



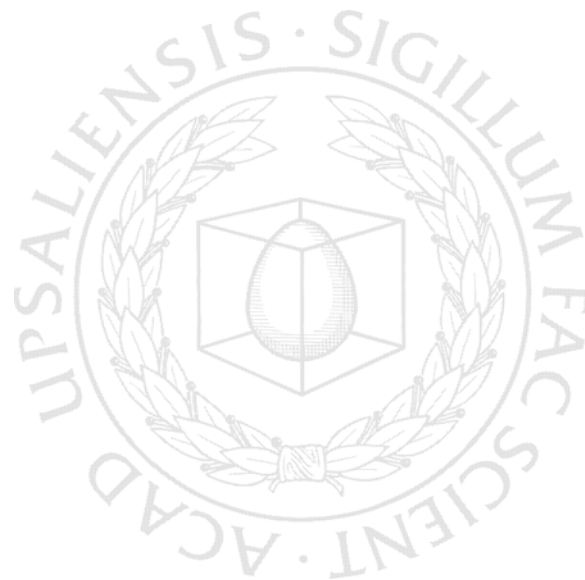
UPPSALA
UNIVERSITET

*Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Science and Technology 43*

Cryptic Female Choice and Male Mating Behaviour

Sexual Interactions in Beetles

MARTIN EDVARDSSON



ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2005

ISSN 1651-6214
ISBN 91-554-6225-1
urn:nbn:se:uu:diva-5753

Dissertation presented at Uppsala University to be publicly examined in Zootissalen, Evolutionary Biology Centre, Uppsala, Friday, May 13, 2005 at 15:00 for the degree of Doctor of Philosophy. The examination will be conducted in English.

Abstract

Edvardsson, M. 2005. Cryptic Female Choice and Male Mating Behaviour. Sexual Interactions in Beetles. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 43. 42 pp. Uppsala. ISBN 91-554-6225-1.

The importance of cryptic female choice, i.e. female post-copulatory influence over male reproductive success, in driving the evolution of male traits remains controversial. The main aim of this thesis was to understand the post-copulatory consequences of sexual interactions and the importance of cryptic female choice in two species of beetle.

Males of the red flour beetle *Tribolium castaneum* use their legs to rub the lateral edges of the female elytra during mating. When manipulating female perception of this behaviour, I found that females preferentially use the sperm of males with vigorous leg rubbing when they mate with more than one male. Leg rubbing also appeared to increase female rate of oviposition. Females do not seem to gain any indirect benefits by preferring males with an intense leg rubbing behaviour since this behaviour was found to have very low narrow sense heritability and did not appear to be condition dependent in its expression.

Males of the bruchid beetle *Callosobruchus maculatus* have spiny genitalia that harm their mates. Females kick males during copulation and when prevented from kicking, suffered reduced lifetime offspring production as a consequence of more extensive injuries. Males were not able to delay female remating, increase rate of oviposition or increase sperm precedence by inflicting relatively severe injuries to non-kicking females. Hence, the injuries appear to be side effects of male efforts to remain in copula. When copulation duration was manipulated, ejaculate size and female lifetime offspring production increased with the length of copulation. Females reduced their mating rate when they had access to water, suggesting that they obtain water from the large ejaculates and trade-off their need for additional water against the costs of mating. Males may then reduce the benefits of remating by providing their mates with a large amount of water. Females did not increase their remating propensity to avoid inbreeding when they had mated to brothers. Together, these studies reveal the complexity of sexual interactions and the importance of post-copulatory processes for the fitness of both males and females.

Keywords: Cryptic female choice, Copulatory courtship, Harmful male traits, Nuptial gifts, Sperm competition, Sexual selection, *Tribolium castaneum*, *Callosobruchus maculatus*

Martin Edvardsson, Department of Ecology and Evolution, Norbyvägen 18 D, Uppsala University, SE-75236 Uppsala, Sweden

© Martin Edvardsson 2005

ISSN 1651-6214

ISBN 91-554-6225-1

urn:nbn:se:uu:diva-5753 (<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-5753>)

List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I Edvardsson, M. and Arnqvist, G. 2000 Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum*. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 559-563.
- II Edvardsson, M. and Arnqvist, G. 2005 The effects of copulatory courtship on differential allocation in the red flour beetle *Tribolium castaneum*. *Journal of Insect Behavior* In press.
- III Edvardsson, M. and Arnqvist, G. No apparent indirect genetic benefits to female red flour beetles preferring males with intense copulatory courtship. Submitted manuscript.
- IV Edvardsson, M and Tregenza, T. 2005 Why do male *Callosobruchus maculatus* harm their mates? *Behavioral Ecology* In press.
- V Edvardsson, M. and Canal, D. The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*. Submitted manuscript.
- VI Edvardsson, M., Tregenza, T. and Rodríguez-Muñoz, R. No evidence that female bruchid beetles *Callosobruchus maculatus* use remating to reduce costs of inbreeding. Submitted manuscript.
- VII Edvardsson, M. Female *Callosobruchus maculatus* mate when they are thirsty: resource rich ejaculates as mating effort in a beetle. Submitted manuscript.

Paper I is reprinted with permission from the publisher.

Cover photograph by Fleur Champion de Crespigny.

Contents

Introduction.....	7
Sexual interactions and cryptic female choice	7
Mechanisms of cryptic female choice	8
Evolution of cryptic preferences.....	9
Male traits	11
The study species	13
<i>Tribolium castaneum</i>	14
<i>Callosobruchus maculatus</i>	14
Thesis aims.....	16
Results and discussion	17
Copulatory courtship and sperm precedence in <i>T. castaneum</i>	17
Copulatory courtship and differential allocation in <i>T. castaneum</i>	19
Heritability of the leg rubbing behaviour of <i>T. castaneum</i>	20
Male inflicted harm in <i>C. maculatus</i>	21
The effects of copulation duration in <i>C. maculatus</i>	22
Inbreeding and female mating behaviour in <i>C. maculatus</i>	24
Water and female mating behaviour in <i>C. maculatus</i>	25
Conclusions.....	28
Sammanfattning på svenska.....	30
Acknowledgements.....	35
References.....	37

Introduction

Sexual interactions and cryptic female choice

Interactions with individuals of the opposite sex will inevitably have a strong impact on the reproductive success of sexually reproducing animals. The study of these interactions in the context of sexual selection was for a long time more or less exclusively concerned with processes occurring before mating has started. These processes include competition among males over mating opportunities, male pre-mating courtship and female choice of males to mate with. The realisation that females of many species typically mate with more than one male has, over the past 35 years, led to an increased appreciation of the importance of processes taking place after mating has started (e.g. Eberhard 1996; Birkhead and Møller 1998; Simmons 2001; Birkhead and Pizzari 2002). In 1970, Parker pointed out that when females mate with more than one male during a reproductive cycle, sperm of different males compete to fertilise the eggs. Sperm competition, as it was termed by Parker (1970), is now well established as an important process that has led to a wide range of male adaptations such as ejaculates containing large numbers of sperm, genitalia able to remove sperm from previous matings and the transfer of substances that reduce female mating propensity (e.g. Smith 1984; Birkhead and Møller 1998; Ben-Ari 1999; Simmons 2001).

Sperm competition is the post-mating continuation of male-male competition over access to females and their eggs. Following similar rationale, it was suggested that female choice of males also continues after mating has started (Thornhill 1983; Eberhard 1985). In contrast to the rapid acceptance of the importance of sperm competition, the idea that female controlled post-copulatory mechanisms are important in determining paternity is still controversial despite twenty years of speculation and research (e.g. Eberhard 1996; Ben-Ari 2000). Dubbed “cryptic female choice” by Thornhill in 1983, it has later been defined as “a female-controlled process or structure that selectively favors paternity by conspecific males with a particular trait over that of others that lack the trait when the female has copulated with both types” (Eberhard 1996). Like other young and developing fields of evolutionary biology, cryptic female choice has had its share of semantic confu-

sion. The word “choice” makes most people think of females evaluating the available males carefully and then choosing the best one. However, with the broad definition above, female choice does not necessarily involve any deeper contemplation or even the nervous system at all. Cryptic female choice is then simply a matter of any female trait biasing paternity towards males with certain other traits when females mate with more than one male.

