipositing females than for ovipositing females. Ovipositing SM females lived an average of 2 days longer than MM females and non-ovipositing SM females lived an average of 5.9 days longer than MM females. Furthermore, the significant difference in longevity between MM and SM treatments was greater when seeds were absent compared to when seeds were present.

Number of matings: South-India-strain females

The longevity of the South-India-strain females was unaffected by the number of mating opportunities. MM females showed neither increased nor decreased longevity over SM females (Table 3.5). This contrasts with the significant difference in longevity caused by mating opportunity observed for the Brazil-strain females.

Table 3.4. Mean longevity in days for three analyses where MM Brazil-strain females have significantly reduced longevity over SM Brazil-strain females. SED values are standard errors of the difference between the means. The data were log transformed.

Seed availability	Mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
Seeds present (1)	SM	13.330	2.590 (0.0449)	$F_{(1,75)} = 6.90$
	MM	11.280	2.423 (0.0449)	p<0.05
Seeds absent (0)	SM	25.483	3.238 (0.0503)	$F_{(1,75)} = 13.44$
	MM	19.570	2.974 (0.0503)	p<0.001
(1) & (0) combined	SM	18.486	2.917 (0.0336)	$F_{(1,151)} = 21.70$
	MM	14.820	2.696 (0.0336)	p<0.001

Full ANOVA tables are shown in Tables 1, 3 and 4, appendix 1.

Table 3.5. Mean longevity in days for SM and MM South-India-strain females. SED values are standard errors of the difference between the means. The data were log transformed.

Seed availability	Mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
Seeds present (1)	SM	8.776	2.172 (0.0290)	$F_{(1,75)} = 0.03$
	MM	8.837	2.179 (0.0290)	p>0.05
Seeds absent (0)	SM	12.782	2.548 (0.0576)	$F_{(1,75)} = 1.79$
	MM	14.253	2.657 (0.0576)	p>0.05
(1) & (0) combined	SM	10.602	2.361 (0.0321)	$F_{(1,151)} = 1.52$
	MM	11.212	2.417 (0.0321)	p>0.05

Full ANOVA tables are shown in Tables 2, 5 and 6, appendix 1.

Number of males: Brazil-strain females

The number of males encountered by a female did not significantly affect longevity when seeds were provided ($F_{(1,75)} = 0.05$, p>0.05). When seeds were absent, however, the number of males that a female was exposed to did have a significant effect on longevity and females exposed to only one male lived significantly longer than those exposed to seven males ($F_{(1,75)} = 5.58$, p<0.05). Means for longevity in days are summarised in Table 3.6. Non-ovipositing females exposed to only one male lived an average of 3.7 days longer than those exposed to seven males.

Number of males: South-India-strain females

The number of males encountered by South India strain females had no significant effect on the longevity of ovipositing ($F_{(1,75)} = 0.34$, p>0.05) nor non-ovipositing females ($F_{(1,75)} = 0.10$, p>0.05). Means for longevity in days are shown in Table 3.7.

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Table 3.6. Mean longevity in days for Brazil-strain females exposed to one or seven males. SED values are standard errors of the difference between the means. The data were log transformed.

Seed availability	Number of males encountered	Back transformed mean	Transformed mean (SED)	Significance level
Seeds present (1)	1	12.34	2.513 (0.0449)	$F_{(1,75)} = 0.05$
	7	12.18	2.500 (0.0449)	$p>0.05$
Seeds absent (0)	1	24.26	3.189 (0.0498)	$F_{(1,75)} = 5.58$
	7	20.55	3.023 (0.0498)	$p<0.05$
1&0 combined	1	17.32	2.852 (0.0336)	$F_{(1,151)} = 3.64$
	7	15.82	2.761 (0.0336)	$p>0.05$

Full ANOVA tables are shown in Tables 1, 3 and 4, appendix 1.

Table 3.7. Mean longevity in days for South-India-strain females exposed to one or seven males. SED values are standard errors of the difference between the means. The data were log transformed.

Seed availability	Number of males encountered	Back transformed mean	Transformed mean (SED)	Significance level
Seeds present (1)	1	8.750	2.169 (0.0290)	$F_{(1,75)} = 0.10$
	7	8.864	2.182 (0.0290)	$p>0.05$
Seeds absent (0)	1	13.184	2.579 (0.0576)	$F_{(1,75)} = 0.34$
	7	13.818	2.626 (0.0576)	$p>0.05$
1&0 combined	1	10.75	2.375 (0.0321)	$F_{(1,151)} = 0.36$
	7	11.06	2.403 (0.0321)	$p>0.05$

Full ANOVA tables are shown in Tables 2, 5 and 6, appendix 1.

Individual treatment and parent female longevity

The significance of emergence mass as a predictor of longevity was found to be variable with seed availability, strain and individual treatment and was excluded from the following analyses for simplicity and consistency of tests used. Therefore, comparisons of individual treatments were by T test.

Brazil strain: Ovipositing females

MM females exposed to one (D) or seven (C) males lived significantly less long than SM females were exposed to only one (A) male ($T_{(33)}=3.53$, $p<0.01$ and $T_{(37)}=2.07$, $p<0.05$ respectively). There was no significant difference in longevity between SM females exposed to one (A) or seven (B) males ($T_{(36)}=1.28$, $p>0.05$) or between MM females exposed to one (D) or seven (C) males ($T_{(33)}=1.11$, $p>0.05$). There was also no significant difference between MM females exposed to one (D) or seven (C) males

and SM females exposed to seven (B) males ($T_{(29)}$=1.55, p>0.05 and $T_{(36)}$=0.54, p>0.05 respectively).

Brazil strain: Non-ovipositing females

MM females that were exposed to either one (D) or seven (C) males lived significantly less long than females who SM and were exposed to only one (A) male ($T_{(30)}$=2.25, p<0.05 and $T_{(23)}$=3.66, p<0.01 respectively). The number of males encountered by SM females did not significantly affect female longevity ($T_{(37)}$=1.48, p>0.05). Similarly the number of males encountered by MM females did not significantly affect female longevity ($T_{(31)}$=1.96, p>0.05). There was also no significant difference between MM females exposed to one male (D) and SM females exposed to seven males (B) ($T_{(31)}$=1.15, p>0.05). MM females exposed to seven males (C), however, lived significantly less long than SM females exposed to seven males (B) ($T_{(24)}$=2.92, p<0.01).

Survival curves for ovipositing and non-ovipositing Brazil-strain females are shown in Figure 3.2. It can be seen that the largest effect on longevity was when seeds were absent (-). Females in MM treatments began to die at days 8 (C) and 10 (D) compared to females in SM treatments who began to die at days 18 (B) and 22 (A).

South India: Ovipositing and non-ovipositing females

The individual treatment did not affect female longevity significantly when seeds were present (F $_{(1,75)}$ = 0.13, p>0.05) or absent (F $_{(1,75)}$ = 0.92, p>0.05). Survival curves for ovipositing and non-ovipositing South-India-strain females are shown in Figure 3.3.

Figure 3.2. Survival curves for Brazil-strain females exposed to one of four mating treatments: SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D); when seeds were either present (+) or absent (-).
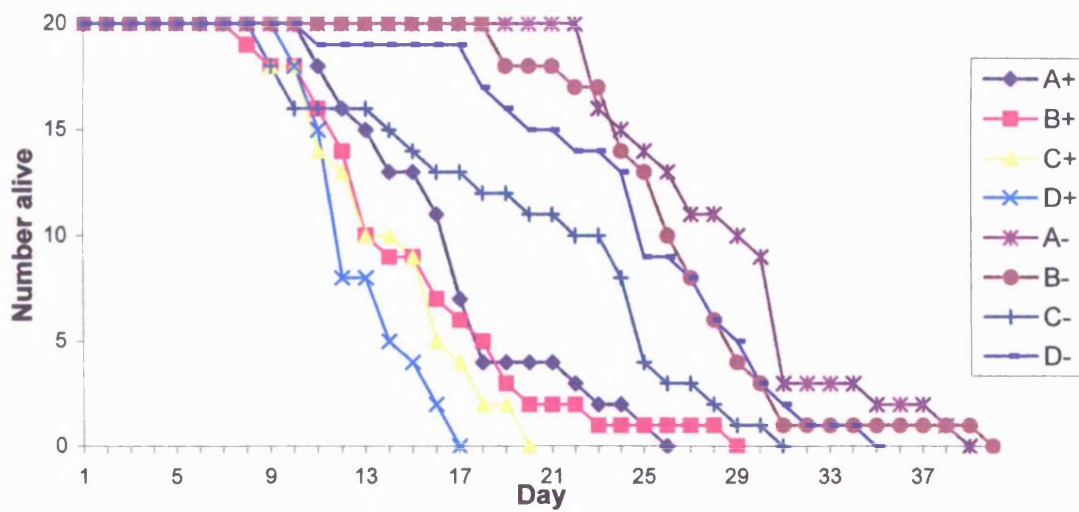
Figure 3.3. Survival curves for South-India-strain females exposed to one of four mating treatments: SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D); when seeds were either present (+) or absent (-).
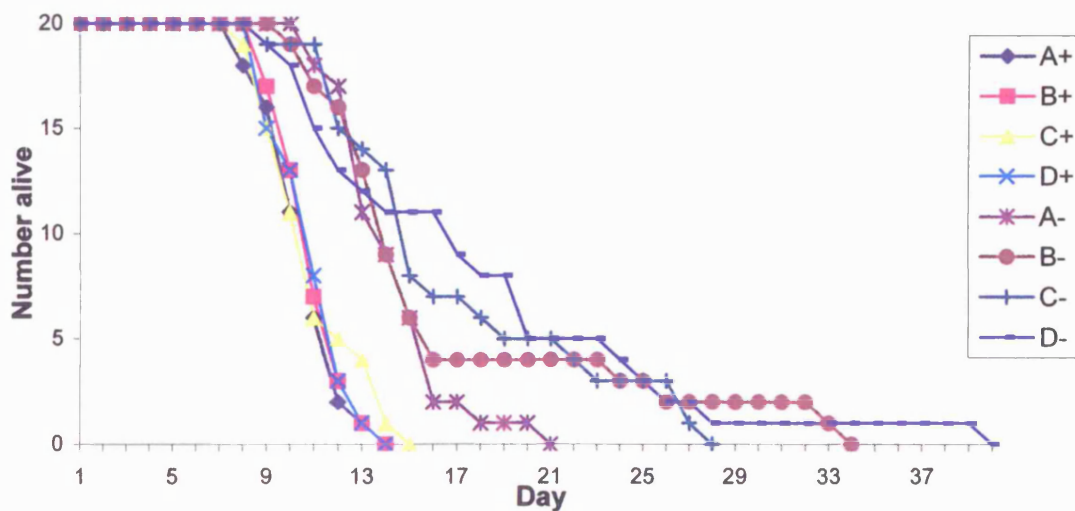
3.4.2 The effect of MM by parent females on the longevity of the F_1 offspring

All F_1 offspring were treated identically so that any observed effect on longevity would represent carry-over effects from the treatment incurred by the parent female.

Seed availability: F_1 Brazil-strain females

The presence of seeds and hence the opportunity to oviposit had a highly significant effect on longevity with non-ovipositing females living an average of 9 days longer than ovipositing females ($F_{(1,151)}$ = 452.60, p<0.001). Within the four mating treatments seed availability had a significant effect on longevity with non-ovipositing females living significantly longer than ovipositing females (Table 3.8). Non-ovipositing females lived an average of 8 to 10 days longer than ovipositing females.

Seed availability: F_1 South-India-strain females

Non-ovipositing females lived significantly longer than ovipositing females ($F_{(1,151)}$ = 118.07, p<0.001) with non-ovipositing females living an average of 4 days longer (Table 3.9). The effect of the parent mating treatment in conjunction with seed availability also affected longevity. F_1 females with MM mothers (treatments C and D) and SM mothers exposed to seven males (treatment B) lived an average of 4 to 5 days longer when seeds were absent compared to F_1 females with seeds. When F_1 females had SM mothers kept alone (treatment A), however, there was no significant difference in the longevity of their ovipositing and non-ovipositing female F_1 offspring ($F_{(1,37)}$ = 2.57, p>0.05).

Table 3.8. Mean longevity in days for SM F_1 Brazil-strain females when seeds were present or absent and whose female parent had experienced one of four different mating treatments.

(a)   Means for log transformed data and SED values.   SED values are standard errors of the difference between the means.

Female parent mating opportunity	Significance level	Seeds present	Seeds absent	SED
SM alone (A)	$F_{(1,37)} = 121.79$ p<0.001	2.282	2.962	0.0421
SM 7 males (B)	$F_{(1,37)} = 182.00$ p<0.001	2.321	3.010	0.0363
MM 7 males (C)	$F_{(1,37)} = 55.75$ p<0.001	2.202	2.823	0.0592
MM 1 male (D)	$F_{(1,37)} = 191.62$ p<0.001	2.258	2.883	0.0315
Average*	$F_{(1,151)} = 452.60$ p<0.001	2.267	2.918	0.0216

*See Table 7, appendix 1 for full ANOVA table.

(b)   Back transformed means.

Female parent mating opportunity	Seeds present	Seeds absent	Difference in longevity
SM alone (A)	9.796	19.337	9.540
SM 7 males (B)	10.186	20.287	10.102
MM 7 males (C)	9.043	16.827	7.784
MM 1 male (D)	9.564	17.868	8.304
Average*	9.650	18.504	8.854

Table 3.9. Mean longevity in days for SM F_1 South-India-strain females when seeds were present or absent and whose female parent had experienced one of four different mating treatments.

(a)   Means for log transformed data and SED values.   SED values are standard errors of the difference between the means.

Female parent mating opportunity	Significance level	Seeds present	Seeds absent	SED
SM alone (A)	$F_{(1,37)} = 2.57$ p>0.05	2.273	2.387	0.0503
SM 7 males (B)	$F_{(1,37)} = 47.34$ p<0.001	2.205	2.586	0.0392
MM 7 males (C)	$F_{(1,37)} = 52.93$ p<0.001	2.171	2.649	0.0468
MM 1 male (D)	$F_{(1,37)} = 46.66$ p<0.001	2.148	2.602	0.0468
Average*	$F_{(1,151)} = 118.07$ p<0.001	2.200	2.554	0.0230

*See Table 8, appendix 1 for full ANOVA table.

(b)   Back transformed means.

Female parent mating opportunity	Seeds present	Seeds absent	Difference in longevity
SM alone (A)	9.708	10.881	1.172
SM 7 males (B)	9.070	13.277	4.206
MM 7 males (C)	8.767	14.140	5.373
MM 1 male (D)	8.568	13.491	4.923
Average*	9.025	12.858	3.833

Number of matings by female parent: Brazil-strain F_1.

The mating opportunity experienced by the female parent had an effect on longevity that was carried over into the longevity of the F_1 offspring. Female offspring of SM female parents lived significantly longer than female offspring of MM female parents irrespective of whether F_1 females had access to seeds or not (Table 3.10). Ovipositing F_1 females of SM female parents lived an average of 0.65 days longer than F_1 offspring of MM female parents. For non-ovipositing females this increased to an average of 2.25 days longer to live for F_1 offspring of SM female parents. The parent-mating opportunity also significantly affected the longevity of the F_1 male offspring. F_1 offspring from SM females lived significantly longer than those F_1 offspring of MM females (Table 3.11). Male F_1 offspring of SM females lived an average of 2.8 days longer than male offspring from MM females.

Number of matings by female parent: South-India-strain F_1

The parent mating treatment had a significant effect on F_1 female longevity but whether females lived more or less long depended upon seed availability. For ovipositing F_1 female offspring of MM mothers, longevity was decreased by 0.6 days over F_1 female offspring of SM mothers ($F_{(1,75)} = 8.76$, p <0.05). Conversely, for non-ovipositing F_1 female offspring of MM mothers, longevity was increased by 1.7 days over F_1 female offspring of SM mothers ($F_{(1,75)} = 5.52$, p<0.05). Mean longevity in days is shown in Table 3.10. The longevity of South-India-strain F_1 male offspring was unaffected by the mating treatment of the parent female (Table 3.11). This contrasts with the effect seen on the longevity of the Brazil- and South-India-strain F_1 female offspring and also the Brazil-strain F_1 male offspring.

Table 3.10. Mean longevity in days for SM F_1 females whose female parent was provided with an opportunity for either SM or MM. SED values are standard errors of the difference between the means. The data were log transformed.

(a)   Brazil strain.

Seed availability	Female parent mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
Seeds present (1)	SM	10.014	2.304 (0.023)	$F_{(1,75)} = 4.08$
	MM	9.365	2.237 (0.023)	$p<0.05$
Seeds absent (0)	SM	19.609	2.976 (0.036)	$F_{(1,75)} = 5.54$
	MM	17.357	2.854 (0.036)	$p<0.05$
1&0 combined	SM	14.013	2.640 (0.022)	$F_{(1,151)} = 9.78$
	MM	12.743	2.545 (0.022)	$p<0.05$

Full ANOVA tables are shown in Tables 7, 8 and 9, appendix 1.

(b)   South India strain.

Seed availability	Female parent mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
Seeds present (1)	SM	9.469	2.248 (0.023)	$F_{(1,75)} = 8.76$
	MM	8.908	2.154 (0.023)	$p<0.05$
Seeds absent (0)	SM	12.013	2.486 (0.040)	$F_{(1,75)} = 5.52$
	MM	13.749	2.621 (0.040)	$p<0.05$
1&0 combined	SM	10.665	2.367 (0.023)	$F_{(1,151)} = 0.44$
	MM	10.892	2.388 (0.023)	$p>0.05$

Full ANOVA tables are shown in Tables 10, 11 and 12, appendix 1.

Table 3.11. Mean longevity in days for SM F_1 males kept in the absence of seeds, whose female parent was provided with an opportunity for either SM or MM. SED values are standard errors of the difference between the means. The data were log transformed.

(a)   Brazil strain.

Female parent mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
SM	18.746	2.931 (0.048)	$F_{(1,32)} = 6.03$
MM	15.975	2.771 (0.048)	$p<0.05$

Full ANOVA table is shown in Table 13, appendix 1.

(b)   South India strain.

Female parent mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
SM	16.216	2.786 (0.046)	$F_{(1,115)} = 1.26$
MM	17.444	2.859 (0.046)	$p>0.05$

Full ANOVA table is shown in Table 14, appendix 1.

Number of males encountered by female parent: Brazil-strain F_1

The number of males that a female parent was exposed to had no significant effect upon the longevity of the F_1 female offspring whether the F_1 females were able to

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oviposit ($F_{(1,75)}$ = 0.00, p>0.05) or not ($F_{(1,75)}$ = 0.09, p>0.05). There was also no effect of female parent treatment on the longevity of the F_1 male offspring either ($F_{(1,32)}$ = 0.01, p>0.05). Mean longevities in days are shown in Tables 3.12 for the female F_1 and 3.13 for the male F_1 offspring.

Number of males encountered by female parent: South-India-strain F_1

The number of males that a female parent was exposed to had a significant effect only on non-ovipositing F_1 offspring. Non-ovipositing F_1 female offspring of female parents that were exposed to seven males lived 1.6 days longer than offspring of female parents exposed to only one male ($F_{(1,75)}$ = 4.61, p<0.05). In contrast when F_1 females were able to oviposit, the female parent treatment had no significant effect upon offspring longevity ($F_{(1,75)}$ = 0.79, p>0.05). No effect of female parent treatment was observed on the male F_1 offspring ($F_{(1,115)}$ = 0.80, p>0.05). Mean longevities in days are shown in Tables 3.12 for the female F_1 and 3.13 for the male F_1 offspring.

Table 3.12. Mean longevity in days for SM F_1 females whose female parent was exposed either to one or seven males. SED values are standard errors of the difference between the means. The data were log transformed.

(a)   Brazil strain.

Seed availability	Number of males mother exposed to	Back transformed mean	Transformed mean (SED)	Significance level
Seeds present (1)	1	9.689	2.271 (0.024)	$F_{(1,75)}$ = 0.00
	7	9.679	2.270 (0.024)	p>0.05
Seeds absent (0)	1	18.302	2.907 (0.036)	$F_{(1,75)}$ = 0.09
	7	17.357	2.922 (0.036)	p>0.05
1&0 combined	1	13.330	2.590 (0.022)	$F_{(1,151)}$ = 0.04
	7	13.410	2.596 (0.022)	p>0.05

Full ANOVA tables are shown in Tables 7, 8 and 9, appendix 1.

(b)   South India strain.

Seed availability	Number of males mother exposed to	Back transformed mean	Transformed mean (SED)	Significance level
Seeds present (1)	1	9.16	2.215 (0.023)	$F_{(1,75)}$ = 0.79
	7	8.91	2.187 (0.023)	p>0.05
Seeds absent (0)	1	12.09	2.492 (0.040)	$F_{(1,75)}$ = 4.61
	7	13.67	2.615 (0.040)	p<0.05
1&0 combined	1	10.52	2.353 (0.023)	$F_{(1,151)}$ = 2.18
	7	11.05	2.402 (0.023)	p>0.05

Full ANOVA tables are shown in Tables 10, 11 and 12, appendix 1.

Table 3.13. Mean longevity in days for SM F_1 males kept in the absence of seeds, whose female parent was exposed either to one or seven males. SED values are standard errors of the difference between the means. The data were log transformed.

(a)   Brazil strain.

Number of males female parent exposed to	Back transformed mean	Transformed mean (SED)	Significance level
1	17.236	2.847 (0.048)	$F_{(1,32)} = 0.01$
7	17.374	2.855 (0.048)	p>0.05

Full ANOVA table is shown in Tables 13, appendix 1.

(b)   South India strain.

Number of males female parent exposed to	Back transformed mean	Transformed mean (SED)	Significance level
1	16.314	2.792 (0.047)	$F_{(1,115)} = 0.80$
7	17.340	2.853 (0.047)	p>0.05

Full ANOVA table is shown in Tables 14, appendix 1.

Individual treatment of female parents and F_1 female offspring longevity

Survival curves for F_1 Brazil- and South-India-strain female offspring are shown in Figures 3.4 and 3.5 respectively.

Brazil-strain females

When seeds were present, F_1 female offspring of MM mothers exposed to seven males (C) lived significantly less long than those F_1 offspring of SM females exposed to one (A) or seven males (B) ($T_{(37)}$=2.15, p<0.05 and $T_{(36)}$=2.41, p<0.05 respectively). All other treatments with seeds did not differ significantly (p>0.05). When seeds were absent, F_1 female offspring of MM mothers exposed to one (D) or seven males (C) lived significantly less long than F_1 female offspring of SM mothers exposed to seven males (B) ($T_{(31)}$=2.36, p<0.05 and $T_{(32)}$=2.21, p<0.05 respectively). All other treatments without seeds did not differ significantly (p>0.05).

South-India-strain females

The female parent treatment had a significant effect on the longevity of the F_1 offspring when seeds were provided (F $_{(1,75)}$ = 3.72, p<0.05) and when seeds were absent (F $_{(1,75)}$ = 4.06, p<0.05). The effect of the female parent treatment on the longevity of the F_1 offspring depended on whether F_1 females had access to seeds or not. When seeds were provided, offspring of MM female parents exposed to one or seven males lived significantly less long than offspring of SM females exposed to only one male ($T_{(36)}$=2.04, p<0.05 and $T_{(36)}$=2.49, p<0.05 respectively). There were

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no significant differences in longevity in other treatments (p>0.05). When seeds were absent however, offspring of females SM exposed to one male lived significantly less long than offspring of females exposed to seven males or of MM females (A/B $T_{(37)}$=2.32, p<0.05, A/C $T_{(37)}$=3.16, p<0.005 and A/D $T_{(37)}$=2.51, p<0.05). This is in contrast to the situation observed in the Brazil strain.

Figure 3.4. Survival curves for F_1 Brazil-strain females when seeds were either present (+) or absent (-) and whose female parents were exposed to one of four mating treatments: SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D).

Figure 3.5. Survival curves for F_1 South-India-strain females when seeds were either present (+) or absent (-) whose Female parents were exposed to one of four mating treatments: SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D).
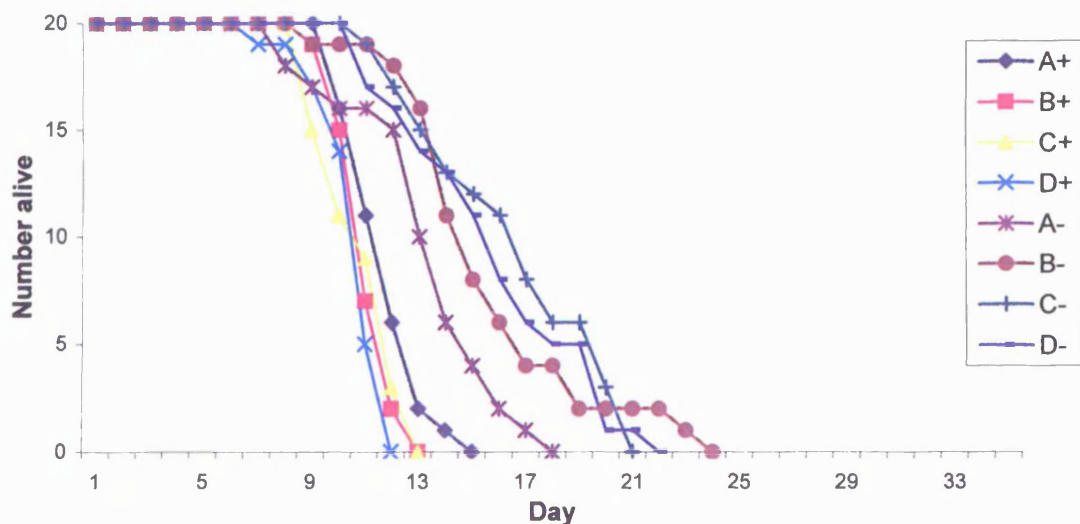
3.4.3 The effect of MM by the grandmother on the longevity of the F_2 offspring

Seed availability: F_2 offspring

The effect of seed availability and hence oviposition on longevity was not examined for the F_2 offspring with males and females being kept in the absence of seeds for the duration of the experiment. Only the effect of the grandmothers mating treatment will be considered here.

Number of matings by grandmother: Brazil-strain F_2

The number of matings that a grandmother had did not significantly affect the longevity of either the female ($F_{(1,75)} = 0.85$, p>0.05) or male ($F_{(1,75)} = 2.31$, p>0.05) F_2 offspring. Mean longevity in days for female and male F_2 offspring is shown in table 3.14.

Number of matings by grandmother: South-India-strain F_2

Similar to the response seen for Brazil-strain F_2 offspring above, the number of matings that the grandmother had did not significantly affect the longevity of either the female ($F_{(1,75)} = 1.40$, p>0.05) or male ($F_{(1,75)} = 2.52$, p>0.05) F_2 offspring. Mean longevity in days for female and male F_2 offspring is shown in table 3.15.

Table 3.14. Mean longevity in days for SM Brazil-strain F_2 offspring whose mothers (F_1) SM and whose grandmothers were provided with SM or MM opportunities. SED values are standard errors of the difference between the means. The data were log transformed.

(a)   Female F_2.

Female parent mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
SM	20.025	2.997 (0.048)	$F_{(1,75)} = 0.85$
MM	21.349	3.061 (0.048)	p>0.05

Full ANOVA table is shown in Tables 15, appendix 1.

(b)   Male F_2.

Female parent mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
SM	13.957	2.636 (0.047)	$F_{(1,75)} = 2.31$
MM	15.425	2.736 (0.047)	p>0.05

Full ANOVA table is shown in Tables 16, appendix 1.

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Table 3.15. Mean longevity in days for SM South India strain F_2 offspring whose mothers (F_1) SM and whose grandmothers were provided with a SM or MM opportunity. SED values are standard errors of the difference between the means. The data were log transformed.

(a)   Female F_2.

Female parent mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
SM	22.511	3.114 (0.038)	$F_{(1,75)} = 1.40$
MM	24.047	3.180 (0.038)	p>0.05

Full ANOVA table is shown in Tables 17, appendix 1.

(b)   Male F_2.

Female parent mating opportunity	Back transformed mean	Transformed mean (SED)	Significance level
SM	17.409	2.857 (0.028)	$F_{(1,75)} = 2.52$
MM	16.379	2.796 (0.028)	p>0.05

Full ANOVA table is shown in Tables 18, appendix 1.

Number of males encountered by the grandmother: Brazil-strain F_2 offspring

The number of males that the grandmother was exposed to did not significantly affect the longevity of either the female ($F_{(1,75)}$ = 0.14, p>0.05) or male ($F_{(1,75)}$ =0.68, p>0.05) F_2 offspring. Mean longevity in days for female and male F_2 offspring is shown in Table 3.16.

Number of males encountered by the grandmother: South-India-strain F_2 offspring

The number of males that the grandmother was exposed to did not significantly affect the longevity of either the female ($F_{(1,75)}$ =1.16, p>0.05) or male ($F_{(1,75)}$ = 0.64, p>0.05) F_2 offspring. Mean longevity in days for female and male F_2 offspring is shown in Table 3.17.

Individual treatment experienced by grandmother and F_2 offspring longevity: Brazil-strain

The individual treatment experienced by the grandmother had no significant effect on the longevity of the male ($F_{(1,75)}$ = 1.27, p>0.05) or female ($F_{(1,75)}$ = 0.86, p>0.05) F_2 offspring. Survivorship curves for male and female F_2 offspring are shown in Figure 3.6.

Individual treatment experienced by grandmother and F_2 offspring longevity: South-India-strain

The individual treatment experienced by the grandmother had no significant effect on the longevity of the male ($F_{(1,75)} = 1.20$, $p>0.05$) or female ($F_{(1,75)} = 0.38$, $p>0.05$) F_2 offspring. Survivorship curves for male and female F_2 offspring are shown in Figure 3.7.

Table 3.16. Mean longevity in days for SM Brazil strain F_2 offspring whose mothers (F_1) SM and whose grandmothers were exposed to one or seven males. SED values are standard errors of the difference between the means. The data were log transformed.

(a)   Female F_2.

Number of males grandmother exposed to	Back transformed mean	Transformed mean (SED)	Significance level
1	20.409	3.016 (0.049)	$F_{(1,75)} = 0.14$
7	20.947	3.042 (0.049)	$p>0.05$

Full ANOVA table is shown in Tables 15, appendix 1.

(b)   Male F_2.

Number of males grandmother exposed to	Back transformed mean	Transformed mean (SED)	Significance level
1	15.074	2.713 (0.047)	$F_{(1,75)} = 0.68$
7	14.282	2.659 (0.047)	$p>0.05$

Full ANOVA table is shown in Tables 16, appendix 1.

Table 3.17. Mean longevity in days for SM South India strain F_2 offspring whose mothers (F_1) SM and whose grandmothers were exposed to one or seven males. SED values are standard errors of the difference between the means. The data were log transformed.

(a)   Female F_2.

Number of males grandmother exposed to	Back transformed mean	Transformed mean (SED)	Significance level
1	23.951	3.176 (0.038)	$F_{(1,75)} = 1.16$
7	22.601	3.118 (0.038)	$p>0.05$

Full ANOVA table is shown in Tables 17, appendix 1.

(b)   Male F_2.

Number of males grandmother exposed to	Back transformed mean	Transformed mean (SED)	Significance level
1	17.167	2.843 (0.028)	$F_{(1,75)} = 0.64$
7	16.627	2.811 (0.028)	$p>0.05$

Full ANOVA table is shown in Tables 18, appendix 1.

Figure 3.6. Survival curves for Brazil strain F_2 males (M) and females (F) when seeds were absent whose mothers (F_1) were exposed to one treatment (A) and whose grandmothers were exposed to one of four mating treatments: SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D).
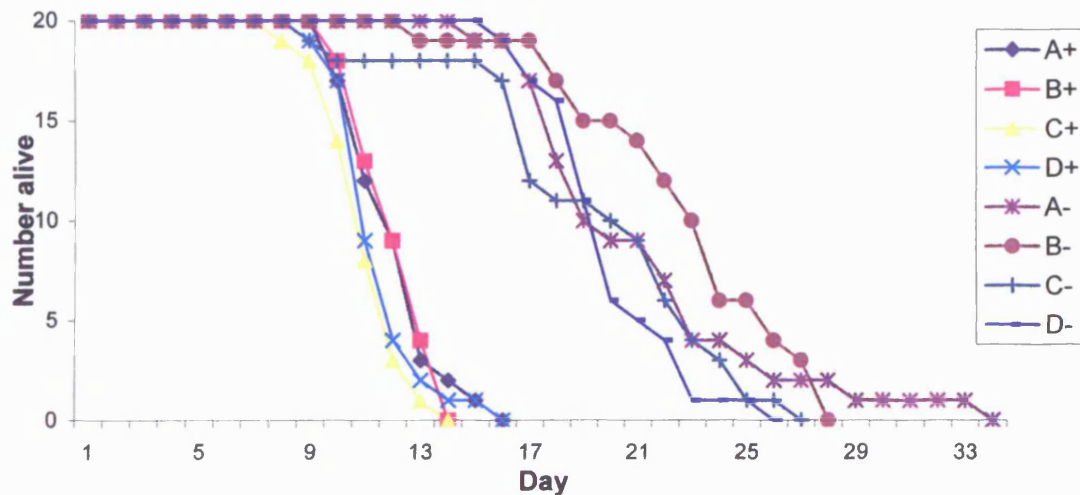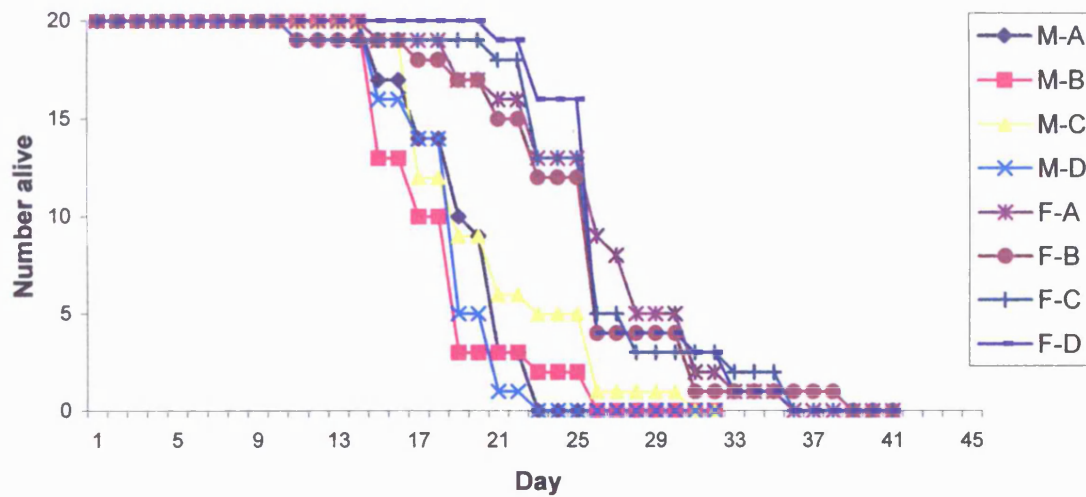

Figure 3.7. Survival curves for South India strain F_2 males (M) and females (F) when seeds were absent, whose mothers (F_1) were exposed to one treatment (A) and whose grandmothers were exposed to one of four mating treatments: SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D).

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3.5   DISCUSSION

The effect of MM on longevity of females

Seed availability had a significant effect on longevity for both the Brazil- and South-India-strain females.   Ovipositing females (seeds present) showed significantly reduced longevity when compared to non-ovipositing females (seeds absent).   The most likely explanation for this is in terms of a reproductive trade-off between fitness components where an increase in lifetime fecundity may be traded for a decrease in longevity (Møller *et al.*, 1989a).   Ovipositing females would invest time, energy and resources into maturing eggs, searching for suitable oviposition sites and ovipositing, and such behaviours may be performed at the expense of longevity.   Conversely, non-ovipositing females did not incur such costs and may have reabsorbed eggs and utilised them as energy source therefore extending longevity.   Egg absorption in times of food shortage has been observed in *Prostephanus truncatus* (Scholz, 1997) and although no obvious resorption bodies have been observed in *C. maculatus* it was assumed that egg reabsorption may be possible (Credland and Wright, 1989).

Brazil-strain longevity was greatly increased (by approximately 10 days) when females were unable to oviposit when compared to ovipositing females.   In contrast, the longevity of South-India-strain females was only increased by 4.6 days by preventing oviposition.   This may be due to a difference in the life-history strategies of the two strains since the Brazil strain tends to live for a shorter time but lays more eggs in comparison to the supposedly longer-lived South India strain that lays fewer eggs.   In this way the selection pressure acting on the two strains is quite different with Brazil-strain females investing most of their energy into fecundity whereas selection pressure on South-India-strain females is expected to be on increased longevity.   As the South-India-strain larvae actively compete with each other, so that only one larva per seed is victorious, it does not benefit females to lay more eggs if seeds are limiting or already have eggs laid on them.   Instead they would benefit from being able to live longer with more time to search for more profitable oviposition sites, lay fewer eggs but invest more resources per egg.   The larvae of the scramble competitor, Brazil, passively compete for the resources in a seed and several larvae can feed and survive to emergence within one seed. Females therefore, need not be as discriminating about where they lay their eggs and even lay more than one egg per seed. It should be noted that it might not be valid to compare mean longevity directly

3.5 DISCUSSION

The effect of MM on longevity of females

Seed availability had a significant effect on longevity for both the Brazil- and South-India-strain females. Ovipositing females (seeds present) showed significantly reduced longevity when compared to non-ovipositing females (seeds absent). The most likely explanation for this is in terms of a reproductive trade-off between fitness components where an increase in lifetime fecundity may be traded for a decrease in longevity (Møller *et al.*, 1989a). Ovipositing females would invest time, energy and resources into maturing eggs, searching for suitable oviposition sites and ovipositing, and such behaviours may be performed at the expense of longevity. Conversely, non-ovipositing females did not incur such costs and may have reabsorbed eggs and utilised them as energy source therefore extending longevity. Egg absorption in times of food shortage has been observed in *Prostephanus truncatus* (Scholz, 1997) and although no obvious resorption bodies have been observed in *C. maculatus* it was assumed that egg reabsorption may be possible (Credland and Wright, 1989).

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in the two strains as the experiments were separated in time. The South India strain, however, did not live as long as expected or as long as seen in a repeat experiment and the reason for this is unknown. Previously, mean longevities for ovipositing and non-ovipositing South India females were recorded as 12.77 ± 1.43 days and 29.81 ± 1.83 days respectively and 8.47 ± 1.84 days and 14.16 ± 3.53 days respectively for ovipositing and non-ovipositing Brazil strain females (see Tables 3.2 and 3.3 for means in the present study).

