Polyandry and the evolution of reproductive divergence in insects

Tina Nilsson

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Department of Ecology and Environmental Science
Umeå University
SE-901 87 Umeå
Sweden

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Title: Polyandry and the evolution of reproductive divergence in insects

Abstract
Multiple mating by females is common in nature. Yet, the evolution and maintenance of polyandry remains a bit of an evolutionary puzzle. It was my aim in this thesis to reach a greater understanding of this phenomenon as well as to investigate the consequences of polyandry on the evolution of reproductive divergence in insects. In an extensive meta analysis addressing the direct effects of multiple mating on female fitness in insects, I found that insects gain from multiple matings in terms of increased lifetime offspring production. In species without nuptial feeding, increased mating rate leads to decreased female lifespan and my results strongly support the existence of an intermediate optimal female mating rate. However, results from an experimental study where I examined the relationship between female fitness and mating rate in the bean weevil (Callosobruchus maculatus) showed that female fitness was maximized at two alternative mating rates, indicating that some species may exhibit a more complex relationship between the costs and benefits of mating. In the meta analysis on species with nuptial feeding, I found only positive effects of increased mating rate and the puzzle is rather what constrains the actual mating rates of females in these groups.

Sexual selection is a very potent driver of rapid evolutionary change in reproductive characters. Most research has focussed on precopulatory sexual selection, but in promiscuous species sexual selection continues after copulation and variance in male fertilization success gives rise to postcopulatory sexual selection. In this thesis I found that three allopatric populations of the red flour beetle (Tribolium castaneum) have diverged in traits related to reproduction. Male genotype affected all aspects of female reproduction, but more interestingly, males and females interacted in their effect on offspring production and reproductive rate, showing that the divergence was due to the evolution of both male and female reproductive traits.

When studying postcopulatory sexual selection, sperm competition has been put forward as the main source of variance in fertilization success. The results from a set of double-mating experiments, using the same populations of flour beetles, provided strong evidence that cryptic female choice is also important in generating variance in male fertilization success. I found not only main effects of female genotype on male fertilization success but also male-female interactions which provide more unambiguous evidence for cryptic female choice. Finally, I attempted to uncover which male signals-female receptors are involved in the reproductive divergence observed in the Tribolium populations. In a double-mating experiment I manipulated female perception of two male reproductive signals, copulatory courtship and cuticular hydrocarbons, and the results indicate that, within populations, both signals are sexually selected. However, only male cuticular hydrocarbons seem to be involved in the reproductive divergence between the populations.

In conclusion, multiple mating by female insects can be understood solely in terms of direct fitness benefits resulting from increased offspring production. I have shown that postcopulatory sexual selection can lead to rapid divergence in reproductive traits related to mating and that cryptic female choice plays an important role in this divergence.

Key Words: Bruchidae, cryptic female choice, multiple mating, sexual conflict, sexually antagonistic coevolution, sexual selection, speciation, sperm competition, Tenebrionidae

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LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


II. Arnoqvist, G., Nilsson, T. & Katvala, M. Mating rate and fitness in female bean weevils. *Submitted manuscript*


V. Nilsson, T. & Andres, J.A. Postcopulatory sexual selection: the role of copulatory courtship and cuticular hydrocarbons in the reproductive divergence between strains of the red flour beetle (*Tribolium castaneum*). *Manuscript*

Papers I, III and IV are published with the kind permission of the publishers.
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7. REFERENCES
1. INTRODUCTION

The need to reproduce is common to all species. For most animals, apart from asexuals, this involves finding a mate, fusion of the gametes and the production of offspring. The traditional view of reproduction was governed by harmony and cooperation between the sexes, i.e. that males and females worked together in producing offspring (see Eberhard 1996). This view was partly due to an overestimation of the number of monogamous species in nature. It has become increasingly clear though, that most species have females and/or males that mate with several mates within a reproductive cycle (Gowaty 1994; Birkhead & Møller 1998) and that the evolutionary interest between males and females rarely coincide, resulting in intersexual conflicts (Trivers 1972; Parker 1979). Because of this earlier misconception, it has been necessary to re-evaluate how we interpret the interactions between the sexes with respect to mating.

1.1 Polyandry and optimal mating rates

Multiple mating by females (polyandry) is a taxonomically widespread phenomenon but despite this it still remains a bit of an evolutionary puzzle (Keller & Reeve 1995; Yasui 1998; Jennions & Petrie 2000; Hosken & Stockley 2003). Understanding the evolution and maintenance of female mating rates lies at the heart of reproductive biology, as female mating rates determine the pattern of sexual selection and therefore have implications on a species’ behaviour, morphology and life history.

The primary function of mating is the transfer of sperm to females. Since each mating offers an opportunity to father offspring, males can generally increase their reproductive success by mating with many females. A female’s reproductive success, however, is limited by the number of viable eggs produced and rarely by the number of matings (but see Wedell et al. 2002). This basic asymmetry between the sexes suggests that male fitness increase monotonically with increased mating rate, while one or a few matings are sufficient for females to maximize their reproductive success (Bateman 1948). The fact that many female insects engage in multiple mating goes contrary to the theoretical predictions and the question remains: why do females mate multiply? In species where males provide the female with apparent benefits such as nutritional donations, nest sites or protection, polyandry is understandable. However, in species where males provide the female with nothing more than their ejaculate, and where females nevertheless engage in multiple matings, implies that one or a few matings are in fact not sufficient to ensure maximal fitness. In order to reach a greater understanding of what governs polyandry we need to examine the costs as well as the benefits of mating in much greater detail.

The costs associated with mating are many (Daly 1978). Not only is it time and energy consuming (Thornhill & Alcock 1983), mating can also increase predation risk because pairs in copula are more conspicuous to predators, have reduced mobility and/or reduced vigilance (Arnqvist 1989). Mating also increases the risk of disease transmission (Hurst et al. 1995) and in Drosophila melanogaster it has a negative effect on female lifespan due to toxic substances in the male ejaculate (Chapman et al. 1995). The strength of these
Table 1. The benefits a female might gain from multiple mating are usually divided into direct (those that a female obtains for herself) and indirect (benefits that a female obtain for her offspring).

<table>
<thead>
<tr>
<th>Direct fitness benefits</th>
<th>Indirect fitness benefits</th>
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<tr>
<td>Fertility insurance</td>
<td>Genetic diversity</td>
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<tr>
<td>Courship feeding</td>
<td>Genetic complementarity</td>
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<td>Ejaculatory nutrients</td>
<td>Genetically more viable offspring</td>
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<td>Increased parental care</td>
<td>Genetically more attractive offspring</td>
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<td>Protection from harassment</td>
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costs varies between species but it is unlikely that polyandry would persist if there were not any benefits involved. The benefits to female multiple mating have generally been divided into direct, material benefits and indirect, genetic benefits (Table 1), but they are not mutually exclusive and thus may occur at the same time.