Mechanisms of cryptic female choice

The reproductive tracts of females are generally complex structures and several female responses, such as transport of sperm and ovulation, must take place before insemination can lead to fertilisation (Eberhard 1996; Bloch Qazi et al. 1998; Hellriegel and Ward 1998). Hence, females could potentially possess a number of mechanisms that would qualify as cryptic female choice. Eberhard (1996) lists 20 possible mechanisms of cryptic female choice that may influence male reproductive success. The mechanisms can be divided into four main groups.

1. Manipulation and discrimination of sperm. The female manipulates the sperm after the male has inseminated them or attached a spermatophore.
2. Control of male access to the female reproductive tract and to sperm from previous matings. The female controls how deeply the male intromittent organ may penetrate her reproductive tract.
3. Control of own reproductive investment. The female varies her reproductive investment after being inseminated by different males or her investment in offspring fathered by different males.
4. Control of remating. The female varies the time until she mates with another male.

Evolution of cryptic preferences

Material benefits

Females may sometimes use mate choice to gain material benefits such as paternal care, food or access to a high quality territory (Andersson 1994). Material benefits are less likely to be important to the evolution of cryptic female choice than to pre-copulatory choice. In contrast with pre-mating female choice where the male knows whether the female has mated with him or not, males have no means of controlling the outcome of but a few of the cryptic female choice mechanisms. Therefore, it is generally considered unlikely that a system where female co-operation, in terms of cryptic female choice, is rewarded with male provisioning of some resource would evolve (Eberhard 1996). However, when females change their mating behaviour in response to material resources provided by males this may occur (paper VII).

Sensory exploitation

Female cryptic preferences may originate as side effects of female sensory or neural characteristics, which males have evolved to exploit (West-Eberhard 1984; Christy 1995; Eberhard 1996; Ryan 1998). Males may provide females with stimuli that mimic stimuli females respond to in other contexts. When the female response somehow has a positive effect on male fertilisation success males will be selected to trigger it. Córdoba-Aguilar (1999) showed that in the damselfly *Calopteryx haemorrhoidalis asturica* males mimic the stimulus of an egg that passes through the female reproductive tract with their genitalia. This triggers the female sperm storage organ to release sperm from previous matings that can then be removed from the female reproductive tract by the male (Córdoba-Aguilar 1999).

Genetic benefits

Male traits involved in sexual interactions may serve as honest indicators of male quality that enable females to increase the fitness of their offspring by favouring high quality males. Females may benefit because these traits indicate that males possess genes that will make their offspring attractive or vi-

able (Andersson 1994; Houle and Kondrashov 2002). It may also be important for females to favour males with compatible genes and hence avoid closely related males or males of the wrong species (Andersson 1994). There is evidence suggesting that female sand lizards *Lacerta agilis* (Olsson et al. 1996; Olsson et al. 2004), field crickets of the species *Gryllus bimaculatus* (Tregenza and Wedell 2002; Bretman et al. 2004) and *Gryllodes supplicans* (Stockley 1999), red junglefowl *Gallus gallus* (Pizzari et al. 2004), and possibly also females of the fly *Drosophila melanogaster* (Mack et al. 2002) have the ability to favour sperm from unrelated males over sperm from related males.

Females would also benefit from using cryptic female choice if offspring fitness depended on an interaction between genotype and environment and the type of environment that offspring will encounter could not be predicted at the time of mating (Ward et al. 2002). Larval growth in the yellow dung fly, *Scathophaga stercoraria*, is influenced by an interaction between the variable environmental conditions of the cowpats larvae grow in and larval phosphoglucosmutase (PGM) genotype (Ward 1998). Females have been found to bias sperm use to match the PGM genotype of their offspring to expected environmental conditions when they have mated with males with different PGM genotypes (Ward 2000; Ward et al. 2002).

There is no reason why pre-copulatory and post-copulatory choice mechanisms cannot be in operation at the same time (Eberhard 1996). However, under certain circumstances females may be restricted in the extent to which they can use pre-copulatory mate choice mechanisms. Females may receive information about males during copulation and from their ejaculates after copulation. This could generate selection for cryptic female choice especially if females are unable to detect any reliable signals before copulation starts, for instance regarding the genetic compatibility of their partner (Jennions and Petrie 2000). Sometimes, cryptic female choice may even represent the only possible choice mechanism. Females that are inseminated forcibly may be unable to exert pre-copulatory choice (Birkhead 1998). This often occurs in the feral fowl *Gallus gallus domesticus* and females have been found to discriminate against subordinate males by ejecting their sperm after copulation (Pizzari and Birkhead 2000). Sessile organisms such as ascidians that receive gametes from a variety of males are not able to control the source of these gametes (Wirtz 1997). In the compound ascidian *Diplosoma listerianum*, the passage through the oviduct of self sperm and sperm from certain incompatible crosses is blocked (Bishop 1996).

Male traits

Copulatory courtship

Whenever there is female post-copulatory influence over male reproductive success, males that can manipulate this influence to their advantage will be favoured by selection. Several male traits are thought to have evolved to manipulate females in response to cryptic female choice (Eberhard 1996). However, direct experimental evidence is often lacking. For instance many males perform behaviours during copulation that appear to be aimed solely at stimulating the females (Eberhard 1991, 1994, 1996). These behaviours occur in a wide variety of species and include various forms of tactile stimulation by male extremities, mouthparts or genitalia as well as acoustic and visual signals. These behaviours seem to be the equivalent of pre-copulatory courtship aimed at enticing females to mate and would make very little sense in the absence of cryptic female choice. In spite of the apparent prevalence of copulatory courtship, very few studies have attempted to link it with male reproductive success in a rigorous way (paper I). However, recent studies of two beetle species have found that female perception of male leg rubbing in *Tribolium castaneum* (paper I) and stroking with antennae in *Diabrotica undecimpunctata howardi* (Tallamy et al. 2003) affects male fertilisation success.

Harmful male traits

Some male traits involved in sexual interactions appear to be harmful to females and reflect a conflict between individuals of the two sexes. Conflicting evolutionary interests of individual males and females appear to be a common and important driving force behind the evolution of traits involved in reproduction (Parker 1979; Partridge and Hurst 1998; Chapman et al. 2003). The underlying reason for these sexual conflicts is that the optimal outcome of an interaction between potential mates often differs between males and females. As a consequence, male traits that manipulate female reproductive processes can evolve even if they are harmful to females. This may lead to sexually antagonistic coevolution with males evolving traits to manipulate females and females evolving resistance to male traits (Parker 1979; Holland and Rice 1998). Traits that have been suggested, and in some cases shown, to be involved in sexual conflicts include rate of offspring production and female remating propensity (Parker 1979; Partridge and Hurst 1998; Rice 2000; Chapman et al. 2003). Males that can somehow stimulate

female offspring production will increase the number of eggs they fertilise before the females remate. Similarly, males that can prevent their mates from remating will also increase the number of offspring they father. This appears to have led to the evolution of complex male ejaculates that contain substances that manipulate female reproduction (Eberhard 1996; Simmons 2001). It has been shown that some of these substances, which include various proteins and hormones, are capable of altering females' rate of egg production and their receptivity to courting males (Eberhard 1996; Simmons 2001). Females are best served by maintaining an optimal rate of reproductive output to maximise their lifetime offspring production and by mating at a rate that maximises benefits of mating such as replenished sperm stores and nutrients from male nuptial gifts (Arnqvist and Nilsson 2000). Manipulative seminal substances have been studied extensively in the fly *Drosophila melanogaster* and it appears that receiving these substances is harmful for females (Fowler and Partridge 1989; Chapman et al. 1995). To keep their optimal mating frequency and rate of egg production females should evolve resistance to the male secondary substances or other pre- or post-mating male stimuli that act in similar ways (Gavrilets et al. 2000; Jennions and Petrie 2000). Males will then be selected to overcome female resistance. This can lead to an arms race of antagonistic co-evolution between the sexes where males refine their means of manipulation and females strengthen their resistance (Holland and Rice 1998; Gavrilets et al. 2000). If males differ in their ability to overcome female resistance they will also differ in fertilisation success. This qualifies as cryptic female choice according to the definition above and will result in sexual selection on males (Eberhard 1996).