Another contributory factor to a decrease in longevity may be the effect of the seeds themselves. Fox and Tatar (1994) demonstrated that virgin female *C. maculatus* kept on seeds suffered a significantly reduced longevity over those kept in the absence of seeds. This phenomenon has been observed for both virgin females and males of both strains kept on cowpeas (pers.obs). It is unlikely however, that this alone has accounted for the large difference seen in the Brazil strain but it may account for the differences seen in the South-India-strain females where the difference in longevity between ovipositing and non-ovipositing females was smaller than that observed with Brazil. Alternatively, South-India-strain females may have been unable to reabsorb eggs and so not benefited from using these as an additional energy source.

The strain of *C. maculatus* was the most important factor in determining the effect of mating opportunity on females' longevity. MM or SM had no effect on the longevity of South-India-strain females. In contrast, SM Brazil-strain females lived significantly longer than MM Brazil-strain females. The effect of MM or SM on female longevity will be considered first for the Brazil strain and second for the South India strain.

Brazil-strain females

As well as fitness component trade-offs reducing female longevity, an additional reduction relating to the number of times a female mated was also observed. Both SM ovipositing and non-ovipositing females lived significantly longer than MM females. This shows that MM had a cost to females in terms of longevity. The observed decrease in longevity may be caused by an increase in numbers of eggs laid by MM females compared to eggs laid by SM females (see Savalli and Fox, 1999a) through fitness trade-offs discussed above. However, this would not explain why the reduction in longevity was observed for non-ovipositing females. Indeed, the

reduction in longevity was larger when females did not oviposit and this cost to longevity cannot be attributed to fecundity (also observed for *D. melanogaster* by Chapman *et al.*, 1995). Longevity, however, was measured to the nearest day, which may have given too much measurement error relative to the means for ovipositing females since these means were small therefore masking any effects of the mating treatment. So why did these non-ovipositing females incur a cost to their longevity? Possible reasons include physical damage caused by repeated courtship and copulation, the energetic cost of re-mating or a physiological effect brought about by mechanical stimulation or some component of the ejaculate.

Males and females may incur physical damage from repeated courtship and copulation. Damage may occur during rejection or when males are fighting for a female. Obvious physical damage resulting in death was only observed for less than 10 females and males from over 300 observed matings. In nine of these cases the females' ovipositor became permanently extruded following copulation. These females were unable to oviposit and died up to 12 hours later. One extreme case was observed where the male's aedeagus broke off while the female was kicking the male during copulation. In this case neither the male nor female died immediately, but they were not capable of additional matings and the female was unable to oviposit. These incidents are few, however, and are unlikely to be responsible for the large decrease in longevity seen in MM females. It is not known from this present study whether *C. maculatus* beetles incur any internal damage from mating such as that observed for the house fly, *Musca domestica* (Leopold *et al.*, 1971) and the blowfly *Lucilia sericata* (Lewis and Pollack, 1975).

Re-mating can be costly since time may be spent assessing a potential mate and a male must chase and court a female, which often ends in rejection. Females incur energetic costs from re-mating as energy is expended in rejecting unsuitable males either by kicking or running away. In addition, even during a successful mating opportunity the female will eventually begin to reject the male by kicking. The male however, does not usually withdraw at this point so the female may be left kicking and walking around dragging the male still *in copula* for up to 2 to 4 minutes. In this way an individual mating several times will use more energy than those mating only once and it appears that male *C. maculatus* can mate a large number of times. Ofuya

(1995) reported that in a Nigerian population, male *C. maculatus* were capable of mating with up to 10 different females daily for the first three days and mating with 25 to 73 different females during their adult lives. It is unlikely, however, that equivalent mating rates would be observed per female although actual re-mating rates for females confined with males are not known. When looking at the individual treatments it was observed that the longevity of ovipositing MM females was not significantly different from the longevity of females that mated once only but were exposed to seven cauterised males implying that any energetic cost of re-mating was no more costly than male harassment. Furthermore, SM females exposed to one male did not live significantly longer than SM females exposed to seven males suggesting that male harassment itself was not very costly in terms of longevity.

It has been suggested that females may obtain a nutritional contribution from the male or use the excess ejaculate as a nutritional source, which may extend longevity or enable increased egg production (Fox, 1993a; Fox, 1993b; Eady, 1994a). Indeed male *C. maculatus* do transfer oversized ejaculates that could contribute nutritionally to female somatic maintenance (see section 3.1). Fox (1993a) found that MM female *C. maculatus* beetles maintained under starvation conditions had increased longevity over SM females possibly from ejaculate-derived nutrients. This increase in longevity disappeared, however if females had access to a yeast and sugar water solution. This contrasts with the present study in that despite being fed a decrease in longevity was still observed for MM females suggesting that if there were any nutritional gain from metabolising ejaculate it did not offset the cost of MM or result in increased longevity. Certainly females in this present study were not energy limited although they may have been nutrient limited. This delivery of an oversized ejaculate may have additional consequences for longevity if some component of what was transferred was in fact toxic or contained a product or products that stimulated some aspect of a female's physiology.

The presence of accessory gland products has been observed in several species (e.g. Chapman *et al.*, 1995, Huignard, 1970, Huignard *et al.*, 1977; Rice, 1996) with consequences for female survival (Partridge *et al.*, 1987). Savalli and Fox (1999a) found that female *C. maculatus* who mated with four virgin males experienced greater mortality than females mated singly or with non-virgins. Females mated to virgin

males also produced more eggs and Savalli and Fox (1999a) argue that the increased mortality may be due to these increased egg production rates. This may be true, however, it does not account for the decrease in longevity that was observed when females did not oviposit in this present study. It could be argued that since virgin males transfer larger spermatophores than non-virgin males it may also reflect a larger contribution from the accessory gland indicating a dose dependent response. A substance present in the ejaculate that stimulated egg production could also have a toxic side effect and females mating with virgins received proportionally more, further reducing longevity. Ovipositing females may not live long enough for any toxic effect of a male accessory gland product to become apparent certainly when oviposition substrates were not limited as in this present study. The number of times that a female mated may become more important when oviposition sites are limited or females disperse to new oviposition sites. Furthermore, a toxic effect may be reduced through oviposition as spermatophore material is utilised and does not remain within the female's system for as long as when females do not oviposit.

Female *C. maculatus* exposed to males may also undergo physiological changes due to male pheromones or mechanical stimulation during copulation and courtship. These changes may induce a female to alter the allocation of resources within her body. The presence of males may prevent eggs from being reabsorbed and therefore unavailable as energy source. There may also be other physiological changes that alter the movement of resources around the body at the expense of somatic repair. The stimulation of oogenesis by mating has been observed in *C. maculatus* (Ofuya, 1995) and consequences may arise if oviposition is prevented. However, non-ovipositing MM females exposed to seven males lived significantly less long than SM females exposed to seven males. This suggests that the effect on longevity was mediated through copulation and not by male presence.

Finally, frequent mating has also been observed to increase female metabolic rate resulting in accelerated senescence (see Sohal, 1981). Although this may well be a contributory factor for the parent females it does not account for the decrease in longevity observed in the F_1 and is therefore unlikely to be the main cause of decreased longevity.

South-India-strain females

The mating opportunity of South-India-strain females had no effect on their longevity, which is in contrast to that seen for the Brazil-strain females. It may be that South-India-strain females were not accepting further matings from males despite being presented with the opportunity. If there were products transferred from the male accessory gland that could stimulate oviposition, egg maturation or decrease a female's desire to re-mate, they may simply not be transferred by South-India-strain males. Alternatively, if such products are transferred, South-India-strain females may have evolved ways of countering the stimulatory effects of such products if they result in increased oviposition rates. This is particularly important for this strain whose larvae actively compete for the resources in a seed. Such competition may have already driven females to alter their egg spacing behaviour as a response to this competition to ensure an even distribution of larvae between seeds (Smith and Lessells, 1985) (see section 1.2.1). In this way an oviposition stimulant might encourage females to lay eggs at inappropriate sites leading to a loss of fitness if eggs are laid where the larva has no chance of survival. To counter this the female may raise the response threshold to such products and therefore regain control over her bodily processes until a suitable site could be found (Eberhard, 1996). Additionally, if the reaction is dose dependent, South-India-strain males may transfer a smaller volume of stimulatory product or even transfer smaller spermatophores compared with Brazil-strain males.

The presence of males may affect female longevity in two ways. Males may harass females in an attempt to obtain a mating and in doing so may cause females to incur either energetic or fitness costs. Females may have to discourage or to try to escape from courting males; males may physically prevent females from laying eggs, disrupt searching for oviposition sites and even damage eggs that are in the process of being laid. The second way that a male may affect a female is during copulation and from ejaculates delivered (as discussed previously). It is clear from the results that the number of males a female was exposed to was less important in terms of affecting longevity than the number of matings. The effect of the number of males encountered by females will first be discussed for the Brazil strain and second for the South India strain.

Brazil-strain females

Exposure to males significantly affected longevity only when seeds were absent. Females exposed to seven males lived significantly less long than females exposed to only one male. It might be expected that non-ovipositing females experienced a greater degree of male harassment since there were fewer opportunities for females to escape a male's attention by hiding in the seeds. However, females placed with cauterised males would be subject to the same levels of harassment as those females exposed to intact males. The longevity of MM females exposed to one male was not significantly different from SM females exposed to seven cauterised males. In contrast, MM females exposed to seven males lived significantly less long than SM females exposed to seven cauterised males. This was not due to differences in mating frequencies between females exposed to the same or to different males, however (see section 5.4.3), which suggests two things. First, that mating was more important as a cause of decreased longevity than harassment, and second, mating multiply with different males may have been more costly than mating multiply with the same male (e.g. STPs, Westneat and Rambo, 2000).

South-India-strain females

In contrast to the Brazil strain, the longevity of the South-India-strain females was unaffected by the number of males encountered. There was no cost or benefit to South-India-strain female longevity associated with the number of males she encountered. Were these females being harassed less by males?

The effect of MM by female parents on the longevity of their F_1 and the F_2 offspring

Female F_1 offspring from both strains experienced a significant decrease in longevity when seeds were available compared to when seeds were absent. As discussed previously for the parent females this decrease in longevity was probably due to fitness-component trade-offs between longevity and fecundity.

Brazil-strain males and females

MM by the female parent resulted in a decrease in longevity for both the male and female F_1 offspring. Thus, not only was there a cost to longevity of the female parent from MM but there was an additional cost in terms of reduced longevity in the F_1 offspring also. There may be two possible reasons for this. First, accessory gland

products or their metabolites may be passed to the egg along with sperm and if such products are indeed toxic it might explain the resulting decrease in fitness observed for the offspring. Substances may be present in the male ejaculate that have evolved to damage or destroy rival males' sperm inside the female and these products may also have a genotoxic effect on the offspring. This would concur with the view that the main cause of decreased longevity in the parent females was some component of the male ejaculate and not an increase in metabolism, energetic cost from re-mating or male harassment. Second, if oviposition rates were elevated by MM then it is possible that females who MM and were stimulated to increase oviposition invested quantitatively rather than qualitatively in eggs. This assumes that the resources available to females to invest in egg production are finite. It has been documented that larvae hatching from smaller eggs are at a disadvantage over those hatching from larger eggs (Fox, 1993c) therefore investing quantitatively at the expense of quality would not appear to be the best option. It is worth considering, however, that the amount of toxin present in an egg compared to the eventual size of an adult would be relatively small. In addition, it is well documented that the host plants produce a variety of potentially toxic compounds, such as alkaloids, saponins, glycosides, haemagglutinins and free amino acids, which can confer resistance to attack by insects (Smartt, 1977). The biological adaptations of insects and presence of highly effective detoxification systems to such compounds is also documented (e.g. *Caryedon brasiliensis*, Rosenthal *et al.*, 1977 and Rosenthal, 1990; *Drosophila sechellia*, Jones, 1998; *C. maculatus*, Desroches *et al.*, 1997). Although the ability to detoxify or adapt to the presence of plant toxins is present in *C. maculatus* it is unknown whether this ability would aid in the detoxification of male-derived toxins.

South-India-strain males and females

The longevity of the female but not the male F_1 offspring was affected by the mating history of the parent female. Could the female parent selectively alter nutrient provisioning to their eggs depending on the sex of the offspring? Offspring from SM females demonstrated reduced longevity when not ovipositing and this may be due to parent females laying smaller eggs. Since selection acting on South-India-strain females will favour fewer but larger eggs to be laid in preference to a greater number of smaller eggs, females are more likely to metabolise the ejaculate from males and to use it to produce larger eggs. It may follow that F_1 offspring from MM females are

larger, have more reserves available and may be more likely to invest in their own eggs resulting in a decrease in longevity when seeds are available. In contrast, when seeds were absent, females lived longer due to the increased resources accrued from their MM mothers. Offspring from SM females exposed to seven males, however, lived significantly longer than offspring from SM females exposed to one male. If females were investing in eggs, the presence of males may stimulate aspects of female physiology enabling the mobilisation and metabolism of their own somatic reserves to be invested in offspring. So there may be both a copulatory effect and an effect of male presence possibly through pheromonal or external tactile means.

The number of males encountered by the female parent did not significantly affect the longevity of the Brazil-strain male or female F_1 offspring or the male F_1 offspring of the South India strain. As seen for the female parents, the number of matings was more important in determining a cost to longevity than the number of males. In contrast, non-ovipositing South-India-strain F_1 female offspring lived significantly longer when the female parent had been exposed to seven different males. Again, this indicates a positive effect of exposure to multiple males not necessarily mediated through copulation.

Finally, no effect of the original female-parent mating treatment was carried over in the F_2 generation of either the Brazil or South India strain. The effects observed on longevity from a mating experience were therefore greatest in the parent females with some transmissible effects observed in the F_1 offspring and finally no observable effect on the F_2 offspring.

Summary

A cost of MM in terms of reduced longevity was observed for Brazil-strain females and this cost was carried over into the SM F_1 male and female offspring who also suffered a decrease to longevity. Furthermore, this cost was present for both ovipositing and non-ovipositing females, therefore, can not be solely attributed to an increased fecundity-longevity trade-off that may or may not be mediated by an accessory gland product. It is likely that some product was passed as part of the ejaculatory contribution of the male that may have a toxic side effect and resulted in an observable decrease in longevity. This product may be passed along with the egg to the F_1 offspring, which also suffer reduced longevity. In contrast, no cost of MM

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in terms of longevity was observed for the South-India-strain females. This may be due to a behavioural or physiological difference between the two strains. South-India-strain females may have refused additional matings and therefore mated fewer times than Brazil-strain females since oviposition stimulation is to be avoided for this strain. Alternatively, South-India-strain males may not pass stimulatory products, may pass less ejaculate or South-India-strain females may have evolved resistance to stimulatory products.

The effect of MM on the numbers and size of eggs laid by females is examined in Chapter 4.

CHAPTER 4

CHAPTER 4: EFFECT OF MULTIPLE MATING ON FECUNDITY OF FEMALES AND THEIR OFFSPRING

4.1 INTRODUCTION

Chapter three dealt with the effects of MM on longevity. This chapter is concerned with the effects of MM on fecundity. In the literature, an increase in female fecundity has been measured either as an increase in the total number of eggs laid (e.g. Bonato and Gutierrez, 1999) or enhanced oogenesis and oviposition (e.g. Lachmann, 1998). In the present study fecundity was measured as the total number of eggs laid by a female over her lifetime. Furthermore, half of the females were provided with an excess of oviposition sites (e.g. newly colonised grain store) to estimate potential fecundity, and to compare it with realised fecundity such as when oviposition sites were poor or restricted (e.g. depleted grain store or ancestral environment).

The effect of MM on fecundity has been studied extensively with a range of effects being observed between species. An increase in fecundity has been observed in the Bruchids *Bruchidus dorsalis* (Takakura, 1999) and *Callosobruchus analis* (Wilson *et al.*, 1999), in the moths *Heliocoverpa armigera* (Hou and Sheng, 1999) and *Utetheisa ornatrix* (LaMunyon, 1997), the bean bug *Reptortus clavatus* (Sakurai, 1996) and four species of spider mite (Bonato and Gutierrez, 1999). In contrast, a decrease in fecundity was observed in *Callosobruchus subinnotatus* following four matings (Mbata *et al.*, 1997), the mite *Caloglyphus berlesei* (Radwan and Rysinska, 1999) and a seed-eating true bug *Neacoryphus bicrucis* (McLain and Pratt, 1999). Finally, no effect on fecundity from MM was observed in the moth *Plodia interpunctella* (Cook, 1999), the grasshopper *Chorthippus parallelus* (Reinhardt and Kohler, 1999), two species of predatory mite (Rasmy and Hussein, 1996) and the beetle *Homichloda barkeri* (Jacoby) (Nahrung and Merritt, 1999).

In *C. maculatus* fecundity has been observed either to increase (Savalli and Fox, 1999a; Wilson *et al.*, 1999; Ofuya, 1995; Fox, 1993a) or to be unaffected by female MM when compared with SM females (Fox, 1993a). Certainly in *C. maculatus* there is more evidence to suggest that MM increases female fecundity. Furthermore, ovarian production in *C. maculatus* females has been observed to increase following

repeat matings (Ofuya, 1995). But what process or processes could generate this increase in fecundity?

First, females may be re-mating to replenish sperm stores, ensuring that they are not sperm limited, which would restrict the numbers of fertilised eggs that were laid (e.g. leaf cutter ant queens *Atta columbica*, Fjerdingstad and Boomsma, 1998). There is little evidence in the literature, however, to point to this as the most likely cause for re-mating in *C. maculatus*. Furthermore, *C. maculatus* ejaculates are oversized, containing 85% more sperm than a female can effectively store in her spermatheca (Eady, 1994b). Indeed, doubly mated *C. maculatus* females contain no more sperm in their spermathecae than SM females (Eady, 1992). Similarly, experiments performed by Fox (1993a) suggested that females were not sperm limited after just SM but may have been energetically constrained and failed to mature sufficient eggs to utilise all the available sperm.

Second, fecundity may be elevated by mechanical stimulation from repeated copulation (Boucher and Huignard, 1987) or by substances with a stimulatory component being transferred as part of the male ejaculate (e.g. Hihara, 1981 cited in Eberhard, 1996). These processes may alter a female's physiology or behaviour in a way that results in oviposition stimulation, elevated egg-maturation rates or even affecting resource mobilisation and distribution. Yasui (1997) reported that MM by females of the mite *Parasitus fimetorum* might be a necessary stimulus for continued oogenesis and that some physiological factors for this stimulus may exist in spermatophores (see also Lachmann, 1998). Hihara (1981) demonstrated that an accessory gland product and not the presence of sperm in ejaculates of the fruit fly, *Drosophila melanogaster*, was responsible for stimulating oviposition. Female *D. melanogaster*, however, have been shown to need both sperm and seminal fluid to initiate and maintain normal receptivity and rates of egg production (Fowler and Partridge, 1989; Manning, 1967; Scott, 1987 see also Chapman *et al.*, 1995). Eberhard (1996) suggested that such processes could quite feasibly be triggered by the act of copulation or by substances transferred by the male. Failing to respond to these triggers would render mating ineffectual. Similarly, initiating processes such as ovulation, oogenesis and oviposition before mating took place might also be disadvantageous. This could be particularly true for species such as *C. maculatus*

where non-feeding adults have finite resources to invest in reproduction and somatic maintenance. Indeed, trade-offs between longevity and fecundity have already been demonstrated in these beetles (Møller *et al.*, 1989b).

Oogenesis is a nutrient-limited process and is usually triggered only if sufficient nutrients are available from feeding either during the larval or adult stages. Mating may trigger oogenesis through mechanically stimulating mobilisation of reserves or from a sudden nutritional contribution provided by the male (Wheeler, 1996). Oversized ejaculates, such as those produced by *C. maculatus*, may therefore fulfil such a role (see Fox, 1993a and 1993b).

Why does MM fail to result in an increase in fecundity in some species? First, accessory gland products with stimulatory functions may not be transferred. For example, some species of *Photinus* fireflies transfer sperm only and do not transfer spermatophores (van der Reijden *et al.*, 1997). Second, females have evolved resistance to these products or raised their response threshold such that the amount transferred by the male does not elicit a response. This is important to females where egg production is costly, where oviposition following mating is delayed or where suitable oviposition sites are potentially rare. By raising the response threshold to a stimulus the females could regain control over their own bodily processes and refrain from ovipositing until a suitable site could be found (Eberhard, 1996). The alternative for females could mean that eggs were dumped or laid at an inappropriate site, as the stimulus to oviposit was too strong. Coupled with this is the fact, that by not over-stimulating oviposition, females could invest qualitatively rather than quantitatively, metabolising ejaculates and investing more per individual than in total offspring numbers.

A decrease in fecundity suggests that there is a cost to MM. This could be mediated by exposure to toxins present in ejaculates (Chapman *et al.*, 1995). Oogenesis may be stimulated to too great a degree resulting in physiological breakdown or stimulated at a faster rate than females can effectively lay eggs. Eggs may then be reabsorbed or even block female's reproductive structures. In *C. maculatus*, ovaries rapidly fill with matured ova if oviposition is prevented (Wilson and Hill, 1989). In addition, egg viability can also be affected. The incorporation of male-derived nutrients into both female tissues and oocytes has been demonstrated in the Bruchids *Caryedon serratus*

(Boucher and Huignard, 1987) and *Acanthoscelides obtectus* (Huignard, 1983), in the moth *Utetheisa ornatrix* (LaMunyon, 1997) and in the firefly *Photinus marginellus* (van der Reijden *et al.*, 1997). In this way, toxins or toxic by-products from spermatophore metabolism may be transferred to eggs resulting in reduced offspring survival or even infertile eggs.

Egg size is another important factor in determining offspring survival. MM has been shown to increase egg size in *C. maculatus* (Fox, 1993b; Wasserman and Asami, 1985) and offspring size in *Chorthippus parallelus* (Reinhardt *et al.*, 1999). *C. maculatus* offspring from larger eggs have been reported to develop faster and emerge as larger adults than offspring from smaller eggs (Fox, 1993c). Larval survivorship has also been shown to increase with increasing egg size (Wasserman and Asami, 1985; Fox, 1993b). Egg size and offspring performance decrease with increasing maternal age (Fox and Dingle, 1994), however, adult feeding (Fox and Dingle, 1994) or re-mating (Wasserman and Asami, 1985) can compensate for this and increase larval survival. It has been postulated that *C. maculatus* spermatophores possess both a nutritive and a stimulatory role with regards to female fecundity (Wilson *et al.*, 1999).

The aims of this chapter are as follows: First to quantify any effects of MM on the fecundity of females from two strains of *C. maculatus* with differing life-histories measured as total numbers of eggs produced over lifetime, egg survival and egg size; Second, to quantify the effects of MM on singly mated female F_1 offspring fecundity and emergence weight from SM or MM parent females; Finally, the patterns of egg distribution over seeds and also the numbers of eggs laid per day will be examined in order to assess whether females in a particular mating treatment alter their egg spacing or oviposition behaviour as the result of a mating treatment. As in the rest of the thesis the abbreviations MM and SM will be used as a shorthand expression for individuals that mate multiply and mate singly respectively.

4.2   AIMS

This chapter aims to test the following null hypotheses.

In parent females:

- MM has no effect on female fecundity.

- MM has no effect on female oviposition behaviour

- MM has no effect on egg mass or F_1 offspring emergence mass

In F_1 female offspring:

- MM by a female parent has no effect on the fecundity of the F_1 female offspring or their oviposition behaviour.

In F_2 offspring

- MM by a female grandparent has no carry-over effect on the emergence mass of F_2 offspring.

4.3   MATERIALS AND METHODS

4.3.1   The effect of MM on the fecundity of females

Experimental design, number of replicates and treatment of beetles before and during the experiment followed the protocol described in section 3.3.1.   Additional procedures are outlined below.

Total numbers of eggs produced

Total egg numbers laid per female and the survival of those eggs through hatching to adult emergence were analysed in section 4.4.1.   The main effects of SM or MM and exposure to one or seven males on the numbers of eggs produced were also analysed in section 4.4.1.   Treatments A and B (see Figure 4.1) were combined and contrasted against C and D in order to compare the numbers of eggs laid by females experiencing either a SM or a MM opportunity.   Treatments A and D were combined and contrasted against B and C in order to compare the numbers of eggs laid by females who were exposed either to a single male or to seven males.   Similarly, the main effects of SM or MM and exposure of females to one or seven males on egg viability and survival were examined using the combination of treatments outlined above.   The total numbers of eggs that were laid by females were divided into four categories. These were as follows; non-hatch eggs, non-emerge eggs, viable and non-viable eggs. Non-hatch eggs were classified as those eggs that remained clear indicating that no larva had hatched thus no frass was produced (see section 1.1.2) or without the head

case of the first larval instar visible as a black spot (Eady, 1992). Non-emerge eggs were those that had hatched and therefore turned white, but the individual larva died before emerging as an adult. Viable eggs therefore were all those eggs from which an adult beetle emerged from the seed. Finally, non-viable eggs were those eggs that did not result in an adult beetle emerging from the seed (non-hatch and non-emerge eggs combined). Data concerning the numbers of eggs laid per seed, the number of eggs laid per day (one twenty-four hour period) and the number of days over which females laid eggs were collected and analysed in section 4.4.2.

Figure 4.1. Mating treatments imposed upon females where exposure was to one or seven different males with SM and MM.

A: Female mated once and then kept alone until death.

B: Female mated once. Male cauterised and returned to female. The male was replaced with a new cauterised male daily for a further six days after which females were kept alone until death.

C: Female kept with the original male that she mated with. A new male replaced the old male for a further six days after which the female was kept alone until death.

D: Female kept with the same intact male that she originally mated with for seven days. After seven days the male was removed and the female was kept alone until death.

4.3.2 Egg mass

Virgin beetles were obtained as described in section 2.2.3. Males and females were paired at random and observed to mate once before being transferred to one of four

possible treatments. Treatments are as described in Figure 4.1. Seeds were provided and females were allowed to oviposit for one half day. Following this the original seeds were discarded and replaced with fresh seeds. This would ensure that subsequent eggs to be collected and weighed would be more likely to have matured post emergence (see Wilson and Hill, 1989). Five replicates were used per mating treatment and seeds were harvested and eggs weighed every hour on days one, three and four of the females' lay period. Seeds were held in 'blue-tac' on the lid of a 5cm petri dish with the egg uppermost. The egg was removed by cutting into the seed coat at the side of the egg with a scalpel under a Nikon SMZ-1 binocular microscope. The egg could then be 'flicked' from the seed and placed into a small foil dish using watchmaker's forceps. Eggs were weighed on the Cahn C-31 microbalance to $0.1\mu g$. Egg mass was recorded on days one, three and four only, therefore, male exposure treatment refers either to one or four males. The effect of SM or MM and exposure of female to one or four males on egg mass was examined in section 4.4.3.

4.3.3 Parent female treatment and the fecundity of the F_1 female offspring

Source of insects

Seeds containing eggs laid on days three and four by parent females were isolated in 5 x 5 plates. Individuals from day three and four were chosen in order that parent females would have been exposed to the mating treatment for several days. Offspring isolated from seeds bearing one egg were chosen at random from a pool of offspring collected from the 20 females in the same treatment. Offspring from seeds containing more than one egg were discarded in order to avoid any effects of larval competition on an individual's fitness.

Apparatus

As described in section 3.3.1 beetles were held in 120ml Beatson jars containing a food source and sealed with filter paper, gauze and a plastic screw lid as described for culture jars in section 2.1.3. Treatments were replicated 20 times.

Experimental design

Individual beetles from parents exposed to the same treatment were weighed at emergence and paired together at random. No beetles were paired with siblings. Pairs were numbered and observed to mate. After mating males and females were separated to prevent re-mating and the males were transferred to 1.5ml Eppendorf

tubes with holes in the lid to allow for gaseous exchange, and checked daily until death. Females were transferred to a 120ml Beatson jar and kept as females in treatment A (Figure 4.1). They were not fed, but were observed daily until death.

Analyses of the main effects of SM or MM by parent females and their exposure to one or seven males on the fecundity of the SM F_1 offspring were analysed as described for parent females in section 4.3.1. The numbers of eggs produced over the lifetime of F_1 offspring from females exposed to treatments A and B were combined and contrasted against C and D in order to compare the numbers of eggs laid by F_1 females whose female parent had experienced a SM or a MM opportunity. Similarly, the number of eggs produced over the lifetime of F_1 offspring from females exposed to treatments A and D were combined and contrasted against B and C in order to compare the numbers of eggs laid by F_1 females whose female parent was exposed either to a single male or to seven males. Egg survival and viability were also analysed as described for parent females in section 4.3.1.

The total numbers of eggs that were laid by the F_1 female offspring were recorded as a single egg per seed, two eggs per seed or more than two eggs per seed. The data were analysed according to the treatment experienced by the parent female (A, B, C or D) (Figure 4.1), mating opportunity of the parent (SM or MM) and number of males that the female parent was exposed to (one or seven males) (section 4.4.5).

4.3.4 Parent female treatment and the emergence mass of the F_1 offspring

Male and female F_1 offspring were isolated on emergence from seeds containing a single egg according to the parent mating treatment (A, B, C or D) (Figure 4.1) and weighed using a Cahn C-31 microbalance in mg. The mass of offspring emerging from eggs laid on day one and also on eggs laid on days three and four were recorded and analysed in section 4.4.6.

4.3.5 Grandmother's treatment and the emergence mass of the F_2 offspring

Seeds containing single eggs laid on day one by the F_1 generation were isolated into 5 x 5 plates and held in the CTH room until the beetles emerged. On emergence, virgin beetles were sexed and then weighed using the Cahn C-31 microbalance. Data were analysed according to the treatment experienced by the grandmother (section 4.4.7).

4.4 RESULTS

4.4.1 The effect of MM on the fecundity of females

The total number of eggs laid by a female was recorded over her lifetime. The data were analysed as GLM ANOVA with emergence mass of the female taken as a covariate as it has been reported that emergence mass is an important predictor of fecundity (e.g. Møller *et al.*, 1989a). Data on total numbers of eggs produced were untransformed since no transformation could correct for the bimodal distribution of the results.

Total eggs produced

The number of males a female was exposed to did not affect total lifetime egg production for females of either strain. There was no significant difference in terms of the number of eggs laid by Brazil or South India strain females when exposed either to one or seven males (Brazil: $F_{(1,68)} = 0.04$, $p > 0.05$; South India: $F_{(1,75)} = 1.72$, $p > 0.05$). Mean numbers of eggs produced over the female's lifetime are shown in Table 4.1 for the Brazil and South India strain.

Brazil-strain females

Seven out of forty SM females did not lay any eggs at all and were excluded from the analysis. In contrast, all MM females did lay eggs. Emergence weight was found to be a significant factor in terms of the total numbers of eggs laid by females ($F_{(1,68)} = 22.73$, $p < 0.001$). In addition, MM significantly increased the total number of eggs laid, resulting in approximately 15 more eggs being laid over the female's lifetime ($F_{(1,68)} = 5.63$, $p < 0.05$).

South-India-strain females

All South India females laid eggs irrespective of the mating opportunity provided. Emergence mass was also found to be a significant factor in terms of the total numbers of eggs laid by South-India-strain females ($F_{(1,75)} = 42.80$, $p < 0.001$). In contrast to the Brazil-strain, however, MM by South-India-strain females did not result in an increase in the total numbers of eggs laid, thus the numbers of eggs laid by SM and MM females were not significantly different ($F_{(1,75)} = 1.21$, $p > 0.05$). The standard errors for South India were smaller than for Brazil (Table 4.1), showing that the lack of significance was not a result of higher variability.

Table 4.1. Mean (and SE) numbers of eggs laid by females that were provided with either a SM or MM opportunity and were exposed either to one or seven males. SE values are standard errors of the means. The data were untransformed.

(a)   Brazil strain.

	SM	MM	Mean
Exposed to 1 male	75.22 (6.553)	92.76 (6.209)	83.99 (4.515)
Exposed to 7 males	75.92 (7.177)	89.45 (6.210)	82.68 (4.747)
Mean	75.57 (4.854)	91.10 (4.390)	

Full ANOVA is shown in Table 1, appendix 2.

(b)   South India strain.

	SM	MM	Mean
Exposed to 1 male	107.41 (4.930)	98.70 (4.875)	108.98 (3.464)
Exposed to 7 males	110.55 (4.869)	106.34 (4.897)	102.52 (3.464)
Mean	108.44 (3.453)	103.06 (3.453)	

Full ANOVA is shown in Table 2, appendix 2.

Individual female treatment and total numbers of eggs laid: Brazil-strain females

Differences were also observed between individual treatments (interaction means). Numbers of eggs laid by females experiencing one of four possible treatments are shown in Figure 4.2. Results were analysed as ANCOVA with female emergence mass as a covariate.

MM females exposed to one male (D) laid significantly more eggs than both SM treatments, whether SM females were exposed to one (A) or seven males (B) ($F_{(1,35)}$ = 4.82, $p<0.05$ and $F_{(1,32)}$ = 6.20, $p<0.05$ respectively). All other treatments did not differ significantly ($p>0.05$). MM females exposed to seven males (C) therefore did not lay significantly more eggs than SM females exposed either to one or seven males ($F_{(1,35)}$ = 1.84, $p>0.05$ and $F_{(1,32)}$ = 1.60, $p>0.05$, respectively). Treatments A and B did not differ significantly in terms of total numbers of eggs produced ($F_{(1,30)}$ = 0.01, $p>0.05$,) and C and D did not differ significantly ($F_{(1,37)}$ = 0.12, $p>0.05$).

Figure 4.2. Total number of eggs laid by Brazil-strain females who were either mated singly and exposed to one male (A), mated singly and exposed to seven males (B), mated multiply and exposed to seven males (C) or mated multiply and exposed to one male (D). Bars are standard errors of means. The data were untransformed.
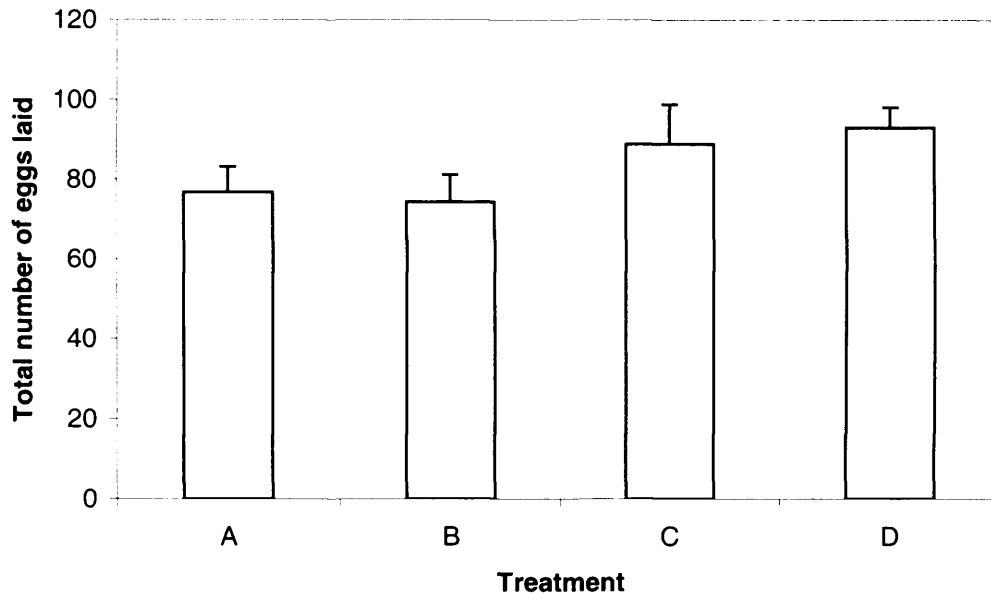
Treatment C is more variable than the others (Table 4.2, Fmax = 3.72, p $\cong$ 0.05) because of bimodality (three females laid only a few infertile eggs). Significance tests involving treatment C should be treated with caution.

Table 4.2. The standard deviation and range of the total numbers of eggs produced by Brazil-strain females experiencing one of four different treatments: mated singly and exposed to one male (A), mated singly and exposed to seven males (B), mated multiply and exposed to seven males (C) or mated multiply and exposed to one male (D). The data were untransformed.

Treatment	N	SD	Minimum number of eggs laid	Maximum number of eggs laid	Range
A	18	27.32	24	108	84
B	15	26.45	40	136	96
C	20	44.38	13	152	139
D	20	23.01	57	139	82

Individual female treatment and total numbers of eggs laid: South-India-strain females

Numbers of eggs laid by females experiencing one of four different treatments are shown in Figure 4.3. There was no significant difference in total numbers of eggs laid between treatments ($F_{(1,75)}$ = 1.42, p>0.05).

Figure 4.3. Total number of eggs laid by South-India-strain females that were either mated singly and exposed to one male (A), mated singly and exposed to seven males (B), mated multiply and exposed to seven males (C) or mated multiply and exposed to one male (D). Bars are standard errors of the mean. The data were untransformed.
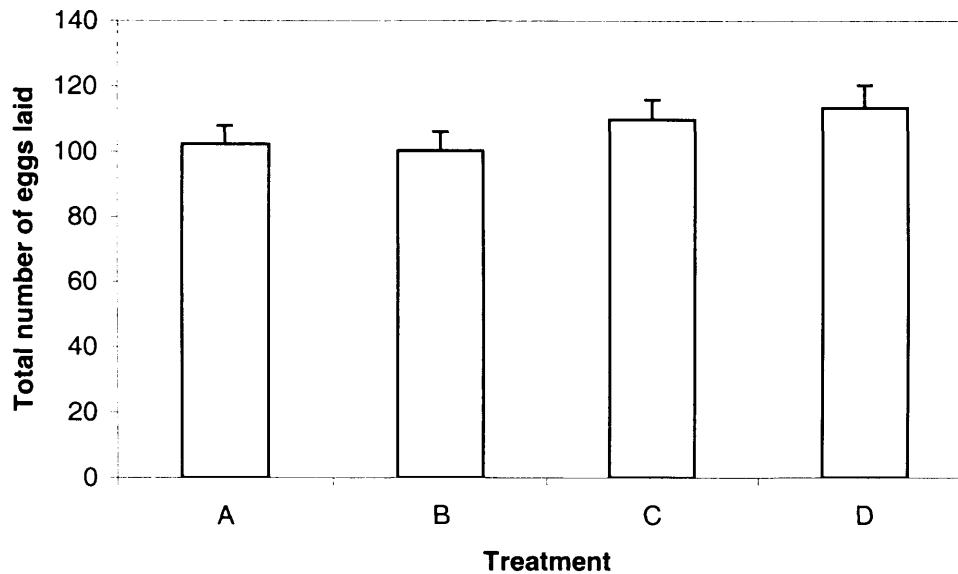
In contrast to the Brazil strain there was less variability between treatments for the South-India-strain females in terms of the standard deviation and the range of numbers of eggs produced. No treatment was more variable than the others (Table 4.3, Fmax=1.59, p>0.05).