Direct benefits are those that a female obtains for herself and have positive effects on her fitness through increased offspring production and/or offspring viability. Such benefits include for example sperm replenishment (Thornhill & Alcock 1983) and nutritional donations (Vahed 1998). The indirect benefits a female gains from mating with several males, on the other hand, are benefits obtained for her offspring. That is, a female can achieve higher fitness either through the production of sons with a higher fertilization success, due to increased competitiveness/attractiveness (“sexy sons”), or through the production of more genetically viable offspring (“good genes”) (Jennions & Petrie 2000) (see section 1.3.1 for further details). Taking into account the different costs and benefits of mating, there should exist an optimal mating rate where female fitness is maximized (see Paper I). This optimal female mating rate is likely to differ between species but perhaps also within species, e.g. between allopatric populations. Due to the inherent difference between males and females (e.g. in gamete investment) the costs and benefits of mating, and hence the optimal mating rate, are likely to differ for the sexes. This could result in sexual conflict over mating rates and it is still not clear if and in what sense female mating rates are optimal (Paper I), or whether females are constrained (Wiklund et al. 2001) or manipulated by males (Holland and Rice 1998) into mating sub-optimally.

1.2 Sexual selection and speciation

The process by which new species arise, speciation, is a long studied phenomenon mainly because of its obviously central role in evolutionary biology. Speciation results from the evolution of reproductive isolation, mediated by divergence in traits related to reproduction. Divergence in sexual traits can occur as a result of random processes such as genetic drift but also as a side effect of adaptations to new environmental conditions (natural selection). Lately, sexual selection, i.e. selection that arises from differences in
reproductive success caused by competition over mates (Andersson 1994), has gained popularity as a key mechanism in speciation because it can generate rapid evolutionary changes in reproductive characters (Lande 1981; West-Eberhard 1983; Anderson 1994; Coyne & Orr 1998). Most research on sexual selection has focused on events leading up to mating, i.e. the mating success of individuals (precopulatory sexual selection). In precopulatory sexual selection a male can increase his reproductive success either by out-competing other males directly (male-male contest) or by making females prefer him over other mates (female choice) with e.g. extreme ornaments or vocalization. In monandrous species, this focus on precopulatory events is valid since mating success constitutes the main source of variance in reproductive success. In polyandrous species, however, the competition between males continues after copulation since sperm from multiple males will be present at the same time inside the female reproductive tract. That is, males compete for access to the female’s eggs which creates an additional source of variance in reproductive success, namely fertilization success. That is, in polyandrous species a male’s net reproductive success is determined both by his success at acquiring mates and copulations (mating success), and by the number of eggs fertilized at each mating (fertilization success).

One prediction of postcopulatory sexual selection is that traits related to reproduction should show a higher rate of evolution compared to non-reproductive traits. It has been shown in several different taxa that proteins mediating sexual reproduction have a higher rate of evolution compared to proteins from non-reproductive tissues (Civetta & Singh 1995; Vacquier 1998; Chapman 2001; Swanson & Vacquier 2002). Sexual conflicts, sperm competition and/or cryptic female choice (see section 1.3) have been suggested as the selective forces driving this rapid evolution of reproductive proteins (Rice 1996; Swanson & Vacquier 2002). A comparative study on the divergent evolution of male genitalia found that in polyandrous species, genital evolution was more than twice as divergent compared to monandrous species where the opportunity for postcopulatory sexual selection is weak or absent (Arnqvist 1998). Another comparative study showed that polyandrous groups of insects exhibited speciation rates four times as high as related monandrous groups, and again postcopulatory sexual selection driven by sexual conflicts were held responsible for this (Arnqvist et al. 2000). In summary, evidence from different taxa suggests that postcopulatory sexual selection is responsible for the rapid evolution of both physiological and morphological traits related to reproduction. This rapid evolution of reproductive traits is expected to give rise to reproductive incompatibilities when allopatric populations come into secondary contact (Parker & Partridge 1998; Swanson & Vacquier 2002).

1.3 Processes behind postcopulatory sexual selection

In order to understand postcopulatory sexual selection and its influence on reproductive divergence we need to understand the processes that generate variance in male fertilization success, i.e. what determines which of the males that the female mates with will sire her offspring. The processes through which male and female sexual traits
influence paternity can broadly be categorized as “sperm competition” and “cryptic female choice”. The outcome of these processes is likely to be influenced both by the resolution of conflicts between the sexes and the partial control each sex has over certain reproductive processes (Eberhard 1998; Simmons & Siva-Jothy 1998). It is important to recognize that the interactions between the sexes that determine the paternity of the offspring start when the pair is in copula, and this is therefore considered as a part of postcopulatory sexual selection.

Parker (1970) defined sperm competition as the “competition within a single female between the sperm from two or more males for the fertilization of the ova”. For a long time sperm competition was considered to constitute the main source of variance in male fertilization success and females were seen as passive arenas for male combats. This view was partly due to a general male-centred tradition in biology (see Eberhard 1996) and the observation that there is greater selection on males to ensure paternity than there is on females to manipulate paternity (Parker 1984). A further complication has been the methodological difficulty involved in following the fate of sperm inside the female reproductive tract. There is, however, increasing evidence that females are in fact not passive recipients of sperm but can influence the paternity of their offspring (e.g. Price 1997; Wilson et al. 1997; Clark et al. 1999; Pizzari & Birkhead 2000; Edvardsson & Arnqvist 2000; Tallamy et al. 2002) and may thus contribute significantly to variance in male fertilization success. This process, cryptic female choice (Thornhill 1983; Eberhard 1996), is broadly defined as any postcopulatory process that biases the paternity of some males over others when females mate with more than one male. This is equivalent to classic female choice in precopulatory sexual selection. There are numerous stages from the initiation of copulation to the fertilization of the ova where females have the potential to affect the fate of sperm (Fig. 1). Experiments have shown female control over some of these stages, e.g. insemination success (Pizzari & Birkhead 2000) and the transport of sperm to storage organs (Bishop 1996; Bloch Qazi et al. 1998; Hellriegel & Bernasconi 2000). Cryptic female choice could also cause variance in fertilization success if females invest differently in offspring production depending on which male she mates with (differential allocation: Burley 1986, 1988; Sheldon 2000).