Seminal substances are not the only male traits that harm females in connection with mating. Males of some species harm females physically during copulation. For example, males of the bruchid beetle *Callosobruchus maculatus* and the dung fly *Sepsis cynipsea* have spines on their intromittent organs that harm the female reproductive tract during mating and leave scars (Crudgington and Siva-Jothy 2000; Blanckenhorn et al. 2002). It has been suggested that these harmful male traits, as well as toxic seminal fluids, have evolved because males benefit from harming females by altering their mate's optimal reproductive strategy in favour of greater investment in offspring from their current mate (Constantz 1984). Recent treatments (Johnstone and Keller 2000; Lessells 1999, 2005) show that this is theoretically possible and may happen in two ways. First, if there are escalating costs to females of repeated matings, then mate harm can be maintained because it reduces female remating propensity (Johnstone and Keller 2000). Second, females may perceive the injuries as a threat to their survival and respond by increasing their current reproductive effort in order to maximise offspring production before they die (Michiels 1998; Lessells 1999). In both cases, males would benefit through the increased number of eggs fertilised by their sperm before the females remate. The harm caused by these traits may also be side effects

of other functions that are sufficiently beneficial to males to outweigh any negative effects on the offspring production of their mates. Recent studies of *C. maculatus* (paper **IV**) and *S. cynipsea* (Hosken et al. 2003) indicate that the harm caused by the spiny male genitalia of these species does not benefit males.

Nuptial gifts

Many males, particularly among insect and bird species, provide females with ‘nuptial gifts’ in the form of prey, seminal products or parts of the male body (Thornhill and Alcock 1983; Andersson 1994; Vahed 1998). Besides playing a role in pre-mating female choice and functioning as paternal investment aimed at increasing the offspring production of females, some of these gifts appear to be selected through cryptic female choice. The function of some nuptial gifts, in particular those consumed by females during mating (e.g. prey items and parts of externally attached male ejaculates) appears to be to manipulate females to allow long ejaculate transfer (Gwynne 1997; Vahed 1998). For example, some female scorpionflies (Mecoptera) allow males to transfer sperm for longer periods of time when they receive relatively large arthropod food gifts (Thornhill 1976, 1983). Similarly, the spermatophylax, a large spermless gelatinous mass that is part of the externally attached spermatophore of some male bushcrickets functions to prolong sperm transfer (Orthoptera: Tettigoniidae) (Wedell and Arak 1989; Wedell 1991; Sakaluk 2000). Females first remove and consume the spermatophylax and then the sperm containing ampulla thereby terminating sperm transfer.

It has also been suggested that when females derive material benefits from nuptial gifts, males may manipulate female remating by providing large gifts that decrease the benefits of receiving additional gifts and delay female remating (Johnson et al. 1999; Weddle and Sakaluk 2003). This may be especially important when females trade-off substantial costs of mating against the benefits of nuptial gifts (paper **VII**).

The study species

The papers in this thesis concern two beetle species; the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) (papers **I**, **II** and **III**) and the cowpea seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae) (papers **IV**, **V**, **VI** and **VII**).

Tribolium castaneum

The red flour beetle is a 3-4 mm long chestnut brown, cosmopolitan pest of stored grain (Sokoloff 1972). It infests a wide variety of cereal products in granaries and homes. This species is easy to maintain in the laboratory and it is usually reared on a medium consisting of a mix of whole-wheat flour and dry brewer's yeast at temperatures around 30°C (Sokoloff 1972). Female oviposition, larval development and pupation all takes place in the medium. Generation time depends on environmental variables such as temperature but is generally 24-32 days (e.g. Sokoloff 1974). Males and females are relatively long lived and can survive for over a year under ideal conditions (Sokoloff 1974).

Females are promiscuous, mating frequently when given the opportunity (Sokoloff 1974) and may produce more than 1000 eggs over their lifespan (Nilsson et al. 2002). Nevertheless, they can store and use sperm from a single mating for up to 140 days (Bloch Qazi et al. 1996). Males emit a pheromone that is attractive to both males and females (Faustini et al. 1981; Lewis and Austad 1994) but there is no obvious pre-copulatory courtship (Wojcik 1969). The male stands on top of the female during copulation and rubs the lateral edges of her elytra with the tarsi of his legs (Wojcik 1969). This behaviour is thought to function as copulatory courtship aimed at influencing cryptic female choice (Eberhard 1996).

Callosobruchus maculatus

The cowpea seed beetle (sometimes referred to as the “cowpea weevil” or “bean weevil” in spite of not being a weevil at all) is a pest on legume seeds. It is widespread in the warmer parts of the Old World and has spread to the Americas in seeds (Southgate 1979). Females attach their eggs to the surface of beans and peas and the larvae burrow inside feeding on the cotyledons. Pupation takes place just below the surface of the seed and the adult beetle emerges after eclosion (Southgate 1979). Adults are typically only a few millimetres in length, can reproduce without access to food or water and live for about a week to ten days depending on the temperature (Giga and Smith 1983; Dick and Southgate 1984). Longevity is prolonged if the beetles are provided with nutrients and/or water (e.g. Tatar and Carey 1995). The generation time also depends on temperature and generally varies from just over three weeks at 30°C up to four weeks at 25°C (e.g. Giga and Smith 1983). *C. maculatus* can be maintained in the laboratory on wide variety of beans and peas. The black-eyed bean (also known as the cowpea) *Vigna unguiculata* was used in all studies reported in this thesis.

Females emit a pheromone that attracts males (Qi and Burkholder 1982; Shu et al. 1996). Males antennate females vigorously before mating and while they are attempting to insert their genitalia (Rup 1986). During copulation, the male remains behind the female in an upright position only holding on to the female with his genitalia (see cover picture). The copulatory interactions of this species have three intriguing components. First, males transfer large ejaculates in spermatophores weighing up to 10% of the males' body weight in virgin males (Savalli and Fox 1998). Females appear to derive material benefits from these ejaculates (Fox 1993; Ofuya 1995; Savalli and Fox 1999). Second, the male genitalia bear spines that unfurl during copulation and puncture the female reproductive tract (Crudgington and Siva-Jothy 2000) (figure 1). Third, females kick their mates during the last part of the copulation (Qi and Burkholder 1982). If they are prevented from kicking, copulations last longer and the injuries they sustain are more extensive (Crudgington and Siva-Jothy 2000).