Table 4.3. The standard deviation and range of the total numbers of eggs produced by females experiencing one of four different treatments: mated singly and exposed to one male (A), mated singly and exposed to seven males (B), mated multiply and exposed to seven males (C) or mated multiply and exposed to one male (D). The data were untransformed.

Treatment	N	SD	Minimum number of eggs laid	Maximum number of eggs laid	Range
A	20	24.73	56	147	91
B	20	25.54	48	143	95
C	20	27.41	71	162	91
D	20	31.17	66	168	102

Mating treatment and egg viability

All counts of non-emerge, non-hatch, non-viable and viable eggs were taken as a proportion of the total eggs laid over the females lifetime and angular transformed to achieve a normal distribution.

Brazil-strain-females

The emergence mass of the female was a significant factor in terms of the numbers of eggs laid for the non-emerge eggs only ($F_{(1,68)}$ = 4.97, p<0.05,). It was not a significant factor for numbers of non-hatch ($F_{(1,68)}$ = 0.35, p>0.05,), non-viable ($F_{(1,68)}$ = 2.07, p>0.05,) or viable ($F_{(1,68)}$ = 0.97, p>0.05,) eggs. The numbers of eggs in each of the four categories were recorded and analysed as GLM ANOVA with female emergence mass as a covariate. Mean numbers of eggs produced are shown in Tables 4.4 and 4.5. Differences within treatments were analysed by T test if the emergence mass was not shown to be a significant factor. Egg survival was not affected by the number of times that a female mated. There was no significant difference in terms of the numbers of non-emerge, non-hatch, non-viable or viable eggs laid when females were SM or MM ($F_{(1,68)}$ = 1.43, p>0.05; $F_{(1,68)}$ = 1.16, p>0.05; $F_{(1,68)}$ = 0.10, p>0.05 and $F_{(1,68)}$ = 0.14, p>0.05 respectively). Similarly, the numbers of males that a female was exposed to did not significantly affect the numbers of non-emerge, non-hatch, non-viable or viable eggs that were laid ($F_{(1,68)}$ = 0.07, p>0.05; $F_{(1,68)}$ = 0.10, p>0.05; $F_{(1,68)}$ = 0.05, p>0.05 and $F_{(1,68)}$ = 0.03, p>0.05 respectively).

Table 4.4. Proportion of non-emerge, non-hatch, non-viable and viable eggs that were laid by MM or SM Brazil-strain females.

(a)   Angular transformed proportions and SE values. SE values are standard error of the mean.

Mating opportunity	Non-emerge eggs (SE)	Non-hatch eggs (SE)	Non-viable eggs (SE)	Viable eggs (SE)
SM	0.344 (0.020)	0.277 (0.037)	0.470 (0.035)	0.932 (0.035)
MM	0.312 (0.018)	0.331 (0.034)	0.484 (0.031)	0.914 (0.032)

Full ANOVA is shown in Table 3, appendix 2.

(b)   Back transformed proportions.

Mating opportunity	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
SM	0.113	0.075	0.205	0.645
MM	0.094	0.106	0.217	0.627

Table 4.5. Proportion of non-emerge, non-hatch, non-viable and viable eggs that were laid by Brazil-strain females exposed either to one or seven males.

(a)   Angular transformed proportions and SE values. SE values are standard errors of the means.

Number of males	Non-emerge eggs (SE)	Non-hatch eggs (SE)	Non-viable eggs (SE)	Viable eggs (SE)
1 male	0.331 (0.019)	0.296 (0.035)	0.472 (0.032)	0.928 (0.033)
7 males	0.324 (0.019)	0.312 (0.037)	0.483 (0.034)	0.919 (0.034)

Full ANOVA is shown in Table 3, appendix 2.

(b)   Back transformed proportions.

Number of males	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
1 male	0.106	0.085	0.206	0.640
7 males	0.104	0.094	0.215	0.632

An analysis of significance between treatments gave some interesting results. The numbers of non-emerge eggs laid by Brazil-strain females were not significantly different between treatments ($F_{(1,68)}$ = 0.72, $p>0.05$). Additionally, there was no significant interaction between the number of males that a female was exposed to and the number of matings a female had ($F_{(1,68)}$ = 0.58, $p>0.05$). This is in contrast to the other three treatments where a significant interaction between number of matings and exposure to males was observed for non-hatch ($F_{(1,68)}$ = 4.57, $p<0.05$,), non-viable ($F_{(1,68)}$ = 5.50, $p<0.05$) and viable eggs ($F_{(1,68)}$ = 4.83, $p<0.05$).

Females that MM and were exposed to seven males laid the most non-hatch eggs followed by SM females exposed to one male, then MM females exposed to one male and finally SM females exposed to seven males which laid the least number of non-hatch eggs (Table 4.6). In addition, females that MM and were exposed to seven males laid significantly more non-hatch eggs than SM females exposed to seven males ($T_{(26)}$=2.38, $p<0.05$) indicating that mating with several males has a greater detrimental effect on egg survival than simply being exposed to seven males.

Females who were MM and exposed to seven males and also SM females exposed to one male laid the highest numbers of non-viable eggs. There was however, no

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significant difference between the numbers of non-viable eggs laid by MM females exposed to seven males and SM females exposed to seven males ($T_{(28)}=1.92$, $p>0.05$).

MM females exposed to seven males and SM females exposed to one male laid the least number of viable eggs. Overall however, the numbers of viable eggs laid by females were not significantly different when comparing within treatments ($F_{(1,68)} = 1.66$, $p>0.05$). Therefore, despite MM females laying more eggs in total than SM females, they did not lay a significantly higher proportion of viable eggs. This observation was also repeated when raw numbers of viable eggs produced were analysed by Mann Whitney ($W=1067.5$, $p>0.05$).

Table 4.6. Proportion of non-emerge, non-hatch, non-viable and viable eggs laid by Brazil-strain females experiencing one of four different mating treatments.

(a)   Angular transformed proportions and SE values. SE values are standard errors of the means.

Treatment	Non-emerge eggs (SE)	Non-hatch eggs (SE)	Non-viable eggs (SE)	Viable eggs (SE)
SM alone (A)	0.357 (0.027)	0.323 (0.051)	0.519 (0.047)	0. 884 (0.047)
SM 7 males (B)	0.330 (0.029)	0.231 (0.055)	0.421 (0.051)	0.980 (0.052)
MM 7 males (C)	0.318 (0.025)	0.393 (0.048)	0.545 (0.044)	0.858 (0.045)
MM 1 male (D)	0.305 (0.025)	0.2691 (0.048)	0.424 (0.044)	0.971 (0.045)

(b)   Back transformed proportions.

Treatment	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
SM alone (A)	0.122	0.101	0.246	0.598
SM 7 males (B)	0.105	0.052	0.167	0.689
MM 7 males (C)	0.098	0.147	0.268	0.572
MM 1 male (D)	0.090	0.071	0.169	0.681

South-India-strain females

The emergence mass of the female was not found to be a significant factor in terms of the numbers of non-emerge ($F_{(1,75)} = 1.42$, $p>0.05$,), non-hatch ($F_{(1,75)} = 0.07$, $p>0.05$),

non-viable ($F_{(1,75)}$ = 0.16, p>0.05) or viable ($F_{(1,75)}$ = 0.15, p>0.05) eggs laid. The numbers of eggs in each of the four categories were recorded and analysed as GLM ANOVA with female emergence mass as a covariate. Mean numbers of eggs produced are shown in Tables 4.7 and 4.8. Differences within treatments were analysed by ANOVA since the emergence weight was not shown to be a significant factor.

South-India-strain females' egg survival was not affected by the number of times that a female mated. There was no significant difference in terms of the numbers of non-emerge, non-hatch, non-viable and viable eggs laid when females were SM or MM ($F_{(1,75)}$ = 0.33, p>0.05; $F_{(1,75)}$ = 0.68, p>0.05; $F_{(1,75)}$ = 0.08, p>0.05 and $F_{(1,75)}$ = 0.24, p>0.05 respectively).

Similarly, the numbers of males that a female was exposed to did not significantly affect the numbers of non-emerge, non-hatch, non-viable and viable eggs that were laid ($F_{(1,75)}$ = 0.43, p>0.05; $F_{(1,75)}$ = 0.60, p>0.05; $F_{(1,75)}$ = 0.47, p>0.05 and $F_{(1,75)}$ = 0.85, p>0.05, respectively).

Table 4.7. Proportion of non-emerge, non-hatch, non-viable and viable eggs that were laid by MM or SM South-India-strain females.

(a)   Angular transformed proportions and SED values. SED values are standard errors of the difference between the means.

Mating opportunity	Non-emerge eggs (SED)	Non-hatch eggs (SED)	Non-viable eggs (SED)	Viable eggs (SED)
SM	0.244 (0.015)	0.140 (0.027)	0.306 (0.024)	1.062 (0.023)
MM	0.231 (0.015)	0.172 (0.027)	0.315 (0.024)	1.046 (0.023)

Full ANOVA is shown in Table 4, appendix 2.

(b)   Back transformed proportions.

Mating opportunity	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
SM	0.058	0.020	0.091	0.763
MM	0.053	0.029	0.096	0.749

Table 4.8. Proportion of non-emerge, non-hatch, non-viable and viable eggs that were laid by South-India-strain females exposed either to one or seven males.

(a)   Angular transformed proportions and SED values. SED values are standard errors of the difference between the means.

Number of males	Non-emerge eggs (SED)	Non-hatch eggs (SED)	Non-viable eggs (SED)	Viable eggs (SED)
1 male	0.230 (0.015)	0.171 (0.027)	0.322 (0.024)	1.039 (0.023)
7 males	0.245 (0.015)	0.141 (0.027)	0.299 (0.024)	1.069 (0.023)

Full ANOVA is shown in Table 4, appendix 2.

(b)   Back transformed proportions.

Number of males	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
1 male	0.052	0.029	0.100	0.743
7 males	0.059	0.020	0.087	0.769

There was no significant difference between treatments in terms of the numbers of non-emerge, non-hatch, non-viable and viable eggs that were laid by females ($F_{(1,75)}$ = 0.51, $p > 0.05$; $F_{(1,75)}$ = 0.52, $p > 0.05$; $F_{(1,75)}$ = 0.48, $p > 0.05$; $F_{(1,75)}$ = 0.69, $p > 0.05$ respectively) (see Table, 4.9). In addition, there were no significant interactions between the number of males a female was exposed to and the number of matings a female had for non-emerge, non-hatch, non-viable and viable eggs ($F_{(1,75)}$ = 0.51, $p > 0.05$; $F_{(1,75)}$ = 0.21, $p > 0.05$; $F_{(1,75)}$ = 0.88, $p > 0.05$ and $F_{(1,75)}$ = 0.95, $p > 0.05$ respectively).

Table 4.9. Proportion of non-emerge, non-hatch, non-viable and viable eggs laid by South-India-strain females experiencing one of four different mating treatments.

(a)   Angular transformed proportions and SE values. SE values are standard errors of the mean.

Treatment	Non-emerge eggs (SE)	Non-hatch eggs (SE)	Non-viable eggs (SE)	Viable eggs (SE)
SM alone (A)	0.229 (0.022)	0.146 (0.039)	0.302 (0.034)	1.063 (0.032)
SM 7 males (B)	0.258 (0.021)	0.134 (0.039)	0.310 (0.034)	1.061 (0.032)
MM 7 males (C)	0.231 (0.021)	0.148 (0.039)	0.288 (0.034)	1.077 (0.032)
MM 1 male (D)	0.232 (0.021)	0.196 (0.038)	0.343 (0.034)	1.016 (0.032)

(b)   Back transformed proportions.

Treatment	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
SM alone (A)	0.051	0.021	0.088	0.763
SM 7 males (B)	0.065	0.018	0.093	0.762
MM 7 males (C)	0.052	0.022	0.080	0.775
MM 1 male (D)	0.053	0.038	0.113	0.723

4.4.2 Oviposition behaviour

The strain of the female has been shown to be important in determining whether a particular mating treatment will affect the total number of eggs that a female will lay over her lifetime. MM Brazil-strain females have been observed to lay significantly more eggs than SM females (section 4.4.1). Thus, it is the effect of the number of matings a female has that significantly alters the number of eggs laid and not the number of males a female is exposed to, for the Brazil strain at least. This will highlight whether females simply lay more eggs per day or if females lay for a longer number of days. This may have important consequences as we have already seen in Chapter three that MM Brazil-strain females show reduced longevity over SM females. The numbers of eggs laid per female per day on days one, two, three, four and more than four (5+) were recorded and analysed according to whether females were provided with a SM or MM opportunity or were exposed either to one or seven males. Daily egg totals were analysed as GLM ANOVA with female emergence mass

as a covariate since this has been shown to be a significant factor in terms of numbers of eggs laid (section 4.4.1).

The numbers of eggs laid per day for the first three days for both SM and MM females show a decrease from approximately 26 and 22 eggs for SM and MM females respectively to 12 and 14 eggs for SM and MM females respectively. For SM females this decrease in numbers continued on day four also when females laid an average of 9 eggs. It did not continue, however, for MM females who were observed to lay more eggs on day four (16) than on day three. Significance values for numbers of eggs laid per day are shown in Table 4.10. When comparing the numbers of eggs laid per day for SM and MM females it can be seen that days one, two and three are not significantly different. MM females did, however, lay significantly more eggs on day four and laid significantly more eggs on day five and beyond than SM females (Figure 4.4).

The number of males that a female was exposed to did not significantly affect the number of eggs that were laid on any day. Significance values for each day are shown in Table 4.11. The number of eggs laid per day for the first four days by females exposed to only one male decreased daily from 25 eggs on day one to 12 eggs on day four. Similarly, the numbers of eggs laid per day for the first four days by females exposed to seven different males decreased from 23 eggs on day one to approximately 13 eggs on day three. An average of just over 13 eggs was laid on day four by these females (Figure 4.5).

Figure 4.4. Number of eggs laid per female per day by Brazil-strain females provided with the opportunity to SM or MM. Bars are standard errors of the means.
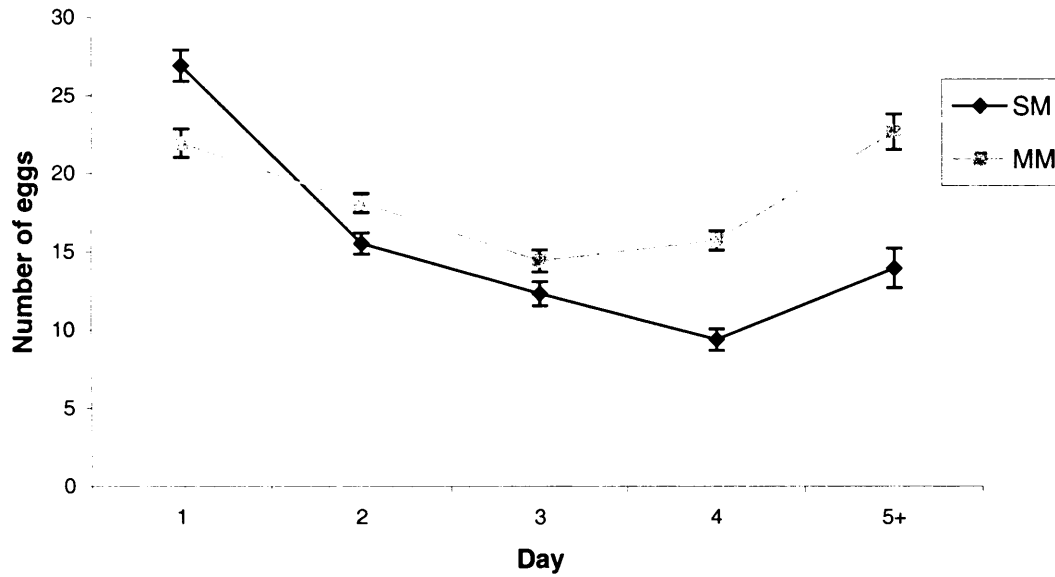
Figure 4.5. Number of eggs laid per female per day by Brazil-strain females exposed either to one or seven males. Bars are standard errors of the means.
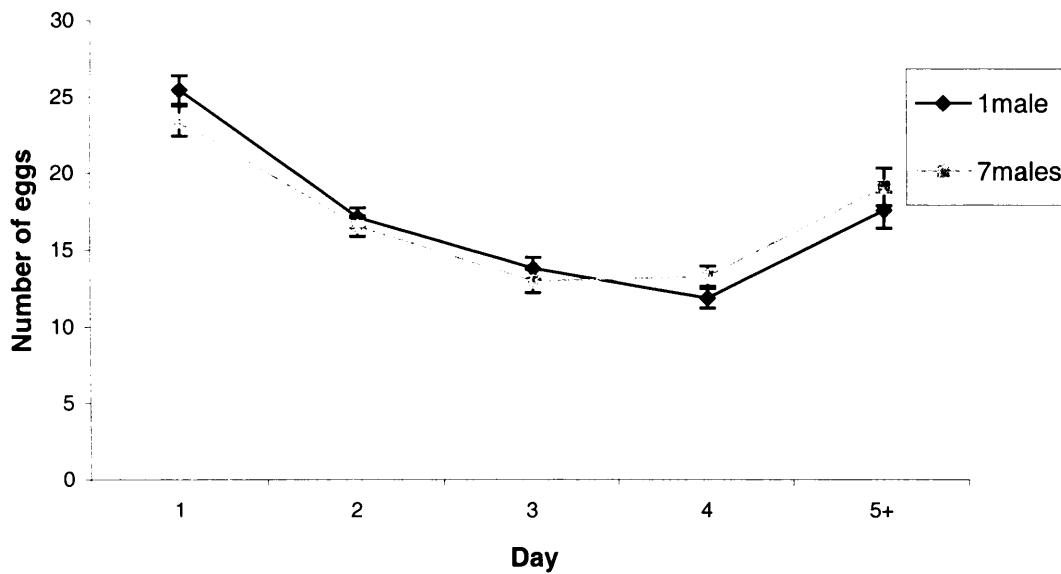
Table 4.10. Significance values for the numbers of eggs laid by Brazil-strain females on a particular day when comparing singly and MM females (Matings) and females exposed either to one or seven males (Males). The data were untransformed.

Day	1	2	3	4	5+
Matings	$F_{(1,64)} = 3.37$ $p > 0.05$	$F_{(1,64)} = 1.96$ $p > 0.05$	$F_{(1,64)} = 1.00$ $p > 0.05$	$F_{(1,64)} = 11.71$ $p < 0.01$ *	$F_{(1,64)} = 6.57$ $p < 0.05$ *
Males	$F_{(1,64)} = 0.57$ $p > 0.05$	$F_{(1,64)} = 0.09$ $p > 0.05$	$F_{(1,64)} = 0.16$ $p > 0.05$	$F_{(1,64)} = 0.60$ $p > 0.05$	$F_{(1,64)} = 0.20$ $p > 0.05$

* Indicates a significant result

Full ANOVA is shown in Table 5, appendix 2.

South-India-strain females

The number of eggs laid per day for the first four days for both SM and MM females decreased daily from approximately 36 and 37 eggs for SM and MM females respectively to 15 eggs for both SM and MM females. SM females laid fewer eggs on day four than MM females. However, no significant difference between the daily numbers of eggs laid was observed on any given day when SM and MM females were compared (Figure 4.6). Significance values for numbers of eggs laid per day are shown in Table 4.11.

The numbers of eggs laid per day for the first three days decreased daily from an average of 37 eggs on day one to 17 eggs on day three irrespective of the number of males that females were exposed to. On day four the number of eggs laid by females exposed to one male remained at 17 whereas for females exposed to seven males this number decreased to 13 eggs. After day four females exposed to only one male laid a further 19 eggs whereas females exposed to seven males laid a further 14 eggs. The number of males that a female was exposed to did not significantly affect the number of eggs that a female laid on day one, two, three or days five and beyond (Figure 4.7). Significance values for each day are shown in Table 4.11. Females exposed to only one male laid significantly more eggs on day four, however, than females exposed to seven different males ($F_{(1,74)} = 4.57$, $p<0.05$).

Figure 4.6. Number of eggs laid per female per day by South-India-strain females provided with the opportunity to SM or MM. Bars are standard errors of the means.
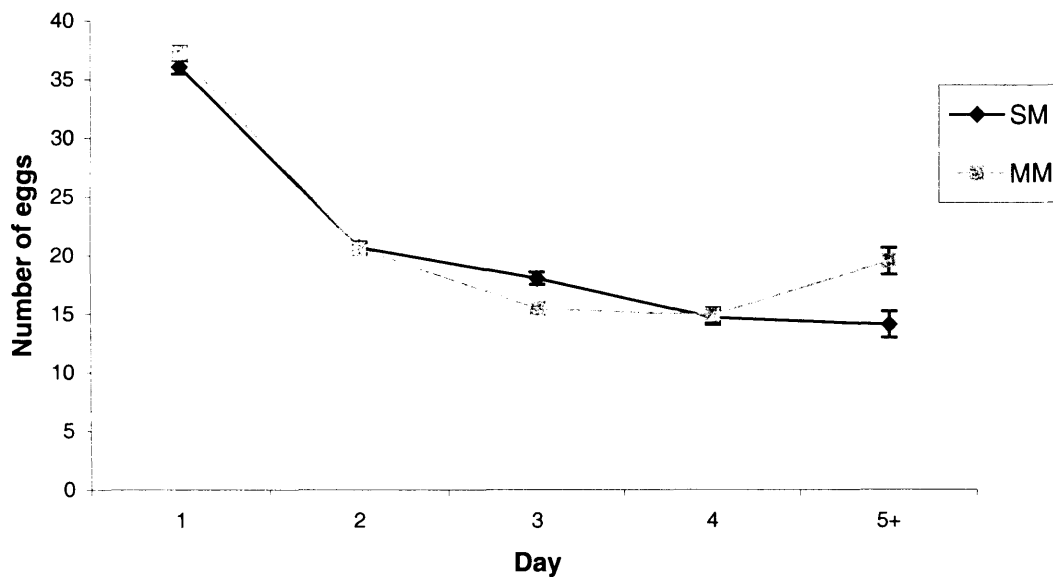
Figure 4.7. Number of eggs laid per female per day by South-India-strain females exposed either to one or seven males. Bars are standard errors of the means.
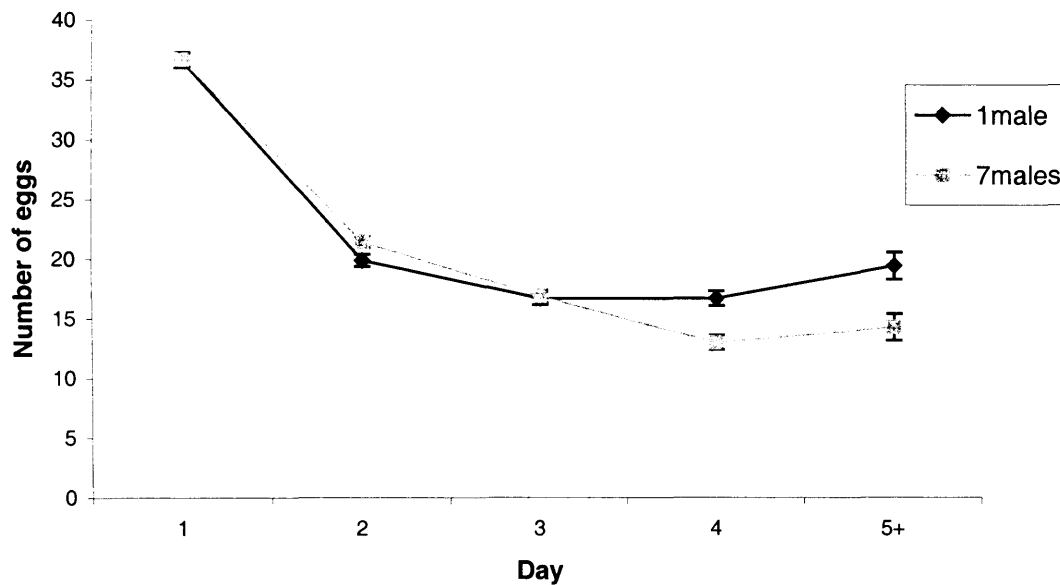
Table 4.11. Significance values for the numbers of eggs laid by South-India-strain females on a particular day when comparing SM and MM females (Matings) and females exposed either to one or seven males (Males). The data were untransformed.

Day	1	2	3	4	5+
Matings	$F_{(1,74)} = 0.48$ $p>0.05$	$F_{(1,74)} = 0.01$ $p>0.05$	$F_{(1,74)} = 2.87$ $p>0.05$	$F_{(1,74)} = 0.02$ $p>0.05$	$F_{(1,74)} = 2.89$ $p>0.05$
Males	$F_{(1,74)} = 0.00$ $p>0.05$	$F_{(1,74)} = 1.18$ $p>0.05$	$F_{(1,74)} = 0.02$ $p>0.05$	$F_{(1,74)} = 4.57$ $p<0.05$ *	$F_{(1,74)} = 2.55$ $p>0.05$

* Indicates a significant result
Full ANOVA is shown in Table 6, appendix 2.

Lay period for Brazil-strain females

The lay period is defined here as the number of days over which a female lays eggs. The number of eggs laid by the South-India-strain females was not significantly different between treatments; therefore it is only the Brazil-strain female lay period that will be examined here.

The lay period of MM Brazil-strain females was significantly longer than for SM females ($T_{(69)}$=2.17, p<0.05). The lay period of MM females was 0.79 days or 14% longer than for SM females (Table 4.12). In contrast, the number of males that a female encountered did not have a significant effect on female lay period ($T_{(69)}$=0.79, p>0.05) (Table 4.13).

Table 4.12. Mean number of days on which Brazil-strain females who are provided with either a MM or SM opportunity will lay eggs. SE values are standard errors of the mean. The data were untransformed.

Mating opportunity	N	Mean number of days	SE
SM	33	5.64	0.26
MM	40	6.43	0.25

Table 4.13. Mean number of days on which females laid eggs after being exposed either to one or seven males. SE values are standard errors of the mean. The data were untransformed.

Males encountered	N	Mean number of days	SE
1 male	38	6.21	0.25
7 males	45	5.91	0.28

Distribution of eggs over seeds

Does distribution of eggs over seeds vary significantly between treatments? To answer this the individual treatments A, B, C and D were compared using contingency table tests. A comparison between the numbers of eggs laid per seed by Brazil- and South-India-strain females was also performed.

The total numbers of eggs that were laid as either a single egg, two eggs or more than two eggs per seed when females experienced one of four different mating treatments were recorded. The individual mating treatment did not significantly affect the egg distribution behaviour of females of either strain (Brazil: $\chi^2_{(6)}$= 4.514, p>0.05, Table 4.14; South India: $\chi^2_{(6)}$= 6.337, p>0.05, Table 4.15). South-India-strain females were found to be significantly more discriminatory than Brazil-strain females, however, and laid fewer multiple eggs per seed ($\chi^2_{(2)}$= 21.785, p<0.001) (Table 4.16).

Table 4.14. Numbers of seeds with single, two or more than two eggs per seed when Brazil-strain females were mated singly and exposed to only one male (A), mated singly and exposed to seven males (B), mated multiply and exposed to seven males (C) or mated multiply and exposed to only one male (D).

	A	B	C	D	Egg/seed total
1 egg/seed	1383	1116	1778	1860	6137
2 eggs/seed	124	80	149	156	509
More than 2 eggs/seed	11	15	16	19	61
Treatment total	1518	1211	1943	2035	6707

$\chi^2 =$ 0.026 +   0.057 +   0.000 +   0.002 +

0.672 +   1.542 +   0.016 +   0.016 +

0.570 +   1.443 +   0.158 +   0.013   = 4.514

Table 4.15. Numbers of seeds with single, two, or more than two eggs per seed when South-India-strain females were mated singly and exposed to only one male (A), mated singly and exposed to seven males (B), mated multiply and exposed to seven males (C) or mated multiply and exposed to only one male (D).

	A	B	C	D	Egg/seed total
1 egg/seed	1763	1691	1835	1914	7203
2 eggs/seed	133	144	174	167	618
More than 2 eggs/seed	6	9	4	6	25
Treatment total	1902	1844	2013	2087	7846

$\chi^2 =$ 0.163 +   0.002 +   0.092 +   0.002 +

1.887 +   0.011 +   1.504 +   0.042 +

0.001 +   1.661 +   0.909 +   0.064   = 6.337

Table 4.16. Numbers of seeds with single, two, or more than two eggs per seed laid by Brazil- and South-India-strain females. Expected values are in parentheses below observed values.

	Brazil strain	South India strain	Egg/seed total
1 egg/seed	6137 (6147.97)	7203 (7192.03)	13340
2 eggs/seed	509 (519.40)	618 (607.60)	1127
More than 2 eggs/seed	61 (39.63)	25 (46.37)	86
Strain total	6707	7846	14553

$$\chi^2 = \begin{matrix} 0.020 + & 0.017 + \\ 0.208 + & 0.178 + \\ 11.517 + & 9.845 \end{matrix} \quad = 21.785$$

4.4.3 Egg mass

The mass of individual eggs (in mg) laid by females of both strains on day one and also on days three and four combined were recorded. Data were analysed untransformed as GLM ANOVA with female emergence mass as a covariate. Mean egg mass for day one and days three and four combined are shown in Tables 4.18 and 4.19.

Both the strain of the female and the day had a significant effect on the mass of eggs laid (Table 4.17; Figure 4.8). South-India-strain females laid the largest eggs compared to those laid by Brazil-strain females ($F_{(1,321)} = 5.85$, $p<0.05$). Eggs laid on days three and four were smaller than those laid on day one by females of both strains ($F_{(1,321)} = 18.01$, $p<0.001$).

Table 4.17. Mean egg mass (SE) in mg for eggs laid by Brazil- and South-India-strain females on day 1 and days 3 and 4 of a lay period. SE values are standard errors of the means. The data were untransformed.

	Brazil	South India	Mean
Day 1	0.0258 (0.0007)	0.0278 (0.0007)	0.0268 (0.0005)
Days 3 and 4	0.0232 (0.0007)	0.0252 (0.0006)	0.0242 (0.0004)
Mean	0.0245 (0.0005)	0.0265 (0.0005)	

Full ANOVA is shown in Table 7, appendix 2.

Figure 4.8. Change in egg mass over time for eggs laid by Brazil-strain and South-India-strain females. Error bars are standard errors of the mean. Note also that the Y-axis does not start at zero.
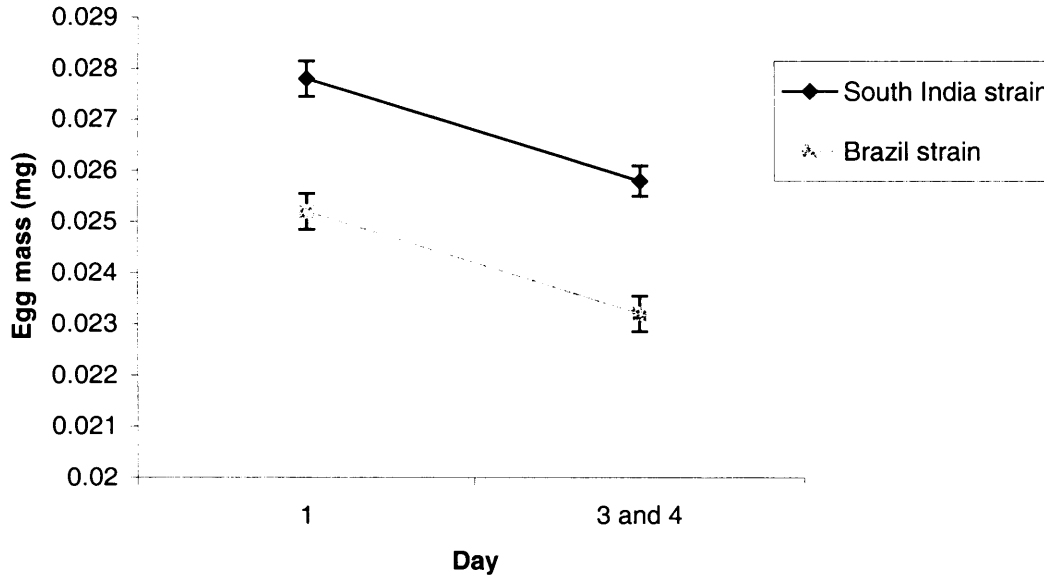
Eggs laid on day 1

Female emergence mass had a significant effect on the mass of eggs laid by females of both strains with larger females laying larger eggs (Brazil: $F_{(1,60)} = 5.22$, $p<0.05$; South India: $F_{(1,69)} = 8.77$, $p<0.005$). For Brazil-strain females only, the number of matings or the number of males a female encountered was not significant in affecting egg mass ($F_{(1,60)} = 2.24$, $p>0.05$ and $F_{(1,60)} = 0.87$, $p>0.05$ respectively). There was a significant interaction, however, between the number of matings and the number of males encountered with SM females exposed to only one male laying the smallest eggs compared to the other three treatments ($F_{(1,60)} = 5.55$, $p<0.05$).

In contrast, the number of matings but not the number of males encountered affected the mass of eggs laid by South-India-strain females (males: $F_{(1,69)} = 1.16$, $p>0.05$). Females that had the opportunity to MM on day one laid significantly larger eggs than SM females ($F_{(1,69)} = 5.52$, $p<0.05$). A significant interaction between the number of matings and the number of males encountered was also observed for the South India strain with SM females exposed to only one male laying the smallest eggs ($F_{(1,69)} = 9.37$, $p<0.005$).

Eggs laid on days 3 and 4

The masses of eggs laid by females that were SM or MM and exposed either to one or four males are shown in Table 4.18. Female emergence mass was a significant factor in terms of egg mass for the South-India-strain females ($F_{(1,87)}$ = 6.59, $p<0.05$) but not for the Brazil-strain females ($F_{(1,90)}$ = 0.42, $p>0.05$). In addition, for Brazil-strain females, the effect of the number of matings, the number of males encountered and their interaction on egg mass was not significant ($F_{(1,90)}$ = 0.05, $p>0.05$; $F_{(1,90)}$ = 1.27, $p>0.05$; $F_{(1,90)}$ = 0.26, $p>0.05$ respectively). For South-India-strain females, the number of matings a female had was highly significant in terms of egg mass with MM females laying significantly heavier eggs ($F_{(1,87)}$ = 22.88, $p<0.001$). The number of males encountered did not affect egg weight ($F_{(1,87)}$ =1.07, $p>0.05$), however, there was a highly significant interaction between the number of matings and the number of males ($F_{(1,87)}$ = 18.07, $p<0.001$).

The masses of eggs laid by females in each individual treatment are presented in Table 4.19. Analyses between South-India-strain female treatments were carried out as ANCOVA with female emergence weight as the covariate. South-India-strain MM females exposed to only one male laid significantly larger eggs than females in all other treatments (SM/exposed to one male: $F_{(1,41)}$ = 34.69, $p<0.001$; SM/exposed to seven males: $F_{(1,45)}$ = 8.98, $p<0.005$; MM/exposed to seven males: $F_{(1,49)}$ = 12.18, $p<0.005$). SM South-India-strain females exposed to only one male laid the smallest eggs compared to females in all other treatments (SM/exposed to seven males: $F_{(1,37)}$ = 8.79, $p<0.001$; MM/exposed to seven males: $F_{(1,41)}$ = 18.14, $p<0.001$). Finally, egg mass was not significantly different between MM or SM South-India-strain females exposed to seven males ($F_{(1,45)}$ = 0.15, $p>0.05$).

Table 4.18. Mean (and SE) mass of eggs (mg) laid by Brazil- and South-India-strain females on day one or days three and four following mating and provided with either MM or SM opportunities or exposure either to one or four males over a four day period. SE values are standard errors of the means. The data were untransformed.

	Brazil Day 1	Brazil Day 3+4	South India Day 1	South India Day 3+4
MM	0.0263 (0.0008)	0.0229 (0.0012)	0.0289 (0.0004)	0.0265 (0.0003)
SM	0.0245 (0.0009)	0.0225 (0.0013)	0.0276 (0.0004)	0.0241 (0.0004)
1 male	0.0248 (0.0009)	0.0217 (0.0013)	0.0279 (0.0004)	0.0251 (0.0004)
4 males	0.0260 (0.0009)	0.0237 (0.0012)	0.0285 (0.0004)	0.0256 (0.0003)

Full ANOVA tables are shown as Tables 8, 9, 10 and 11, appendix 2.

Table 4.19. Mean (and SE) mass of eggs (mg) laid by Brazil- and South-India-strain females on day one or days three and four following mating and exposed to one of four possible treatments: SM and exposed to one (A) or to four different males (B); MM and exposed either to one (D) or to four different males (C). SE values are standard errors of the means. The data were untransformed.

Treatment	Brazil Day 1	Brazil Day 3+4	South India Day 1	South India Day 3+4
A	0.0223 (0.0013)	0.0209 (0.0019)	0.0264 (0.0005)	0.0228 (0.0005)
B	0.0267 (0.0011)	0.0240 (0.0016)	0.0287 (0.0006)	0.0255 (0.0005)
C	0.0254 (0.0014)	0.0237 (0.0016)	0.0283 (0.0005)	0.0273 (0.0005)
D	0.0273 (0.0014)	0.0220 (0.0016)	0.0294 (0.0006)	0.0256 (0.0005)

4.4.4 Parent female treatment and the fecundity of the F_1 female offspring

The total numbers of eggs laid by the F_1 females were recorded over their lifetime. The data were analysed untransformed as GLM ANOVA with emergence mass of the F_1 female taken as a covariate. Transformation could not correct for data skew.

Emergence mass of the F_1 female offspring was found to be a significant factor in terms of the total numbers of eggs laid by both Brazil-strain females ($F_{(1,75)}$ = 7.27, $p<0.01$) and South-India-strain females ($F_{(1,75)}$ = 24.85, $p<0.001$).

The number of matings by the parent female had no effect on the total lifetime egg production of F_1 female offspring of either strain. The numbers of eggs laid by the F_1 offspring were not significantly different for offspring of SM or MM females (Brazil: $F_{(1,75)}$ = 2.36, $p>0.05$; South India: $F_{(1,75)}$ = 2.44, $p>0.05$). The number of males that the parent female encountered did not affect total lifetime egg production of F_1 female

offspring of either strain (Brazil: $F_{(1,75)}$ = 0.53, p>0.05, Table; South India: $F_{(1,75)}$ = 0.59, p>0.05) (Table 4.20).

Table 4.20. Mean numbers of eggs (and SE) laid by SM female F_1 offspring whose parent female was provided with either a SM or MM opportunity and were exposed either to one or seven males. SE values are standard errors of the means. The data were untransformed.

(a)   Brazil-strain F_1 offspring.

Parent treatment	SM	MM	Mean
Exposed to 1 male	106.7 (3.104)	112.5 (2.939)	109.6 (2.110)
Exposed to 7 males	110.1 (2.969)	113.5 (2.948)	111.8 (2.110)
Mean	108.4 (2.090)	113.0 (2.090)	

Full ANOVA is shown in Table 12, appendix 2.