Fig. 1. A schematic model of the different stages from copulation to post-fertilization events. (Adapted from Birkhead & Møller 1998)
As mentioned above, sperm competition is generally accepted as an important component of postcopulatory sexual selection (Birkhead & Møller 1998), but at present this can hardly be said about cryptic female choice (Telford & Jennions 1998; Birkhead 2000; Simmons 2001). Part of this problem arises from a lack of consistency in the definitions of both sperm competition and cryptic female choice. For example, sperm competition, in its broad sense, is defined as compromising both the differential competitiveness of ejaculates and the differential utilization of sperm by females (Simmons & Siva-Jothy 1998). The second part of this definition in fact falls under the more inclusive definition of cryptic female choice (see above). Sperm competition sensu stricto, however, involves only the direct interactions among sperm from different males (e.g. dilution or raffle competition) and direct interactions between males and sperm from other males (e.g. physical replacement) (Eberhard 1998). Regardless of the definitions of sperm competition and cryptic female choice, the processes are highly associated, not least because they all take place inside the female reproductive tract and are therefore very difficult to differentiate between. In fact, Eberhard (1998) argues that sperm competition sensu stricto is unlikely to occur and that most postcopulatory male competition probably involves cryptic female choice.

Observing that populations have diverged in reproductive traits, and identifying the responsible processes (cryptic female choice/sperm competition), gives only a partial understanding of how speciation may occur. To obtain a more complete picture it is also necessary to identify the forces driving the divergence. One way of visualizing this is to think of the interaction between males and females at mating as being mediated by a series of signals and receptors. Reproductive signals produced by one sex, usually males, are aimed at and received by female receptors and trigger a reproductive response. These male signals could include a wide range of traits, for example copulatory courtship, pheromones and substances in the male ejaculates. The associated reproductive response in females, behavioural or physiological, will affect the reproductive success of the sender. If male signals and female receptors coevolve and diverge, they may contribute to the evolution of reproductive isolation. There are many processes that can lead to coevolution in the signal-receptor system, such as natural selection and random genetic processes including genetic drift and/or founder effects. These processes could be important in speciation, but sexual selection and more specifically intersexual selection has been put forward as an especially potent force (Kirkpatrick & Ravigné 2002). The models of coevolution concerning intersexual selection can be broadly classified into those where divergence is driven by indirect selection on female preferences, and those where it is driven by direct selection on female preferences.

1.3.1 Divergence driven by indirect selection

Females may gain indirect benefits by having preferences for certain male traits, and this can lead to coevolution of the male signal – female receptor system (Kirkpatrick & Ryan 1991; Jennions and Petrie 2000). The preference becomes associated with genes that confer higher competitiveness/attractiveness in the sons (“sexy sons” – Fisherian process) and/or more viable offspring (“good genes” process). In the Fisherian runaway model, females have an initial preference for a male signal so the female preference receptors and
the male signals will become genetically associated in their offspring due to assortative mating (Lande 1981; Andersson 1994). This leads to a positive feedback loop where the male signal and female receptor increase in the population in a runaway coevolutionary process, which can cause substantial evolution of the signals and receptors in a small number of generations (Kirkpatrick & Ryan 1991). In the other process where female preference is under indirect selection, i.e. the good genes model (Kirkpatrick & Ryan 1991; Andersson 1994), females benefit by responding to male signals that confer a viability advantage to her offspring. The male signal has to be an indicator of male quality, and females with receptors that can discriminate high quality males will increase in the population. As in the Fisherian runaway model, the female preference receptor will evolve because it becomes genetically associated with a male signal that is under selection.

1.3.2 Divergence driven by direct selection

Evolution of the signal-receptor system can also occur if a female’s response to certain male signals affects her fitness directly, i.e. the preference itself is under natural selection (Kirkpatrick & Ryan 1991; Iwasa & Pomiankowski 1999). One form of direct selection arises if females with the appropriate receptors gain direct benefits such as resources, better territories and/or greater male parental care (Price et al. 1993). The model of direct benefits is equivalent to the good genes model in that male signals have to be indicators of quality. Another form of direct selection arises as a result of sexual conflicts between the sexes. These conflicts arise whenever the evolutionary interest between males and females differ (Parker 1979) and are very common in nature. In fact, very rarely, at least among promiscuous species, does the male and female evolutionary interest coincide (Trivers 1972; Parker & Partridge 1998; Rice 2000). Sexual conflicts can lead to sexually antagonistic coevolution. A male signal that increases his relative fitness is selected for, but if that signal reduces female fitness then females are under selection to reduce that cost. One way of reducing the cost is by decreasing the receptors’ sensitivity to male signals. Males are then under selection to increase the signal or, alternatively, produce a different signal to maximize their relative fitness. This will lead to a perpetual antagonistic coevolution between the sexes (Parker 1979; Rice 1996, 1998; Holland & Rice 1998; Gavrilets et al. 2001).

Although the models based on indirect selection on female preferences, as well as selection driven by direct benefit all have the potential to drive coevolution between the sexes, sexually antagonistic coevolution has been put forward as an especially strong force in driving rapid evolutionary change in reproductive characters (e.g. Parker & Partridge 1998; Rice 1998; Panhuis et al. 2001; Chapman et al. 2003; Martin & Hosken 2003). This is supported by recent models (Gavrilets 2000; Gavrilets et al. 2001), comparative evidence (Arnvist et al. 2000) and experiments (Martin & Hosken 2003) indicating sexual conflicts as an important driver of fast evolutionary change. As a final note, although sexually antagonistic coevolution is an important process, non of the above mentioned processes are mutually exclusive and may therefore be important at different stages of the evolution of reproductive divergence and in different species.
2. AIMS OF THE THESIS

The purpose of this thesis was to reach a greater understanding of the general occurrence of female polyandry in insects. I also wanted to examine the consequences of multiple mating on the evolution of reproductive divergence with a focus on postcopulatory events. The main questions I tried to answer were:

✓ Can the common occurrence of polyandry in insects be understood by looking only on the direct effects multiple mating has on female fitness? (Paper I)
✓ What effects does elevated mating rate have on female fitness? (Paper II)
✓ Can relatively short-term population isolation result in genetic divergence in the effects mating has on female reproductive performance? (Paper III)
✓ What are the roles of males and females in generating variance in male fertilization success? (Paper IV)
✓ Which male traits are involved in generating reproductive divergence in flour beetles? (Paper V)

3. MATERIAL AND METHODS

3.1 Study organisms

When studying polyandry and postcopulatory sexual selection an obvious prerequisite is that females mate with several males during their reproductive period. As many insects are promiscuous, i.e. both males and female mate with several different mates during their lifespan, they make suitable study organisms. Also, since most female insects have special, and sometimes multiple, sperm storage organs it increases the opportunity for cryptic female choice and sperm competition (Eberhard 1996). Moreover, many insects are easily reared in a laboratory environment and have relatively short generation times and lifespans. In conclusion, insects as a group are very suitable to study polyandry and its evolutionary consequences.