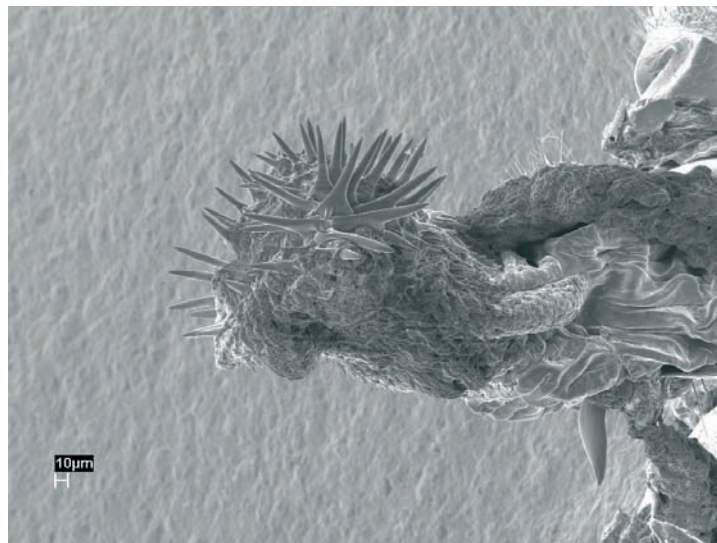


Figure 1. The spiny male genitalia of *C. maculatus*. Photograph by Johanna Rönn.

Thesis aims

Male and female traits may evolve in response to one another when sexual interactions affect the fitness of individual males and females. The main aim of this thesis was to understand the post-copulatory consequences of sexual interactions and to what extent cryptic female choice has driven the evolution of male traits. The specific questions posed by the studies of the two beetle species were:

Tribolium castaneum

- Do females favour males with a vigorous leg rubbing behaviour through preferential use of their sperm? (Paper **I**)
- Do females increase their offspring production when they have mated to males with a vigorous leg rubbing behaviour? (Paper **II**)
- Do females benefit from preferring males with a vigorous leg rubbing behaviour? Is the leg rubbing behaviour heritable and condition dependent in its expression? Do males with a vigorous leg rubbing behaviour father offspring with a high viability or sons with a vigorous leg rubbing behaviour? (Paper **III**)

Callosobruchus maculatus

- Do males benefit from harming their mates? Are the injuries the spines on male genitalia inflict costly to females? Do females benefit from kicking their mates? (Paper **IV**)
- Do the male spines and the female kicking behaviour reflect a sexual conflict over copulation duration? (Paper **V**)
- Do females change their remating propensity to avoid inbreeding? (Paper **VI**)
- Is the large male ejaculate maintained because females change their mating rate depending on their need for water? (Paper **VII**)

Results and discussion

Copulatory courtship and sperm precedence in *T. castaneum*

The aim of paper I was to assess whether or not females use cryptic female choice to increase the relative fertilisation success (i.e. sperm precedence) of males with a vigorous leg rubbing behaviour. There is very little direct experimental evidence linking copulatory courtship and sperm precedence. Simply establishing an association between the intensity of copulatory courtship and sperm precedence is not sufficient since the copulatory courtship behaviour may be correlated to other important male traits such as sperm production. One way to avoid such confounding factors is to manipulate female perception of the copulatory courtship behaviour independently of the behaviour itself.

During copulation, male *T. castaneum* rub the lateral edges of the female elytra with the tarsi of their legs. This behaviour is performed in bouts of one to a few strokes with either one leg alone or with two legs simultaneously. It can involve any leg but the fore legs are rarely used (Wojcik 1969; personal observation). In paper I, female perception of this behaviour was manipulated by shortening one or two male legs. Males appear to use shortened legs in the copulatory courtship behaviour just like normal legs. However, the shortened legs do not reach the lateral edges of the female elytra. Therefore, females should not be able to perceive any rubbing performed by shortened legs. Females were first mated to males homozygous for a mutation making the beetles black instead of their wildtype chestnut brown (Sokoloff et al. 1960). Twenty-four hours later, they were mated to wildtype males that were either normal or had had one or two legs ablated. We quantified the intensity of the leg rubbing behaviour at this mating by simply recording the number of rubbing bouts performed by each leg and measuring copulation duration. Females were then allowed to oviposit for seven days. All adult offspring were collected and scored for body colour. Offspring of black males and brown females are intermediate in colour (Sokoloff et al. 1960) and it was therefore possible to assign paternity to all offspring.

The results showed that the proportion of offspring fathered by the second male to mate (P2) increased with the intensity of his leg rubbing behaviour in normal males but not in males with shortened legs (figure 2). These re-

sults were not artefacts of males with ablated legs transferring a reduced amount of sperm since this was measured in a follow up experiment. Together, these findings suggest that female perception of the leg rubbing behaviour is important to male fertilisation success and that this behaviour is selected through cryptic female choice. The underlying mechanism is not known but one possibility is that the tactile stimulation of female elytra somehow affects how much sperm females transport into storage and how much they dump. Sperm dumping appears to occur regularly in this species (Bloch Qazi et al. 1996; personal observation) and females play an active role in transportation of sperm into their storage organs (Bloch Qazi et al. 1998).

Direct experimental evidence linking copulatory courtship and cryptic female choice has now also been found in the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* (Coleoptera: Chrysomelidae) where males stroke females with their antennae during copulation until they gain access to the bursa copulatrix (Tallamy et al 2002). Tallamy et al. (2003) showed that females favour males with a fast stroking behaviour and refuse to accept spermatophores from males with one ablated antenna.

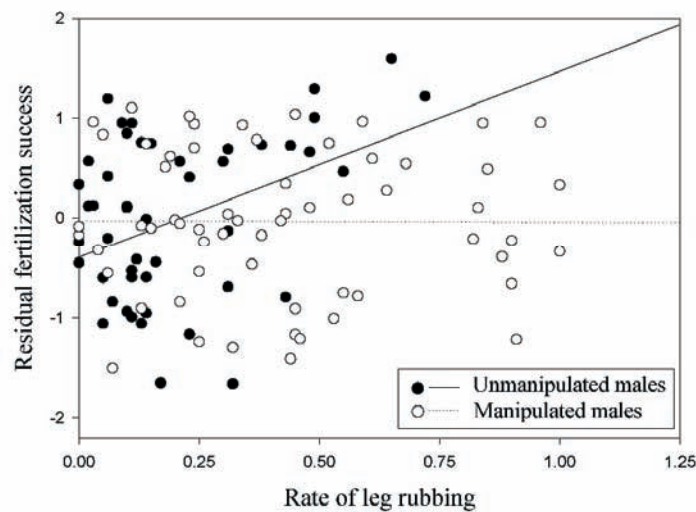


Figure 2. The relationship between rate of leg rubbing and fertilisation success of the second male to mate in *T. castaneum* (paper I). Unmanipulated males had normal legs and manipulated males had one or two shortened legs that did not reach the female elytra.

Copulatory courtship and differential allocation in *T. castaneum*

The aim of paper **II** was to test whether or not female *T. castaneum* increase their offspring production (differential allocation) or produce a higher proportion of sons (sex allocation) when they have mated with males with a relatively vigorous leg rubbing behaviour. Altering reproductive investment, such as number of eggs or the investment in each offspring, in response to male characters is one way through which females can favour males with certain traits through cryptic female choice (Thornhill 1983; Eberhard 1996). According to the differential allocation hypothesis, females that face trade-offs between investment in current and future reproduction may benefit from varying their reproductive investment depending on the attractiveness of their partner and hence the expected value of each offspring (Burley 1986; Sheldon 2000). Males of polyandrous species on the other hand, will benefit from any trait that increases female egg production since this will elevate the number of offspring they sire before their mates remate with other males. This may lead to male manipulations that increase the rate of female offspring production above what is optimal for females (Arnqvist and Nilsson 2000; Chapman et al. 2003). In addition, when the expected fitness of male and female offspring differs, both parents will benefit from investing differently in sons and daughters depending on which sex has the highest expected fitness (Trivers and Willard 1973; Charnov 1982; Frank 1990). Males generally have a greater potential reproductive rate than females and hence also a larger variance in fitness with the fittest males being fitter than the fittest females. Therefore, if attractiveness were important to male fitness and also heritable, then sons of attractive males would be expected to have a higher fitness than daughters of attractive males. Females would then benefit from skewing the sex ratio of their offspring towards sons if they had mated with a male that was relatively attractive (Ellegren et al. 1996).