(b)   South-India-strain F_1 offspring.

	SM	MM	Mean
Exposed to 1 male	106.6 (2.394)	102.6 (2.387)	104.6 (1.688)
Exposed to 7 males	104.5 (2.390)	101.0 (2.401)	102.8 (1.688)
Mean	105.6 (1.696)	101.8 (1.696)	

Full ANOVA is shown in Table 13, appendix 2.

Individual parent female treatment and total numbers of eggs laid by the F_1 offspring

The individual parent treatment (A, B, C or D) did not significantly affect the fecundity of F_1 female offspring of both strains (Brazil: $F_{(1,76)}$ = 0.96, p>0.05, Figure 4.9; South India: $F_{(1,76)}$ = 1.01, p>0.05, Figure, 4.10).

The standard deviations and ranges of total numbers of eggs produced by the F_1 female offspring of both strains are also much smaller than those observed for their parents (section 4.4.1). These are illustrated in Table 4.21.

Figure 4.9. Total number of eggs laid by SM Brazil-strain F_1 female offspring whose parent female was either SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D). Bars are standard errors of the means. The data were untransformed.
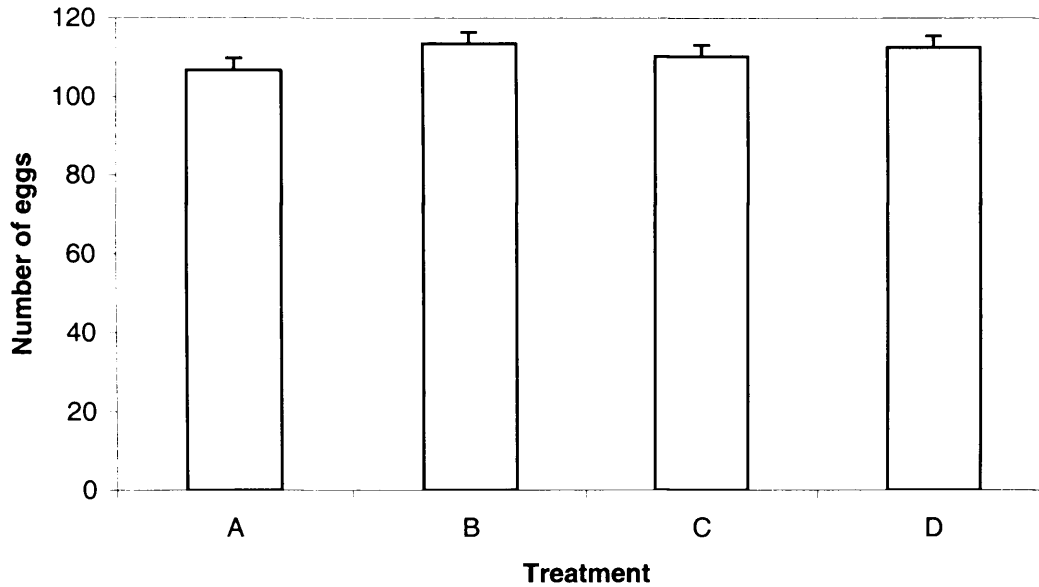
Figure 4.10. Total number of eggs laid by SM South-India-strain F_1 female offspring whose parent female was either SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D). Bars are standard errors of the means. The data were untransformed.
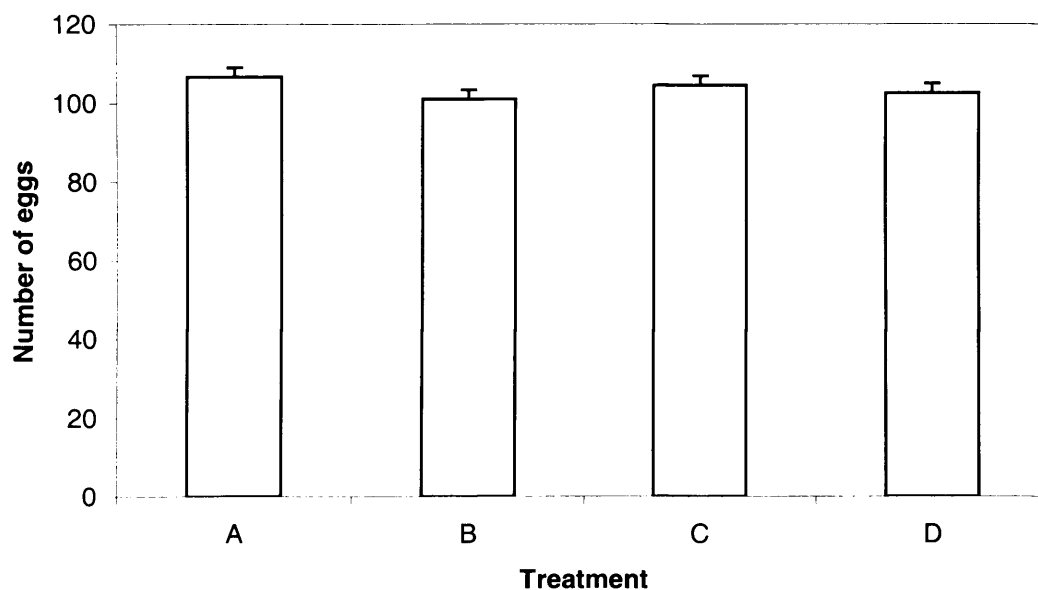
Table 4.21. The standard deviation and range of the total numbers of eggs produced by SM F_1 female offspring whose parent female experienced one of four different treatments: SM and exposed to one male (A), SM and exposed to seven males (B), MM and exposed to seven males (C) or MM and exposed to one male (D). The data were untransformed.

(a)   Brazil-strain F_1 female offspring.

Parent treatment	N	SD	Minimum number of eggs laid	Maximum number of eggs laid	Range
A	20	15.11	62	131	69
B	20	13.88	91	143	52
C	20	13.35	76	128	52
D	20	11.87	98	138	40

(b)   South-India-strain F_1 female offspring.

Parent treatment	N	SD	Minimum number of eggs laid	Maximum number of eggs laid	Range
A	20	10.64	86	127	41
B	20	15.31	72	125	53
C	20	11.32	79	124	45
D	20	11.17	82	131	49

Parent female treatment and egg viability of the F_1 female offspring: Brazil-strain F_1 female offspring

The emergence mass of the female was not found to be a significant factor in terms of the numbers of non-emerge ($F_{(1,75)} = 0.84$, $p > 0.05$), non-hatch ($F_{(1,75)} = 0.89$, $p > 0.05$), non-viable ($F_{(1,75)} = 1.19$, $p > 0.05$) or viable eggs laid by females ($F_{(1,75)} = 1.30$, $p > 0.05$). The numbers of eggs in each of the four categories were recorded and analysed as ANCOVA with the F_1 female emergence mass as the covariate. Mean numbers of eggs produced are shown in Tables 4.22, 4.23 and 4.24. Differences within treatments were analysed by T test since the emergence mass was not shown to be a significant factor.

For SM F_1 Brazil-strain females, egg survival was not affected by the number of times that the parent female mated. There was no significant difference in terms of the numbers of non-emerge, non-hatch, non-viable and viable eggs laid by the SM F_1 female offspring when parent females were SM or MM ($F_{(1,75)} = 0.06$, $p > 0.05$; $F_{(1,75)} = 1.65$, $p > 0.05$; $F_{(1,75)} = 0.52$, $p > 0.05$ and $F_{(1,75)} = 0.48$, $p > 0.05$ respectively).

The number of males that a parent female was exposed to did, however, have a significant effect on the number of non-hatch eggs laid by the F_1 offspring. In this

case, F_1 female offspring from parent females exposed to seven males laid significantly more eggs that failed to hatch than F_1 females from parent females exposed to only one male ($F_{(1,75)} = 5.32$, $p<0.05$).

The numbers of non-emerge, non-viable and viable eggs laid by the female F_1 offspring were unaffected by the number of males that the parent female encountered with no significant difference being observed when parent females were exposed either to one or seven males ($F_{(1,75)} = 0.01$, $p>0.05$; $F_{(1,75)} = 1.36$, $p>0.05$ and $F_{(1,75)} = 1.26$, $p>0.05$ respectively).

Table 4.22. Proportion of non-emerge, non-hatch, non-viable and viable eggs that were laid by SM F1 Brazil-strain female offspring whose parent female was provided either with a MM or SM opportunity.

(a)   Angular transformed proportions and SE values. SE values are standard errors of the mean.

Female parent mating opportunity	Non-emerge eggs (SE)	Non-hatch eggs (SE)	Non-viable eggs (SE)	Viable eggs (SE)
SM	0.238 (0.017)	0.221 (0.012)	0.333 (0.012)	1.044 (0.013)
MM	0.232 (0.017)	0.199 (0.012)	0.314 (0.018)	1.056 (0.013)

Full ANOVA is shown in Table 14, appendix 2.

(b)   Back transformed proportions.

Female parent mating opportunity	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
SM	0.056	0.048	0.107	0.747
MM	0.053	0.039	0.096	0.758

Table 4.23. Proportion of non-emerge, non-hatch, non-viable and viable eggs that were laid by SM F_1 Brazil-strain female offspring whose parent female was exposed either to one or seven males.

(a)   Angular transformed proportions and SE values. SE values are standard errors of the mean.

Number of males	Non-emerge eggs (SE)	Non-hatch eggs (SE)	Non-viable eggs (SE)	Viable eggs (SE)
1 male	0.234 (0.017)	0.190 (0.012)	0.308 (0.018)	1.061 (0.013)
7 males	0.237 (0.017)	0.230 (0.012)	0.339 (0.018)	1.039 (0.013)

Full ANOVA is shown in Table 14, appendix 2.

(b)   Back transformed proportions.

Number of males	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
1 male	0.054	0.036	0.092	0.762
7 males	0.055	0.052	0.110	0.743

There was no significant difference between treatments in terms of the numbers of non-emerge, non-viable and viable eggs that were laid by females ($F_{(1,75)}$ = 0.85, $p > 0.05$; $F_{(1,75)}$ = 0.92, $p > 0.05$; and $F_{(1,75)}$ = 0.85, $p > 0.05$ respectively). In contrast, F_1 offspring from parent females that SM and were exposed to seven males laid significantly more eggs that failed to hatch (non-hatch eggs) than the F_1 offspring from MM parent females exposed to only one male ($T_{(33)}$=2.46, $p < 0.05$).

Finally, no significant interactions were observed between the number of males the parent female was exposed to and the number of matings the parent female had in terms of non-emerge, non-hatch, non-viable and viable eggs laid by the F_1 female offspring ($F_{(1,75)}$ = 1.63, $p < 0.05$; $F_{(1,75)}$ = 0.14, $p > 0.05$; $F_{(1,75)}$ = 0.56, $p > 0.05$ and $F_{(1,75)}$ = 0.49, $p > 0.05$ respectively).

Table 4.24. Proportion of non-emerge, non-hatch, non-viable and viable eggs laid by SM F_1 Brazil-strain female offspring whose female parent experienced one of four different mating treatments.

(a) Angular transformed proportions and SE values. SE values are standard errors of the mean.

Parent treatment	Non-emerge eggs (SE)	Non-hatch eggs (SE)	Non-viable eggs (SE)	Viable eggs (SE)
SM alone (A)	0.253 (0.025)	0.198 (0.018)	0.327 (0.027)	1.048 (0.019)
SM 7 males (B)	0.224 (0.024)	0.244 (0.017)	0.338 (0.025)	1.039 (0.019)
MM 7 males (C)	0.249 (0.024)	0.216 (0.017)	0.339 (0.025)	1.039 (0.018)
MM 1 male (D)	0.215 (0.024)	0.182 (0.017)	0.289 (0.025)	1.074 (0.018)

(b) Back transformed proportions.

Parent treatment	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
SM alone (A)	0.063	0.039	0.103	0.750
SM 7 males (B)	0.049	0.058	0.110	0.743
MM 7 males (C)	0.061	0.046	0.111	0.743
MM 1 male (D)	0.046	0.033	0.081	0.773

Parent female treatment and egg viability of the F_1 female offspring : South-India-strain F_1 female offspring

The emergence mass of the F_1 females was not found to be a significant factor in terms of the numbers of non-emerge ($F_{(1,75)}$ = 2.58, p>0.05), non-hatch ($F_{(1,75)}$ = 0.48, p>0.05) or non-viable ($F_{(1,75)}$ = 3.76, p>0.05) eggs laid by females. It was found to be significant for the numbers of viable eggs laid, however ($F_{(1,75)}$ = 3.98, p<0.05). The numbers of eggs in each of the four categories were recorded and analysed as ANCOVA with F_1 female emergence mass as the covariate. Mean numbers of eggs produced are shown in Tables 4.25, 4.26 and 4.27. Differences within treatments were analysed by T test where emergence mass was not shown to be a significant factor.

There was no significant difference in terms of the numbers of non-emerge, non-hatch, non-viable and viable eggs laid by the SM F_1 females when parent females

were SM or MM ($F_{(1,75)}$ = 0.29, p>0.05; $F_{(1,75)}$ = 0.04, p>0.05; $F_{(1,75)}$ =0.01, p>0.05 and $F_{(1,75)}$ =0.01, p>0.05 respectively) (Table 4.25). The number of males that a parent female was exposed to did, however, have a significant effect on the number of non-viable and viable eggs laid by the F_1 female offspring. In this case, offspring from parent females exposed to seven males laid significantly fewer non-viable eggs ($F_{(1,75)}$ = 5.22, p<0.05) and significantly more viable eggs ($F_{(1,75)}$ = 5.11, p<0.05) than F_1 offspring from parent females exposed to only one male. The number of non-emerge and non-hatch eggs laid by the female F_1 offspring was unaffected by the number of males that the parent female had encountered with no significant difference being observed when parent females had been exposed either to one or seven males ($F_{(1,75)}$ = 2.53, p>0.05 and $F_{(1,75)}$ = 3.19, p>0.05 respectively).

Table 4.25. Proportion of non-emerge, non-hatch, non-viable and viable eggs that were laid by SM F_1 South-India-strain females whose female parents were provided either with a MM or SM opportunity.

(a)   Angular transformed proportions and SED values. SED values are standard errors of the difference between the means.

Female parent mating opportunity	Non-emerge eggs (SED)	Non-hatch eggs (SED)	Non-viable eggs (SED)	Viable eggs (SED)
SM	0.271 (0.013)	0.143 (0.012)	0.311 (0.013)	1.061 (0.009)
MM	0.261 (0.013)	0.146 (0.012)	0.312 (0.013)	1.060 (0.009)

Full ANOVA is shown in Table 15, appendix 2.

(b)   Back transformed proportions.

Female parent mating opportunity	Non-emerge eggs (SE)	Non-hatch eggs (SE)	Non-viable eggs (SE)	Viable eggs (SE)
SM	0.071	0.020	0.093	0.762
MM	0.067	0.021	0.094	0.761

Table 4.26. Proportion of non-emerge, non-hatch, non-viable and viable eggs that were laid by SM F_1 South-India-strain females whose female parent was exposed either to one or seven males.

(a) Angular transformed proportions and SED values. SED values are standard errors of the difference between the means.

Number of males	Non-emerge eggs (SED)	Non-hatch eggs (SED)	Non-viable eggs (SED)	Viable eggs (SED)
1 male	0.280 (0.013)	0.159 (0.012)	0.332 (0.013)	1.046 (0.009)
7males	0.252 (0.013)	0.130 (0.012)	0.291 (0.013)	1.074 (0.009)

Full ANOVA is shown in Table 15, appendix 2.

(b) Back transformed proportions.

Number of males	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
1 male	0.076	0.025	0.106	0.749
7males	0.062	0.017	0.082	0.773

There was no significant difference between parent female treatments in terms of the numbers of non-emerge and non-hatch eggs that were laid by F_1 female offspring ($F_{(1,75)} = 1.19$, $p>0.05$ and $F_{(1,75)} = 1.31$, $p>0.05$ respectively). In terms of the numbers of non-viable and viable eggs that were laid however, a significant difference was observed. F_1 female offspring from MM parent females exposed to seven males laid significantly more viable eggs and significantly fewer non-viable eggs than the F_1 female offspring from SM parent females exposed to only one male ($T_{(37)}=2.14$, $p<0.05$ and $T_{(37)}=2.14$, $p<0.05$ respectively). No other combinations of treatments were significantly different ($p>0.05$).

Finally, no significant interactions were observed between the number of males the parent female was exposed to and the number of matings the parent female had in terms of non-emerge, non-hatch, non-viable and viable eggs laid by the F_1 female offspring ($F_{(1,75)} = 0.19$, $p>0.05$; $F_{(1,75)} = 0.59$, $p>0.05$; $F_{(1,75)} = 0.01$, $p>0.05$ and $F_{(1,75)} = 0.03$, $p>0.05$ respectively).

Table 4.27. Proportion of non-emerge, non-hatch, non-viable and viable eggs laid by SM F_1 South-India-strain female offspring whose female parent experienced one of four different mating treatments.

(a)   Angular transformed proportions and SED values. SED values are standard errors of the difference between the means.

Parent treatment	Non-emerge eggs (SED)	Non-hatch eggs (SED)	Non-viable eggs (SED)	Viable eggs (SED)
SM alone (A)	0.281 (0.018)	0.164 (0.017)	0.330 (0.018)	1.048 (0.012)
SM 7 males (B)	0.260 (0.018)	0.122 (0.017)	0.291 (0.018)	1.074 (0.012)
MM 7 males (C)	0.243 (0.018)	0.138 (0.017)	0.291 (0.018)	1.075 (0.012)
MM 1 male (D)	0.279 (0.018)	0.155 (0.017)	0.333 (0.018)	1.045 (0.012)

(b)   Back transformed proportions.

Parent treatment	Non-emerge eggs	Non-hatch eggs	Non-viable eggs	Viable eggs
SM alone (A)	0.077	0.027	0.105	0.751
SM 7 males (B)	0.066	0.015	0.082	0.772
MM 7 males (C)	0.058	0.019	0.082	0.773
MM 1 male (D)	0.076	0.024	0.107	0.748

4.4.5   Parent female treatment and F_1 female offspring egg distribution

The distribution of eggs over seeds by F_1 female offspring whose parent female experienced one of four possible treatments (A, B, C and D) was compared using contingency table tests. Treatments A and B were combined and contrasting with treatments C and D to examine the effect of SM or MM by parent females on the egg distribution of the F_1 female offspring. Similarly, combining treatments A and D and contrasting them with treatments B and C examined the effect of number of males a parent female was exposed to on the egg distribution of the F_1 female offspring. Finally, a comparison between the total numbers of eggs laid per seed by F_1 Brazil- and South-India-strain females was performed. The numbers of seeds with a single egg, two eggs or more than two eggs per seed laid on them by SM F_1 female offspring whose female parent experienced one of four different mating treatments were recorded.

Brazil-strain F_1 female offspring

The number of times that a parent female mated (SM or MM) and the number of males that she encountered (1 male or 7 males) had no effect on the egg distribution of the F_1 female offspring ($\chi^2_{(2)}=1.097$, $p>0.05$ and $\chi^2_{(2)}=2.166$, $p>0.05$ respectively). The individual treatment experienced by the P female did, however, affect the egg distribution of the F_1 female offspring ($\chi^2_{(6)}=12.885$, $p<0.05$) (see Table 4.28).

SM female F_1 offspring from SM parent females exposed to one male (A) laid 45% fewer multiple eggs per seed than expected and F_1 female offspring from SM parent females exposed to seven males (B) laid 44% more multiple eggs per seed than expected. Numbers of eggs laid by F_1 females from MM parent females exposed to seven males (C) did not differ significantly from expected. However, those offspring from MM parent females exposed to one male (D) laid 6.8% more double eggs per seed than expected

Table 4.28. Chi-square approximation with expected (in parentheses) and observed values of numbers of seeds with single, two or more than two eggs per seed laid by SM F_1 Brazil-strain female offspring when the parent female was exposed to one of four different treatments: SM and exposed to only one male (A); SM and exposed to seven males (B); MM and exposed to seven males (C); MM and exposed to only one male (D).

	A	B	C	D	Eggs/seed total
1 egg/seed	1677 (1651.54)	1593 (1602.66)	1682 (1677.26)	1619 (1639.53)	6571
2 eggs/seed	236 (250.83)	243 (243.41)	253 (254.74)	266 (249.01)	998
More than 2 eggs/seed	13 (23.63)	33 (22.93)	21 (23.99)	27 (23.45)	94
Treatment total	1926	1956	1869	1912	7663

$$\chi^2 = \quad 0.393 + \quad 0.058 + 0.013 + \quad 0.257 +$$
$$0.877 + \quad 0.001 + 0.012 + \quad *1.159 +$$
$$*4.779 + \quad *4.426 + 0.374 + \quad 0.536 \qquad = 12.885$$

*Indicates an influential result.

South-India-strain F_1 female offspring

The individual treatment experienced by the parent female and the number of times that the parent female mated significantly affected the egg distribution of the SM F_1 South-India-strain female offspring ($\chi^2_{(6)}=12.883$, $p<0.05$ and $\chi^2_{(2)}=8.418$, $p<0.05$).

The number of males encountered by a parent female, however, did not affect the SM F_1 females egg distribution ($\chi^2_{(2)}$=3.611, p>0.05).

F_1 female offspring from SM parent females exposed to seven male (B) laid 68% fewer multiple eggs per seed than expected whereas F_1 female offspring from MM parent female exposed to one male (D) laid 94% more multiple eggs per seed than expected. F_1 female offspring from MM parent females exposed to seven males (C) laid 12% fewer double eggs per seed than expected (Table 4.29).

SM F_1 female offspring from MM parent females laid 7.5% fewer double eggs per seed and 45% more multiple eggs per seed than expected. SM F_1 female offspring from SM parent females laid 7.6% more double eggs per seed and 44% fewer multiple eggs per seed than expected (Table 4.30).

Table 4.29. Chi-square approximation with expected (in parentheses) and observed values of numbers of seeds with single, two or more than two eggs per seed laid by South-India-strain F_1 female offspring when the parent female was exposed to one of four different treatments: mated singly and exposed to only one male (A); mated singly and exposed to seven males (B); mated multiply and exposed to seven males (C); mated multiply and exposed to only one male (D).

	A	B	C	D	Egg/seed total
1 egg/seed	1764 (1773.43)	1747 (1754.15)	1768 (1749.56)	1733 (1734.86)	7012
2 eggs/seed	162 (151.24)	161 (149.60)	131 (149.21)	144 (147.95)	598
More than 2 eggs/seed	5 (6.32)	2 (6.24)	6 (6.24)	12 (6.19)	25
Treatment total	1931	1905	1910	1889	7635

$\chi^2 =$ 0.050 + 0.029 + 0.194 + 0.002 +
0.765 + 0.869 + *2.222 + 0.106 +
0.277 + *2.894 + 0.374 + *5.466 = 12.883
*Indicates an influential result.

Table 4.30. Chi-square approximation with expected and observed values for the numbers of seeds with single, two or more than two eggs per seed laid by SM F_1 South-India-strain female offspring whose parent female was provided with either a SM or MM opportunity.

	SM	MM	Eggs/seed total
1 egg/seed	3511 (3527.58)	3501 (3484.42)	7012
2 eggs/seed	323 (300.84)	275 (297.16)	598
More than 2 eggs/seed	7 (12.58)	18 (12.42)	25
Treatment total	3841	3794	7635

$$
\chi^2 = \quad
\begin{array}{ll}
0.078 + & 0.079 + \\
*1.632 + & *1.652 + \\
*2.473 + & *2.504 + \quad = 8.418
\end{array}
$$

*Indicates an influential result.

The strain of the F_1 female offspring had a significant effect on the distribution of eggs over seeds ($\chi^2_{(2)}$=200.682, p<0.0001). South-India-strain females were more discriminating and laid fewer multiple eggs per seed than expected. In contrast, the F_1 Brazil-strain females laid significantly more multiple eggs per seed than expected (Table 4.31).

Table 4.31. Numbers of seeds with single, two or more than two eggs per seed laid by SM F_1 Brazil- and South-India-strain females. Expected values are in parentheses below observed values.

	Brazil strain	South India strain	Egg/seed total
1 egg/seed	6571 (6847.96)	7012 (6735.04)	13583
2 eggs/seed	1098 (855.05)	598 (840.95)	1696
More than 2 eggs/seed	94 (59.99)	25 (59.01)	119
Strain total	7763	7635	15398

$$
\chi^2 = \quad
\begin{array}{lll}
& 11.201 & + \quad 11.389 \\
+ & 69.031 & + \quad 70.189 \\
+ & 19.275 & + \quad 19.598 \quad = 200.682
\end{array}
$$

4.4.6 Parent female mating treatment and the emergence mass of the F_1 offspring

Eggs laid by parent females were separated according to the day on which they were laid. To examine the effect of the parent treatment on F_1 offspring emergence mass, offspring emergence mass was recorded for those individuals that had emerged from eggs laid on day one and also on days three and four combined. Similar numbers of individuals emerging from eggs laid on days three and four were used and the two days were amalgamated due to a smaller number of eggs being laid on these days.

The emergence mass of the Brazil-strain parent female could not be analysed as a covariate for the emergence weight of the Brazil-strain F_1 offspring for males hatching from eggs laid on day one and male and female offspring hatching from eggs laid on days three and four. The emergence mass of the Brazil-strain parent female was analysed separately, however, and was not found to be significantly different between treatments ($F_{(1,76)} = 0.13$, p>0.05) (Table 4.32). It was therefore assumed that the emergence mass of the Brazil-strain F_1 offspring would be unaffected by differences in parent female mass between treatments.

Table 4.32. Mean (and SE) mass of Brazil-strain parent females at emergence (in mg) analysed according to the treatment that they were later exposed to. SE values are standard errors of the mean. The data were untransformed.

Parent female treatment	Emergence mass (SE)	N
SM alone (A)	5.3078 (0.678)	20
SM 7 males (B)	5.1999 (0.818)	20
MM 7 males (C)	5.1526 (0.806)	20
MM 1 male (D)	5.2417 (0.941)	20

Brazil-strain offspring emerging from eggs laid on day one

The emergence mass of the parent female was analysed as a covariate for the emergence mass of the F_1 female offspring and had a significant effect on the emergence weight of the offspring ($F_{(1,155)} = 20.78$, p<0.001). The number of matings that a parent female had did not significantly affect the emergence mass of the F_1 female ($F_{(1,155)} = 2.15$, p>0.05) or male offspring ($F_{(1,156)} = 0.51$, p>0.05). Similarly, the number of males that a female was exposed to was not significant in determining female ($F_{(1,155)} = 0.00$, p>0.05) and male offspring emergence mass ($F_{(1,156)} = 0.01$,

$p > 0.05$). Mean emergence masses for F_1 female and male offspring are shown in Table 4.33.

Table 4.33. Mean (and SE) adult emergence masses (mg) for Brazil-strain F_1 offspring hatched from eggs laid on day one whose female parent was provided with either a SM or MM opportunity and exposed either to one or seven males. SE values are standard errors of the mean. The data were untransformed.

(a) F_1 female offspring.

	SM	MM	Mean
Exposed to 1 male	6.272 (0.076)	6.097 (0.078)	6.184 (0.054)
Exposed to 7 males	6.214 (0.077)	6.156 (0.076)	6.185 (0.054)
Mean	6.243 (0.055)	6.126 (0.055)	

Full ANOVA is shown in Table 16, appendix 2.

(b) F_1 male offspring.

	SM	MM	Mean
Exposed to 1 male	3.875 (0.062)	3.956 (0.062)	3.916 (0.044)
Exposed to 7 males	3.918 (0.062)	3.925 (0.062)	3.922 (0.044)
Mean	3.897 (0.044)	3.941 (0.044)	

Full ANOVA is shown in Table 17, appendix 2.

Brazil-strain F_1 offspring emerging from eggs laid on days three and four

The number of matings the parent female had did not significantly affect the emergence mass of the F_1 female ($F_{(1,212)} = 0.30$, $p > 0.05$) or male offspring ($F_{(1,178)} = 0.96$, $p > 0.05$). Similarly, the emergence mass of F_1 female offspring was unaffected by the number of males that the parent female was exposed to ($F_{(1,212)} = 0.97$, $p > 0.05$). In contrast, male F_1 offspring from parent females exposed to seven males were significantly larger (3.7% or 0.145mg larger) than F_1 males from parent females exposed to only one male ($F_{(1,178)} = 8.25$, $p < 0.01$). Mean adult emergence masses for F_1 male and female offspring are shown in Table 4.34.

The individual treatment of the parent female also had a significant effect on the adult emergence mass of the F_1 male offspring and was analysed with T test. F_1 male offspring from SM parent females exposed to seven males were significantly larger than F_1 male offspring from SM or MM parent females exposed to one male ($T_{(83)} =$

2.84, p<0.01 and $T_{(89)}$=3.02, p<0.005 respectively). No other combination was significantly different (p>0.05).

Table 4.34. Mean (and SE) adult emergence masses (mg) for F_1 Brazil-strain offspring hatched from eggs laid on days three and four whose female parent was provided with either a SM or MM opportunity and was exposed either to one or seven males. SE values are standard errors of the mean. The data were untransformed.

(a) F_1 female offspring.

	SM	MM	Mean
Exposed to 1 male	6.385 (0.077)	6.236 (0.077)	6.310 (0.054)
Exposed to 7 males	6.202 (0.077)	6.268 (0.077)	6.235 (0.054)
Mean	6.293 (0.054)	6.252 (0.054)	

Full ANOVA is shown in Table 18, appendix 2.

(b) F_1 male offspring.

	SM	MM	Mean
Exposed to 1 male	3.866 (0.051)	3.886 (0.050)	3.876 (0.036)
Exposed to 7 males	4.080 (0.050)	3.962 (0.050)	4.021 (0.035)
Mean	3.973 (0.036)	3.924 (0.035)	

Full ANOVA is shown in Table 19, appendix 2.

South-India-strain F_1 offspring emerging from eggs laid on day one

The emergence mass of the parent female was analysed as a covariate in GLM ANOVA and was found to have a significant effect on the emergence mass of the F_1 female offspring ($F_{(1,99)}$ = 7.34, p<0.01). F_1 female offspring from MM parent females were significantly larger than F_1 female offspring from SM parent females ($F_{(1,99)}$ = 9.19, p<0.01). The number of males that a parent female was exposed to, however, did not affect the emergence mass of the F_1 female offspring ($F_{(1,99)}$ = 0.03, p>0.05). Mean emergence mass for F_1 female offspring is shown in Table 4.35.

Differences within treatments were analysed as ANCOVA with the parent female emergence weight as the covariate. F_1 female offspring from MM parent females exposed to seven males were significantly larger than F_1 female offspring from SM parent females who were exposed either to one or seven males ($F_{(1,49)}$ = 5.20, p<0.05; 0.238mg or 3.4% larger and $F_{(1,49)}$ = 15.05, p<0.001; 0.403mg or 6.1% larger, respectively). F_1 female offspring from MM parent females exposed to one male were significantly larger than F_1 females offspring from SM parent females exposed

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to seven males ($F_{(1,49)}$ = 4.40, p<0.05; 0.268mg or 4% larger) but not from SM parent females exposed to only one male ($F_{(1,49)}$ = 0.84, p>0.05). All other combinations were not significantly different (p>0.05).

The emergence mass of the parent female did not have a significant effect on the emergence mass of the F_1 male offspring ($F_{(1,119)}$ = 1.76, p>0.05). The emergence mass of the F_1 male offspring was not significantly affected by the number of matings that a parent female had or by the number of males that she was exposed to ($F_{(1,119)}$ = 0.06, p>0.05 and $F_{(1,119)}$ = 2.82, p>0.05, respectively. Mean emergence mass for F_1 male offspring are shown in Table 4.35.

The parent female treatment was observed to have a significant effect on the emergence mass of the F_1 male offspring. F_1 male offspring from MM parent females exposed to seven males were significantly larger than F_1 offspring from SM or MM parent females exposed to only one male ($T_{(59)}$=2.15, p<0.05 and $T_{(46)}$=2.15, p<0.05 respectively). No other combinations were found to be significantly different (p>0.05).

Table 4.35. Mean (and SE) adult emergence masses (mg) for F_1 South-India-strain offspring hatched from eggs laid on day one whose female parent was provided with either a SM or MM opportunity and was exposed either to one or seven males. SE values are standard errors of the mean. The data were untransformed.

(a) F_1 female offspring.

	SM	MM	Mean
Exposed to 1 male	6.788 (0.085)	6.891 (0.083)	6.840 (0.060)
Exposed to 7 males	6.623 (0.084)	7.026 (0.083)	6.825 (0.060)
Mean	6.706 (0.059)	6.959 (0.059)	

Full ANOVA is shown in Table 22, appendix 2.

(b) F_1 male offspring.

	SM	MM	Mean
Exposed to 1 male	4.487 (0.065)	4.423 (0.064)	4.455 (0.046)
Exposed to 7 males	4.517 (0.064)	4.612 (0.064)	4.565 (0.046)
Mean	4.502 (0.045)	4.518 (0.045)	

Full ANOVA is shown in Table 23, appendix 2.

South-India-strain F_1 offspring emerging from eggs laid on days three and four

The emergence mass of the female parent was not found to be a significant factor on the emergence mass of the F_1 female offspring ($F_{(1,75)} = 0.46$, p>0.05). The number of matings that a female parent had and the number of males she encountered did not significantly affect the emergence mass of her F_1 female offspring ($F_{(1,75)} = 1.49$, p>0.05 and $F_{(1,75)} = 0.09$, p>0.05 respectively). Mean adult emergence masses for F_1 female offspring hatched from eggs laid on days three and four are shown in Table 4.36.

The emergence mass of the female parent had a significant effect on the emergence mass of the F_1 male offspring ($F_{(1,115)} = 4.06$, p<0.05). The number of matings that a female parent had was not found to significantly affect the emergence mass of the F_1 male offspring ($F_{(1,115)} = 0.48$, p>0.05). The number of males that a female parent was exposed to, however, did have a significant effect on the emergence mass of the F_1 male offspring. F_1 offspring from parent females exposed to seven males were significantly larger (5.69% or 0.248mg larger) than offspring from female parents exposed to only one male ($F_{(1,115)} = 8.83$, p<0.005). Mean adult emergence masses for F_1 male offspring hatched from eggs laid on days three and four are shown in Table 4.36.

The pattern of emergence masses for F_1 males hatched from eggs laid on days three or four follows that already seen for F_1 males hatched from eggs laid on day one. The differences are now much greater, however. F_1 male offspring from MM female parents exposed to seven males were significantly larger than offspring from SM parent females exposed either to one or seven males ($F_{(1,87)} = 7.97$, p<0.005 and $F_{(1,87)} = 4.18$, p<0.05 respectively) and MM female parents exposed to only one male ($F_{(1,87)} = 12.90$, p<0.005). Interestingly, the F_1 male offspring from MM female parents exposed to only one male were the smallest when compared to males in all other treatments.

Table 4.36. Mean (and SE) adult emergence masses (mg) for F_1 South-India-strain offspring hatched from eggs laid on days three and four whose female parent was provided with either a SM or MM opportunity and was exposed either to one or seven males. SE values are standard errors of the mean. The data were untransformed.

(a) F_1 female offspring.

	SM	MM	Mean
Exposed to 1 male	6.676 (0.114)	6.771 (0.112)	6.723 (0.080)
Exposed to 7 males	6.668 (0.113)	6.846 (0.112)	6.757 (0.080)
Mean	6.672 (0.079)	6.809 (0.079)	

Full ANOVA is shown in Table 24, appendix 2.

(b) F_1 male offspring.

	SM	MM	Mean
Exposed to 1 male	4.419 (0.086)	4.295 (0.082)	4.357 (0.058)
Exposed to 7 males	4.486 (0.083)	4.724 (0.082)	4.605 (0.058)
Mean	4.452 (0.058)	4.510 (0.058)	

Full ANOVA is shown in Table 25, appendix 2.

4.4.7 Grandmother's treatment and the emergence mass of the F_2 offspring

F_1 female offspring were mated singly and kept alone and the resulting eggs laid on day one were isolated and the adult emergence masses of the F_2 male and female offspring recorded. The data were analysed according to the mating treatment experienced by the grandmother (parent female to F_1). All F_1 females had experienced the same mating treatment, SM and exposed to one male (A). Data was analysed as GLM ANOVA.

It was not possible to take the F_1 female emergence mass as a covariate in the analyses. Emergence masses of F_1 females from grandmothers experiencing treatments B, C and D were not significantly different. In contrast, however, emergence masses of F_1 females from grandmothers experiencing treatment A were significantly heavier than the other F_1 females ($F_{(1,75)}$ = 4.28, p<0.01) and this may have contributed to the significant result observed for F_2 offspring from SM grandmothers below. Mean emergence masses for the F_1 females used are shown in Table 4.37.

Table 4.37. Emergence mass of F_1 Brazil-strain females (in mg) that gave rise to the F_2 offspring. SE values are standard errors of the mean. The data were untransformed.

Parent female treatment	Mean emergence weight in mg (SE)	N
SM alone (A)	6.412 (0.323)	20
SM 7 males (B)	6.032 (0.475)	20
MM 7 males (C)	5.991 (0.474)	20
MM 1 male (D)	6.062 (0.387)	20

Brazil-strain F_2 offspring

The number of males encountered by the grandmother did not affect the adult emergence mass of the F_2 female offspring ($F_{(1,76)} = 0.02$, $p>0.05$) by F_1 females. The number of matings that the grandmother had did, however, have a significant effect on the adult emergence mass of the F_2 female offspring. F_2 female offspring from SM grandmothers were significantly larger (4.02% or 0.244mg larger) than F_2 female offspring from MM grandmothers ($F_{(1,76)} = 5.78$, $p<0.05$). Mean adult emergence masses for F_2 female offspring are shown in Table 4.38. Additionally, when comparing the individual treatments, F_2 female emergence mass was not significantly different irrespective of the grandmothers treatment ($F_{(1,76)} = 2.04$, $p>0.05$).

The number of matings that the grandmother had did not significantly affect the adult emergence mass of the male F_2 ($F_{(1-76)} = 2.82$, $p>0.05$). The number of males encountered by the grandmother did, however, have a significant effect on the adult emergence mass of the F_2 male offspring. F_2 Male offspring whose grandmothers were exposed to seven males were significantly larger (5.74% or 0.214mg larger) than F_2 males whose grandmothers were exposed to only one male ($F_{(1,76)} = 7.60$, $p<0.01$). Mean adult emergence masses for F_2 male offspring are shown in Table 4.38. In addition, F_2 males from SM grandmothers exposed to seven males were significantly larger than F_2 males from MM grandmothers exposed to only one male ($T_{(37)}=3.05$, $p<0.005$; 9.25% larger or a difference of 0.343mg) and SM grandmothers exposed to only one male ($T_{(37)}=3.04$, $p<0.005$; 8.05% larger or a difference of 0.302mg). They were not significantly different to F_2 males from MM grandmothers exposed to seven males ($T_{(37)}=1.99$, $p>0.05$).