3.1.1 Callosobrucus maculatus – the bean weevil

The bean weevil (Coleoptera: Bruchidae) is a pest that infests stores of legume seeds such as black eye beans and chickpeas in both the temperate and tropical part of the world. The female bean weevil lays eggs on the surface of the seed and the larvae burrow into the seed, remaining there through four instars and pupation, after which the adults emerge. The generation time (egg to adult) varies with temperature but ranges between 22-30 days. Adults are well adapted to storage conditions and do not require food or water to reproduce. Under normal conditions the bean weevil’s lifespan is around 10 days (Dick & Credland 1984) but beetles given access to nutrients and/or water generally live longer.
Female lifetime fecundity depends on many factors, e.g. size at emergence, availability of oviposition sites and temperature (for references, see Credland & Wright 1989), and may also vary between allopatric populations (see Paper II).

Both males and females *Callosobruchus maculatus* are promiscuous. At mating, the male mounts the female and strokes her sides with his antennae (Rup 1986). After this courtship behaviour, the male inserts the aedagus and remains in copula for an average of three minutes (Rup 1986). The female terminates the copulation by kicking the male with her large hind legs (Rup 1986). An interesting feature of this species is that the male genitalia bears strongly sclerotized spines, which are everted inside the female’s genital tract during copulation. These spines cause considerable damage to the female tissues and Crudgington & Siva-Jothy (2000) suggested that genital damage may be responsible for the reduction in lifespan they found in doubly mated females compared to singly mated females. The benefits a male gains from damaging the females is as yet not resolved but two explanations have been proposed by Crudgington & Siva-Jothy (2000): damaging the female may reduce the risk of sperm competition if females willingness to remate is decreased and/or female damage might increase her immediate oviposition rates because the female perceives the damage as a threat to her survival, hence invests more in current reproduction; this being beneficiary to the male since eggs produced later in life are most likely fertilized by another male (but see Morrow et al. in press).

In conclusion, *C. maculatus* is an ideal study organism for several reasons, including that it is easily reared in the laboratory, has a relatively simple and short life cycle, short lifespan and interesting mating behaviours.

### 3.1.2 Tribolium castaneum – the red flour beetle

The red flour beetle (Coleoptera: Tenebrionidae) is a world-wide pest that infests a wide range of stored grain products such as flour, cereals, dried pet foods, pasta, nuts, seeds etc. It is also a common pest of homes and grocery stores. The females lay eggs in the medium, in which they live and after several instars the larvae pupate and later emerge as adults. Generation time varies with temperature and humidity but is generally around 23-30 days (Sokoloff 1974). Females lay on average 10 eggs per day and although egg production drops later in life, a female can produce a total of more than 1000 eggs during her lifespan of around 4-6 months (see Paper III). Both male and female lifespans, however, vary greatly with levels of stress and food supply and in ideal conditions they may live for over a year (see Sokoloff 1974).

Flour beetles are highly promiscuous with frequent remating (Sokoloff 1974). The copulation is initiated by the male, who mounts the female, grasps the female pronotum (prothorax) with his fore legs and extrudes the aedagus (Lewis & Iannini 1995). For copulation to occur, the female needs to lower her last sternite to allow successful intromission (Lewis & Iannini 1995). During copulation, the male rubs the sides of the female elytra with the tarsi of his mid- and hind legs (copulatory courtship). The duration of the copulation varies greatly, from about 30 s to 30 min, and Edvardsson & Arnvist (2000) showed that no copulation lasting less than 36 s resulted in any offspring production. During mating the male transfers a large number of sperm packed in a spermatophore which he deposits in the female bursa copulatrix. Shortly thereafter, the
sperm sack ruptures and released sperm are transported to the female spermatheca where they are stored. Stored sperm from a single mating can be used to fertilize eggs for up to 140 days (Bloch Qazi et al. 1996).

In conclusion, flour beetles are very easily reared in the laboratory and their highly promiscuous mating system makes them an ideal study organism for postcopulatory sexual selection. In addition, due to their devastating effect on stored grain products, much research has been done on the life history and mating behaviours of several *Tribolium* species in attempts to reduce infestations. Different wild type and mutation strains are also easily accessible through the *Tribolium* stock center at the Grain Marketing and Production Research Center in Manhattan, Kansas, USA. However, due to their long lifespans, flour beetles are not the most suitable insect species for studies measuring lifetime fitness, as we became well aware of in the experiments behind Paper III.

### 3.2 P2-experiments – limitations and possibilities

Studies designed to measure postcopulatory sexual selection have mainly focused on the outcome of sperm competition. This has lead to the extensive use of a design where the proportion of eggs fathered by the second male to mate has been measured in a controlled double-mating experiment (Boorman & Parker 1976). This proportion is termed the P2-value; “P” for paternity and “2” for the second male to mate. Species have then been classified as showing either first or second male sperm precedence, i.e. if either the first or the second male to mate obtained highest fertilization success. The pattern of sperm utilization within species has been used to interpret the underlying mechanisms behind the P2-value but it has been proven not to be so straightforward (for a discussion on this matter see Simmons & Siva-Jothy 1998).

Although measuring P2 can provide us with valuable information regarding postcopulatory sexual selection it is important not to use it as the sole measure of variance in male fertilization success (see Paper IV). One reason why it is insufficient is that postcopulatory sexual selection generates opposing evolutionary pressures on males; males are selected to achieve fertilizations when mating with already mated females (P2 – male offense ability) and also selected to ensure that other males do not fertilize the eggs of their mates (P1 – male defense ability) (Parker 1970; Rice & Holland 1997). Phenotypic traits in males that increase their offense ability do not necessarily affect their defense ability. For example, Clark et al. (1995) showed that these two components of fertilization success were uncorrelated in *Drosophila melanogaster* and stressed that both should therefore be assessed. Similarly, a recent study of *Tribolium castaneum* (Bernasconi & Keller 2001) reported a potential trade-off between these components, suggesting that male investment in defense ability may even be negatively correlated to that in offense. If this is the case, it is likely that male defense ability (P1) can have a great effect on male fertilization success and should be measured independently of P2.

In addition, female postcopulatory behaviour can be important for male fertilization success but is not captured by typical measures of P1 and P2. For example, the ability of
males to induce a period after mating during which females are unceptive to further matings (hence, refractiveness) will influence male fertilization success (Simmons & Gwynne 1991; Eberhard 1996). Similarly, female reproductive rate following mating will also affect male fertilization success (cf. Burley 1988; Sheldon 2000). A male which is able to increase the reproductive rate of his mates immediately after copulation will have a greater overall fertilization success than males unable to do so (Chapman et al. 1995; Eberhard 1996). To summarize, in order to avoid some of the limitations with traditional uses of $P_2$, attempts should be made to use measures of variance in male fertilization success that are more inclusive. This can be achieved by simultaneously acquiring independent measures of $P_1$, $P_2$, female refractiveness and female reproductive rate.