As in paper **I**, it is necessary to manipulate female perception of the male leg rubbing behaviour to isolate any effects on differential allocation or sex allocation while avoiding effects of any correlated traits. In this study, virgin females were mated to either a normal male or to a male with shortened mid-legs. The leg rubbing behaviour was quantified and females were allowed to oviposit for 12 days after mating. The adult offspring were counted and sexed by inspecting their extruded genitalia. Whereas there was no effect of rate of leg rubbing on offspring production of females that had mated to males with shortened legs, offspring production increased with rate of leg rubbing in females that had mated to normal males. However, the positive association between offspring production and rate of leg rubbing was not quite significant. When the data from paper **I** were reanalysed, we found that females mated to normal males at their second mating produced significantly more offspring than females mated to males with shortened legs. Taken to-

gether, these two studies provide some support for an effect of copulatory courtship on offspring production in *T. castaneum*. There was no effect of leg rubbing on offspring sex ratio. Hence, females do not seem to vary the sex ratio of their offspring depending on the attractiveness of their mates.

Heritability of the leg rubbing behaviour of *T. castaneum*

Females may gain indirect genetic benefits by preferring males with certain traits if these males possess genes that will make their offspring relatively attractive or viable (Andersson 1994; Houle and Kondrashov 2002). Several studies have found correlations between male traits used by females in pre-copulatory choice and viability of offspring (Møller and Alatalo 1999). This is a controversial issue however since it requires a mechanism that maintains additive genetic variance in the preferred male traits. Condition dependence of male traits has been put forward as a mechanism that could maintain the necessary genetic variance in male traits by linking their expression to the supposedly large number of genes affecting condition (Rowe and Houle 1996). The aim of paper III was to measure the heritability of the leg rubbing behaviour of *T. castaneum* and to assess whether or not it is condition dependent in its expression. We measured the intensity of the leg rubbing behaviour of males and their sons and used parent offspring regression to estimate the narrow sense heritability of this behaviour. The leg rubbing behaviour of males reared under high-density conditions, where competition for food should be substantial, and under low-density conditions, where food availability should be virtually unlimited, was compared to assess any effects of condition. We also recorded the proportion of surviving offspring in the two density treatments to test whether or not the leg rubbing behaviour of males can be used as an indicator of offspring viability.

We found genetic variance in larval to adult survival and in the rate of leg rubbing in males. However, the rate of leg rubbing in sires was not significantly related to offspring survival or condition dependent in its expression. This fits the results of Pai and Yan (2003) who found that the leg rubbing behaviour was not affected by tapeworm infection. The genetic variance in leg rubbing was mostly non-additive with very low narrow sense heritability. Therefore, we failed to document any indirect genetic benefits to choosy females either through viability of their offspring or through attractiveness of their sons. Results from other studies show that female *T. castaneum* may increase the reproductive success of their sons by being polyandrous but whether the leg rubbing behaviour plays a role in this or not is not known (Bernasconi and Keller 2001; Pai and Yan 2002).

The female cryptic preference for males with intense leg rubbing may be maintained in the absence of apparent genetic benefits if it is neutral to fe-

males. It may represent a side effect of female sensory or neural characteristics, which males have evolved to exploit (Ryan 1998). However, the situation appears to be different in the spotted cucumber beetle, *D. undecimpunctata howardi*. In the only similar study on copulatory courtship, Tallamy et al. (2002) found that females may gain indirect genetic benefits by favouring males with a vigorous copulatory courtship behaviour since sons of these males inherit their fathers vigorous behaviour and mating success (Tallamy et al., 2003).

Male inflicted harm in *C. maculatus*

The aim of paper **IV** was to assess whether or not male *C. maculatus* benefit from using their spiny genitalia to harm their mates and to evaluate the importance of the female kicking behaviour in reducing the costs of these injuries. The extent of the injuries caused by male genitalia can be increased by shortening the female hind-legs and thereby preventing females from kicking their mates (Crudginton and Siva-Jothy 2000). It has been suggested that males may reduce the remating propensity of their mates or increase their rate of offspring production by harming them (Michiels 1998; Lessells 1999; Johnstone and Keller 2000). We compared remating propensity and rate of oviposition in females that had their hind-legs shortened prior to their first mating (and hence were unable to kick their mates, non-kicking females) with that of females that had their hind-legs shortened immediately after their first mating (and hence were able to kick their mates, kicking females). We also mated other females twice and manipulated their ability to kick at the second mating to assess any effects of male inflicted harm on sperm precedence.

Males that mated with females that were unable to kick them did not benefit through delayed female remating or increased rate of oviposition. Nor were they more successful than males that had mated with normal females when competing with sperm from a previous mating. This suggests that the spines have evolved for reasons other than harming females. One possibility is that they serve as an anchor during copulation. Males often try to interfere with copulating pairs and avoiding being dislodged may be more important to males than any negative effects of harming their mates. We compared the lifetime offspring production of non-kicking females and kicking females. Females that were not able to kick their mates during copulation suffered reduced lifetime fecundity. This suggests that the injuries males inflict really are costly to females and that the kicking behaviour mitigates these injuries.

The effects of copulation duration in *C. maculatus*

Control over copulation duration is likely to be a cause of sexual conflict in many species. Males of polyandrous species are expected to prefer longer copulations than females since they may benefit from transferring large numbers of sperm, large quantities of manipulative substances and/or from mate guarding (Parker 1974; Eberhard 1996; Simmons 2001). Females, on the other hand, may prefer copulations that last just long enough for them to receive the sperm they need to minimise the costs of mating such as time waste, energetic costs, increased predation risk and injuries caused by male behaviour (Daly 1978; Watson et al. 1998; Simmons 2001). The aim of paper V was to determine whether or not the spiny male genitalia and the female kicking behaviour reflect a sexual conflict over copulation duration in *C. maculatus*. We manipulated copulation duration experimentally and quantified its effects on the size of the transferred ejaculate, female lifetime offspring production, female remating propensity and sperm precedence. Copulating males are attached firmly to females by their genitalia and are virtually impossible to dislodge. However, severing the abdomen of a copulating male with a pair of micro-scissors will cause the male genitalia to deflate and it is then not difficult to pull them out of the female. We used this technique to manipulate copulation duration. Matings were conducted at 21°C. Females usually start to kick after about six minutes at this temperature and matings normally last for about eight minutes (personal observation). We shortened the hind legs of all females to be able to extend copulations beyond their normal length (see above). To assess the effects of copulation duration on size of the transferred ejaculate and female lifetime offspring production, females were allowed to mate once for six, eight or ten minutes.

Size of the transferred ejaculate was measured by weighing males and females immediately before and immediately after mating. It increased with copulation duration even when copulations were extended beyond their normal length. Females with relatively long copulation durations did not suffer from reduced lifetime offspring production. Instead, females that copulated for long times produced significantly more offspring than did females that copulated for short times. This was most likely due to material resources that females derive from the large ejaculates since sperm limitation should not be a problem for females (Eady 1995) and because there was no effect of copulation duration on the rate of oviposition, which would suggest an effect of manipulative substances in the ejaculates.

We conducted two experiments to evaluate the effects of copulation duration on sperm precedence. First, virgin females were mated to virgin males for six, eight or ten minutes and then, 48 hours later, to another virgin male for eight minutes. Second, virgin females were mated to virgin males for eight minutes and then, 48 hours later, to another virgin male for six, eight or

ten minutes. The second male to mate in the first experiment and the first male to mate in the second experiment had been sterilised by irradiation prior to mating (Parker 1970; Boorman and Parker 1976; Eady 1991). This enabled us to calculate the proportion of eggs fertilised by the first male to mate (P1) in the first experiment and the second male to mate in the second experiment (P2) by recording the number of viable and non-viable eggs. Viable eggs can be readily distinguished from non-viable eggs because they fill up with shavings from the bean and turn a milky white as the larvae burrow into the bean. The hatched larvae also leave tunnels in the beans that are easy to see when eggs are removed.