It should be noted that it was not possible to take the F_1 female emergence mass as a covariate in the analyses for the male offspring. As explained above F_1 females taken

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from treatments B, C and D were not significantly different, however, F_1 females taken from treatment A were significantly heavier than the other females ($F_{(1,76)}$ = 4.28, p<0.01) (Table 4.37). This does not account for the significant difference in masses observed for F_2 males, however, as it is the F_2 male offspring from treatment B and not A that are larger.

Table 4.38. Mean (and SE) adult emergence masses (mg) for F_2 Brazil-strain offspring hatched from eggs laid by SM F_1 females on day one whose grandmother was provided with either a SM or MM opportunity and was exposed either to one or seven males. SE values are standard errors of the mean. The data were untransformed.

(a) F_2 female offspring.

	SM	MM	Mean
Exposed to 1 male	6.350 (0.101)	6.049 (0.101)	6.200 (0.072)
Exposed to 7 males	6.277 (0.101)	6.090 (0.101)	6.184 (0.072)
Mean	6.314 (0.072)	6.070 (0.072)	

Full ANOVA is shown in Table 20, appendix 2.

(b) F_2 male offspring.

	SM	MM	Mean
Exposed to 1 male	3.750 (0.077)	3.709 (0.077)	3.729 (0.055)
Exposed to 7 males	4.052 (0.077)	3.833 (0.077)	3.943 (0.055)
Mean	3.901 (0.055)	3.771 (0.055)	

Full ANOVA is shown in Table 21, appendix 2.

South-India-strain F_2 offspring

Data was analysed as GLM ANOVA with the F_1 parent emergence weight as the covariate. The emergence mass of the F_1 females was found to be a significant factor on the emergence mass of the F_2 female ($F_{(1,75)}$ = 11.48, p<0.005) and the F_2 male offspring ($F_{(1,75)}$ = 11.61, p<0.005). The number of matings that the grandmother had did not significantly affect the emergence mass of the F_2 female ($F_{(1,75)}$ = 1.32, p>0.05) or male offspring ($F_{(1,75)}$ = 0.30, p>0.05) nor did the number of males that the grandmother was exposed to (F_2 female: $F_{(1,75)}$ = 0.18, p>0.05; F_2 male: $F_{(1,75)}$ = 0.14, p>0.05). Mean adult emergence masses for F_2 offspring are shown in Table 4.39.

Table 4.39. Mean (and SE) adult emergence masses (mg) for F_2 South-India-strain offspring hatched from eggs laid by SM F_1 females on day one whose grandmother was provided with either a SM or MM opportunity and was exposed either to one or seven males. SE values are standard errors of the mean. The data were untransformed.

(a) F_2 female offspring.

	SM	MM	Mean
Exposed to 1 male	6.424 (0.125)	6.244 (0.125)	6.334 (0.884)
Exposed to 7 males	6.443 (0.125)	6.331 (0.126)	6.387 (0.884)
Mean	6.433 (0.890)	6.288 (0.890)	

Full ANOVA is shown in Table 26, appendix 2.

(b) F_2 male offspring.

	SM	MM	Mean
Exposed to 1 male	4.353 (0.089)	4.326 (0.088)	4.340 (0.625)
Exposed to 7 males	4.343 (0.088)	4.271 (0.089)	4.307 (0.625)
Mean	4.348 (0.628)	4.299 (0.628)	

Full ANOVA is shown in Table 27, appendix 2.

4.4.8   Inter-strain comparison

In the present study, average female longevity was not found to differ significantly between the two strains (Chapter 3) and South-India-strain females laid more total eggs than expected with respect to previous studies (see Table 1.1, page 9). However, an ANCOVA comparing the strain of the female and the mating treatment does show that the two strains do differ in their responses to MM opportunities (Table 4.40).

Table 4.40. ANCOVA showing a significant interaction between strain and matings in terms of (a) fecundity (log transformed) and (b) longevity (log transformed). Female emergence mass was added as the covariate.

(a)   Fecundity

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Emergence mass	1	0.86891	0.55457	0.55457	34.98	$p<0.001$
Strain	1	0.10008	0.11650	0.11650	7.35	$p<0.01$
Matings	1	0.15162	0.16474	0.16474	10.39	$p<0.01$
Strain*Matings	1	0.06939	0.06939	0.06939	4.38	$p<0.05$
Error	145	2.29856	2.29856	0.01585		
Total	149	3.48856				

(b)   Longevity

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Emergence mass	1	0.07271	0.22295	0.22295	7.71	p<0.01
Strain	1	2.74511	2.79587	2.79587	96.67	p<0.001
Matings	1	0.10620	0.10694	0.10694	3.70	n.s.
Strain*Matings	1	0.29254	0.29254	0.29254	10.12	p<0.01
Error	315	9.11008	9.11008	0.02892		
Total	319	12.32663				

4.5   DISCUSSION

MM and female parent fecundity

MM and the fecundity of female insects has received much attention and effects ranging from very positive to negative have been observed.   In the Bruchid, *Bruchidius dorsalis*, Takakura (1999) reported that females who copulated ten times displayed fecundity that was eight times higher than for SM females.   In contrast, female *Caloglyphus berlesei* mites showed a decrease in fecundity when MM compared to SM females (Radwan and Rysinska, 1999).   In *C. maculatus*, effects of MM on fecundity have also been observed; however, it has not been previously documented that two strains of the same species have shown a markedly different response to MM in terms of fecundity as was observed in this study.

The provision of a MM opportunity significantly increased the numbers of eggs that were laid by Brazil-strain females compared to females who SM.   In contrast, no such difference was observed for South-India-strain females.   In addition, it was also clear that for Brazil females at least, the number of matings experienced by that female was a more important factor in terms of stimulating the numbers of eggs laid than the numbers of males encountered.   An increase in lifetime fecundity for MM female Brazil strain *C. maculatus* has been widely reported (Savalli and Fox, 1999a; Savalli and Fox, 1999b; Wilson *et al.*, 1999; Ofuya, 1995 and Fox, 1993c).   Possible explanations for this increase include mechanical stimulation from repeated copulation (Boucher and Huignard, 1987) or that substances with a stimulatory component are being transferred as part of the male ejaculate (e.g. Yasui, 1997; Hihara, 1981; Chapman *et al.*, 1995; Chen, 1996; Eberhard and Cordero, 1995; Eberhard, 1996) or receipt of a large nutrient donation in the form of a spermatophore (see Wheeler, 1996 and Filippi *et al.*, 2000).   Certainly female *C. maculatus* do not

appear to begin egg laying until they have mated since virgin females isolated on seeds rarely lay any eggs at all and when they do, not more than six eggs have been found (pers. obs.). These few eggs that are laid by virgins may have already matured before the adult female eclosed (see Wilson and Hill, 1989) and an increase in the number of matings does lead to an increase in ovarian production (Ofuya, 1995). This adds to the suggestion that it could be mechanical stimulation or products present in the male ejaculate that triggers processes such as ovulation and egg maturation in these beetles, which might render these processes at risk from modification by males.

The oversized ejaculate of *C. maculatus* has already been described in section 3.1 and it is possible that females who MM may use this excess male ejaculate as a nutrient or energy source or as a triggering factor resulting in increased egg production (Fox, 1993a; Fox, 1993b; Wheeler, 1996).

Fox (1993a; 1993b) reported no observed difference in lifetime fecundity for MM or SM *C. maculatus* females. Fox (1993c) found a stimulatory effect of MM on fecundity when females were mated every 48 hours but not when confined with males for life. However, Wilson *et al.* (1999) found that fecundity in *C. maculatus* was increased following a second mating but not a third. In contrast, the fecundity of *C. subinnotatus* females was actually found to decrease after four matings compared to two or three matings (Mbata *et al.*, 1997). Interestingly, an energy excess negatively affected egg production in hens (Walzem *et al.*, 1994). An excess of male derived nutrient may have been responsible for the lack of increase in fecundity observed by Mbata *et al.* (1997) and Fox (1993a; 1993b), however, it is more likely to be attributed to a deleterious effect of MM and furthermore that mating with several different males may increase this cost, especially as the cost was not observed for MM females confined with the same male in this study. In the present study, females that MM with the same male laid significantly more eggs than SM females exposed either to one or seven males. In contrast, females who had the opportunity to MM with up to seven different males did not lay significantly more eggs than the SM females exposed either to one or seven males indicating that there may have been an additional cost in terms of the total numbers of eggs laid to those females who MM with different males (this cost was also observed for Brazil-strain female longevity in section 3.4.1). There may be consequences of sperm mixing or some factor resulting

from competition between males within the female, which resulted in damage to eggs or inhibited or prevented the female from ovipositing due to interactions between the opposing males spermatophores or seminal fluid (Prout and Clark, 2000). However, there were three females given the opportunity to MM with seven males that demonstrated poor rates of egg laying from day one. It is unknown whether these females had an existing problem or if it was caused by the treatment itself. All females given the MM treatment and exposed to seven males exhibited the most extreme differences between the least and most numbers of eggs laid and consequently the largest range and standard deviation compared with any of the females in the other three treatments. MM with different males, therefore, may have a different effect depending on the individual female and the number of times mated and may either inhibit or stimulate depending on these factors. Wilson *et al.* (1997) reported that sperm competition in *C. maculatus* was strongly affected by female genotype and that the percentage of offspring fathered by the second male to mate (P2) was only repeatable when a pair of males was mated to three different but genetically similar (full sisters) females. P2 was not repeatable or marginally so when the females were unrelated. It may be possible that if female genotype can affect sperm competition it may also be able to influence the degree of stimulation of factors responsible for fecundity. Conversely, females in the present study who MM with the same male exhibited the smallest range between lowest and highest numbers of eggs laid and also the smallest standard deviation. This further indicates that it was a potential interaction between the products transferred by different males that altered a female's response to the treatment.

In contrast to the Brazil strain, the mating opportunity presented to South-India-strain females did not result in an increase in the number eggs laid irrespective of whether that opportunity enabled MM or SM. It may be that these MM opportunities were not being taken and that male mating attempts were rejected thus oviposition was, therefore, not being further stimulated in these females. If these females were in fact accepting additional matings but oviposition stimulation failed to result it presents three possible explanations. First, if accessory gland products exist in these beetles as suggested in section 3.5 (see also Wilson *et al.*, 1999 and Savalli and Fox, 1999a) it may be that, unlike Brazil-strain males, the South-India-strain males do not transfer them to females as part of an ejaculate. Second, the South-India-strain males may

simply be transferring smaller quantities. Third, South-India-strain males are transferring products but South-India-strain females may have evolved resistance or raised their response threshold such that the amount transferred by the male does not elicit a response (see Eberhard, 1996). This would seem sensible for South-India-strain females whose larvae are fiercely competitive within a seed. If oviposition were induced females might be forced to lay eggs at inappropriate sites such as on seeds with eggs already laid upon them. Furthermore, South-India-strain females are generally more limited by the time they have to search for suitable oviposition sites and not by the number of eggs that they can lay. The ability to lay more eggs, therefore, would not increase fitness if oviposition sites were limited or were of poor quality. It is worth bearing in mind that the fecundity measured in this study was based on fecundity when seeds were not limited and may, therefore, be different to actual fecundity when females have to search for oviposition sites. By raising the response threshold to a stimulus the females could regain control over their own bodily processes and refrain from ovipositing until a suitable site could be found (Eberhard, 1996). Coupled with this is the fact that by not stimulating oviposition females could invest more resources into each egg and not into laying more eggs by metabolising ejaculates. An additional factor could be delaying oviposition after mating. Wilson and Hill (1989) and Credland and Wright (1989) reported that female *C. maculatus* ovaries rapidly filled with mature ova after mating and eggs accumulate when oviposition sites are withheld in the first few days after emergence. It is not known whether such processes could damage eggs but a female's chance of successfully ovipositing if she deferred oviposition after mating does decrease in line with female age (Credland and Wright, 1989). In a strain such as South India where deference of oviposition is likely, maturing more eggs than are required could be costly and affect a female's future fecundity.

When seeds were absent females did not lay eggs irrespective of strain or mating treatment therefore MM females did not appear forced to lay eggs regardless of oviposition site availability (pers. obs.). Seven out of forty of the SM Brazil strain females provided with seeds did not lay any eggs. It is possible that these matings had failed and that no sperm was transferred before the beetles separated or that the males were actually sterile. Male sterility is unlikely, however, since all of the females confined with the same male in the MM treatment laid fertile eggs. What is

more likely is that the mating itself failed in some way and that no sperm was transferred or was ejected after copulation possible through some mechanism of female choice (see Birkhead and Møller, 1993). In contrast, all of the singly and MM South India females laid eggs. This may indicate that matings are less likely to fail in this strain, that South-India-strain females are much less choosy about their mates and are less likely to reject the male's sperm after mating or that they are incapable of rejecting sperm (e.g. female *Ozophora baranowski* lygaed bugs sometimes eject spermatophores containing live sperm following mating, Rodriguez, 1999).

The number of males encountered by a female had no detectable effect on the numbers of eggs laid by females for either the Brazil- or South-India-strain females. This indicates that the increased oviposition observed was triggered by an effect mediated through copulation and not by any external tactile or pheromone influence from the male.

Egg survival

Overall, the presence of either one or seven males did not affect the hatchability or subsequent survival of the Brazil-strain larvae to emergence. Egg survival was similarly unaffected by the number of matings that a female had. However, the interaction between these treatments was found to be important. MM Brazil-strain females exposed to different males laid significantly more eggs that failed to hatch than SM females exposed to only one male. This indicates that there may be a cost to egg survival mediated through mating with several males and not simply from exposure to several males. Some process was occurring whereby offspring from MM females exposed to different males were at a disadvantage over offspring from SM females. It may be that components of different males' ejaculates are mixing within the female and resulting in damaged sperm or that toxic products from male ejaculates or toxic by-products of sperm mixing are being transferred along with the egg (e.g. male seminal fluid in *Drosophila* reduced egg hatch and may incapacitate rival males sperm, Prout and Clark, 2000). The observed non-hatch eggs laid by the Brazil-strain females in the present study could also be the result of early embryo death through too many sperm entering the egg especially if male gametes are "under strong selective pressure for 'aggressive' ability to enter the egg and fuse with the nucleus" (Eberhard, 1996). Despite laying more eggs in total, MM Brazil-strain females did not lay more

viable eggs than SM females and this may be due to the consequences discussed above. The extremes of the mating treatments also appeared to be more costly to the Brazil-strain females in terms of egg survival as a significant interaction between the numbers of times a female mated and the number of males she encountered was found. MM Brazil-strain females exposed to seven males and SM Brazil-strain females exposed to one male laid the least numbers of viable eggs and the most numbers of non-viable eggs when compared to MM females exposed to one male or SM females exposed to seven males. This indicates that, for MM females, exposure to seven different males is more costly to egg survival than exposure to one male and that for SM females, exposure to seven males is less detrimental than exposure to only one male. This suggests that MM and exposure to different males is beneficial to egg survival but not when combined and that exposure to seven males in the absence of mating may be beneficial.

In contrast, the hatchability and subsequent survival of South-India-strain females eggs were unaffected irrespective of the number of matings a female had or the number of males that she encountered. This again reinforces the contrast between the two strains.

Egg distribution

The mating treatment was insignificant in determining female egg distribution patterns. Egg distribution may be an important factor due to larval competition strategy. Laying more than one egg per seed is detrimental to the fitness of South-India-strain females (section 1.2). For Brazil-strain females, however, laying more eggs per seeds is less costly to a point since larvae are scramble competitors and will share the resource. High larval densities may become detrimental to the survival of scramble competition larvae (Giga and Smith, 1991) and although mating treatment did not alter egg spacing behaviour when seeds were unlimited the fact that Brazil-strain females are being induced to lay more eggs may increase the numbers of eggs laid per seed when seeds are limiting.

Number of eggs laid per day

MM Brazil-strain females exhibited a longer egg laying period and also laid more eggs on later days than SM females. These effects were not as a result of the number of males encountered but of the number of matings. The number of eggs laid on days

one to three were not significantly different with respect to number of matings suggesting that the stimulatory effect expected on oviposition did not lead to an increase in numbers of eggs laid on these early days when typically most eggs are laid (see Eady, 1992). It may be that females were not re-mating until day three or four when a second mating results in a further 'burst' of stimulation on oviposition. Females may invest all their time on egg laying in these early days when an unlimited resource is available and may reject further matings or it may be that males pass substances with an anti-aphrodisiac property as part of the ejaculate in an attempt to acquire a higher percentage of paternity (see Ringo, 1996; Kalb *et al.*, 1993). Sheer volume and size of an ejaculate has also been shown to prevent females re-mating. Stretch receptors in the bursa may indicate that females have sufficient sperm and do not need to re-mate (Sugawara, 1979). Since sperm competition in these beetles favours the second male to mate (Eady, 1991) preventing females from re-mating for as long as possible will ensure the first male gains more paternity. Passing inhibitory substances as part of the ejaculate or passing an oversize ejaculate would both be valid options for *C. maculatus* males. Alternatively, egg maturation and egg laying rates of females may already be at their maximum and therefore unable to be further stimulated by males in the first three days after mating. It may not be until days three or four that oviposition rates would naturally begin to fall. Any anti-aphrodisiac effect may also wear off after this time (e.g. Some oviposition stimulants in *Drosophila funebris* have relatively short half lives, Baumann, 1974).

Once again, in contrast to the Brazil strain, the numbers of eggs laid per day by South-India-strain females were unaffected by the mating opportunity except for a significant decrease in numbers of eggs laid on day four by females exposed to seven males. The reason for this is unknown.

Egg size

Nutritional contribution by males and the incorporation of male derived nutrients into both female tissues and oocytes has been documented in a variety of species (Boucher and Huignard, 1987; Huignard, 1983; Castro *et al.*, 1997). In one extreme example, male *Bruchidius dorsalis* transfer massive spermatophores to females representing 7% of the male body weight and it is suggested that these males pay most of the nutritional cost of egg production (Takakura, 1999). Increased egg size in MM *C.*

maculatus females has been observed previously (Fox, 1993b; Wasserman and Asami, 1985) and the benefits of this to the offspring are clear (Fox, 1993c; see also McLain, 1998, male *Nezara viridula* offspring from larger eggs have greater mating success as adults).

In the present study, the weight of eggs laid by Brazil-strain females was unaffected by the number of matings or the number of males encountered which is in contrast to Fox (1993b) and Wasserman and Asami (1985). What this does indicate, however, is that despite MM females laying more eggs than SM females they are not smaller. Females may be utilising male derived nutrients or their own somatic tissue to produce 'normal' weight eggs (e.g. LaMunyon, 1997). In contrast, MM increased the size of eggs laid by South-India-strain females and this effect was observable from day one of egg laying. South-India-strain females are expected to invest qualitatively rather than quantitatively as predicted from their larval competition strategy (see section 2.1) and they may have used spermatophore materials in order to do this. This would also suggest that South-India-strain females should re-mate more frequently to obtain a larger nutritional contribution from males.

For both strains, SM females exposed to only one male produced the lightest eggs, indicating a positive effect of male presence in the other three treatments. The presence of males may encourage females to utilise more of their own somatic reserves in egg production since they may perceive a MM treatment even if they have only mated once. Finally, South-India-strain females that MM with the same male produced the heaviest eggs compared to the other three treatments. SM females exposed to more than one male may have the additive positive effect of male presence but will still lay smaller eggs due to the lack of spermatophore material available. Similarly, any reactions between rival males' spermatophores and seminal fluid within MM females exposed to several males may render some portions of that material unavailable for incorporation into eggs.

MM by female parents and fecundity of SM F_1 offspring

The fecundity of the SM Brazil- and South-India-strain F_1 offspring was unaffected by the parent females mating treatments. Despite F_1 Brazil-strain females demonstrating a cost to longevity when their parent female MM (see section 3.4.1) it did not affect the numbers of eggs that these females could lay when oviposition sites

were unlimited. In terms of egg survival, the mating treatment experienced by the parent female did have an observable effect in the F_1 offspring. The numbers of eggs that were laid by F_1 Brazil-strain females showed significantly reduced hatchability when the parent female had been exposed to seven males. However, this observed cost is harder to explain since the treatment involving exposure to seven males included both the SM and MM females. A cost when females had the opportunity to mate with several males may be explained in terms of products transferred along with the egg that reduced the fitness of the F_1 resulting in poor egg maturation, although this is unlikely (see section 3.5). The cost to F_1 females could not have been mediated by MM parent females producing smaller eggs, however, as egg size was not significantly different between treatments. Interactions between accessory gland products or sperm of different males' may have prevented the female from using excess sperm as a nutrient source and eggs may have been nutrient deficient. The physiology of SM females exposed to seven cauterised males may have been influenced in some way by the presence of those males, which resulted in the production of F_1 offspring whose eggs showed reduced hatchability. What is clear, however, is that the effect was due to the presence of different males but could not mediated entirely by internal mechanical stimulation of copulation or from the transfer of any substances from the male as part of an ejaculate. Therefore, an effect on the females' physiology through male pheromones or external tactile behaviour such as courtship that manifested in the F_1 is the most likely explanation. The effect was not seen for offspring from females who were exposed to only one male but who had either a single or MM opportunity further emphasising that it was a male effect and not one of copulation frequency. Utida (1941) reported that *C. chinensis* males may adversely affect eggs through trampling and interference with the female when she was trying to oviposit, however, Credland and Wright (1989) did not observe this in *C. maculatus*. Furthermore, it is unclear how this could adversely affect the F_1 offspring and reduce egg hatchability of these females.

In contrast to the Brazil-strain, South-India-strain F_1 offspring laid fewer non-viable eggs and more viable eggs if their parent female was exposed to seven males. This indicates that there was a benefit from mating with several males and also a smaller benefit from being exposed to several males. Furthermore, females who MM with seven males gave rise to offspring that laid significantly more viable eggs and

significantly fewer non-viable eggs than offspring from females who SM and were exposed to one male. It appears that MM in the South India strain resulted in an observable benefit to the F_1 offspring. South-India-strain parent females may have metabolised excess ejaculate transferred by the male and used it to provision eggs resulting in larger eggs. Indeed South-India-strain male and female offspring from MM females were larger than those from SM parent females.

Emergence mass of the F_1 and F_2 offspring

The effect of the parent-female mating treatment on the emergence mass of the F_1 and F_2 offspring is summarized in table 4.40 for the Brazil and South India strain. The absence of a significant difference in the mass of male and female F_1 Brazil-strain offspring from eggs laid on day one suggests that any cost or benefit of a mating treatment was not yet observable at that time. For eggs laid on days three and four, however, only the mass of the male offspring displayed an effect from the parent treatment. In contrast, offspring from MM South-India-strain females were larger from day one than offspring from SM females. This effect disappeared for females laid on day three and four but male size at this time increased even further with male offspring from MM females exposed to seven males being larger than males from all other treatments.

Brazil-strain parent females may be laying at a maximal rate on the first three days of oviposition and if anti-aphrodisiacs are present in the male ejaculate then females may not re-mate until the third or even the fourth day. As a result, fewer male-derived nutrients would be available to invest into producing larger eggs thus larger offspring would not result. Conversely, South-India-strain females may mate more than once on the first day and consequently have more available nutrient resources to invest into egg quality and size. Male size might be easier to influence due to their smaller body size at emergence when compared with females; thus a smaller contribution from the mother may be required to influence overall male size. Conversely, a larger contribution would be needed to give a corresponding increase in female emergence mass hence the disappearance of effect on days three and four for the South India strain and no effect on the Brazil strain. Also consider the fact that male ejaculates, and consequently potential nutrient value, are larger from virgin males and thereafter decline in size with each subsequent mating and also with increasing male age (Fox *et*

al., 1995b; Eady, 1995; Birkinshaw, 1998). Brazil-strain females re-mating on day three or four could have an additional, albeit smaller, input of nutrients that might be used to provision eggs resulting in an increase in male offspring size. Similarly, for the South-India-strain females, re-mating on day one would potentially provide a more than sufficient male-derived nutrient donation that when transmitted to both the male and female eggs might result in the increases observed.

There was no significant difference in the emergence masses of the South-India-strain F_2 offspring indicating that the benefit accrued from the original parent treatment was passed only to the F_1 females and did not pass to the F_2 offspring. The trend in mass observed for the Brazil-strain F_1 offspring did continue in the F_2 generation. This trend appeared to relate to the size of the male and may indicate that offspring size was influenced more by male size and less by female size (e.g. *Coelopa frigida*, Wilcockson *et al.*, 1995). An additional factor may be a consequence of the size of male-ejaculate contribution as larger males have larger ejaculates (Savalli and Fox, 1999a). It is also possible, however, that there was a positive effect on the F_1 female physiology that resulted in the production of larger sons.

Egg distribution

Egg distribution over seeds by the F_1 offspring was observed to change relating to the parent females' mating treatment. It is unclear why female offspring would respond in this way and requires further study.

Summary

MM by Brazil-strain females increased lifetime fecundity when seeds were not limiting over SM females. It did not result in the production of more surviving offspring, however, and eggs of MM females exposed to seven males had the lowest hatchability. MM females laid more eggs on later days and also laid for longer than SM females. These effects may be mediated by stimulatory products present in the male ejaculate, which may increase oviposition or egg maturation rates, decrease egg viability through mixing and have anti-aphrodisiac qualities. The decreased longevity observed for F_1 offspring in chapter three could not have been due to females laying smaller eggs as egg size was not observed to decrease. MM females laid more eggs but they were not smaller as a result.

F_1 Brazil-strain offspring showed reduced hatchability of eggs when parent females were exposed to seven males. Egg distribution pattern was also affected and the reason for this is unknown. The parent mating treatment affected the mass of males emerging from eggs laid on later days but had no observable effect on the female emergence mass. Emergence weights of the F_2 Brazil-strain offspring followed those patterns observed in the F_1 offspring and may indicate a positive effect either of male size on offspring size or on the F_1 female physiology that resulted in the production of larger sons. In contrast, MM by South-India-strain females had no effect on the total numbers of eggs laid when seeds were not limiting or on the numbers of eggs laid per day compared to SM females but they did lay larger eggs. South-India-strain males may not transfer stimulatory substances in their ejaculates or females may have evolved a resistance to these substances in a dose dependent nature. MM by female parents does result in more viable eggs and fewer non-viable eggs being laid by the SM F_1 offspring and this may be mediated by metabolism of ejaculates and provisioning of larger eggs resulting in larger fitter offspring. These positive effects are not observed in the SM F_2 offspring suggesting that the benefits of MM are passed to one generation only and that the subsequent generation would need to re-mate in order to pass any such benefits onto their offspring.

Table 4.40 Summary of differences observed in F_1 and F_2 offspring emergence mass. Note that F_2 offspring emerged from eggs laid on day one by the F_1 offspring that emerged from eggs laid on days three and four (3+) by the original female (grandmother to F_2).

(a)   Brazil strain.

	Eggs laid on Day 1		Eggs laid on Day 3+	
	Male emergence mass	Female emergence mass	Male emergence mass	Female emergence mass
F_1 offspring	X	X	B+C >A+D B > A and D	X
F_2 offspring			Larger when grandmother SM B > A and D	Larger when grandmother SM

X indicates that no effect on emergence mass was seen

(b)   South India strain.

	Eggs laid on Day 1		Eggs laid on Day 3+	
	Male emergence mass	Female emergence mass	Male emergence mass	Female emergence mass
F_1 offspring	C > A and D	larger from MM females C > A and B D > B	C > A, B and D	X
F_2 offspring			X	X

X indicates that no effect on emergence mass was seen

CHAPTER 5

CHAPTER 5: MATING BEHAVIOUR OF THE TWO STRAINS

5.1 INTRODUCTION

It has been shown in the previous two chapters that providing the opportunity for MM does not result in the same outcome in terms of longevity and fecundity for females of the two *C. maculatus* strains. It was postulated in Chapters 3 and 4 that Brazil-strain males may be transferring additional substances as part of the spermatophore that resulted in an increase in lifetime fecundity but a decrease in longevity. Conversely, South-India-strain males might not transfer such products or South-India-strain females may have evolved resistance to them as no effect on longevity or fecundity was observed. Alternatively, South-India-strain females may be avoiding additional matings and therefore not being exposed to more products. In other words, are there differences in spermatophore components between the two strains or in female re-mating frequency or physiology that could explain these longevity and fecundity differences?

The duration of copulation varies considerably both between and within insect species. For example, copulating pairs of the red flour beetle, *Tribolium castaneum*, displayed a range of thirty seconds to thirty-two minutes (Bloch *et al.*, 1996) and a range of thirty minutes to three hours was recorded in the damselfly, *Ceriagrion tenellum* (Andres and Rivera, 2000). Two extreme examples within Coleopteran species are the Milkweed leaf beetles, *Labidomera clivicollis clivicollis* and *Acanthoscelides obtectus*. Milkweed leaf beetles remained coupled for an average of 0.75 days (Dickinson, 1988) whereas *Acanthoscelides obtectus* copulated for an average of 1 minute (Halstead, 1973). In contrast, *Stegobium paniceum* copulated for 60 minutes (Barratt, 1977) and pairs of the rice weevil, *Sitophilus oryzae*, remained *in copula* for approximately two hours (Holloway and Smith, 1987). Several possible explanations have been put forward to explain differences in copulation duration and particularly to explain longer copulations. Increased time may enable males to transfer more sperm (e.g. provisioning shield bugs *Parastrachia japonensis*, Fillipi *et al.*, 2000 and *Bittacus apicalis*, Thornhill, 1976), to complete ejaculate transfer (e.g. *Coptaspis sp.*, Wedell, 1998 and fishflies, Hayashi, 1996) or to allow the transfer of additional accessory gland products (e.g. *Stegobium paniceum*, Barratt, 1977).

Removal of a rival male's sperm (e.g. the dragonfly *Sympetrum danae*, Michiels, 1992), transfer of mating plugs to prevent re-mating (e.g. *Coproica vagans*, Lachmann, 1998) or the male physically preventing the female engaging in further matings (e.g. the damselfly *Enallagma hageni*, Fincke, 1982) are also possible reasons. Longer copulations are not always needed, however, in order to transfer more sperm nor does the time spent *in copula* necessarily reflect amount transferred. Gilchrist and Partridge (2000) suggested that copulations in *D. melanogaster* that lasted for more than twice the duration needed for sperm transfer to be completed served to delay female re-mating. Pitnick *et al.* (1991 cited in Eberhard, 1996) found that copulation in *D. pachea* was relatively long at 41 minutes yet males transferred the smallest ejaculate of any *Drosophila* species. In contrast, *D. mojavensis* transferred about fourteen times as much in just two and a half minutes. Finally, the amount of sperm transferred in the lizard, *Anolis sagrei*, did not correlate with copula duration or male or female body size but remained constant (Tokarz, 1999).

Accessory gland products transferred as part of a spermatophore have wide ranging effects on female physiology and behaviour (see Eberhard, 1996 and section 3.5 for a fuller discussion). Such products can alter female mating behaviour with the result that females are more likely or less likely to re-mate. For example, components of seminal fluid of the housefly, *Musca domestica*, decreased female receptivity for most if not all of the rest of her life after just a single mating (Riemann *et al.*, 1967). Similarly, female receptivity to re-mating in the mosquito *Aedes aegypti*, was inhibited by two stimuli found in the male seminal product (Fuchs and Hiss, 1970). Control over female physiology and behaviour in these two examples is by chemical means, though control can also be exerted by mechanical stimulation. For example, the filling of the bursa of female *Aedes aegypti* with seminal fluid resulted in a short-term inhibitory effect on female receptivity (Gwadz *et al.*, 1971). Similarly, mate rejection by female *Pieris rapae* butterflies was triggered by spermatophore size through the response of stretch receptors and frequency of wave contractions in the bursa (Sugawara, 1979).

In *C. maculatus*, males are known to produce oversized ejaculates transferring 85% more sperm than a female can effectively store in her spermatheca (Eady, 1994b). This has consequences for sperm competition since it may ensure that males are more

likely to pre-empt a rival male's sperm and less likely to be pre-empted as sperm competition operates on last male sperm precedence in these beetles (Eady, 1995). It is possible that spermatophore size is also important in delaying female receptivity through mechanical stimulation such as the degree of bursal stretching. Alternatively, larger ejaculates may contain proportionally more products with antiaphrodisiac properties.

Male-body size, female-body size, male age, previous mating experience and social conditions, such as the presence of other males, have been shown to influence the amount of ejaculate transferred (larger males produced larger ejaculates, e.g. coccinellid beetles, *Harmonia axyridis*, Ueno, 1994 and *Stator limbatus*, Fox *et al.*, 1995b; larger females received larger ejaculates, e.g. spiny lobsters, MacDiarmid and Butler, 1999 and the cricket *Acheta domestica*, Gage and Barnard, 1996; older males produced smaller ejaculates, e.g. Fox *et al.*, 1995b; ejaculate size decreased with increasing number of matings, e.g. Eady, 1995 and Birkinshaw, 1998; increasing sperm number per ejaculate in response to perceived competition, e.g. Gage, 1991). In *C. maculatus* ejaculate size has been shown to decrease with increasing male age (Fox *et al.*, 1995a) and with increasing numbers of matings (Savalli and Fox, 1999a). Neither the time spent *in copula* nor the mass of the male nor the mass of the female were found to influence spermatophore size in *C. maculatus* by Eady (1994b). In contrast, however, male size was found to be an important factor by Fox *et al.* (1995a) and Savalli and Fox (1999a) with larger, younger males producing larger spermatophores.

This chapter examines the mating behaviour of the two strains to investigate putative differences that may account for the disparity of effect on longevity and fecundity observed in chapters 3 and 4. First, total copulation duration and in-copula behaviour between same-strain pairs were investigated followed by between strain pairs. The pre-copulatory phases of courtship in *C. maculatus*, general behaviour in-copula and also the total time spent in-copula were reviewed and described by Rup (1986), however, a direct comparison of mating behaviour in the two strains used in the present study has not been done. For the purposes of this study only the phases *in copula* were examined. Second, did females from one strain re-mate more readily than those from the other strain and was re-mating affected by male status (virgin,

non-virgin, different strain) or access to males (controlled or continual)? Final, did the males of both strains invest equally in terms of the amount of spermatophore transferred to a female? As in the rest of the thesis the abbreviations MM and SM will be used as a shorthand expression for individuals that mate multiply and mate singly respectively.

5.2 AIMS

- To contrast some aspects of mating behaviour in the Brazil and South India strains

- To contrast re-mating behaviour in the two strains when females experienced controlled daily exposure to the same male, to virgin males or to males of the opposite strain

- To contrast re-mating behaviour in the two strains when females were confined with a single male (constant access)

- To compare male-spermatophore investment in the two strains

5.3 MATERIALS AND METHODS

Source of insects

All beetles used in these experiments were virgins and isolated as described in section 2.2.3. Both South India and Brazil strains were used and all beetles had emerged less than twelve hours before being used in the experiment. Beetles were selected and paired randomly.

5.3.1 Components of mating behaviour in the two strains

Number of insects

20 males and 20 females per strain were used.

Apparatus

Pairs were observed inside an arena consisting of a circular (5cm diameter, 2cm deep) lidded petri dish.

Same strain pairs

Experimental design

Pairs were observed for 20 minutes after which time beetles that did not mate were discarded. The arena was cleaned with 70% industrial methylated spirit and allowed to dry before new pairs were added.

Individual male and female beetles of the same strain were paired at random and placed inside the arena. Pairs of both strains were observed simultaneously in separate arenas. Mating itself was broadly divided into four main behavioural stages and the time taken to complete stages 2, 3 and 4 was recorded using a stopwatch. These are as follows:

1. Number of attempts made by male before a successful mating occurs

A male attempt was defined as the male placing his aedeagus beneath a female's abdomen prior to insertion. A successful attempt would lead to insertion whereas a failed attempt did not.

2. Time taken from male placing partially everted aedeagus beneath female's abdomen to aedeagus insertion.

Aedeagus insertion was defined as the time when the aedeagus was fully inserted into the female. This stage is also defined as phase 1.

3. Aedeagus insertion to onset of female kicking

Female kicking was defined as the time when a female began repeatedly to kick or push the male with her hind legs. This stage is also defined as phase 2.

4. Time of onset of female kicking to aedeagus removal

Aedeagus removal was defined as the time that the male retracted his aedeagus and the male and female separated. This stage is also defined as phase 3.

Mixed strain pairs

Experimental design

Individual beetles were chosen at random and paired with a male or female from the opposite strain. A South-India-strain female paired with a Brazil-strain male was observed at the same time as a Brazil-strain female paired with a South-India-strain male. As described previously under the heading 'same strain pairs', mating

behaviour was divided into four stages and the time taken for each stage recorded with a stopwatch. Pairs that had not mated after twenty minutes were discarded.

5.3.2 How many times will a female re-mate when presented with a controlled mating opportunity?

Experimental design

Three males and three females were chosen at random and placed together inside an arena and observed until mating took place. Pairs *in-copula* were isolated to a separate arena and marked. Marking consisted of applying a small amount of acrylic paint to the elytra of both the male and female of the copulating pair. Three colours were used so that each individual could be distinguished from the others in their group of six (male or female and blue, yellow or red). When mating was completed, male and female pairs were separated into all male and all female arenas. Beetles were labelled as a group of three males and three females to represent one replicate. The next day, males and females were placed together within their replicate group and observed for 30 minutes at 10am and 4pm. Any matings and the male and female involved were recorded. After 30 minutes the males and females were returned to their respective all male or all female arenas. Beetles were observed for seven days.

Apparatus

Matings were observed inside arenas consisting of circular (5cm diameter, 2cm deep) lidded petri dishes.

Re-mating opportunity with the same three males per three females twice daily for seven days

Number of insects

45 males and 45 females (15 replicates) per strain were used. South-India-strain females were paired with South-India-strain males and Brazil-strain females were paired with Brazil-strain males.

Re-mating opportunity with three different virgin males per three females twice daily for seven days

Number of insects

21 males and 21 females (7 replicates) per strain were used. South-India-strain females were paired with three different virgin South-India-strain males daily and Brazil-strain females were paired with three different virgin Brazil-strain males daily.

Re-mating in mixed strain pairs

Number of insects

21 males and 21 females (7 replicates) per strain were used. South-India-strain females were paired with the same three Brazil-strain males daily and Brazil-strain females were paired with the same South-India-strain males daily.

5.3.3 How many times will a female re-mate when opportunities are constantly available?

Number of insects

24 pairs of Brazil-strain males and females and 24 pairs of South-India-strain males and females were used.

Apparatus

Insects were held in pairs in the cells of a 5 x 5 plate (see section 2.2.3). Video recordings were taken using a Vista (Japan) NCL1100 CCTV camera with a Vista (Japan) CCTV lens, 3.5mm, F1.4. This was linked to a Hitachi time-lapse video recorder (model VT-L2000E). The tape speed was set at 240, which meant that a 3-hour tape lasted for 243 hours at 0.31 frames per second.