3.3 Description of the studies

3.3.1 Multiple mating and female fitness (Paper I)

There have been a great number of studies performed on many different insect species that have looked at the effect mating has on female reproductive performance. Individual studies have reported positive, negative or no effects of multiple mating on female fitness. In an attempt to assess the general effect of polyandry on female fitness we used a meta analytical approach to the problem. We performed an extensive search for experiments which addressed the effects of varying mating rates on female reproductive performance in insects. We were able to locate 122 experiments, on species belonging to ten insect orders, where all of our five criteria were met (see Paper I), which we then analysed using mixed model meta analysis. Based on the experimental design and the actual mating frequencies used in the experiments, we could predict the outcome of an experiment a priori using a simple model (Fig. 2). The model predicts the direct effect increasing mating rate has on female fitness by assuming an asymptotic benefit curve (diminishing rewards of e.g. sperm replenishment) and a linear or exponential cost curve. Furthermore, the optimal female mating rate will generally be higher in species in which nuptial feeding occurs (Fig. 2, line I) when compared to species without nuptial feeding (Fig 2, line II), owing to the added positive nutritional effect.
Fig. 2. A simple model of the effects of increased mating rate on female fitness. In species in which nuptial feeding occurs (I), female fitness is expected to increase with mating rate. As females become saturated, however, the net effects of further elevated mating rate should be insignificant. In species without nuptial feeding (II), in contrast, female fitness should be maximised at a relatively well defined optimal mating rate (see text in Paper I for a discussion). Thus, the actual mating rates used in any study relating the degree of multiple mating to female fitness (A, B or C) should be critical to the outcome of the experiment.

3.3.2 Female fitness and mating rate (Paper II)

Our results in Paper I supported the existence of an intermediate optimal mating rate in insects without nuptial feeding. To further investigate this pattern we decided to perform a study on a species with a relatively complex relationship between the costs and benefits of mating, the bean weevil (*Callosobruchus maculatus*), where we tested the effects of varying mating rates on female fitness. Experimental female were exposed to virgin males according to the following scheme during their entire lifetime: (i) once only, (ii) every 6th day, (iii) every 4th day, (iv) every 3rd day, (v) every 2nd day and (vi) every day. All females (< 24 h of age) were mated the first time during the first day of the experiment and copulation usually occurred within 15 min after introducing a virgin male to the female. Females that were subjected to additional mating attempts (i.e., treatment ii-vi) were given the chance to mate for a maximum of one hour during each mating opportunity. We used the following procedure: one virgin male was introduced to the female in the mating vial and if no mating took place within 20 min, the male was replaced with another virgin male. This was repeated once more if no copulation occurred, resulting in a maximal exposure to three males at each mating opportunity. If mating did take place, the male was removed directly after the termination of the copulation and the female was not offered another virgin male within a given mating opportunity. To control for the potentially confounding effects of exposure to males per se, all females not scheduled to mate a given day were instead exposed for 15 min to a male whose aedagus had been ablated. Ablated males harass females (i.e. chase and mounted females) but are unable to insert their aedagus in the female genitalia. To estimate female fitness we measured hatching rate of eggs, female lifespan and lifetime offspring production.
3.3.3 Effects of mating on female reproductive performance (Paper III)

Speciation is an inherently difficult process to study because it generally takes place over a long period of time. It is, however, possible to study the early signs of speciation, i.e. the evolution of divergence in reproductive traits. In this study we used three strains of the red flour beetle, *Tribolium castaneum*, to assess whether postcopulatory sexual selection had caused divergence in the effect mating has on female reproductive performance.

Females from the three strains were mated to males of the different strains at two different frequencies and in all possible reciprocal combinations throughout the entire female lifespan. The two mating frequencies were: *high* - three times a week, and *low* - once every second week. At each mating event, females were transferred to a small petri dish together with three males, and were given the opportunity to mate for three hours. The mating frequency of the females was assayed during the second week of the experiment by observing the females continuously during the three-hour mating period. We measured offspring production on a weekly basis as well as female lifespan. To control for any genetic incompatibilities between the strains we also performed a separate experiment where we measured female fertility in all possible crosses.

3.3.4 Variance in male fertilization success (Paper IV)

Postcopulatory sexual selection is generated by variance in male fertilization success. When measuring this variance the main focus has been on the outcome of sperm competition and then only measured as the proportion of offspring fathered by the second male to mate in a controlled double-mating experiment, i.e. the P2-value. We believe this is insufficient for several reasons and that instead, attempts should be made to use more inclusive measures of variance in male fertilization success (see section 3.2), which was the aim of this study. To test the importance of various sources in generating variance in male fertilization success we used the same three strains of *T. castaneum* as in Paper III, as well as one phenotypic marker strain (Black) which enabled us to determine the paternity of the offspring. Each female was mated once to a wild type male and once to a Black male in all possible reciprocal combinations. The experiment was divided into two parts, aimed at independently assessing: (1) male defense ability (measured as P1 with wild type males mating as the first mate) and (2) male offense ability (measured as P2 with wild type males as the second mate). In addition we also measured female willingness to remate (female refractiveness) in the defense experiment as the time until copulation after introduction of the second male (Black). Offspring production was scored separately for each of two weeks and we measured the copulation duration of wild type males.

3.3.5 The role of two specific signals in reproductive divergence (Paper V)

In Paper III and Paper IV we found that the three strains of *T. castaneum* have diverged in many traits related to reproduction. Nevertheless, finding that our strains had diverged provided us with no information on which specific traits they had diverged in, and this was the focus of Paper V. We tested the role of two specific male signals which have
been shown to have some influence on the pattern of sperm precedence in flour beetles, i.e. male production of cuticular hydrocarbons (pheromones) and copulatory courtship (Lewis & Austad 1994; Edvardsson & Arnqvist 2000).

We used the same experimental set-up as in Paper IV with three exceptions: (1) we used only two of the three wild type strains to increase the number of possible replicates and hence the power of the experiment, (2) we used a different marker strain, Reindeer (Rd) and, more importantly, (3) we experimentally manipulated female perception of male cuticular hydrocarbons and copulatory courtship by ablating the female antenna at the base and the male mid-legs at the midpoint of the tibia, respectively. As in Paper IV we measured \( P_1 \) and \( P_2 \) independently (one week only) as well as female refractiveness and copulation duration of wild type males.