Surprisingly, long copulations were associated with lowered paternity success for males mating as first males. Why P1 decreases with increasing copulation duration of the first male is unclear and contrasts with many studies that have found the opposite pattern (Simmons 2001). However, there was also a similar effect of copulation duration on P1 in paper I. There was no significant effect of copulation duration on P2. We used the proportion of females that remated after 48 hours as a measure of the effect of copulation duration on female remating propensity. The proportion of females that remated decreased with copulation duration but this pattern was not significant.

Stimuli provided by copulating males or their ejaculates often trigger a decrease in female receptivity to courting males (Eberhard 1996). It has been suggested that some males may prolong copulations to ensure that enough time passes for these stimuli to take effect (Parker 1970). To test this hypothesis in *C. maculatus*, we recorded the proportions of females from three experimental groups that remated. All females had intact hind legs in this experiment. In the first group, copulations were interrupted after six minutes and females were placed with new males directly. Females of the second group also had their first copulations interrupted after six minutes but were not placed with new males until four minutes after the first copulation had been interrupted. In the third group, copulations were not interrupted and the females were placed with new males immediately after the first copulation had ended. There were no differences in female remating propensity among the three groups.

We found little evidence for a conflict over copulation duration in *C. maculatus* since females do not suffer from relatively long copulations but instead benefit from the large ejaculates obtained from them. Apparently, the female mate kicking behaviour mitigates the injuries caused by the spiny male genitalia (Crudginton and Siva-Jothy 2000; paper IV) but not through a shortening of copulation duration. However, female *C. maculatus* typically mate with more than one or two males and it is important to note that we cannot rule out the possibility that copulation duration has negative effects when injuries accumulate over more than two matings. Copulating males are attached firmly to their mates and the main function of the spines appears to

be to act as an anchor. Spiny genitalia may have evolved because decreasing the risk of copulations being interrupted prematurely through dislodgement by other males outweighs the costs of reduced female fecundity associated with the injuries caused by the spines. When males compete with sperm from future matings they suffer from long copulations. However, we found a trend suggesting that long copulations and the resulting large ejaculates reduce female remating propensity. Other studies have found similar results in *C. maculatus* (Eady 1995; Savalli and Fox 1999) and this may be more important to males than the reduced success in sperm competition when females do remate.

Inbreeding and female mating behaviour in *C. maculatus*

In spite of the often dramatic negative effects of inbreeding on offspring fitness (Thornhill 1993; Falconer and Mackay 1996; Pusey and Wolf 1996), matings between closely related individuals sometimes occur (Thornhill 1993). This may be because females are unable to recognise related males before mating with them. As an alternative to pre-copulatory choice, polyandrous females could avoid inbreeding through cryptic female choice mechanisms if they can assess mate relatedness during or after copulation. These mechanisms include increasing remating propensity and decreasing rate of offspring production in response to incestuous matings. The aim of paper VI was to evaluate whether or not female *C. maculatus* use their mating behaviour or rate of offspring production to avoid the negative effects of inbreeding. We conducted four experiments in which virgin females were offered virgin males that were either their brothers or unrelated to them. Virgin females did not discriminate against brothers and did not appear to exercise any pre-copulatory choice at all since only 12 out of 149 females that were offered a brother and 13 out of 143 females that were offered an unrelated male refused to mate. Females were allowed to oviposit on black-eyed beans (*Vigna unguiculata*) and were offered new males for ten minutes after 6 hours in the first experiment, after 24 hours in the second experiment and after 12, 16 and 20 hours in the third experiment. In the fourth experiment, females were kept without access to oviposition substrate. Between 7 and 24 hours after the first mating they were offered new males for 5 minutes every hour and then 26, 27, 31, 35, 40, 51, 64, 75 and 98 hours after the first mating.

We recorded the proportion of eggs laid in between the two mating opportunities that resulted in adult offspring in the first experiment and found a significant effect of the relatedness of parents. Offspring from incestuous matings had an 18% lower egg to adult survival than offspring from females mated to unrelated males. In spite of this cost of inbreeding, females did not

change their remating propensity in response to incestuous matings in any of the four experiments. It is still uncertain how important inbreeding avoidance has been in the evolution of insect behaviour in general. Our results suggest that it has not been a major driving force in the evolution of the mating behaviour of *C. maculatus*. However, female *C. maculatus* may use sperm choice, another mechanism of cryptic choice, to discriminate against closely related males. This has been documented in another insect, the field cricket *Gryllus bimaculatus* (Tregenza and Wedell 2002; Bretman et al. 2004).

Water and female mating behaviour in *C. maculatus*

Male *C. maculatus* transfer large ejaculates weighing up to 10% of the males' body weight in virgin males (Savalli and Fox 1998; personal observation). Females seem to derive material benefits from these ejaculates (Fox 1993; Ofuya 1995; Savalli and Fox 1999; paper V). There are also substantial costs of mating to females because of the spines on the male genitalia. The trade-off between the costs and benefits of mating may be important in shaping female mating behaviour. While the costs of mating are likely to be additive or even multiplicative (Arnqvist and Nilsson 2000; Johnstone and Keller 2000) any material benefits should show diminishing returns (Arnqvist and Nilsson 2000). Gaining additional nutrients or water from nuptial gifts will be more valuable to females that are starved or dehydrated than to females that are well fed or hydrated. Hence, if females can adjust their mating behaviour actively in response to their need for additional resources, they should only mate when the benefits outweigh the costs. This provides males with an opportunity to influence female mating behaviour. Males that donate large gifts will make it less beneficial for their mates to remate and will therefore reduce the risk of future sperm competition. Interestingly, this is analogous to the suggestion put forth by Johnstone and Keller (2000) that males may evolve harmful traits because when costs of mating are escalating, the more a male harms his mate the more costly it will be for her to remate.

The aim of paper VII was to assess whether or not female *C. maculatus* change their mating behaviour in response to their need for water. This would indicate that the material resources they derive from male ejaculates are, at least partly, water. The large male ejaculate may then be maintained because providing females with a large amount of water will delay their remating. Results from earlier studies suggest that females do delay their remating when they receive relatively large ejaculates (Eady 1995; Savalli and Fox 1999). However, these results could be caused by manipulative sub-

stances in the ejaculates that reduce female remating propensity rather than any material resources.

C. maculatus inhabits areas that are arid for large parts of the year (Labeurie 1981) and is generally reared without access to water in the laboratory. Water could therefore be an important factor limiting female reproductive output and lifespan. I found that giving females access to deionised water in Eppendorf tubes sealed with cotton wool increased their longevity and lifetime offspring production both when they were kept with and without a pair of males (figure 3). Presence of males had negative effects on female longevity and offspring production, which was most likely due to female inability to escape male harassment in the Petri dishes they were kept in.

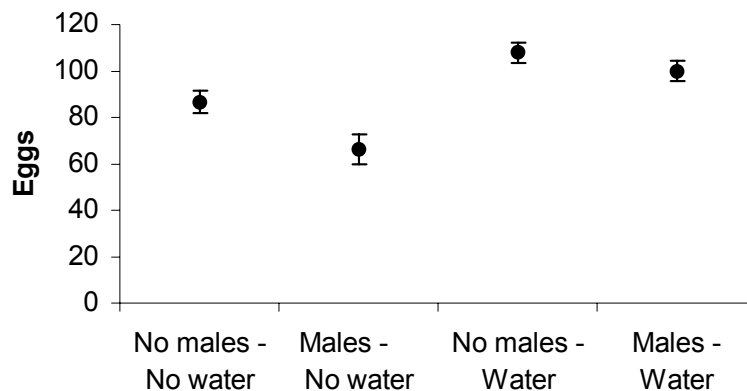


Figure 3. Average lifetime egg production (\pm standard error of mean) of females of the four experimental groups from the second experiment.