Experimental design

Males and females were chosen at random and placed as pairs into individual cells of a 5 x 5 plate. Six Brazil-strain pairs could be observed at the same time as six South-India-strain pairs. Behaviour was recorded continuously for seven days. Three Brazil- and three South-India-strain pairs were kept with the same male for the full seven days and three Brazil- and three South India-strain-pairs had a different male every day. Recordings were replicated four times.

5.3.4 Male investment: spermatophore size

Number of insects

20 virgin males and 20 virgin females per strain were used.

Apparatus

Observations were carried out in an arena consisting of a circular (5cm diameter, 2cm deep) lidded petri dish. Individual male and female beetles were weighed at emergence using a Cahn C-31 microbalance.

Experimental design

Virgin male and female beetles were paired randomly and transferred to separate arenas and allowed to mate. Following mating the male and female were re-weighed. Pairs that did not mate after twenty minutes or where a mating took place but the female gained no mass were discarded. The mass of ejaculate was calculated using the following formula:

$$\text{Mass of ejaculate} = \frac{(\text{Mass lost by male} + \text{Mass gained by female})}{2}$$

The mass of the ejaculate was also calculated as a proportion of the mass at emergence of the male and also the female.

5.4 RESULTS

5.4.1 Components of mating behaviour in the two strains

Same strain pairs

Three distinct phases to mating were observed and the time spent by pairs on each phase was recorded and presented in Figure 5.1. There was no significant difference between the two strains in the time taken to achieve aedeagus insertion once an attempt had begun ($T_{(37)}$=0.34, p>0.05). Similarly, no significant difference was observed between times from aedeagus insertion to the onset of female rejection or kicking ($T_{(27)}$=0.79, p>0.05). The time taken from the onset of rejection until the aedeagus was removed and the pair separated, however, was significantly different between the two strains. The Brazil-strain males were observed to remain *in copula* for a significantly longer time once the female had begun to kick compared with the

South-India-strain males ($T_{(37)}$=3.68, p<0.001). After mating the male and female separated and no post-copulatory guarding behaviour was observed by males of either strain.

During phase three the male remained passive while the female kicked or walked about the arena and only began to move several seconds before the pair separated. The male would begin to push against the female and might also rotate 90 to 180 degrees to the female before the aedeagus was withdrawn. Separation appeared to be under male control in that it required the male to retract the aedeagus before the pair could separate. None of the females observed succeeded in removing the males by kicking or walking away.

Total time spent *in copula* was significantly longer for Brazil-strain pairs than for South-India-strain pairs ($T_{(32)}$=5.41, p<0.0001). Pairs remained *in copula* for an average of 320 seconds and 266 seconds for the Brazil and South India strains respectively (see Figure 5.2).

The number of attempts made by males before a successful copulation occurred was also noted and found not to be significantly different between the strains ($F_{(1,38)}$=0.02, p>0.05). Mean number of attempts performed by males before a successful copulation are shown in Table 5.1 (page 134).

Figure 5.1. Mean times for the three phases of mating behaviour for Brazil- and South-India-strain pairs. Bars are standard errors of means.
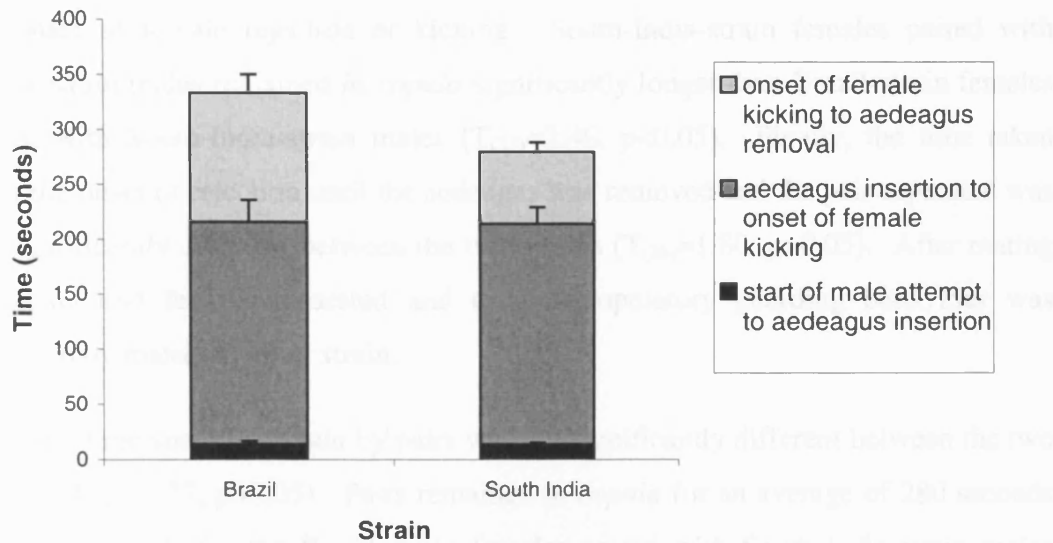
Figure 5.2. Average total time spent *in copula* for Brazil- and South-India-strain pairs. Bars are standard errors of means. Note also that the Y-axis does not start at zero.
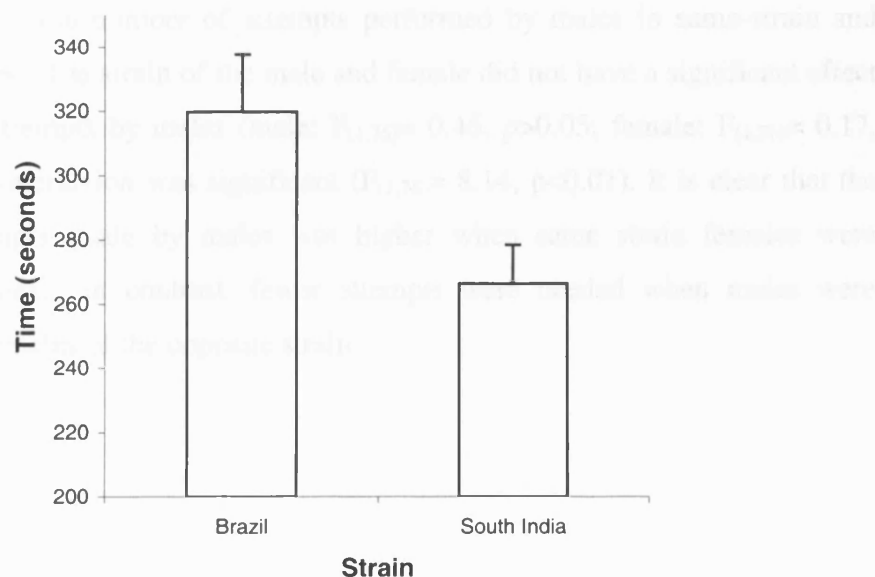
Mixed strain pairs

Again the three distinct phases to mating were observed and the time taken to perform each was recorded and presented in Figure 5.3. No significant difference was observed between the two groups of mixed-strain pairs in the time taken to achieve

aedeagus insertion once an attempt had begun ($T_{(36)}$=0.76, p>0.05). There was a significant difference observed, however, between times from aedeagus insertion to the onset of female rejection or kicking. South-India-strain females paired with Brazil-strain males remained *in copula* significantly longer than Brazil-strain females paired with South-India-strain males ($T_{(33)}$=2.46, p<0.05). Finally, the time taken from the onset of rejection until the aedeagus was removed and the pair separated was not significantly different between the two strains ($T_{(36)}$=1.80, p>0.05). After mating the male and female separated and no post-copulatory guarding behaviour was observed by males of either strain.

The total time spent *in copula* by pairs was not significantly different between the two groups ($T_{(28)}$=1.77, p>0.05). Pairs remained *in copula* for an average of 280 seconds and 313 seconds for the Brazil-strain females paired with South-India-strain males and South-India-strain females paired with Brazil-strain males respectively (Figure 5.4).

The number of attempts made by males before a successful copulation occurred was not significantly different between the mixed strain pairs ($F_{(1,38)}$=3.23, p>0.05). Mean numbers of attempts by males are shown in Table 5.1 (page 134). A GLM ANOVA was performed on the number of attempts performed by males in same-strain and mixed-strain pairs. The strain of the male and female did not have a significant effect on numbers of attempts by males (male: $F_{(1,76)}$= 0.46, p>0.05; female: $F_{(1,76)}$= 0.17, p>0.05) but the interaction was significant ($F_{(1,76)}$= 8.14, p<0.01). It is clear that the number of attempts made by males was higher when same strain females were presented to males. In contrast, fewer attempts were needed when males were presented with females of the opposite strain.

Figure 5.3. Mean times for the three phases of mating behaviour for Brazil- and South-India-strain females paired with South-India- and Brazil-strain males respectively. Bars are standard errors of means.
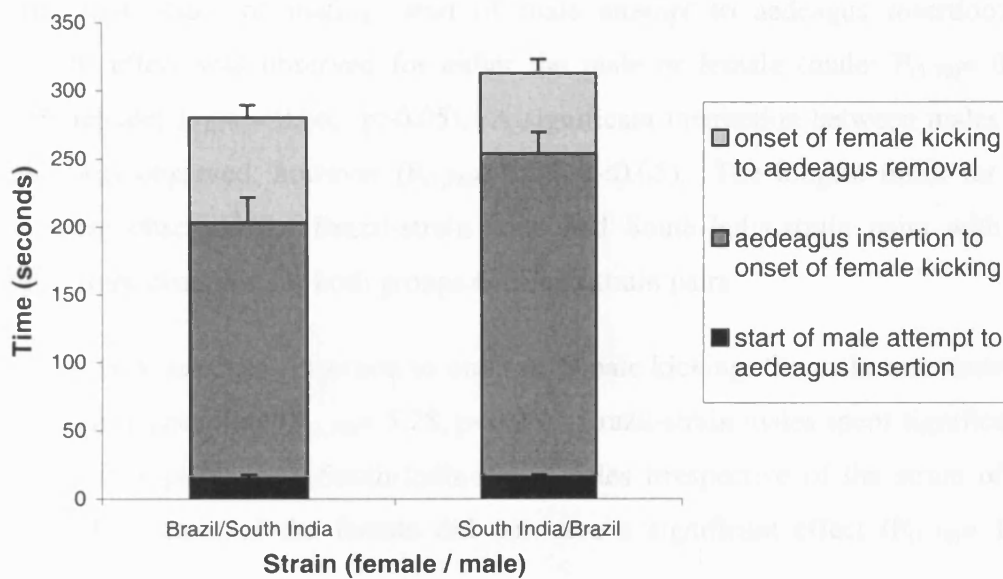
Figure 5.4. Average total time spent *in copula* for Brazil- and South-India-strain females paired with South-India- and Brazil-strain males respectively. Bars are standard errors of means. Note also that the Y-axis does not start at zero.
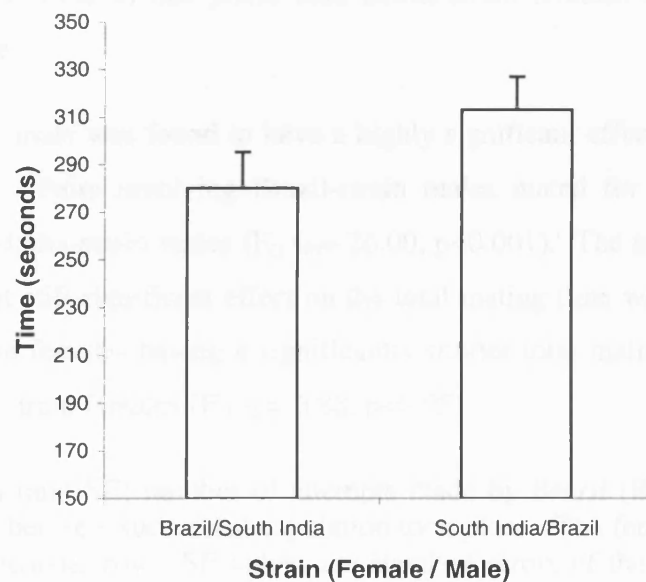
Data on the timings for each phase of mating behaviour and also on the total time spent *in copula* were log transformed and a GLM ANOVA was performed. This

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would reveal whether it was the male or female that was affecting the differences that were observed. Back transformed mean times are shown in Table 5.2.

For the first phase of mating; start of male attempt to aedeagus insertion; no significant effect was observed for either the male or female (male: $F_{(1,76)}= 0.17$, p>0.05; female: $F_{(1,76)}= 0.66$, p>0.05). A significant interaction between males and females was observed, however ($F_{(1,76)}= 4.56$, p<0.05). The longest times for this phase were observed for Brazil-strain pairs and South-India-strain pairs with the shortest times observed for both groups of mixed strain pairs.

For phase two; aedeagus insertion to onset of female kicking; the male was shown to have a significant effect ($F_{(1,76)}= 5.28$, p<0.05). Brazil-strain males spent significantly longer on this phase than South-India-strain males irrespective of the strain of the female. The strain of the female did not have a significant effect ($F_{(1,76)}= 1.41$, p>0.05).

Finally, for phase three; onset of female kicking to aedeagus removal; the female but not the male was shown to have a highly significant effect (female: $F_{(1,76)}= 16.24$, p<0.001; male: $F_{(1,76)}= 3.59$, p>0.05). South-India-strain females spent a significantly shorter amount of time in this phase than Brazil-strain females irrespective of the strain of the male.

The strain of the male was found to have a highly significant effect on the total time spent *in copula*. Pairs involving Brazil-strain males mated for longer than pairs involving South-India-strain males ($F_{(1,76)}= 26.00$, p<0.001). The strain of the female had a smaller but still significant effect on the total mating time with pairs involving South-India-strain females having a significantly shorter total mating time than pairs involving Brazil-strain females ($F_{(1,76)}= 6.88$, p<0.05).

Table 5.1. Mean (and SE) number of attempts made by Brazil (B) and South India (SI) strain males before a successful copulation took place. The female is represented first in the female/male row. SE values are standard errors of the means. The data were untransformed.

Female / male pair	B / B	SI / SI	B / SI	SI / B
Number of attempts by male	1.65 (0.18)	1.70 (0.18)	1.25 (0.18)	1.05 (0.18)

Full ANOVA shown in Table 1, appendix 3

Table 5.2. Times in seconds for pairs of Brazil- (B) and South-India-strain (SI) males and females. Times are for three phases of mating behaviour; start of male attempt to aedeagus insertion (phase 1); aedeagus insertion to onset of female kicking (phase 2); onset of female kicking to aedeagus removal (phase 3) and total time spent mating. The female of the pair is represented first in the female/male column.

(a)   Means and SED values for log transformed data. SED values are standard errors of the difference between the means.

Female / male pair	Phase 1	Phase 2	Phase 3	Total time
B / B	1.22	2.29	1.90	2.49
B / SI	1.10	2.24	1.82	2.41
SI / SI	1.24	2.25	1.39	2.33
SI / B	1.16	2.36	1.64	2.46
SED	0.047	0.036	0.085	0.022

Full ANOVA table is shown Table 2, appendix 3.

(b)   Back transformed means.

Female / male pair	Phase 1	Phase 2	Phase 3	Total time
B / B	16.63	193.20	79.07	311.89
B / SI	12.62	171.79	66.37	257.63
SI / SI	17.39	176.20	24.72	211.84
SI / B	14.42	228.56	43.65	291.07

Same-strain versus mixed-strain pair

The total time *in copula* was significantly longer for Brazil-strain females mated to Brazil-strain males when compared to Brazil-strain females mated to South-India-strain males ($T_{(37)}=2.37$, $p<0.05$). Similarly, time spent in phase 2, and total mating time significantly increased when South-India-strain females mated with Brazil-strain males compared to South-India-strain females mated to South-India-strain males (phase 2: $T_{(36)}=3.16$, $p<0.005$; total mating time: $T_{(37)}=5.41$, $p<0.001$). No other comparisons were significantly different ($p>0.05$).

5.4.2   How many times will a female re-mate when presented with a controlled mating opportunity?

This section examines the number of times that females re-mated when provided with daily mating opportunities with the same males, virgin males or males of the opposite strain. First, we consider female re-mating with the same males of the same strain as the female daily. Second, we consider re-mating when females are presented with newly emerged virgin males of the same strain as the female daily. Third, we consider re-mating when females are provided with the same three males daily of the opposite strain from the female. Analyses are based on the number of re-matings by

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females only and exclude day one, i.e. the original mating. Data on the proportion of females re-mating per day were angular transformed and analysed by ANOVA. Data of the total number of matings per female over seven days were analysed untransformed by Mann-Whitney.

Re-mating opportunity with the same three males per three females twice daily for seven days

The average number of times that a female of either strain mated and the proportion of females mating when provided with a half-hour opportunity twice daily are presented in Tables 5.3 and 5.4. Females were all mated on the first day and the numbers that re-mated on subsequent days decreased (Figure 5.5). Over the seven days that females were observed the proportion of South-India-strain females mating per day was not significantly different from the proportion of Brazil-strain females mating ($F_{(1,12)}$=1.90, p>0.05). When looking at the number of re-matings per female over the seven day period, however, South-India-strain females were observed to mate significantly more times in total than Brazil-strain females (W=1702.5, p<0.005). This difference arose because some South-India-strain females mated twice in a half hour period. None of the Brazil-strain females in any treatments mated more than once per half hour period.

Table 5.3. Mean (SE) number of matings observed per female when provided with two half hour mating opportunities per day for seven days. SE values are standard errors of the means. Data were untransformed.

	Brazil	South India	N
Same males daily, same strain as female	2.022 (0.385)	2.622 (0.274)	45
Different virgin males daily, same strain as female	0.762 (0.153)	3.143 (0.360)	21
Same males daily, different strain to female	1.048 (0.288)	2.762 (0.419)	21

Table 5.4. Proportion of females mating over seven days when provided with two half hour mating opportunities per day.

(a)   Angular transformed proportions and SE values. SE values are standard errors of the means.

	Brazil	South India
Same males daily, same strain as female	0.542 (0.035)	0.628 (0.052)
Different virgin males daily, same strain as female	0.305 (0.060)	0.717 (0.078)
Same males daily, different strain to female	0.368 (0.039)	0.617 (0.079)

(b)   Back transformed proportions.

	Brazil	South India
Same males daily, same strain as female	0.266	0.345
Different virgin males daily, same strain as female	0.090	0.432
Same males daily, different strain to female	0.129	0.335

The proportion of re-mating Brazil-strain females was significantly lower on the second day than the proportion of re-mating South-India-strain females (at 95% C.I.). Following day two, the proportion of re-mating Brazil-strain females gradually increased over the remaining days whereas the pattern for South-India-strain females followed a general decrease. The proportion of females that re-mated was not significantly different between the strains from day three to day eight (at 95% C.I.) (Figure 5.5).

Figure 5.5. Proportion of Brazil- (B) or South-India-strain (SI) females mating on a particular day when provided with two 30 minute mating opportunities daily with three males of the same strain. Error bars are 95% confidence intervals.
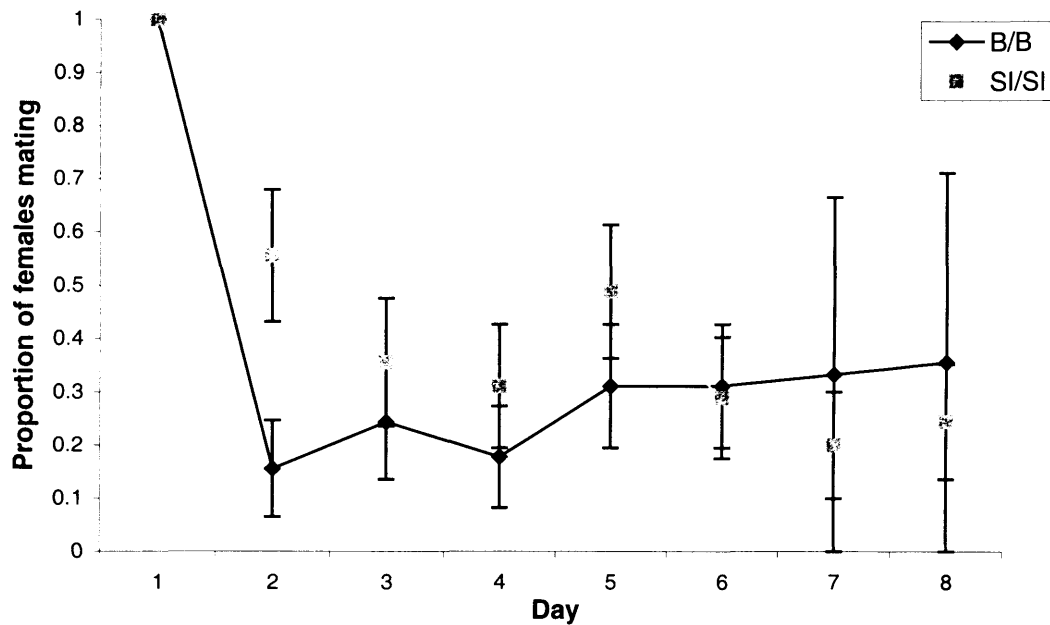
Re-mating opportunity with three different virgin males per three females twice daily for seven days

The proportion of females mating and the number of re-matings per female were both found to be significantly higher for South-India-strain females paired with virgin South-India-strain males when compared with Brazil-strain females paired with virgin Brazil-strain males (Proportion: $F_{(1,12)}$ =17.56, p<0.005, Figure 5.6 and Table 5.4; Matings per female: W=278.0, p<0.0001, Table 5.3). The proportion of mating females was significantly higher for South-India-strain females on days 2, 3 and 4 than for Brazil-strain females (at 95% C.I.). No Brazil-strain females mated at all on day 2 and mating proportions on subsequent days remained low. The highest mating proportions of South-India-strain females were observed on days 3 and 4 showing an increase over day 2. On subsequent days, however, the proportion of mating South-India-strain females decreased (Figure 5.6).

Mating with virgin Brazil-strain males significantly decreased the number of matings per female and also the proportion of females mating when compared to Brazil-strain females paired with the same Brazil-strain males (Proportion: $F_{(1,12)}$ =11.79, p<0.01, Figure 5.7 and Table 5.4; Matings: W=1645.5, p<0.05, Table 5.3). The general trend

for Brazil-strain females presented with the same Brazil-strain males daily was for a decrease in mating proportion after day one followed by a gradual increase over the subsequent days. In contrast, the proportion of Brazil-strain females mating daily when presented with virgin Brazil-strain males remained low. This difference in re-mating proportion between the two treatments became significant at days seven and eight but was not significantly different on previous days (at 95% C.I.) (Figure 5.7).

For South-India-strain females there was no significant difference in either the number of re-matings per female (W=1394.0, p>0.05) (Table 5.3) or in the proportion of females mating ($F_{(1,12)}$ =0.91, p>0.05) (Figure 5.8) irrespective of male status. A significant difference between the two treatments in the proportion of mating South-India-strain females was observed on days three and four (at 95% C.I.). On both of these days females exposed to virgin South-India-strain males mated significantly more times than females exposed to the same males daily (Figure 5.8).

Figure 5.6. Proportion of Brazil- (B) or South-India-strain (SI) females mating on a particular day when provided with two 30 minute mating opportunities daily with three virgin males of the same strain. Error bars are 95% confidence intervals.
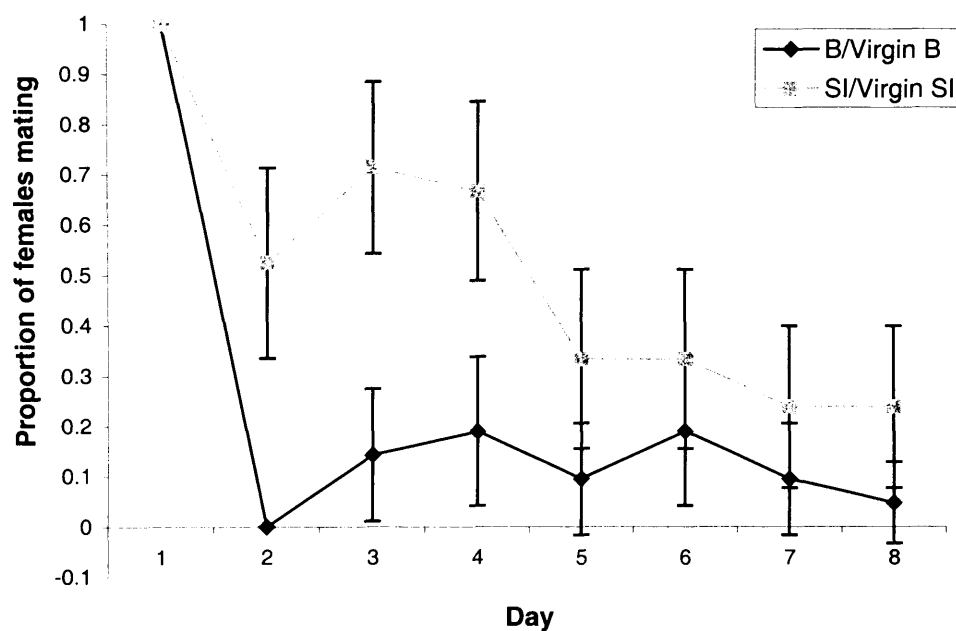
Figure 5.7. Proportion of Brazil-strain (B) females mating on a particular day when provided with two 30 minute mating opportunities daily and presented with either the same males (B/B) or with three different virgin males daily (B/Virgin B). Error bars are 95% confidence intervals.
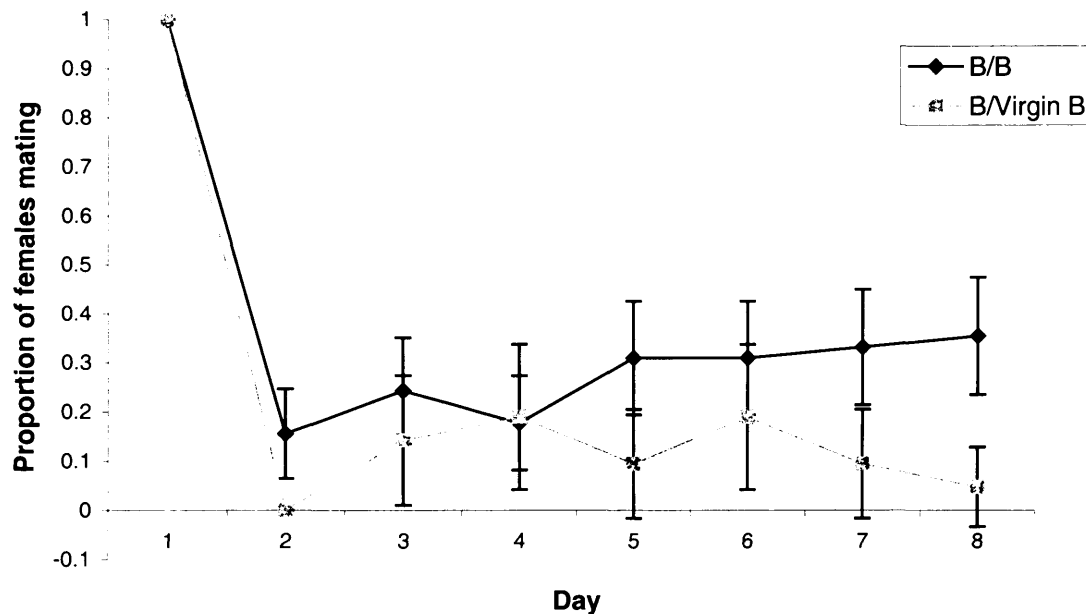
Figure 5.8. Proportion of South India (SI) strain females mating on a particular day when provided with two 30 minute mating opportunities daily and presented with either the same males (SI/SI) or with three different virgin males daily (SI/Virgin SI). Error bars are 95% confidence intervals.
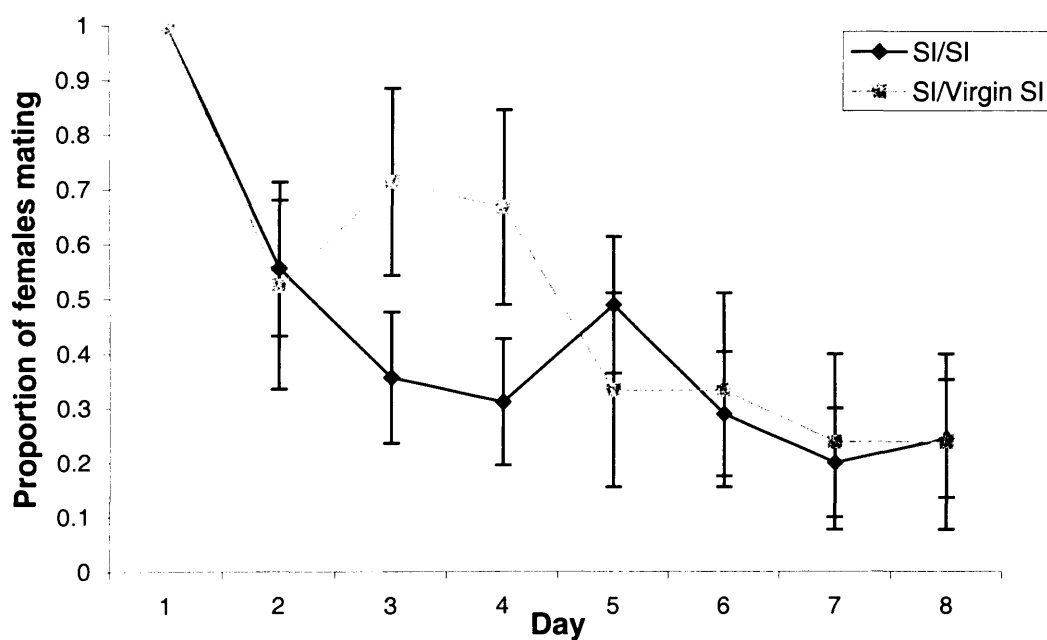
Mixed-strain pairs

The proportion of females mating and the number of re-matings per female were both found to be significantly higher for South-India-strain females paired with Brazil-strain males when compared with Brazil-strain females paired with South-India-strain males (Proportion: $F_{(1,12)}$ =7.96, p<0.05, Figure 5.9; Matings per female: W=315.0, p<0.001, Table 5.3). The proportion of mating South-India-strain females exposed to Brazil-strain males was significantly higher on days two, three and four when compared to Brazil-strain-females mated to South-India-strain males. The proportions of mating females were not significantly different on any other days (at 95% C.I.) (Figure 5.9).

The proportion of Brazil-strain females mating was significantly lower when paired with South-India-strain males compared to Brazil-strain females paired with Brazil-strain males ($F_{(1,12)}$ =11.19, p<0.01) (Figure 5.10). In contrast, the number of re-matings per Brazil-strain female was not significantly different between these two treatments (W=1625.0, p>0.05) (Table 5.3). The general trend in proportion of matings per day for females in the two treatments is very similar with a decrease on day 2 then a gradual increase over subsequent days. A significantly lower proportion of Brazil-strain females mated on day seven when exposed to South-India-strain males compared to Brazil-strain females exposed to Brazil-strain males (at 95% C.I.). No other day was significantly different in terms of the proportion of females that mated (Figure 5.10).

No significant difference was observed between the numbers of matings per South-India-strain female or the proportion of matings irrespective of the strain of the male (Proportion: $F_{(1,12)}$ =0.01, p>0.05, Figure 5.11; Matings per female: W=1495.5, p>0.05, Table 5.3). The general trend for both treatments in the daily proportions of females mating was a gradual decrease over time, which did not differ significantly except on day five. On day five, the proportion of South-India-strain females mating when exposed to Brazil-strain males was significantly lower than that observed for South-India-strain females exposed to South-India-strain males (at 95% C.I.) (Figure 5.11).

Figure 5.9. Proportion of South-India-strain females paired with Brazil-strain males (SI/B) and Brazil-strain females paired with South-India-strain males (B/SI) mating on a particular day when provided with two 30 minute mating opportunities daily with the same males. Error bars are 95% confidence intervals.
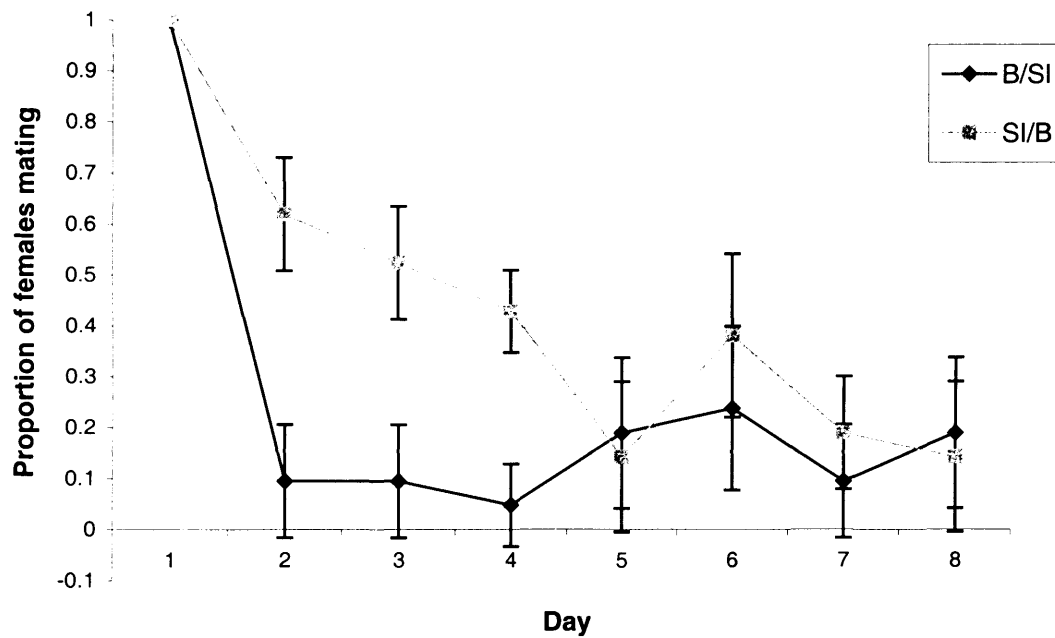
Figure 5.10. Proportion of Brazil-strain females paired with Brazil-strain males (B/B) and Brazil-strain females paired with South-India-strain males (B/SI) mating on a particular day when provided with two 30 minute mating opportunities daily with the same males. Error bars are 95% confidence intervals.
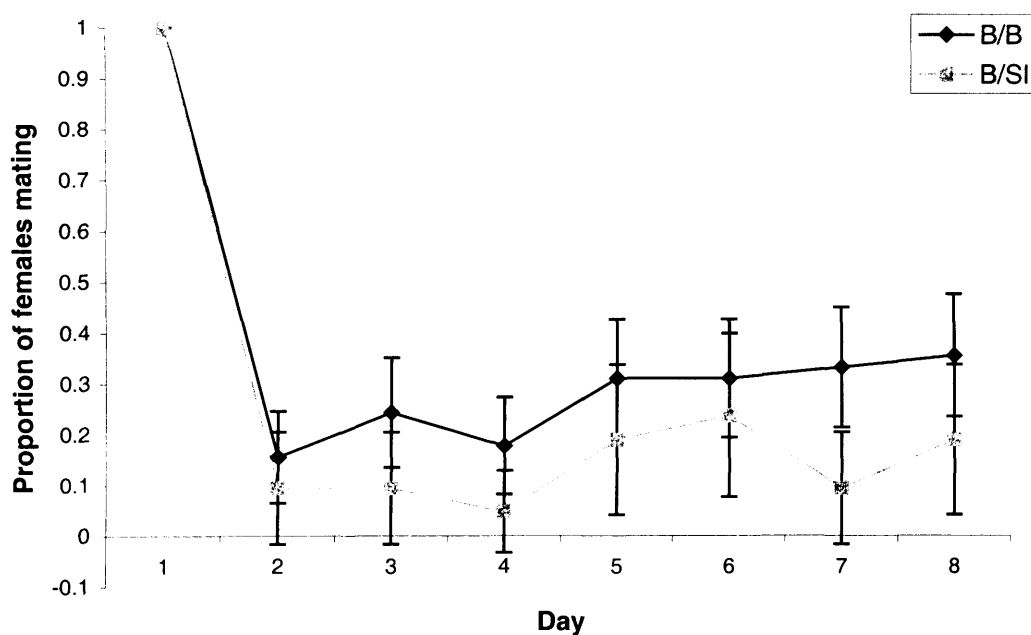
Figure 5.11. Proportion of South-India-strain females paired with South-India-strain males (SI/SI) and South-India-strain females paired with Brazil-strain males (SI/B) mating on a particular day when provided with two 30 minute mating opportunities daily with the same males daily. Error bars are 95% confidence intervals.
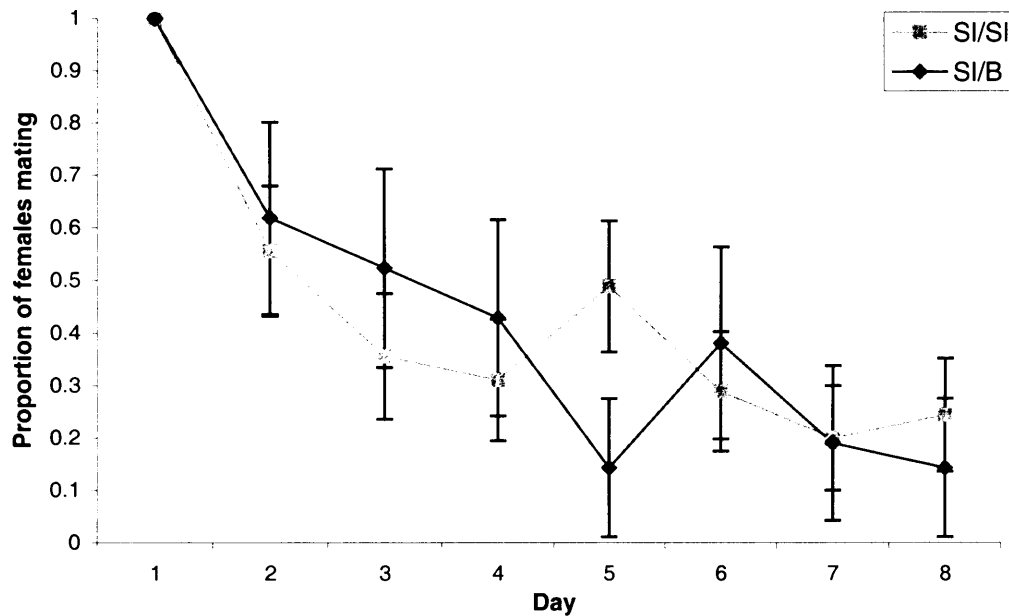
Is re-mating affected more by the strain of the male or the female?

The proportions of Brazil- and South-India-strain females that mated per day, in all treatments, were angular transformed and analysed as GLM ANOVA to determine whether it was the strain of the male or female that had the greater influence on re-mating.