4. MAJOR RESULTS AND DISCUSSION

4.1 Multiple mating and female fitness (Paper I)

Our combined analysis on the effects of multiple mating on female reproduction in insects showed that females gain directly from multiple mating in terms of increased lifetime reproductive success. There were some differences between orders but the main difference was between taxa where nuptial feeding occurs and those in which it does not. I will therefore discuss the results separately for the two groups.

4.1.1 Species with nuptial feeding

In species with nuptial feeding there was a large increase (average 35-85%) in both egg and offspring production with increasing mating rate. There was also a 10% increase in fertility while female longevity was only, if at all, weakly positively affected by multiple mating. Maintenance of polyandry in insects with nuptial feeding is therefore easily understood since we only observed positive effects on female net reproductive fitness. The puzzle is rather what constrains the actual mating rates of females in these groups. Females have been shown to benefit from multiple mating due to nutritious ejaculates in several butterfly genera, e.g. *Papilio* (Watanabe 1988), *Danaus* (Oberhauser 1989) and *Pieris* (Wiklund et al. 1993, 2001). Yet, natural mating frequencies of females in these genera average fewer than two matings per female in the field (Svärd & Wiklund 1988; Wiklund & Forsberg 1991; Karlsson 1995). So the question is not why females mate multiply but rather why they do not mate more frequently. We suggest that sexual conflict over female remating rates, and the resulting antagonistic coevolution between the sexes may be responsible for the origin and maintenance of nutritional ejaculates (see e.g., Simmons & Gwynne 1991; Rice & Holland 1997; Parker & Partridge 1998; Wiklund et al. 2001). Because of sperm competition, males are selected to induce a period of non-receptivity in females and this can be achieved by transferring substances in the ejaculate which induce refractory behaviour in females (see Eberhard 1996). If this non-receptivity effect is dose dependent, which is indeed the case in many insects (Eberhard 1996), it selects for large ejaculates. Although induction of female refractory period might be
beneficiary under some circumstances, if it becomes overly efficient and compromises female interest, females are selected to evolve resistance to male refractory-inducing signals. This can be achieved by either decreasing the sensitivity to the signals or by neutralizing the deleterious effects by metabolising the transferred substances. Male ejaculates may thus become exaggerated by an antagonistic and perpetual coevolutionary arms race between the sexes. Hence nuptial gifts might be a somewhat misleading term for large nutritious ejaculates of many insects since they may be a result of sexual conflicts rather than sexual confluence.

4.1.2 *Species without nuptial feeding*

Multiple mating by females had a positive effect on both egg production and fertility. This positive effect of mating was also seen in studies reporting only offspring production, which increased with an average 50% with increased mating rates. We did, however, find a negative effect of remating on female longevity in species without nuptial feeding, but the positive effects (increased egg production rate and fertility) more than outweigh this decrease in lifespan for moderate mating rates. The positive effects on fertility can, in part, be explained simply by sperm replenishment (Thornhill & Alcock 1983). It is also possible that the increase in fertility is due to indirect benefits since mating with more than one male reduces the probability of genetic incompatibilities (Zeh & Zeh 1996, 1997; Tregenza & Wedell 2002). This assumes that females have some mechanism that enables them to preferentially fertilize their eggs with sperm from genetically compatible males. The positive effect of remating on egg production rate is without doubt due to the well-documented stimulating, gonadotropic effects of mating (see Fig. 3) (Opp & Prokopy 1986; Eberhard & Cordero 1995; Eberhard 1996; Wolfner 1997, 2002; Chapman 2001). It could also stem, in part, from a nutritional effect of male ejaculate substances (Pitnick et al. 1997; Rooney & Lewis 1999). However, doubts can be cast upon nutritious ejaculates being responsible for the positive effect of multiple mating, due to the fact that orders did not differ significantly in their response to multiple mating and that food availability during the experiments did not affect the outcome (see also Vahed 1998). Our analyses also demonstrated that female lifespan decreases with increased mating rate in insects without nuptial feeding. This represents what seems to be a very general cost of polyandry, but because of the complexity of the effects of remating (Fig. 3) it is difficult to make general claims about this effect.

Our analyses also showed that female fitness does not increase monotonically with mating rate in species where males do not provide nuptial gifts. Experiments comparing female reproductive performance under moderate and high mating rates found, in general, negative net effects of increased mating rate on female fitness. Our results thus provide strong support for the existence of an intermediate optimal female mating rate in insects in general; while a single mating is clearly not generally sufficient to maintain a high offspring production through life, high mating rates tend to decrease net reproductive fitness primarily due to a reduction in lifespan.
Fig. 3. A summary of the known effects of mating on female fitness in insects. The act of mating itself, the presence of sperm per se and the transfer of a number of accessory substances with the ejaculate are all known to have various effects on various female fitness components, many of which are antagonistic. Dashed lines represent negative and solid lines positive effects.

In summary, despite the reduction in female survival in species without nuptial gifts, the average net direct fitness gain of multiple mating (measured as lifetime offspring production) was as high as 30-70%. Hence, the near ubiquitous occurrence of polyandry in insects can be understood solely in terms of direct effects, and observations of true monandry in insects, although rare, may actually be more difficult to understand. In any case, it is clear that we do not need to invoke additional indirect benefits to explain the evolutionary maintenance of polyandry in insects.

4.2 Female fitness and mating rate (Paper II)

In this study of two strains of the bean weevil (Callosobruchus maculatus) we have demonstrated that female lifetime offspring production is maximized at either of two alternative mating rates. Those maxima did not vary between populations and occurred at low mating frequencies (a single copulation) and high mating frequencies (≥ 3 copulations). Females mated at intermediate mating rates had lower offspring production. We did not, however, find any effect of mating rate on either female lifespan or egg hatchability.

In Paper I we found that in species where males provide no obvious nutritional benefits and where matings are associated with high costs and only diminishing rewards
(e.g., sperm replenishment), females should mate at an intermediate rate in order to maximize fitness. Some species, however, might exhibit a more complex relationship between the costs and benefits of mating and as a result we may instead expect several alternative fitness peaks. Such a situation could arise if males transfer substances other than sperm in their ejaculate that benefit the females in an accumulative or exponential way (see Fig. 4). We suggest that additional direct benefits of mating (beyond sperm replenishment) may occur in the bean weevil in the form of hydration and/or nutrition effects. Both suggestions are based on the fact that male *C. maculatus* transfer a rather large ejaculate to females at mating (Savelli & Fox 1998). Bean weevils feed little if at all under natural conditions, but females of several bruchid species are known to live longer and/or produce more eggs when provided with water, sugars and/or proteins (Leroi 1981; Tatar & Carey 1995). The water contained in ejaculates could provide a hydration benefit to the female, as previously demonstrated in the cricket *Gryllus sigillaus* (Ivy et al. 1999). In addition, it has been shown in closely related bruchid beetles that substances in the male ejaculate are incorporated into both female somatic and reproductive tissues (Huignard 1983, Boucher & Huignard 1987), and could thus represent a nutrition benefit to the females.