To assess any effects of water on female mating behaviour, I mated virgin females to virgin males and then kept them with or without access to water in Petri dishes with approximately 60 beans. Every 24 hours, females were placed with virgin males in a small Petri dish for ten minutes and I recorded whether or not they mated. This continued until all females were dead. I recorded female lifespan and lifetime offspring production.

In spite of living longer and producing more offspring, females kept with access to water were not only significantly less likely to mate at their first remating opportunity, but also mated significantly fewer times over their entire lifespan than females kept without access to water (figure 4). The finding that females mate more frequently when they do not have access to water suggests that the benefits females obtain from the large ejaculates are, at least partly, hydration benefits. It also suggests that males can delay their

mates' remating by transferring a substantial amount of water during copulation and that this may maintain the large ejaculates. In doing so, they will also increase the amount of their material investment that goes into the production of their own offspring rather than of offspring fathered by other males.

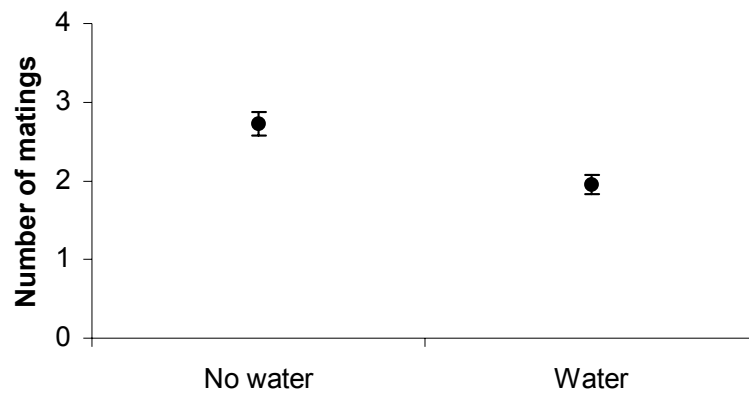


Figure 4. Average total number of matings (\pm standard error of mean) of females kept with or without access to water.

Conclusions

The studies presented in this thesis contribute to the growing body of evidence that supports the importance of post-copulatory processes in mediating the effects of interactions between males and females, in terms of the reproductive success of male and female animals and indeed also of plants (Bernasconi et al. 2004). However, direct experimental evidence for a role of cryptic female choice in the evolution and maintenance of male traits remains scarce. The results of papers **I** and **II** suggest that the leg rubbing behaviour of male *Tribolium castaneum* functions as copulatory courtship and is selected through cryptic female choice. Males with vigorous leg rubbing appear to benefit both through increased sperm precedence and through a slight increase in female offspring production. That we were unable to find any additive genetic variance in this behaviour or an association with viability (paper **III**) indicates that females do not gain any significant indirect genetic benefits from preferring males with a vigorous rubbing behaviour. Female preferences may be maintained even in the absence of apparent genetic benefits to females, if they are neutral to females and represent side effects of female sensory or neural characteristics, which males have evolved to exploit (Ryan 1998). However, this does not exclude the possibility that there were once indirect genetic benefits to choosy females that led to a refinement of the preference. This may be how many female preferences originate, including ones that enable females to find good genes for their offspring (Macías Garcia and Ramirez 2005).

Manipulating female perception of male traits is likely to be a fruitful avenue for future research in cryptic female choice as it has been in studies of pre-copulatory female choice (e.g. Andersson 1994). In addition to studies on *T. castaneum* and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* (Tallamy et al. 2003) a recent study on the guppy *Poecilia reticulata* found that female perception of male coloration affects male insemination success (Pilastro et al. 2003).

Harmful male traits may have evolved to manipulate female post-copulatory processes in the favour of the harming male (Michiels 1998; Lessells 1999; Johnstone and Keller 2000). The results presented in paper **IV** suggest that the injuries inflicted by the spines on the genitalia of male *Callosobruchus maculatus* are costly to females and that the female kicking behaviour has evolved to mitigate these costs. However, males do not seem to benefit from inflicting these costly injuries and a likely function for the

spines seems to be to act as an anchor during copulation. Two other studies on insects have attempted to assess any benefits to males from harming their mates. Hosken et al. (2003) found that the number of previous copulations, and hence the total amount of damage caused by the spiny male genitalia, did not have a positive association either with female reproductive investment or with female reluctance to remate in the dung fly *S. cynipsea*. Morrow et al. (2003) found that harming female insects (*Drosophila melanogaster*, *T. castaneum* and *C. maculatus*) immediately after mating did not cause any responses that would be favourable to the mates of these females. These studies suggest that the injuries caused by harmful male traits are side effects of other functions. More studies are needed to evaluate whether or not male inflicted harm *per se* sometimes evolve to manipulate female controlled post-copulatory processes. Ideally, the harmful male traits should be manipulated to become both more and less harmful rather than just more harmful as in paper IV.

Together, papers IV, V and VII suggest that females trade-off the substantial costs of mating against their need for additional water provided by male ejaculates. Males may therefore delay female remating by transferring ejaculates containing large amounts of water. Similar trade-offs may have been important in the evolution of large ejaculates in many other insect species. Males may first evolve ejaculates that are large enough for females to derive material resources from because of selection on males to transfer large numbers of sperm or manipulative seminal substances to meet sperm competition (Wickler 1985; Simmons and Parker 1989; Cordero 1996; Arnqvist and Nilsson 2000). These ejaculates may then be modified to provide females with larger amounts of resources. More research quantifying female costs of mating as well as determining the composition of ejaculates and the effects of seminal substances on females is required to gain a more complete picture of the evolution of large male ejaculates.

The studies of *T. castaneum* and *C. maculatus* presented in this thesis reveal the complexity of sexual interactions and the importance of post-copulatory processes for the fitness of both males and females. It is clear that at least some male traits evolve in response to cryptic female choice.

Sammanfattning på svenska

Kryptiskt honligt val och hanligt parningsbeteende: Sexuella interaktioner hos skalbaggar.

De flesta djurarter använder sig av sexuell fortplantning. Detta medför att den reproduktiva framgången hos både hanar och honor är beroende av interaktioner med individer av det motsatta könet. Längre ägnade sig biologer som studerade samspelet mellan könen enbart åt företeelser som äger rum före parningen. Man noterade att hanar av de flesta arter investerar mycket lite i sin avkomma och att deras reproduktion därför i stor utsträckning begränsas av hur många honor de kan para sig med. De flesta honor, å andra sidan, begränsas inte av hur många hanar de parar sig med utan av hur mycket de kan investera i produktion av avkomma och är därför mer kräsna i sina partnerval. Detta förklarade varför konkurrens mellan hanar över parningstillfällen, hanligt uppvaktande av honor och honligt val av hanar att para sig med uppenbarligen är viktiga drivkrafter bakom utvecklingen av en mängd hanliga egenskaper.

De senaste 35 åren har man insett mer och mer att eftersom honor av många arter parar sig med flera hanar, kan även företeelser som äger rum efter parningen vara viktiga. Engelsmannen Parker påpekade 1970 att när honor parar sig med flera hanar konkurrerar spermier från dessa hanar med varandra om att befrukta honornas ägg. Denna så kallade spermiekonkurrens accepterades snabbt som ett viktigt fenomen som drivit utvecklingen av en rad hanliga anpassningar. För att bemöta konkurrensen från andra hanars spermier, för många hanar över en enorm mängd spermier vid parning. Vissa hanar använder speciella strukturer på sina könsorgan för att avlägsna spermier från tidigare parningar. Hanar kan också försöka förhindra honor från att para sig igen genom att föra över substanser som minskar deras parningsvillighet.