The strain of the male did not have a significant effect on the proportion of females that mated ($F_{(1,38)}=0.00$, $p>0.05$), however, the strain of the female was found to be a highly significant ($F_{(1,38)}=19.66$, $p<0.001$) and South-India-strain females had a significantly higher proportion of matings compared to Brazil-strain females. Back transformed mean proportions are shown in Table 5.5.

Table 5.5. Proportions of Brazil- and South-India-strain females mating when exposed either to Brazil- or South-India-strain males.

(a)   Angular transformed proportions and SE values. SE values are standard errors of the means.

	Brazil-strain male	South-India-strain male	Mean
Brazil-strain female	0.424 (0.046)	0.368 (0.065)	0.396 (0.040)
South-India-strain female	0.617 (0.065)	0.673 (0.046)	0.645 (0.040)
Mean	0.521 (0.040)	0.520 (0.040)	

Full ANOVA shown in Table 3, appendix 3

(b)   Back transformed proportions.

	Brazil-strain male	South-India-strain male	Mean
Brazil-strain female	0.169	0.142	0.149
South-India-strain female	0.335	0.389	0.361
Mean	0.248	0.247	

5.4.3 How many times will a female re-mate when opportunities are constantly available?

The video recording only showed when a male covered a female and it was impossible to state with any great conviction that an observed 'mating' resulted in intromission.   Male *C. maculatus* could often be seen positioned over a female indicating that mating was taking place and were observed to remain in that position for several minutes.   However, actual observations in the lab showed that males would sometimes take this position over females without intromission and copulation taking place and without a female rejection occurring.   Therefore analyses in this section are based on female rejections, which could be clearly observed from the video, and also apparent matings.   Apparent matings would obviously include actual matings and also when intromission did not take place but a female failed to reject the male.   It is known that some multiple mating did occur as apparent re-matings recorded during the video recordings were confirmed by directly observing the pairs during the day.

Ruth Hayes, a third year BSc. project student working in the laboratory under my supervision, collected the raw data on apparent matings and rejections presented in this section.   Video recordings were made for seven days, however, there were technical problems with the video set-up and a full data set was only obtained for the first four days. The analysis uses only the complete data set for the first four days.

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Females of both strains were found to reject males in similar proportions regardless of whether they were exposed to the same males or to different males (Brazil: $F_{(1,13)}$ = 0.17, p>0.05; South India: $F_{(1,15)}$ = 0.60, p>0.05). Similarly, the number of apparent matings was also unaffected by exposure to the same or to different males (Brazil: $F_{(1,13)}$ = 0.82, p>0.05; South India: $F_{(1,15)}$ = 0.60, p>0.05). The strain of the female was found to be insignificant in determining the number of rejections or apparent matings (Brazil: $F_{(1,28)}$ = 0.07, p>0.05; South India: $F_{(1,28)}$ = 0.01, p>0.05). The average numbers of apparent matings and rejections for the Brazil- and South-India-strain male and female pairs are shown in Figure 5.12.

The number of apparent matings that females had was positively correlated with number of rejections that were recorded (Pearson's correlation: r = 0.733, p<0.001, n=29). A regression line was fitted to the data with the equation y = 8.11 + 0.399 x, where y is the number of matings and x is the number of rejections (r^2=0.538, p<0.0001) (Figure 5.13).

Figure 5.12. Average number of apparent matings and rejections for Brazil- and South-India-strain females exposed either to the same males or to a different male each day for four days. The male and female of the pair were of the same strain. Bars are standard errors of the means. The data were untransformed.
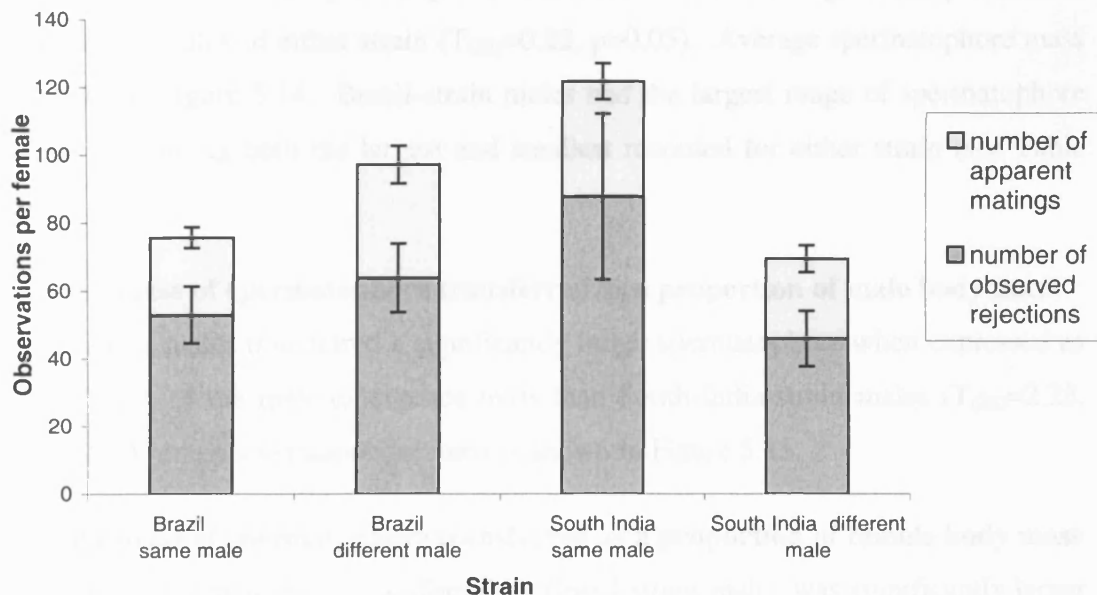
Figure 5.13. Scatter graph to show the correlation between the number of male rejections performed by a female and the number of apparent matings that took place. The data were untransformed.
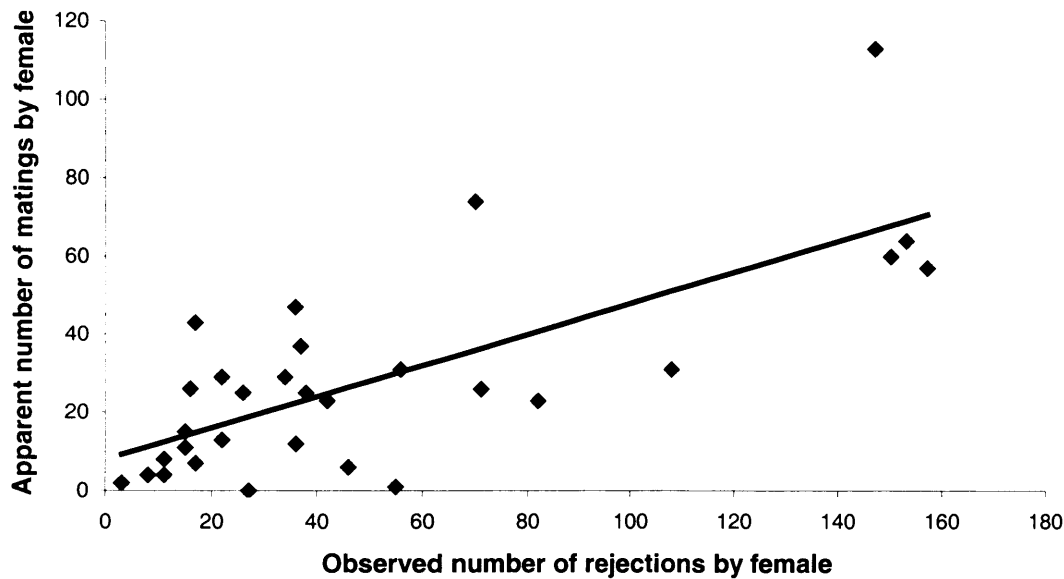
5.4.4 Male investment: spermatophore size

Average mass of spermatophore transferred by males

The average mass of spermatophore transferred was not significantly different between the males of either strain ($T_{(35)}$=0.22, p>0.05). Average spermatophore mass is shown in Figure 5.14. Brazil-strain males had the largest range of spermatophore masses containing both the largest and smallest recorded for either strain (see Table 5.6).

Average mass of spermatophore transferred as a proportion of male body mass

Brazil-strain males transferred a significantly larger spermatophore when expressed as a proportion of the male emergence mass than South-India-strain males ($T_{(28)}$=2.25, p<0.05). Average spermatophore mass is shown in Figure 5.15.

Average mass of spermatophore transferred as a proportion of female body mass

The mass of spermatophore transferred by Brazil-strain males was significantly larger in proportion to the female emergence mass than the mass of spermatophore transferred by the South-India-strain males ($T_{(30)}$=2.53, p<0.05). Average spermatophore mass is shown in Figure 5.16.

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Figure 5.14. Average weight of spermatophore transferred by virgin Brazil and South India strain males. Bars are standard errors of means. Note also that the Y-axis does not start at zero. The data were untransformed.
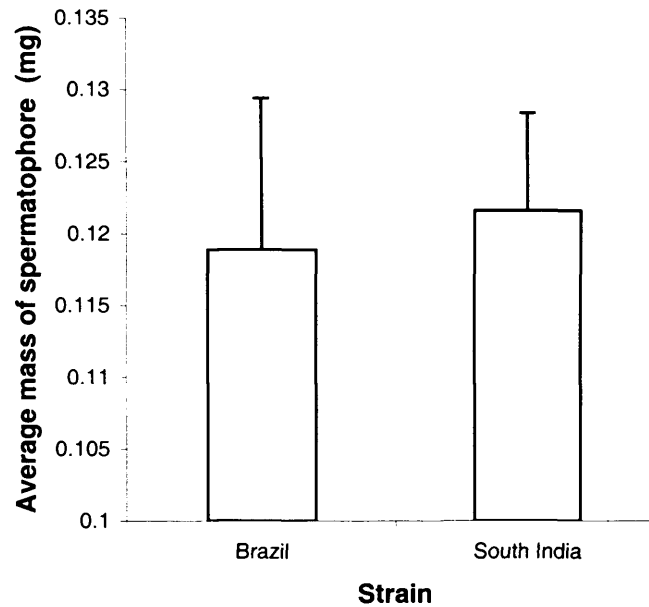
Figure 5.15. Mass of spermatophore transferred by virgin Brazil- and South-India-strain males represented as a proportion of the male emergence mass. Bars are standard errors of means. The data were untransformed.
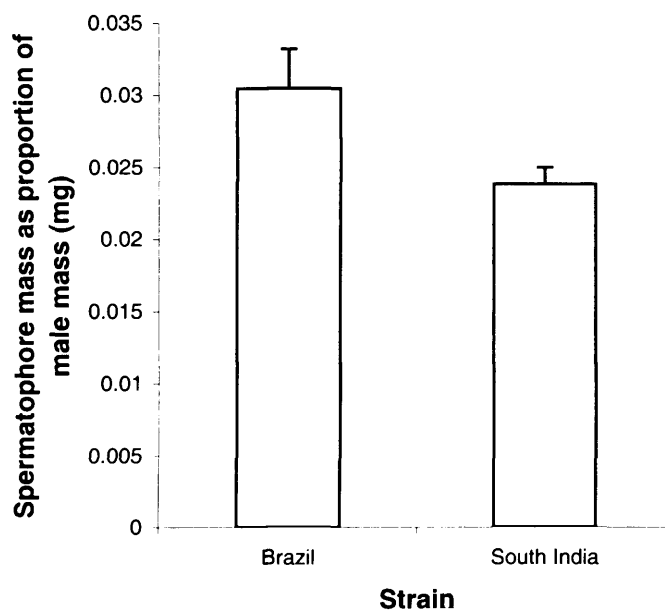
Figure 5.16. Mass of spermatophore transferred by virgin Brazil- and South-India-strain males represented as a proportion of the female emergence mass. Bars are standard errors of means. The data were untransformed.
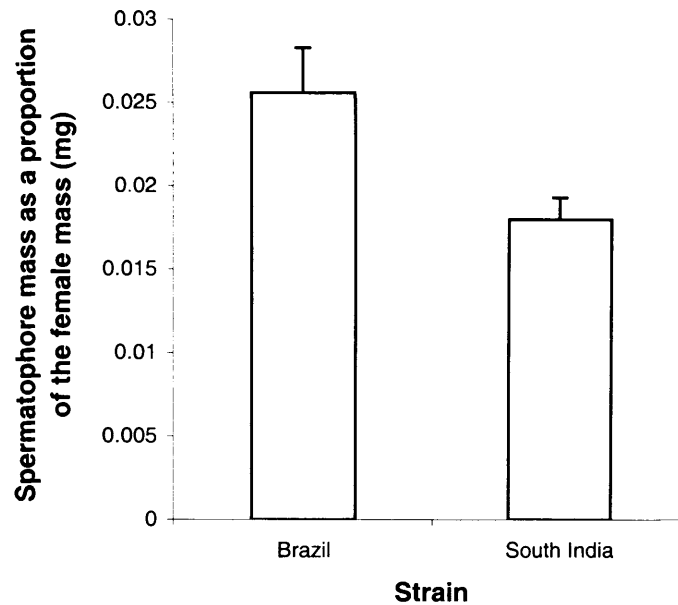
Table 5.6. Standard deviation and range of spermatophore sizes transferred by virgin Brazil- and South-India-strain males. The data were untransformed.

Strain	Minimum spermatophore mass	Maximum spermatophore mass	Range	SD
Brazil	0.0075mg	0.2165mg	0.2090	0.0493
South India	0.0690mg	0.1715mg	0.1025	0.0317

There was no correlation between the emergence mass of the Brazil-strain males (Figure 5.18) or females and the average mass of spermatophore transferred ($r=0.2060$, $p>0.05$ and $r = -0.247$, $p>0.05$ respectively). Similarly, there was no correlation between the emergence mass of the South-India-strain females and the mass of spermatophore transferred by the South-India-strain males ($r = -0.191$, $p>0.05$). There was a correlation, however, between the emergence mass of the South-India-strain males and the mass of the spermatophore transferred by South-India-strain males ($r =0.526$, $p<0.05$) (Figure 5.17). Therefore, larger South-India-strain males transferred heavier spermatophores. A regression line was fitted to the data with the equation $y = 0.026x -0.011$, where y was spermatophore mass and x was male emergence mass ($r^2=0.277$, $p<0.05$).

Figure 5.17. Scatter graph to show the correlation between the emergence mass of the South-India-strain males and the mass of the spermatophore transferred at their first mating. The correlation is significant. The data were untransformed.
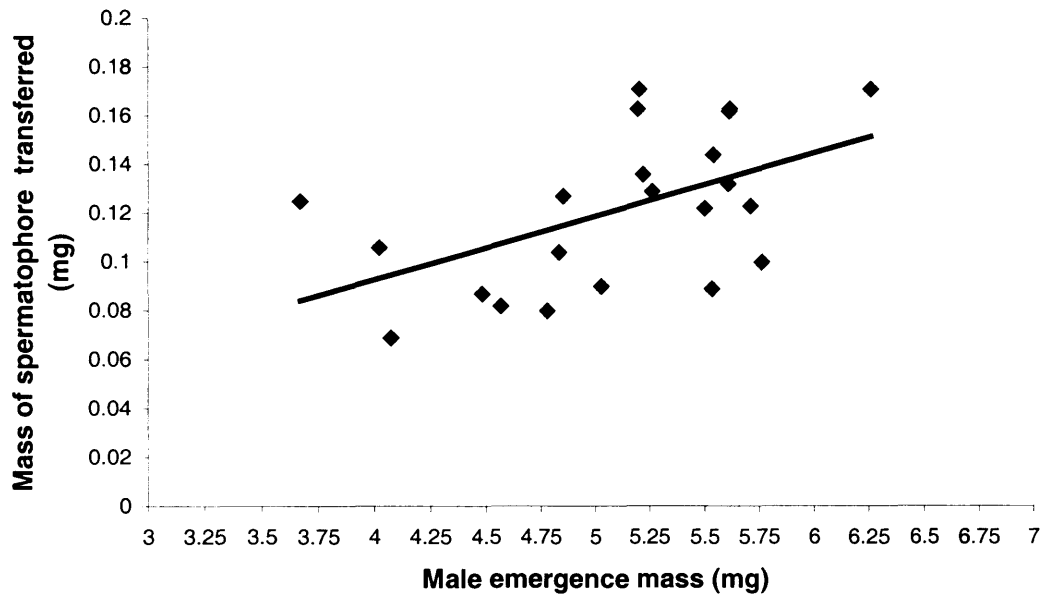
Figure 5.18. Scatter graph to show the correlation between the emergence mass of the Brazil-strain males and the mass of the spermatophore transferred at their first mating. The correlation is not significant. The data were untransformed.
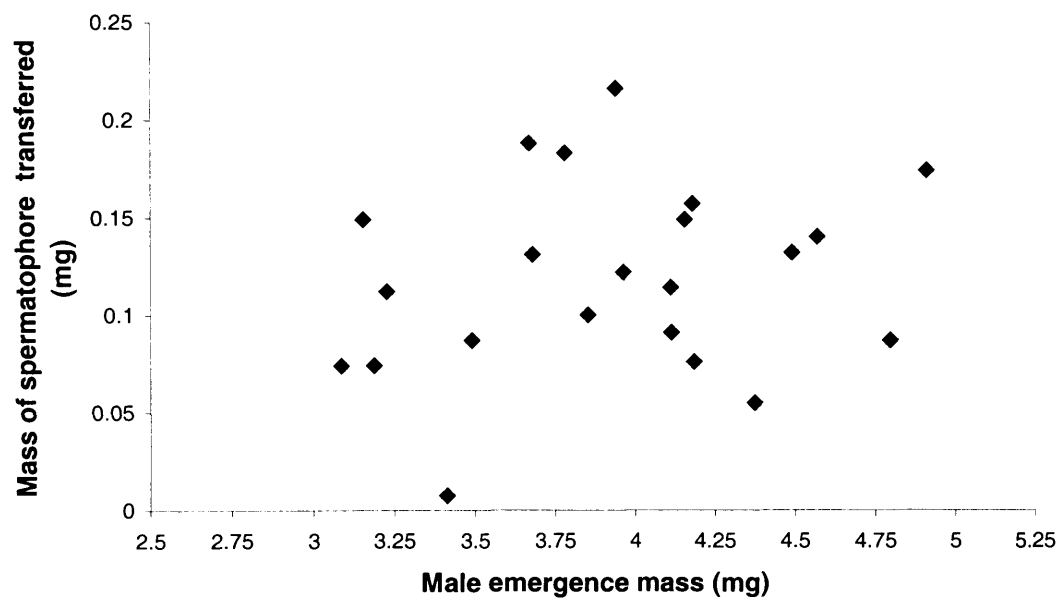
5.5 DISCUSSION

Copulation duration can vary considerably both between species (Hirai and Kimura, 1999 and Dickinson, 1988) and between individuals of the same species (Bloch *et al.*, 1996 and Andres and Rivera, 2000). Variation between Coleopteran species is also common (Halstead, 1973; Barratt, 1977; Holloway and Smith, 1987; Dickinson, 1988) (see section 5.1).

Compared to some of these average times, copulation duration in *C. maculatus* is relatively short. Variability does exist between the two strains of *C. maculatus*, however, and significantly different copulation durations were observed with Brazil-strain pairs mating for the longest time. The average times of 5.3 minutes for the Brazil strain and 4.4 minutes for the South India strain were longer than the average time of 3.17 minutes recorded for *C. maculatus* by Rup (1986) for an unnamed strain of *C. maculatus*. In the present study, the extra time spent *in copula* for the Brazil-strain pairs occurred during phase three of mating; the onset of female kicking to aedeagus removal. Males of both *C. maculatus* strains transferred the same mass of spermatophore, however, the proportion of emergence mass that this represented was significantly different between the two strains. Spermatophores in the Brazil strain represented a significantly larger proportion of the emergence mass of both the male and the female beetle compared with the South India strain. The time spent *in copula* does not always correlate with the amount of sperm transferred, however (Pitnick *et al.*, 1991 described in Eberhard, 1996; Gilchrist and Partridge, 2000 and Tokarz, 1999). Rup (1986) suggested that sperm transfer in *C. maculatus* took place within the first minute of copulation since females were only observed to re-mate if mating was disturbed within the first minute of joining. This suggests that the extra time spent *in copula* by Brazil-strain males may have a function other than simply to transfer a larger amount of sperm. Removal of a rival male's sperm and the passing of a mating plug by males, or even acting as the mating plug himself, have also been proposed to explain longer copulation durations (Michiels, 1992; Lachmann, 1998; Fincke, 1982). However, removal of another male's sperm during mating was not observed or thought possible by Eady (1994a) and the use of the male as a mating plug seems an unlikely function since copulation times were still relatively short in comparison to some species (e.g. Barratt, 1977; Pitnick *et al.*, 1991).

Female kicking behaviour of *C. maculatus* may have been an indication that the bursa had reached its capacity, triggered by stretch receptors. Certainly females of some species use such information in assessing the size of an ejaculate donation (Sugawara, 1979). The extended phase two (aedeagus insertion to onset of female kicking) observed for South-India-strain females paired with Brazil-strain males may be due to a larger bursal volume relative to the smaller Brazil-strain females. Rejection (kicking) in these females could be triggered later suggesting that Brazil-strain males may have taken longer to transfer their spermatophores (more sperm and or additional products). In the South India strain, spermatophore size may have closely matched bursal volume therefore the onset of female kicking naturally preceded copulation termination. In the Brazil strain, however, spermatophore size may have been much larger than the bursal volume so the female began to reject the male, but copulation was not terminated until all spermatophore material had been transferred. This implies two things. First, that males control the timing of this aspect of mating behaviour. Second, that South-India-strain males are either transferring less spermatophore than females can accommodate or Brazil-strain males are transferring excessively large spermatophores relative to the female's physiology, or both.

In summary, it is clear that Brazil-strain males have longer copulation durations and transfer a proportionally larger spermatophore to females than do South-India-strain males. But, it is not clear whether this extra time is utilised in the transfer of additional seminal products to females or whether males are performing additional mating behaviour that may also serve to delay female receptivity (e.g. Gilchrist and Partridge, 2000).

There are two main strategies that can be used by males when attempting to ensure exclusivity of mating to a particular female. One is to change the female's behaviour or receptivity to other males (Riemann *et al.*, 1967) and the second is to alter the perception by other males of the state of that female. Male *Centris adani* bees transfer a distinctive male scent to the abdomens of a female during mating, which makes the female smell like a male and makes her appear unattractive to other males (Frankie *et al.*, 1980). This would seem to be unlikely in the case of *C. maculatus*, however, since re-mating appeared to be more dependent on female willingness than male attempts. Following mating, further courtship attempts by males were

frequently rejected by females who would kick or run away, and not by males approaching and then avoiding a particular female. Certainly the number of matings a female had seemed to be under female control at least when not confined with males.

The first strategy, the alteration of female receptivity to repeat matings following interactions with males, is widely reported (see reviews in Thornhill and Alcock, 1983 and Eberhard, 1996). The results of these interactions can range in effect from a short time to permanent unwillingness to re-mate (Riemann *et al.*, 1967). Courtship stimulation, mechanical stimulation through copulation, the receipt of sperm or seminal fluids or the filling of the bursa or sperm storage organs have all been reported to trigger female non-receptivity (Fuchs and Hiss, 1970; Gwadz *et al.*, 1971 and Sugawara, 1979). This suggests that female re-mating can be controlled by both mechanical and chemical stimulation.

A significant difference in re-mating frequency between the two strains was observed when access to males and therefore mating opportunities were controlled. In contrast, when males were permanently available, there were no observable differences in the numbers of re-matings between females of either strain. This suggests that females of both strains were likely to re-mate more frequently as a consequence of male harassment. Birkinshaw (1998) also observed higher copulation frequencies for female *Prostephanus truncatus* (larger grain borer), when kept in a male-biased group. Copulation rates of 1-3 times per female per 12 hour light period were recorded for groups that included either two or eight females with two males and rates of approximately 20 times per female per day when two females were kept with eight males (Birkinshaw, 1998).

In the controlled male-exposure experiments, South-India-strain females re-mated significantly more frequently over seven days than Brazil-strain females regardless of the strain of the male. Furthermore, South-India-strain females mated more frequently when exposed to virgin males whereas Brazil strain females mated less frequently. Females may have been responding to cues derived from the size of the spermatophore through the degree of stretching of the bursa and used this as an indicator of the need to re-mate (see Eady, 1995). For example, mate rejection by females of the butterfly, *Pieris rapae*, was triggered by the response of stretch receptors and frequency of waves of contraction in the bursa to the size of

spermatophore transferred (Sugawara, 1979). Female chequered white butterflies, *Pieris protodice*, with depleted spermatophores were also much more likely to approach conspecific males than were females with fresh spermatophores (Rutowski, 1980). The dissimilar relative sizes of spermatophores and thus the different degrees of bursal stretching may account for the differences in re-mating frequency observed in the two *C. maculatus* strains.

Alternatively, males may have transferred additional substances as part of the ejaculate, which had an antiaphrodisiac effect and decreased female re-mating frequency. Since last male sperm precedence operates in these beetles, increasing the female refractory period would increase the number of offspring fathered by the first male to mate. Despite this, it would appear that South-India-strain females were either resistant to them or South-India-strain males did not transfer them. The data presented on re-mating frequency in the two strains (section 5.4.2), however, would seem to support the former suggestion rather than the latter. The adaptive significance of this resistance in terms of the life history of the South India strain is discussed more fully in section 4.5. Conversely, Brazil-strain females re-mated more frequently on later days when exposed to the same males but significantly less frequently when exposed to virgins. Furthermore, no Brazil-strain females re-mated on day two when exposed to virgin Brazil-strain males suggesting that females may have been aware of the male's status and avoided mating, which could also indicate a cost to re-mating. The control of re-mating in Brazil-strain females could therefore be due either to the transfer of products having an antiaphrodisiac effect or by the sheer size of the spermatophore itself. This was supported by a general increase in mating frequency over time when females were exposed to the same males. Spermatophore size is known to decrease with increasing male age and the number of times a male has mated (Eady, 1995; Fox, 1995a; Savalli and Fox, 1999a). Since males from both strains transferred the same mass of spermatophore the possibility of re-mating being controlled solely by spermatophore mass cannot be ruled out.

What benefits do South-India-strain females gain from frequent re-mating? It may be that females re-mate in order to obtain extra nutrition from the spermatophores, which could then be invested in egg production or somatic maintenance. This would be beneficial to these females since fecundity is restricted more by oviposition site

quality than the number of eggs they can lay (see section 1.2). If an ejaculate served a solely nutritive role, however, females might be expected to select for males that produced larger ejaculates (see Eady, 1994b). South-India-strain males, therefore, should be capable of producing relatively larger spermatophores than Brazil-strain males but this was not the case. Larger South-India-strain males did transfer larger spermatophores than smaller South-India-strain males, however, thus females may prefer to mate with larger males. It may be that spermatophore size in South-India-strain males was constrained by the cost of oviposition stimulation to larval survival.

Summary

Brazil-male and -female pairs copulated for longer and males transferred proportionally larger spermatophores than was observed for South-India-strain males. Mating with virgins decreased re-mating on later days in the Brazil strain but conversely increased re-mating on earlier days in the South India strain. These differences in re-mating behaviour were suggested to come about either through size of spermatophore or transference of an additional product with an antiaphrodisiac effect. If such products were being transferred it is likely that South-India-strain females have evolved resistance to them, rather than males not transferring them.

CHAPTER 6

CHAPTER 6: DISCUSSION

6.1 REVIEW OF RESULTS

The overall aims of the present study were to quantify the costs and benefits of MM in *C. maculatus* females and their offspring. More specifically, two strains of *C. maculatus* were chosen that displayed differing life histories and the effects of MM on the fitness traits of longevity and fecundity were examined.

Detailed discussions of the costs and benefits and associated behaviours observed relating to MM can be found in sections 3.5, 4.5 and 5.5, however, the main results and their implications are summarised here. MM Brazil-strain females suffered a cost of reduced longevity and a benefit of increased fecundity compared to SM females. Costs were also found to be transmissible to the next generation as F_1 male and female offspring of MM females also displayed reduced longevity compared to those F_1 offspring of SM females. The cost to the longevity of MM females, however, was not mediated by increased fecundity. Similarly, the cost to F_1 offspring longevity of MM female parents was not mediated by decreasing egg size as egg mass did not differ significantly between treatments. MM females also experienced reduced hatchability of eggs. Brazil-strain females did not re-mate readily when seeds were absent and Eady (1995) described unpublished data whereby female *C. maculatus* re-mated less when oviposition sites were unavailable. This would be beneficial if males were transferring substances that stimulated oviposition and egg maturation. The fact that a much higher proportion of South-India-strain females re-mated compared to Brazil-strain females in the absence of an oviposition substrate may highlight the absence of a cost to re-mating in this strain and conversely the presence of a cost in the Brazil strain. It was suggested that Brazil-strain spermatophores had a dual function by stimulating oviposition, probably by chemical means, and by increasing refractory period of females, by chemical or mechanical means.

No costs or benefits in terms of longevity or fecundity (total eggs produced) were observed for South-India-strain females or their F_1 offspring. MM females did, however, lay heavier eggs and the eggs of their F_1 female offspring showed increased hatchability over eggs from F_1 offspring of SM parents. Thus MM females might produce larger eggs from which came larger offspring who would be better

competitors due to faster development (Fox, 1993c) and would accrue more resources at the larval stage to invest in larger, fitter offspring. South-India-strain females were observed to mate more frequently then Brazil-strain females, particularly with virgin South-India-strain males. Excess spermatophore material could then be used to incorporate into eggs (e.g. Rooney and Lewis, 1999).

So why are the two strains so different in their responses to MM? It is unlikely (although still possible) that spermatophores components between the two strains were different (i.e. that stimulatory products were not transferred by South-India-strain males). Certainly Brazil-strain males transferred proportionally larger spermatophores and their size may have functioned in female refractory period and may reflect the transmission of additional components. The evolution of a range of products produced and transmitted by males which alter female physiology in the males favour during competition for increased paternity has been well documented (see reviews in Eberhard, 1996). In *Drosophila* for example, components of male seminal fluid have been shown to increase egg production, oviposition, increase female refractory period and even incapacitate rival males sperm (Prout and Clark, 2000). These wide-ranging functions all help to secure a greater percentage of paternity for the males but may also have the unfortunate side effect of being toxic to the female (Rice, 1996; Chapman *et al.*, 1995). South-India-strain females did not display a decrease in longevity despite mating more frequently (although they did receive proportionally smaller spermatophores) and this may suggest that the decrease observed in the Brazil-strain females was caused not by toxins but from over stimulation of some aspect of female physiology relating to egg production. What is clear, however, is that an oviposition stimulant does exist, in the Brazil strain at least, which South-India-strain females might be resistant to. Resistance would be expected in this strain, as oviposition stimulation could be detrimental to female fitness given their life history and the competitive nature of the larvae.

6.2 EVOLUTION OF LIFE-HISTORY STRATEGIES AND MM

Environmental context and life-history strategy

The differing effects of MM on longevity and fecundity observed in the two strains can be explained by considering the life-history strategies and evolutionary environment of the two strains. Trade-offs between fitness components are central to

the concept of life-history theory. As discussed previously in section 3.1, when resources are limiting one fitness trait cannot be limitlessly increased without a corresponding decrease in a different fitness trait; this is known as the principle of allocation (Sibly and Calow, 1986). The fitness traits of longevity and fecundity are just two possible traits that can be examined when considering trade-offs. Other such traits may be developmental period and adult body mass (Sibly and Antonovics, 1992). The costs and benefits expressed in these traits will depend on the environmental context and are generally only relevant if the life history is considered in the context of that specific environment. For example, killifish (*Cynolebias sp.*) evolved to survive and reproduce in temporary pools present for approximately nine months each year (Axelrod *et al.*, 1987). In this situation, females would not benefit by increasing longevity at the expense of fecundity. Instead, trade-offs resulting in higher fecundity but reduced longevity would be expected. In two species of lampyrid beetles with contrasting life-history characteristics, short-lived, non-feeding *Photinus ignitus* females allocated 62% of spermatophore derived protein into developing oocytes (Rooney and Lewis, 1999). In contrast, the longer-lived, feeding *Ellychnia corrusca* females incorporated 46% of the male-derived protein into female fat body, thus investing in somatic maintenance (Rooney and Lewis, 1999). This illustrates how females may differentially allocate resources between fitness traits resulting in either increased longevity or increased fecundity depending upon the life history. Adaptations in life-history strategies in response to differing environmental conditions have also been reported in the common mussel, *Mytilus edulis* (Bayne, 1986, cited in Sibly and Calow, 1986), different geographical populations of *Drosophila melanogaster* (Parkash and Munjal, 2000) and North American water striders (Blanckenhorn and Fairbairn, 1995).

The importance of considering the environmental context is highlighted by a Yemen strain of *C. maculatus*. This strain was isolated from lentil seeds (*Lens culinaris*) where it develops more successfully than any other *C. maculatus* strain (Credland, 1990). Lentil seeds are relatively small seeds containing large amounts and types of toxic compounds which the Yemen strain invests a lot of energy into detoxifying (Smith, 1990). This coupled with a longer developmental period and larger emergence size (Dick and Credland, 1984), means that individuals must consume larger amounts of seed to satisfy their energy requirements. Small seed size also

ensures that a larva is more likely to encounter other larvae and competition for the resource results in a larval competition strategy that resembles that of a contest type rather than a scramble type (Credland *et al.*, 1986). Smith (1990) argues that the 'evolution of these behavioural traits may be in part a consequence of adaptation to physiological constraints imposed by toxins in the lentil host'.

The two strains of *C. maculatus* used in the present study enabled the comparison of life-history evolution of populations originating from two separate environments. The evolution of these seed predators is closely tied to the evolution of the legume seeds on which they feed. Legume seed evolution has responded to attack by seed predation in one of two ways: First, by decreasing seed size and increasing seed number; Second, by acquiring toxicity without reducing overall seed size (Toquenaga *et al.*, 1994). The production of small wild seeds would have favoured contest-type competition as it ensured the monopoly of a limited resource (Toquenaga *et al.*, 1994; Smith, 1990). Conversely, cultivation by humans and the progression of agriculture resulted in the production of larger seeds and the unintentional selection of scramble type strains, which may have been selected against in the smaller wild type seeds (Smith and Lessells, 1985; Toquenaga *et al.*, 1994).

The ancestral strain of *C. maculatus* is, therefore, believed to have evolved on small wild seeds and would have displayed larval competition typical of the contest type (e.g. South India). As seeds were small, larvae needed to monopolise the resource if they were to grow and survive and contest competition would be favoured (Smith and Lessells, 1985). Furthermore, females would need to search for new host plants and seeds that had not already been colonised to maximise the chances of larval survival. Selection, therefore, would favour individuals that could live longer. In turn this would result in a trade-off between increased longevity at the expense of decreased fecundity, as it would not favour females to lay more eggs if oviposition sites were simply unavailable or seeds already contained eggs or developing larvae. With the advent of agriculture, larger and larger seeds were selected. Oviposition sites also became abundant with the construction of grain stores. As seed size increased more than one larva could feed and emerge from a single seed enabling females to lay more than one egg per seed. This environment would favour the evolution of the scramble-type competitor (e.g. Brazil strain). Indeed increasing seed size has been shown to

favour the scramble-type strategist (Smith, 1990). In terms of the life history, fecundity increased at the expense of longevity, as females would not benefit by laying few eggs but living longer. Females would not need to search for new sites until the grain store had been totally depleted which could be several generations later.

Life-history strategies are not always strictly observed to be either one or the other, however, and the ability to adapt to different environmental conditions has been demonstrated in *C. maculatus*. Female *C. maculatus* display phenotypic plasticity in their ability to trade fecundity and longevity depending on the availability of oviposition sites; they are able to oviposit maximally when seeds are abundant but trade fecundity for longevity when seeds are poor or limited (Møller *et al.*, 1990; Messina and Slade, 1999).

The life-history strategies of the two strains are well documented and when comparing the two strains we see that the Brazil strain is characterised by relatively lower longevity and higher fecundity compared to the longer lived but relatively less fecund South India strain (Table 1.1). Brazil-strain females are not very discriminating in their egg laying patterns, typically characterised as random, unless seeds are unlimited (Messina and Dickinson, 1993). In contrast, South-India-strain females are very discriminating and will lay eggs in a uniform manner (Mitchell, 1991). Finally, and perhaps most important, Brazil-strain larvae are scramble or Avoid competitors whereas South-India-strain larvae are contest or Attack competitors (Smith and Lessells, 1985) (see section 1.2.3).

The fecundity of Brazil-strain females is constrained, therefore, only by the numbers of eggs that they can lay rather than the quantity of oviposition sites since the larvae will share the resources of a seed (low egg dispersal). In contrast, the fecundity of South-India-strain females is constrained by quantity and quality of oviposition sites due to the competitive nature of the larvae where typically only one survives to emerge (high egg dispersal). In this way selection on South-India-strain females is for extending longevity so that females live long enough to enable them to seek out sufficient good quality sites for oviposition thus reducing potential fecundity in favour of realised fecundity. For Brazil-strain females more larvae will survive so they will trade longevity for increased fecundity. This demonstrates why the optimal balance

of reproduction versus adult longevity is determined by the juvenile survivorship (Sibly and Calow, 1986).

Life history and responses to MM

South-India-strain females (contest strategists) evolved in an environment where seeds were a limited resource. Females, therefore, would not benefit by responding to oviposition stimulants if they were delivered by the male during copulation as the competitive nature of the larvae would ensure that fitness did not increase with an increasing number of eggs laid. In contrast, Brazil-strain females (scramble strategist) evolved in an environment where seeds were not limited and these females would benefit by responding to oviposition stimulants as increasing the number of eggs laid would lead to increasing fitness. This may explain why we see an increase in fitness for Brazil-strain females but no increase for South-India-strain females following MM.

MM and male-female conflict

The benefits of MM in South-India-strain females are obvious in the absence of an oviposition stimulant as increasing the number of matings leads to an excess of male-derived nutrients available to invest in increasing egg size. This could increase female fitness by increasing the size and therefore competitive ability of her offspring. The benefits to South-India-strain males are also obvious as mating with several females increases his chance of fathering more offspring. However, it would not benefit either the male or female if the male were to transmit oviposition-inducing substances and the female were to respond to them as offspring survival might then decrease.

In the Brazil strain, females would be expected to mate more frequently when seeds were plentiful as re-mating stimulated oviposition. Similarly females might still gain if oviposition sites were limited as their larvae will coexist (to a point) and share the resource. However, Brazil-strain females do not re-mate frequently, at least when oviposition sites are absent, and this may indicate a cost associated with MM. Males, therefore, are unlikely to get the chance to re-mate and will invest substantially in each mating that they acquire in terms of spermatophore size. This serves two functions: first to pre-empt rival males' sperm and to avoid being pre-empted if a female did re-mate (Eady, 1995); second, to induce a refractory period in the female

to ensure that the majority of eggs laid before the female accepted a re-mating would be fathered by that male.