Fig. 4. In mating systems where the direct costs of mating are significant and approximately additive (C), females will maximize fitness by mating at some intermediate rate (W1) if there are diminishing rewards of a single form of direct benefit of mating (B1; for example, replenishment of sperm supplies). However, if there are additional forms of benefits to females which increase at a low but accelerating rate (B2), two distinct fitness peaks will instead be predicted: females maximize fitness by mating either at a low or a high rate (W1+2).

In summary, our results suggest that multiple mating is associated with several interacting direct benefits and costs (aedagus spines, see section 3.1.1) in *C. maculatus*, which result in two alternative mating rates where female fitness is maximized. Females mating at low rates may efficiently minimize the cost of mating, while females mating at high rates instead may maximize the benefits of mating (see Wedell et al. 2002). However, at this point further studies are needed to provide a clear understanding of this pattern.
4.3 Effects of mating on female reproductive performance (Paper III)

This study documented differences between populations in the effects of mating on female reproductive performance. We found that male genotype affects female lifespan, female lifetime offspring production and female reproductive rate. Male and female genotype also interacted in their effects on female offspring production and reproductive rate. Thus our study has shown that the male signal – female receptor system have diverged between the populations. This divergence involves qualitative and not only quantitative variation across males, as supported by the complex interactions we found between male and female genotypes. There are several different processes that can generate divergence between allopatric populations in traits related to reproduction e.g. random processes, divergence driven by indirect benefits, and sexually antagonistic coevolution (see section 1.3). Several authors (Clark et al. 1999; Andrés & Arnqvist 2001) have pointed out that the pattern of male × female genotype interactions should differ under the various contending processes. In short, random processes should result in a pattern where females exhibit an average relative reproductive response to males from their own population/strain. In contrast, females should evolve resistance to males with which they are coevolved if divergence is driven by sexually antagonistic coevolution, due to fitness costs of male adaptations, and thus responding weaker than average to their “own” males (see also Parker & Partridge 1998). Under the alternative hypothesis, divergence through indirect benefits, females should evolve preference for male signals, thereby responding stronger than average to males with which they are coevolved. However, it is important, as pointed out by Brown and Eady (2001) and Chapman et al. (2003), that these seemingly contrasting predictions should be applied with caution, as their relevance will depend on the absolute magnitude of divergence between populations or genotypes (see also Price 1997; Howard 1999; Gavrilets 2000; Eady 2001). In addition, a recent model of sexually antagonistic coevolution (Rowe et al. 2003) casts doubt on the validity of using patterns of male-female interactions to draw firm conclusions on the underlying processes, but further investigations are necessary in order to corroborate these finding.

The male × female interactions we detected here are complex and not entirely consistent. Furthermore, since our experiment was restricted to only three populations of *T. castaneum*, this does not allow us to critically test the statistical associations mentioned above and hence it is not possible to draw any firm conclusions about evolutionary processes. Nevertheless, when inspecting our patterns we found that in those cases where male genotype had a significant effect on lifetime offspring production and initial reproductive rate, the females responded least strongly to males of their own genotype in three out of four cases, and in no case the strongest. A similar pattern was found when looking at the effect of lifetime offspring production and initial reproductive rate across female genotypes. Males tended to elicit a relatively weak response in females with which they had coevolved in each of the three significant cases. Thus our data fit best with the pattern predicted by sexually antagonistic coevolution. Additional support for sexually antagonistic coevolution as a key process in generating reproductive divergence in flour beetles comes from two correlations across the sexes. Firstly, the genotype with males
most able to achieve matings has females that are least willing to mate. Such correlation between male persistence and female resistance is predicted by sexually antagonistic coevolution theory (Parker 1979). Secondly, the strain with males least able to elicit a high initial reproductive rate among females has females who respond strongest to males in terms of elevated initial reproductive rate. Again, this is predicted by sexually antagonistic coevolution theory (Gavrilets et al. 2001): females can afford a low resistance to males in populations where males are inefficient at manipulating female reproductive rate.

In summary, we have shown that the three strains of *T. castaneum* used in this study have diverged in traits related to reproduction. We also found complex male-female interactions in which the patterns were most consistent with that expected if divergence was driven by sexually antagonistic coevolution. Currently, the validity of using patterns of interactions to infer processes is under debate, mainly due to a lack of solid theoretical background (see Rowe et al. 2003). Nevertheless, our results also include two correlations between male persistence traits and female resistance traits and since our strains showed no genetic incompatibilities, sexually antagonistic coevolution seems to be the most likely process behind the evolution of the observed reproductive divergence.

### 4.4 Variance in male fertilization success (Paper IV)

The genetic divergence previously observed in the three populations of *T. castaneum* (Paper III) was also apparent in the outcome of this experiment where we studied the importance of various sources of variance in male fertilization success. Our results showed both independent and interacting effects of male and female genotypes on several components of variance in male fertilization success. Female genotype influenced male success both in the male offense (P2) and male defense (P1) experiment. Together with other studies, showing both that females actively influence sperm storage (Bloch Qazi et al. 1998) and that female perception of male copulatory courtship influences male fertilization success (Edvardsson & Arnqvist 2000), this suggests that cryptic female choice is important in shaping male fertilization success in flour beetles. More unambiguous evidence for cryptic female choice comes from male × female genotype interactions, as such interactions demonstrate that female characteristics affect relative male fertilization success (Pitnick & Brown 2000). The finding of significant male × female interactions for success both in P2 and the ability to elicit female refractiveness thus add considerable strength to our interpretation of the results. That is, female traits may generally shape the pattern of postcopulatory sexual selection experienced by males.

The pattern of the male × female genotype interactions can be used to understand the underlying processes but is associated with some difficulties (see previous section). In this study the pattern of male × female interactions was highly variable and not entirely consistent, which might reflect the problems with inferring processes from interactions. The pattern of the interaction for male offense ability (P2) was especially difficult to categorize since in no case was the female response to their own males significantly different from the response to the other two male genotypes (focused post-hoc tests;
The pattern of the male × female interaction for female refractiveness, however, showed that males of the females’ own genotype elicited the weakest average response in all genotypes. However, in only one case was the females’ response to their own males significantly different from the response to the other two male genotypes.

To summarize, in this study we found strong support for a females influence (i.e. cryptic female choice) affecting variance in male fertilization success in the red flour beetle. Our data, however, are far from conclusive with regards to the prediction of male-female interactions. Although we cannot exclude that random processes alone have caused the divergence observed here, this seems highly unlikely since the traits involved (components of male fertilization success) should have been exposed to selection. Instead our results, in combination with the results of Paper II, suggest that females may at least in some respects have evolved resistance to the postcopulatory manipulations of males with which they have coevolved, implying sexually antagonistic coevolution as a driving force behind the observed population divergence (Rice 1998; Howard 1999; Arnqvist et al. 2000).

4.5 The role of two specific signals in reproductive divergence (Paper V)

In this study we assessed the role of two different sexual signals, copulatory courtship and male cuticular hydrocarbons, in the reproductive divergence between two previously studied populations of the red flour beetle. We found that copulatory courtship had a significant effect on sperm defense ability ($P_1$) and that male cuticular hydrocarbons affected both the sperm defense ability of males ($P_2$) and female refractiveness. This suggests that both copulatory courtship and male cuticular hydrocarbons are sexually selected within populations (see also Lewis & Austad 1994; Edvardsson & Arnqvist, 2000). Finding that a trait related to reproduction is sexually selected for within a population does not necessarily imply an involvement in the evolution of reproductive divergence between populations. However, if any given signal/receptor system plays an important role in reproductive divergence between population, and is modified so that females can no longer perceive the male signals, then we would expect a different female response after manipulation since the signals or receptors are now lacking. That is to say, there should be an interaction between the male strain and the signal/receptor manipulation treatment. On the other hand, if a given signal/receptor system is not involved in the evolution of the reproductive differentiation, females should show a similar response regardless of manipulation. As a consequence, in this case we would expect no interactions between male strain and the manipulation treatment. We found no interaction between male strain and the manipulation of copulatory courtship, suggesting that although copulatory courtship is sexually selected within populations (see above) it has only a minor role in causing reproductive divergence between the strains. In contrast, we found that manipulation of cuticular hydrocarbons reception did interact with male strain. This indicates that male cuticular hydrocarbons may play a significant role in the evolution of reproductive divergence between our populations. This conclusion is further strengthened by marginally significant male strain × cuticular hydrocarbon interactions in
both P1 and female refractivity (p<0.079 in both cases). The pattern of these interactions is rather puzzling, however, as one would intuitively assume that when females are not able to perceive male cuticular hydrocarbons they would show a similar response to males regardless of genotype, if they indeed are responsible for the divergence. This was not what we found (see Fig. 1, Paper V). A potential explanation for this unexpected pattern might be that a male’s fertilization success is dependent on several interacting signals and receptors and further studies are necessary to evaluate these findings.

In summary, both copulatory courtship and male cuticular hydrocarbons are sexually selected within populations, but we only found support for the involvement of cuticular hydrocarbons in the evolution of reproductive divergence between the two populations of *T. castaneum*. Cuticular hydrocarbons are known to be involved in precopulatory reproductive isolation (Savarit et al. 1999; Howard et al. 2003), but to our knowledge this is the first study specifically showing its involvement in postcopulatory reproductive isolation.

5. CONCLUSIONS

In this thesis I have shown that, in general, female insects benefit directly from multiple mating in terms of increased lifetime offspring production. The benefits of polyandry has been, and is still, a debated subject (e.g. Jennions & Petrie 2000; Hosken & Stockley 2003; Simmons 2003) because mating is associated with costs to females (Daly 1978). In species where males provide females with obvious material benefits, e.g. nutritious donation, polyandry is understandable, and my results supported this view as I found positive effects of increased mating rate on female fitness in a meta-analysis of studies addressing this problem. In species where males provide females with little more than sperm, it is less obvious how females may benefit from polyandry. Indirect benefits, as opposed to direct benefits, has been proposed as a potential mechanism. This it not necessary, however, as my meta-analysis showed that polyandry in species without nuptial gifts can be understood solely in terms of direct benefits to females, although this does not exclude the fact that females may gain indirect benefits by multiple mating as well. For example, if females are able to discriminate between sperm of different males, polyandry can be a way for females to avoid having their eggs fertilized by genetically incompatible males (i.e. inbreeding avoidance, see Tregenza & Wedell 2002). Still, at high mating rates the costs of mating becomes substantial and decreases female lifespan and egg production, suggesting that females should mate at an intermediate rate to maximize fitness. Whilst this might generally be true in insects without nuptial feeding, it is also possible that species with multiple benefits and high costs to mating can show several alternative fitness peaks. One such species is the bean weevil, *Callosobruchus maculatus*; when varying the number of matings to females I found that female fitness was maximized at two alternative mating rates (high and low). Future studies are necessary to see how general such relationships are and whether multiple fitness peaks might explain the large intraspecific variation in the degree of polyandry in e.g. butterflies (Wedell et al. 2002) and between closely related species.
In polyandrous species, the opportunity for postcopulatory sexual selection is strong and this may lead to rapid evolution of reproductive traits. As a result, when allopatric populations come into secondary contact they may show different degrees of reproductive incompatibilities (Parker & Partridge 1998). To test this, I performed two studies where I crossed three populations of the red flour beetle, Tribolium castaneum, and found that they had diverged in many traits related to reproduction. Moreover, male and female genotype interacted in their effect on offspring production, reproductive rate, P2 and female refractiveness, indicating that the male signal – female receptor system has indeed diverged between the populations. These interactions provide strong evidence for cryptic female choice (Pitnick & Brown 2000) and my thesis adds to the growing number of studies of insect species showing that male and female genotypes interact in their effect on male fertilization success (Clark & Begun 1998; Arńqvist & Danielsson 1999; Clark et al. 1999; Andrés & Arńqvist 2001; Brown & Eady 2001; Hosken et al. 2002). As a whole, my findings on the evolution of reproductive divergence are mostly in agreement with that predicted by selection driven by sexually antagonistic coevolution. However, the variability in the pattern of the male and female genotype interactions, as well as the low number of populations studied, make it difficult to draw any firm conclusions regarding selective forces at this point (see Chapman et al. 2003; Rowe et al. 2003).

The reproductive divergence that I found in the T. castaneum populations is most likely mediated by several different male signals and female receptors. As a final part of this thesis I examined the role of two specific male signals in the evolution of reproductive divergence: copulatory courtship and male cuticular hydrocarbons. Both signals are sexually selected within strain, as previously shown (Lewis & Austad 1994; Edvardsson & Arńqvist 2000) but I only found support for the role of cuticular hydrocarbons in the divergence between the two studied populations of flour beetles. It is interesting that cuticular hydrocarbons, which are known to be important in precopulatory isolation in other species (Savarit et al. 1999; Howard et al. 2003) may also play a role in postcopulatory reproductive isolation, but further studies are necessary to confirm this intriguing result.

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