This may represent two possible outcomes of male and female conflict. In the Brazil strain, males mate infrequently but invest heavily in a few copulations and try to exert a greater degree of control over the female's fecundity and hence their own reproductive success. In the South India strain, mating opportunities for males are more frequent so males can 'save' sperm for multiple copulations but consequently may exert less control over female oviposition. Certainly, in a contest strain such as the South India strain, where larvae compete for resources, it may benefit both males and females to mate with several others. The resulting offspring might not be fathered by the same male, which may increase the chances that some males with good competitive ability genes father larvae. This of course assumes that there is no mate choice and females are passive in the receipt of sperm, which is unlikely. It is also unlikely that the receipt of good viability genes is the cause of MM in this strain but it may be one benefit from it.

Sexual conflict in *C. maculatus* ; new evidence

Following the submission of this thesis new evidence has been presented by Crudgington and Siva-Jothy (2000) describing actual physical damage incurred to Brazil-strain females following mating. This work is very timely and may present a mechanism for the decrease in longevity observed following MM. Heavily sclerotised spines present on the aedeagus of male *C. maculatus* were found to penetrate the cuticular lining of the female genital tract during mating and twice mated females died sooner than those that singly mated (Crudgington and Siva-Jothy, 2000). Furthermore, the role of kicking by females as a response intended to limit damage by decreasing copulation duration was also discussed. The benefits to the male could be in increasing a females unwillingness to re-mate, increasing oviposition rates as females perceived a threat to mortality and allowing chemicals present in the spermatophore to gain entry to female haemolymph (Crudgington and Siva-Jothy, 2000). Certainly this shows that damage does occur internally to females and that energy will be expended on repair. Also, that MM females died sooner than SM females potentially as a result of such damage. The presence of spines on the aedeagus of the South India strain of *C. maculatus* was not examined. In this thesis,

no loss of longevity was observed for South-India-strain females observed following MM and females were observed to mate more frequently than the Brazil-strain females. However, copulation duration was significantly shorter for South-India-strain females, which may have limited damage to females' genitalia. Examination of the genitalia of both male and female South-India-strain *C. maculatus* is needed to observe spine structure on the male aedeagus and also damage incurred to females following mating to enable a fuller analysis of the role of spines in the two strains.

6.3 CONCLUSION

When looking at the evolution of life histories and the effect of MM on trade-offs between fitness traits, the environmental context must also be considered. Often the costs and benefits of MM are only relevant to the life history in that specific context. The Brazil and the South India strains of *C. maculatus* are excellent models for comparing evolution in two separate biotic environments and also the costs and benefits of MM. The larval competition strategies found in different strains of *C. maculatus* beetles have already been argued to be a powerful driving force in the evolution of oviposition strategies and life-history trade-offs in adults (Broadhurst, 1996; Smith, 1990). The evidence here suggests that they may also account for driving the responses of adults to MM as well.

6.4 FUTURE WORK

- Examine other strains of *C. maculatus* and also other species with dichotomous juvenile strategies (e.g. *C. chinensis*) to provide further evidence that responses to MM can be driven by such strategies.

- Characterise spermatophore components or stimulated physiological process or processes in Brazil-strain females responsible for the decrease in longevity for MM females and their F_1 offspring.

- Characterisation of spermatophore components responsible for eliciting behaviours such as oviposition stimulation and refractory period in females.

- Investigate the possibility of spermatophore size being responsible for refractory period in Brazil-strain females.

- To compare sperm competition and sperm number in the two strains.

- Investigate mate choice in the two strains to determine whether South-India-strain females prefer larger males.

- Reciprocally crossing the strains to separate theories on the responses of females to MM opportunities.

APPENDICES

Appendix 1

Table 1. GLM ANOVA for Brazil-strain female parent longevity showing the effect of seed availability (U), SM or MM (Matings), exposure to one or seven males (Males) and their interactions. Female mergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.542	0.396	0.396	4.41	<0.05
U	1	14.108	14.052	14.052	156.37	<0.001
Matings	1	1.952	1.950	1.950	21.70	<0.001
Males	1	0.328	0.327	0.327	3.64	n.s.
U*Matings	1	0.120	0.119	0.119	1.32	n.s.
U*Males	1	0.219	0.219	0.219	2.44	n.s.
Matings*Males	1	0.008	0.008	0.008	0.09	n.s.
U*Matings*Males	1	0.342	0.342	0.342	3.81	n.s.
Error	151	13.569	13.569	0.090		
Total	159	31.187				

Table 2. GLM ANOVA for South-India-strain female parent longevity showing the effect of seed availability (U), SM or MM (Matings), exposure to one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.461	0.163	0.396	1.98	n.s.
U	1	6.976	6.942	0.327	84.29	<0.001
Matings	1	0.122	0.125	1.950	1.52	n.s.
Males	1	0.026	0.030	0.030	0.36	n.s.
U*Matings	1	0.103	0.103	0.103	1.25	n.s.
U*Males	1	0.011	0.012	0.119	0.15	n.s.
Matings*Males	1	0.073	0.073	0.008	0.88	n.s.
U*Matings*Males	1	0.020	0.020	0.342	0.24	n.s.
Error	151	12.436	12.436	0.082		
Total	159	20.229				

Table 3. GLM ANOVA for Brazil-strain female parent longevity, when seeds were absent, showing the effect of SM or MM (Matings), exposure to one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.005	0.019	0.019	0.19	n.s.
Matings	1	1.350	1.330	1.330	13.44	<0.001
Males	1	0.551	0.552	0.552	5.58	<0.05
Matings*Males	1	0.131	0.131	0.131	1.33	n.s.
Error	75	7.421	7.421	0.099		
Total	79	9.458				

Table 4. GLM ANOVA for Brazil-strain female parent longevity, when seeds were provided, showing the effect of SM or MM (Matings), exposure to one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.526	0.488	0.488	6.07	<0.05
Matings	1	0.555	0.556	0.556	6.90	<0.05
Males	1	0.004	0.004	0.004	0.05	n.s.
Matings*Males	1	0.221	0.221	0.221	2.74	n.s.
Error	75	6.036	6.036	0.080		
Total	79	7.343				

Table 5. GLM ANOVA for South-India-strain female parent longevity, when seeds were provided, showing the effect of SM or MM (Matings), exposure to one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.049	0.040	0.040	1.19	n.s.
Matings	1	0.001	0.001	0.001	0.03	n.s.
Males	1	0.003	0.003	0.003	0.10	n.s.
Matings*Males	1	0.009	0.009	0.009	0.27	n.s.
Error	75	2.515	2.515	0.034		
Total	79	2.577				

Table 6. GLM ANOVA for South-India-strain female parent longevity, when seeds were absent, showing the effect of SM or MM (Matings), exposure to one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.095	0.146	0.146	1.11	n.s.
Matings	1	0.236	0.237	0.237	1.79	n.s.
Males	1	0.042	0.044	0.044	0.34	n.s.
Matings*Males	1	0.088	0.088	0.088	0.66	n.s.
Error	75	9.890	9.890	0.132		
Total	79	10.358				

Table 7. GLM ANOVA for Brazil-strain (F_1) female longevity showing the effect of seed availability (U) and female parent treatments; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.085	0.304	0.304	8.16	<0.01
U	1	16.870	16.848	16.848	452.60	<0.001
Matings	1	0.366	0.364	0.364	9.78	<0.01
Males	1	0.001	0.001	0.001	0.04	n.s.
U*Matings	1	0.028	0.028	0.028	0.75	n.s.
U*Males	1	0.004	0.004	0.004	0.11	n.s.
Matings*Males	1	0.119	0.119	0.119	3.20	n.s.
U*Matings*Males	1	0.002	0.002	0.002	0.06	n.s.
Error	151	5.621	5.621	0.037		
Total	159	23.096				

Table 8. GLM ANOVA for Brazil-strain (F_1) female longevity, when seeds were provided, showing the effect of female parent treatments; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.192	0.163	0.163	7.73	<0.01
Matings	1	0.092	0.086	0.086	4.08	<0.05
Males	1	0.000	0.000	0.000	0.00	n.s.
Matings*Males	1	0.048	0.048	0.048	2.29	n.s.
Error	75	1.579	1.579	0.021		
Total	79	1.911				

Table 9. GLM ANOVA for Brazil-strain (F_1) female longevity, when seeds were absent, showing the effect of female parent treatments; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.167	0.147	0.147	2.73	n.s.
Matings	1	0.298	0.298	0.298	5.54	<0.05
Males	1	0.005	0.005	0.005	0.09	n.s.
Matings*Males	1	0.079	0.079	0.079	1.46	n.s.
Error	75	4.036	4.036	0.054		
Total	79	4.585				

Table 10. GLM ANOVA for South-India-strain (F_1) female longevity showing the effect of seed availability (U) and female parent treatments; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.273	0.350	0.350	8.26	<0.01
U	1	5.006	5.009	5.009	118.07	<0.001
Matings	1	0.020	0.019	0.019	0.44	n.s.
Males	1	0.094	0.093	0.093	2.18	n.s.
U*Matings	1	0.502	0.503	0.503	11.86	<0.05
U*Males	1	0.224	0.224	0.224	5.28	<0.05
Matings*Males	1	0.017	0.018	0.018	0.43	n.s.
U*Matings*Males	1	0.153	0.153	0.153	3.60	n.s.
Error	151	6.406	6.406	0.042		
Total	159	12.694				

Table 11. GLM ANOVA for South-India-strain (F_1) female longevity, when seeds were absent, showing the effect of female parent treatments; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.131	0.137	0.137	2.09	n.s.
Matings	1	0.364	0.362	0.362	5.52	<0.05
Males	1	0.302	0.302	0.302	4.61	n.s.
Matings*Males	1	0.132	0.138	0.138	2.01	n.s.
Error	75	4.918	4.918	0.066		
Total	79	5.847				

Table 12. GLM ANOVA for South-India-strain (F_1) female longevity, when seeds were provided, showing the effect of female parent treatments; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.179	0.229	0.229	11.65	<0.005
Matings	1	0.172	0.172	0.172	8.76	<0.005
Males	1	0.016	0.015	0.015	0.79	n.s.
Matings*Males	1	0.032	0.032	0.032	1.61	n.s.
Error	75	1.473	1.473	0.020		
Total	79	1.871				

Table 13. GLM ANOVA for SM Brazil-strain (F_1) male longevity showing the effect of female parent treatments; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_1 male emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.006	0.005	0.005	0.14	n.s.
Matings	1	0.229	0.230	0.230	6.03	<0.05
Males	1	0.001	0.000	0.000	0.01	n.s.
Matings*Males	1	0.011	0.011	0.011	0.28	n.s.
Error	32	1.221	1.221	0.038		
Total	36	1.467				

Table 14. GLM ANOVA for SM South-India-strain (F_1) male longevity showing the effect of female parent treatments; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_1 male emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.693	0.749	0.749	6.02	<0.05
Matings	1	0.154	0.157	0.157	1.26	n.s.
Males	1	0.109	0.100	0.100	0.80	n.s.
Matings*Males	1	0.095	0.095	0.095	0.76	n.s.
Error	115	14.311	14.311	0.124		
Total	119	15.361				

Table 15. GLM ANOVA for SM Brazil-strain (F_2) female longevity showing the effect of grandmothers treatment; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_2 female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.482	0.484	0.484	5.07	<0.05
Matings	1	0.081	0.081	0.081	0.85	n.s.
Males	1	0.013	0.013	0.013	0.14	n.s.
Matings*Males	1	0.013	0.013	0.013	0.14	n.s.
Error	75	7.165	7.165	0.096		
Total	79	7.754				

Table 16. GLM ANOVA for SM Brazil-strain (F_2) male longevity showing the effect of grandmothers treatment; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_2 male emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.557	0.537	0.537	6.10	<0.05
Matings	1	0.203	0.203	0.203	2.31	n.s.
Males	1	0.060	0.060	0.060	0.68	n.s.
Matings*Males	1	0.055	0.055	0.055	0.62	n.s.
Error	75	6.603	6.603	0.088		
Total	79	7.477				

Table 17. GLM ANOVA for SM South-India-strain (F_2) female longevity showing the effect of grandmothers treatment; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_2 female emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.078	0.116	0.116	2.02	n.s.
Matings	1	0.081	0.081	0.081	1.40	n.s.
Males	1	0.067	0.067	0.067	1.16	n.s.
Matings*Males	1	0.001	0.001	0.001	0.01	n.s.
Error	75	4.315	4.315	0.058		
Total	79	4.541				

Table 18. GLM ANOVA for SM South-India-strain (F_2) male longevity showing the effect of grandmothers treatment; SM or MM (Matings), exposure to one or seven males (Males) and their interactions. F_2 male emergence mass was added as a covariate (EM). The data were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.459	0.372	0.372	12.83	<0.05
Matings	1	0.076	0.073	0.073	2.52	n.s.
Males	1	0.020	0.018	0.018	0.64	n.s.
Matings*Males	1	0.015	0.015	0.015	0.52	n.s.
Error	75	2.172	2.172	0.029		
Total	79	2.742				

Appendix 2

Table 1. GLM ANOVA for the total number of eggs laid by SM or MM (Matings) Brazil-strain parent females who were exposed to one or seven males (Males) and their interactions. Emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	17438.5	17524.8	17524.8	22.73	<0.001
Matings	1	4379.4	4342.1	4342.1	5.63	<0.05
Males	1	41.4	30.6	30.6	0.04	n.s.
Matings*Males	1	72.2	72.2	72.2	0.09	n.s.
Error	68	52429.1	52429.1	771.0		
Total	72	74360.7				

Table 2. GLM ANOVA for the total number of eggs laid by SM or MM (Matings) South-India-strain parent females who were exposed to one or seven males (Males) and their interactions. Emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	20393.7	20291.1	20291.1	42.80	<0.001
Matings	1	576.9	574.3	574.3	1.21	n.s.
Males	1	833.1	814.1	814.1	1.72	n.s.
Matings*Males	1	101.5	101.5	101.5	0.21	n.s.
Error	75	35553.8	35553.8	474.1		
Total	79	57459.0				

Table 3. GLM ANOVA for the proportion of non-emerge, non-hatch, non-viable and viable eggs laid by SM or MM (Matings) Brazil-strain parent females exposed to either one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were angular transformed.

(a) non-emerge eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0614	0.0641	0.0641	4.97	$p<0.05$
Matings	1	0.0120	0.0184	0.0184	1.43	n.s.
Males	1	0.0005	0.0009	0.0009	0.07	n.s.
Matings*Males	1	0.0075	0.0075	0.0075	0.58	n.s.
Error	68	0.8763	0.8763	0.0129		
Total	72	0.9655				

(b) non-hatch eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0140	0.0159	0.0159	0.35	n.s.
Matings	1	0.0456	0.0533	0.0533	1.16	n.s.
Males	1	0.0129	0.0044	0.0044	0.10	n.s.
Matings*Males	1	0.2091	0.2091	0.2091	4.57	$p<0.05$
Error	68	3.1122	3.1122	0.0458		
Total	72	3.3938				

(c) non-viable eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0747	0.0806	0.0806	2.07	n.s.
Matings	1	0.0018	0.0038	0.0038	0.10	n.s.
Males	1	0.0088	0.0021	0.0021	0.05	n.s.
Matings*Males	1	0.2146	0.2146	0.2146	5.50	$p<0.05$
Error	68	2.6542	2.6542	0.0390		
Total	72	2.9541				

(d) viable eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0350	0.0391	0.0391	0.97	n.s.
Matings	1	0.0033	0.0057	0.0057	0.14	n.s.
Males	1	0.0068	0.0014	0.0014	0.03	n.s.
Matings*Males	1	0.1948	0.1948	0.1948	4.83	$p<0.05$
Error	68	2.7433	2.7433	0.0403		
Total	72	2.9831				

Table 4. GLM ANOVA for the proportion of non-emerge, non-hatch, non-viable and viable eggs laid by SM or MM (Matings) South-India-strain parent females exposed to either one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were angular transformed.

(a) non-emerge eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0153	0.0130	0.0130	1.42	n.s.
Matings	1	0.0032	0.0030	0.0030	0.33	n.s.
Males	1	0.0039	0.0040	0.0040	0.43	n.s.
Matings*Males	1	0.0047	0.0047	0.0047	0.51	n.s.
Error	75	0.6845	0.6845	0.0091		
Total	79	0.7116				

(b) non-hatch eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0025	0.0022	0.0022	0.07	n.s.
Matings	1	0.0207	0.0202	0.0202	0.68	n.s.
Males	1	0.0179	0.0178	0.0178	0.60	n.s.
Matings*Males	1	0.0063	0.0063	0.0063	0.21	n.s.
Error	75	2.2195	2.2195	0.0296		
Total	79	2.2669				

(c) non-viable eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0032	0.0036	0.0036	0.16	n.s.
Matings	1	0.0018	0.0017	0.0017	0.08	n.s.
Males	1	0.0110	0.0108	0.0108	0.47	n.s.
Matings*Males	1	0.0199	0.0199	0.0199	0.88	n.s.
Error	75	1.7029	1.7029	0.0227		
Total	79	1.7388				

(d) viable eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0025	0.0031	0.0031	0.15	n.s.
Matings	1	0.0050	0.0047	0.0047	0.24	n.s.
Males	1	0.0175	0.0172	0.0172	0.85	n.s.
Matings*Males	1	0.0191	0.0191	0.0191	0.95	n.s.
Error	75	1.5098	1.5098	0.0201		
Total	79	1.5538				

Table 5. GLM ANOVA for the total number of eggs laid per day by SM or MM (Matings) Brazil-strain parent females exposed to either one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were untransformed.

(a) Day 1

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	314.5	375.1	375.1	3.00	n.s.
Matings	1	68.3	71.0	71.0	3.37	n.s.
Males	1	421.7	421.5	421.5	0.57	n.s.
Matings*Males	1	0.1	0.1	0.1	0.00	n.s.
Error	64	7993.7	7993.7	124.9		
Total	68	8798.3				

(b) Day 2

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	196.25	197.06	197.06	3.42	n.s.
Matings	1	127.61	112.88	112.88	1.96	n.s.
Males	1	15.41	5.46	5.46	0.09	n.s.
Matings*Males	1	208.84	208.84	208.84	3.62	n.s.
Error	64	3687.89	3687.89	57.62		
Total	68	4236.00				

(c) Day 3

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	124.83	92.77	92.77	1.26	n.s.
Matings	1	61.58	73.71	73.71	1.00	n.s.
Males	1	3.54	11.60	11.60	0.16	n.s.
Matings*Males	1	267.14	267.14	267.14	3.63	n.s.
Error	64	4704.12	4704.12	73.50		
Total	68	5161.22				

(d) Day 4

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	678.34	579.81	579.81	9.98	p<0.005
Matings	1	690.68	680.56	680.56	11.71	n.s.
Males	1	27.91	35.12	35.12	0.60	p<0.005
Matings*Males	1	13.17	13.17	13.17	0.23	n.s.
Error	64	3719.74	3719.74	58.12		
Total	68	5129.83				

(e) Day 5+

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	2984.6	2564.0	2564.0	13.09	p<0.005
Matings	1	1234.3	1286.3	1286.3	6.57	n.s.
Males	1	57.9	39.8	39.8	0.20	p<0.05
Matings*Males	1	283.7	283.7	283.7	1.45	n.s.
Error	64	12533.8	12533.8	195.8		
Total	68	17094.2				

Table 6. GLM ANOVA for the total number of eggs laid per day by SM or MM (Matings) South-India-strain parent females exposed to either one or seven males (Males) and their interactions. Female emergence mass was added as a covariate (EM). The data were untransformed.

(a) Day 1

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	496.78	447.79	447.79	8.06	p<0.01
Matings	1	26.99	26.63	26.63	0.48	n.s.
Males	1	0.19	0.19	0.19	0.00	n.s.
Matings*Males	1	3.21	3.21	3.21	0.06	n.s.
Error	74	4112.91	4112.91	55.58		
Total	78	4640.08				

(b) Day 2

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	105.34	83.89	83.89	2.16	n.s.
Matings	1	0.24	0.27	0.27	0.01	n.s.
Males	1	45.82	45.91	45.91	1.18	n.s.
Matings*Males	1	19.61	19.61	19.61	0.50	n.s.
Error	74	2874.76	2874.76	38.85		
Total	78	3045.77				

(c) Day 3

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	445.98	484.38	484.38	10.77	p<0.005
Matings	1	127.88	129.30	129.30	2.87	n.s.
Males	1	0.69	0.70	0.70	0.02	n.s.
Matings*Males	1	12.31	12.31	12.31	0.27	n.s.
Error	74	3328.47	3328.47	44.98		
Total	78	3915.34				

(d) Day 4

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	1005.85	1132.36	1132.36	19.78	p<0.001
Matings	1	1.26	0.96	0.96	0.02	n.s.
Males	1	261.42	261.41	261.41	4.57	p<0.05
Matings*Males	1	0.03	0.03	0.03	0.00	n.s.
Error	74	4236.20	4236.20	57.25		
Total	78	5504.76				

(e) Day 5+

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	3339.9	3262.0	3262.0	16.61	p<0.005
Matings	1	571.9	567.9	567.9	2.89	n.s.
Males	1	499.7	500.1	500.1	2.55	p<0.05
Matings*Males	1	30.6	30.6	30.6	0.16	n.s.
Error	74	14535.3	14535.3	196.4		
Total	78	18977.4				

Table 7. GLM ANOVA for the effect of strain and day of egg laying on the mass of eggs produced by Brazil- and South-India-strain females. Female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.00063	0.00004	0.00004	1.34	n.s.
Strain	1	0.00013	0.00017	0.00017	5.85	p<0.05
Day	1	0.00053	0.00053	0.00053	18.01	p<0.001
Strain*Day	1	0.00000	0.00000	0.00000	0.00	n.s.
Error	321	0.00938	0.00938	0.00003		
Total	325	0.01066				

Table 8. GLM ANOVA for the mass of eggs laid on day one by SM or MM (Matings) Brazil-strain females who were exposed to either one or seven males (Males). Female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.00025	0.00013	0.00013	5.22	p<0.05
Matings	1	0.00004	0.00006	0.00006	2.24	n.s.
Males	1	0.00005	0.00002	0.00002	0.87	n.s.
Matings*Males	1	0.00014	0.00014	0.00014	5.55	p<0.05
Error	60	0.00150	0.00150	0.00002		
Total	64	0.00197				

Table 9. GLM ANOVA for the mass of eggs laid on day one by SM or MM (Matings) South-India-strain females who were exposed to either one or seven males (Males). Female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	4.4×10^{-5}	4.8×10^{-5}	4.8×10^{-5}	8.77	p<0.005
Matings	1	3.5×10^{-5}	3.0×10^{-5}	3.0×10^{-5}	5.52	p<0.05
Males	1	5.9×10^{-6}	6.4×10^{-6}	6.4×10^{-6}	1.16	n.s.
Matings*Males	1	5.1×10^{-5}	5.1×10^{-5}	5.1×10^{-5}	9.37	p<0.005
Error	69	3.8×10^{-4}	3.8×10^{-4}	5.5×10^{-6}		
Total	73	5.2×10^{-4}				

Table 10. GLM ANOVA for the mass of eggs laid on days three and four by SM or MM (Matings) Brazil-strain females who were exposed to either one or seven males (Males). Female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.00007	0.00003	0.00003	0.42	n.s.
Matings	1	0.00000	0.00000	0.00000	0.05	n.s.
Males	1	0.00008	0.00009	0.00009	1.27	n.s.
Matings*Males	1	0.00002	0.00002	0.00002	0.26	n.s.
Error	90	0.00609	0.00609	0.00007		
Total	94	0.00626				

Table 11. GLM ANOVA for the mass of eggs laid on days three and four by SM or MM (Matings) South-India-strain females who were exposed to either one or seven males (Males). Female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	2.1×10^{-5}	3.4×10^{-5}	3.4×10^{-5}	6.59	$p<0.05$
Matings	1	1.1×10^{-4}	1.2×10^{-4}	1.2×10^{-4}	22.88	$p<0.001$
Males	1	1.7×10^{-6}	5.6×10^{-6}	5.6×10^{-6}	1.07	n.s.
Matings*Males	1	9.4×10^{-5}	9.4×10^{-5}	9.4×10^{-5}	18.07	$p<0.001$
Error	87	4.5×10^{-4}	4.5×10^{-4}	5.2×10^{-6}		
Total	91	6.8×10^{-4}				

Table 12. GLM ANOVA for the total number of eggs laid by SM Brazil-strain F_1 females whose parent females SM or MM (Matings), and were exposed to either one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	957.0	1248.3	1248.3	7.27	$p<0.01$
Matings	1	381.3	405.4	405.4	2.36	n.s.
Males	1	85.5	91.1	91.1	0.53	n.s.
Matings*Males	1	26.6	26.6	26.6	0.15	n.s.
Error	75	12879.2	12879.2	171.7		
Total	79	14329.5				

Table 13. GLM ANOVA for the total number of eggs laid by SM South-India-strain F_1 females whose parent females SM or MM (Matings), and were exposed to either one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	2605.9	2828.6	2828.6	24.85	<0.001
Matings	1	276.0	277.8	277.8	2.44	n.s.
Males	1	67.4	67.3	67.3	0.59	n.s.
Matings*Males	1	2.0	2.0	2.0	0.02	n.s.
Error	75	8536.3	8536.3	113.8		
Total	79	11487.5				

Table 14. GLM ANOVA for the proportion of non-emerge, non-hatch, non-viable and viable eggs laid by SM Brazil-strain F_1 females whose parent female either SM or MM (Matings) and was exposed to either one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were angular transformed.

(a) non-emerge eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0193	0.0096	0.0096	0.84	n.s.
Matings	1	0.0005	0.0007	0.0007	0.06	n.s.
Males	1	0.0004	0.0001	0.0001	0.01	n.s.
Matings*Males	1	0.0187	0.0187	0.0187	1.63	n.s.
Error	75	0.8598	0.8598	0.0115		
Total	79	0.8986				

(b) non-hatch eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0017	0.0049	0.0049	0.89	n.s.
Matings	1	0.0109	0.0091	0.0091	0.65	n.s.
Males	1	0.0289	0.0294	0.0294	5.32	p<0.05
Matings*Males	1	0.0008	0.0008	0.0008	0.14	n.s.
Error	75	0.4140	0.4140	0.0055		
Total	79	0.4563				

(c) non-viable eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0171	0.0149	0.0149	1.19	n.s.
Matings	1	0.0069	0.0065	0.0065	0.52	n.s.
Males	1	0.0184	0.0170	0.0170	1.36	n.s.
Matings*Males	1	0.0071	0.0071	0.0071	0.56	n.s.
Error	75	0.9387	0.9387	0.0125		
Total	79	0.9883				

(d) viable eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0099	0.0087	0.0087	1.30	n.s.
Matings	1	0.0034	0.0032	0.0032	0.48	n.s.
Males	1	0.0092	0.0085	0.0085	1.26	n.s.
Matings*Males	1	0.0033	0.0033	0.0033	0.49	n.s.
Error	75	0.5027	0.5027	0.0067		
Total	79	0.5286				

Table 15. GLM ANOVA for the proportion of non-emerge, non-hatch, non-viable and viable eggs laid by SM South-India-strain F_1 females whose parent female either SM or MM (Matings) and was exposed to either one or seven males (Males) and their interactions. F_1 female emergence mass was added as a covariate (EM). The data were angular transformed.

(a) non-emerge eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0204	0.0163	0.0163	2.58	n.s.
Matings	1	0.0017	0.0018	0.0018	0.29	n.s.
Males	1	0.0160	0.0160	0.0160	2.53	n.s.
Matings*Males	1	0.0012	0.0012	0.0012	0.19	n.s.
Error	75	0.4737	0.4737	0.0063		
Total	79	0.5131				

(b) non-hatch eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0030	0.0026	0.0026	0.48	n.s.
Matings	1	0.0002	0.0002	0.0002	0.04	n.s.
Males	1	0.0175	0.0175	0.0175	3.19	n.s.
Matings*Males	1	0.0032	0.0032	0.0032	0.59	n.s.
Error	75	0.4120	0.4120	0.0055		
Total	79	0.4360				

(c) non-viable eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0271	0.0238	0.0238	3.76	n.s.
Matings	1	0.0001	0.0000	0.0000	0.01	n.s.
Males	1	0.0330	0.0330	0.0330	5.22	p<0.05
Matings*Males	1	0.0001	0.0001	0.0001	0.01	n.s.
Error	75	0.4735	0.4735	0.0063		
Total	79	0.5337				

(d) viable eggs

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.0136	0.0120	0.0120	3.98	p≅0.05
Matings	1	0.0000	0.0000	0.0000	0.01	n.s.
Males	1	0.0153	0.0153	0.0153	5.11	p<0.05
Matings*Males	1	0.0001	0.0001	0.0001	0.03	n.s.
Error	75	0.2253	0.2253	0.0030		
Total	79	0.2544				

Table 16. GLM ANOVA looking at the effect of female parents treatment and their interactions on the emergence mass of SM Brazil-strain F_1 females hatching from eggs laid on day one. Female parents were either SM or MM (Matings) and were exposed to either one or seven males (Males). The parent female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	4.2630	4.7528	4.7528	20.78	p<0.001
Matings	1	0.4802	0.4924	0.4924	2.15	n.s.
Males	1	0.0000	0.0000	0.0000	0.00	n.s.
Matings*Males	1	0.1356	0.1356	0.1356	0.59	n.s.
Error	155	35.4568	35.4568	0.2288		
Total	159	40.3356				

Table 17. GLM ANOVA looking at the effect of female parents treatment and their interactions on the emergence mass of SM Brazil-strain F_1 males hatching from eggs laid on day one. Female parents were either SM or MM (Matings) and were exposed to either one or seven males (Males). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
Matings	1	0.0777	0.0777	0.0777	0.51	n.s.
Males	1	0.0014	0.0014	0.0014	0.01	n.s.
Matings*Males	1	0.0542	0.0542	0.0542	0.36	n.s.
Error	156	23.7184	23.7184	0.1520		
Total	159	23.8518				

Table 18. GLM ANOVA looking at the effect of female parents treatment and their interactions on the emergence mass of SM Brazil-strain F_1 females hatching from eggs laid on days three and four. Female parents were either SM or MM (Matings) and were exposed to either one or seven males (Males). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
Matings	1	0.0940	0.0940	0.0940	0.30	n.s.
Males	1	0.3078	0.3078	0.3078	0.97	n.s.
Matings*Males	1	0.6234	0.6234	0.6234	1.96	n.s.
Error	212	67.5241	67.5241	0.3185		
Total	215	68.5493				

Table 19. GLM ANOVA looking at the effect of female parents treatment and their interactions on the emergence mass of SM Brazil-strain F_1 males hatching from eggs laid on days three and four. Female parents were either SM or MM (Matings) and were exposed to either one or seven males (Males). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
Matings	1	0.1223	0.1113	0.1113	0.96	n.s.
Males	1	0.9448	0.9549	0.9549	8.25	p<0.01
Matings*Males	1	0.2168	0.2168	0.2168	1.87	n.s.
Error	178	20.6119	20.6119	0.1158		
Total	181	21.8959				

Table 20. GLM ANOVA looking at the effect of the grandmothers treatment and their interactions on the emergence mass of SM Brazil-strain F_2 female offspring hatching from eggs laid by SM F_1 females on day one. Grandmothers were either SM or MM (Matings) and were exposed to either one or seven males (Males). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
Matings	1	1.1897	1.1897	1.1897	5.78	p<0.05
Males	1	0.0050	0.0050	0.0050	0.02	n.s.
Matings*Males	1	0.0640	0.0640	0.0640	0.31	n.s.
Error	76	15.6449	15.6449	0.2059		
Total	79	16.9036				

Table 21. GLM ANOVA looking at the effect of the grandmothers treatment and their interactions on the emergence mass of SM Brazil-strain F_2 male offspring hatching from eggs laid by SM F_1 females on day one. Grandmothers were either SM or MM (Matings) and were exposed to either one or seven males (Males). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
Matings	1	0.3372	0.3372	0.3372	2.82	n.s.
Males	1	0.9082	0.9082	0.9082	7.60	p<0.01
Matings*Males	1	0.1572	0.1572	0.1572	1.32	n.s.
Error	76	9.0809	9.0809	0.1195		
Total	79	10.4835				

Table 22. GLM ANOVA looking at the effect of the female parents treatment and their interactions on the emergence mass of SM South-India-strain F_1 females hatching from eggs laid on day one. Female parents were either SM or MM (Matings) and were exposed to either one or seven males (Males). The parent female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	1.4000	1.3187	1.3187	7.34	p<0.01
Matings	1	1.6768	1.6518	1.6518	9.19	p<0.005
Males	1	0.0028	0.0056	0.0056	0.03	n.s.
Matings*Males	1	0.5727	0.5727	0.5727	3.19	n.s.
Error	99	17.7900	17.7900	0.1797		
Total	103	21.4423				

Table 23. GLM ANOVA looking at the effect of the female parents treatment and their interactions on the emergence mass of SM South-India-strain F_1 males hatching from eggs laid on day one. Female parents were either SM or MM (Matings) and were exposed to either one or seven males (Males). The parent female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.3360	0.2244	0.2244	1.76	n.s.
Matings	1	0.0076	0.0079	0.0079	0.06	n.s.
Males	1	0.3616	0.3587	0.3587	2.82	n.s.
Matings*Males	1	0.1961	0.1961	0.1961	1.54	n.s.
Error	119	15.1336	15.1336	0.1272		
Total	123	16.0350				

Table 24. GLM ANOVA looking at the effect of the female parents treatment and their interactions on the emergence mass of SM South-India-strain F_1 females hatching from eggs laid on days three and four. Female parents were either SM or MM (Matings) and were exposed to either one or seven males (Males). The parent female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	0.1540	0.1165	0.1165	0.46	n.s.
Matings	1	0.3732	0.3738	0.3738	1.49	n.s.
Males	1	0.0235	0.0219	0.0219	0.09	n.s.
Matings*Males	1	0.0334	0.0334	0.0334	0.13	n.s.
Error	75	18.7957	18.7957	0.2506		
Total	79	19.3798				

Table 25. GLM ANOVA looking at the effect of the female parents treatment and their interactions on the emergence mass of SM South-India-strain F_1 males hatching from eggs laid on days three and four. Female parents were either SM or MM (Matings) and were exposed to either one or seven males (Males). The parent female emergence mass was added as a covariate (EM). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	1.0669	0.8111	0.8111	4.06	p<0.05
Matings	1	0.0945	0.0966	0.0966	0.48	n.s.
Males	1	1.9210	1.7641	1.7641	8.83	p<0.005
Matings*Males	1	0.9152	0.9152	0.9152	4.58	p<0.05
Error	115	22.9876	22.9876	0.1999		
Total	119	26.9853				

Table 26. GLM ANOVA looking at the effect of the grandmothers treatment and their interactions on the emergence mass of SM South-India-strain F_2 female offspring hatching from eggs laid by SM F_1 females on day one. Grandmothers were either SM or MM (Matings) and were exposed to either one or seven males (Males). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	3.3635	3.5845	3.5845	11.48	p<0.005
Matings	1	0.4151	0.4109	0.4109	1.32	n.s.
Males	1	0.0557	0.0558	0.0558	0.18	n.s.
Matings*Males	1	0.0229	0.0229	0.0229	0.07	n.s.
Error	75	23.4091	23.4091	0.3121		
Total	79	27.2662				

Table 27. GLM ANOVA looking at the effect of the grandmothers treatment and their interactions on the emergence mass of SM South-India-strain F_2 male offspring hatching from eggs laid by SM F_1 females on day one. Grandmothers were either SM or MM (Matings) and were exposed to either one or seven males (Males). The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	Probability
EM	1	1.7576	1.8105	1.8105	11.61	p<0.005
Matings	1	0.0469	0.0471	0.0471	0.30	n.s.
Males	1	0.0216	0.0216	0.0216	0.14	n.s.
Matings*Males	1	0.0099	0.0099	0.0099	0.06	n.s.
Error	75	11.6909	11.6909	0.1559		
Total	79	13.5269				

Appendix 3

Table 1. GLM ANOVA showing the effect of the strain of the female and male and their interaction on the number of attempts made by a male before a successful copulation occurred. The data were untransformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Female	1	0.1125	0.1125	0.1125	0.17	n.s.
Male	1	0.3125	0.3125	0.3125	0.46	n.s.
Female*Male	1	5.5125	5.5125	5.5125	8.14	p<0.01
Error	76	51.4500	51.4500	0.6770		
Total	79	57.3875				

Table 2. GLM ANOVA analysing the effect of the strain of the male (Male), the strain of the female (Female) and their interactions on the time to compete the three phases of copulation and total *in copula* time observed for same- and mixed–strain pairs. The data were log transformed.

(a)   Phase 1: first contact to aedeagus insertion

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Female	1	0.0291	0.0291	0.0291	0.66	n.s.
Male	1	0.0075	0.0075	0.0075	0.17	n.s.
Female*Male	1	0.2001	0.2001	0.2001	4.56	p<0.05
Error	76	3.3340	3.3340	0.0439		
Total	79	3.5706				

(b)   Phase 2: aedeagus insertion to female kick

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Female	1	0.0357	0.0357	0.0357	1.41	n.s.
Male	1	0.1338	0.1338	0.1338	5.28	p<0.05
Female*Male	1	0.0191	0.0191	0.0191	0.75	n.s.
Error	76	1.9260	1.9260	0.0253		
Total	79	2.1145				

(c)   Phase 3: female kick to aedeagus removal

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Female	1	2.3594	2.3594	2.3594	16.24	p<0.001
Male	1	0.5213	0.5213	0.5213	3.59	n.s.
Female*Male	1	0.1460	0.1460	0.1460	1.00	n.s.
Error	76	11.0395	11.0395	0.1453		
Total	79					

(d)   Total time spent *in copula*

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Female	1	0.0647	0.0647	0.0647	6.88	p<0.05
Male	1	0.2447	0.2447	0.2447	26.00	p<0.001
Female*Male	1	0.0151	0.0151	0.0151	1.60	n.s.
Error	76	0.7152	0.7152	0.0094		
Total	79	1.0397				

Table 3. GLM ANOVA analysing the effect of the strain of the male (Male), the strain of the female (Female) and their interactions on the proportion of matings by Brazil- and South-India-strain females exposed to same strain males, to different strain males or to virgin, same strain males over seven days. The data were angular transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Male	1	0.0716	0.0000	0.0000	0.00	n.s.
Female	1	0.5799	0.5799	0.5799	19.66	p<0.001
Male*Female	1	0.0291	0.0291	0.0291	0.98	n.s.
Error	38	1.1212	1.1212	0.0295		
Total	41	1.8018				

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