För drygt 20 år sedan lanserades idén att precis som hanlig konkurrens kan fortsätta efter parningen i form av spermiekonkurrens, kan honligt val också äga rum efter parningen har inletts. Eftersom det är svårt för både hanar och forskare att se vad som försiggår inuti honorna kallade amerikanen

Thornhill denna form av honligt val för kryptiskt honligt val. Kryptiskt honligt val kan definieras som en honlig benägenhet att gynna hanar med vissa egenskaper genom mekanismer som verkar efter det att parningen inletts. Lite mindre kryptiskt skulle man kunna säga att det handlar om honligt inflytande över hur många avkomor varje hane får bli far till som utövas efter det att parningen inletts. Honors fortplantningsorgan är ofta komplexa och flera saker, såsom transport av spermier och ägglossning, måste ske innan inseminering kan leda till befruktning. Man kan därför tänka sig en mängd olika mekanismer som honor kan använda för att gynna eller missgynna hanar som t.ex. upptag och dumpning av spermier, selektivt användande av lagrade spermier från olika hanar, varierande väntetid till nästa parning och ändrad investering i produktion av avkomma. Trots detta är, i motsats till spermiekonkurrens, betydelsen av kryptiskt honligt val fortfarande omdiskuterad.

Syfte

Syftet med den här avhandlingen var att utvärdera betydelsen av kryptiskt honligt val för utvecklingen av hanliga egenskaper samt att öka förståelsen av hur samspelet mellan hanliga och honliga egenskaper påverkar hanlig och honlig reproduktiv framgång. Jag har använt mig av två skalbaggsarter som båda är vitt spridda skadeinsekter i varmare delar av världen. Den kastenjeb-runna mjölbaggen, *Tribolium castaneum*, angriper mjöl och spannmålsprodukter och föds vanligen upp på en blandning av grahamsmjöl och bryggerijäst i laboratoriet. Generationstiden är ungefär en månad och skalbaggen kan leva upp till ett år under gynnsamma förhållanden. Fröbaggen *Callosobruchus maculatus* larver utvecklas inuti bönor och ärtor. I laboratoriet hålls den ofta på bönan *Vigna unguiculata*. Denna skalbagge har en generationstid på drygt tre veckor och lever ungefär 10 dagar som vuxen.

Tribolium castaneum

Hanar av en mängd olika arter utför beteenden under själva parningen som verkar vara ämnade enbart åt att stimulera honorna på olika sätt. De kan bl.a. använda sig av ben, antenner, mundelar eller könsorgan. Det har föreslagits att dessa uppvaktningsbeteenden utvecklats för att påverka kryptiskt honligt val men få studier har försökt påvisa ett samband. För att göra detta krävs att honornas uppfattning av beteendet i fråga kan manipuleras. Att enbart hitta ett samband mellan t.ex. frekvensen av ett beteende och hanlig befruktningsframgång är inte tillräckligt eftersom beteendet kan vara korrelerat med and-

ra viktiga faktor. Man kan t.ex. tänka sig att hanar med ett intensivt beteende också har en hög spermieproduktion. Hanar av *T. castaneum* stryker under parningen sina ben längs ytterkanterna av honornas täckvingar. Honornas uppfattning av detta beteende kan manipuleras genom att hanarnas ben förkortas. Ben som blivit förkortade används precis som vanligt i benstrykningsbeteendet men når inte kanterna av honans täckvingar.

I uppsats **I** undersöktes om honorna använder kryptiskt honligt val för att gynna hanar med ett energiskt benstrykningsbeteende. För att göra detta observerade vi frekvensen av benstrykningar och relaterade den till hur stor andel av en honas avkomma varje hane blev far till när honan parade sig med två hanar. Varje hona fick först para sig med en hane som var bärare av en mutation som gör skalbaggarna svarta istället för bruna. Tjugofyra timmar senare fick honorna para sig med en brun hane som antingen var normal eller hade fått ett eller två ben förkortade före parningen. Eftersom individer som har en svart och en brun förälder är mörkbruna, var det sedan möjligt för oss att se hur stor andel av avkomman varje hane blivit far till. Det visade sig att ju intensivare de bruna hanarnas benstrykningsbeteende var, desto större andel av äggen hade befruktats av deras spermier. Detta gällde dock enbart normala hanar. Hos hanar med förkortade ben fanns inget samband mellan benstrykningsfrekvens och befruktningsframgång. Detta berodde inte på att hanar med förkortade ben av någon anledning för över färre spermier; något vi kontrollerade i ett senare försök. Honor använder alltså kryptiskt honligt val för att gynna hanar med ett intensivt benstrykningsbeteende. Hur detta går till är inte känt, men det är möjligt att benstrykningsbeteendet på något sätt påverkar hur stor andel av sperman som honorna transporterar till sina spermieaggregatsorgan och hur stor andel som dumpas.

I uppsats **II** undersökte vi om honor även gynnar hanar med ett intensivt benstrykningsbeteende genom att höja produktionstakten av avkomma. Detta skulle gynna hanarna eftersom deras spermier då hinner befrukta fler ägg innan de måste konkurrera med spermier från nästföljande parning. Vi fann ett visst stöd för en sådan effekt men den var inte statistiskt signifikant. Emellertid visade det sig då vi analyserade data från uppsats **I**, att honor som vid sin andra parning parat sig med normala hanar producerade signifikant fler avkommor än honor som parat sig med hanar med förkortade ben.

Honor kan tjäna på att föredra hanar med ett intensivt benstrykningsbeteende om deras söner ärver det attraktiva beteendet eller om det är en signal att hanarna är i god form och har goda gener som kommer att ge livskraftig avkomma. I uppsats **III** jämförde vi benbeteendet hos fäder och söner och fann att det hade mycket låg ärftlighet. Det visade sig även att fädernas beteende inte hade något samband med avkommans överlevnadschanser. Honor verkar alltså inte tjäna på att favorisera hanar med ett intensivt benstrykningsbeteende i vår population. Preferenser för hanliga egenskaper kan vara bieffekter av honliga egenskaper som har andra funktioner och finnas kvar om de inte är skadliga för honorna.

Callosobruchus maculatus

Det har föreslagits att hanar kan tjäna på att skada honorna de parar sig med på två sätt. Skadorna kan leda till att honorna blir mer motvilliga att para om sig med andra hanar. Alternativt, kan honorna uppfatta skadorna som ett hot mot sin överlevnad och öka produktionstakten av avkomma för att hinna producera så många avkommor som möjligt innan de dör. I det första fallet gynnas hanarna genom att de undviker spermiekonkurrens och i det andra genom att deras spermier hinner befrukta fler ägg innan honorna parar sig igen. Hanar av *C. maculatus* har taggar på sina könsorgan som skadar honornas fortplantningsorgan under parningen. Honorna sparkar hanarna med sina bakben under parningen. Om de förhindras från att sparka förlängs parningarna och skadorna blir mer omfattande. I uppsats **IV** undersökte vi om hanarna tjänar på att skada honorna och om honornas sparkande verkligen mildrar de negativa effekterna av taggarna. Vissa honor förhindrades från att sparka under parningarna genom att deras bakben förkortades så att de inte kunde nå hanarna. Övriga honor fick istället sina bakben förkortade omedelbart efter parningarna. Resultaten visade att hanarna inte kan förlänga tiden tills honorna parar sig igen eller stimulera deras äggläggning genom att tillfoga dem relativt allvarliga skador. Detta tyder på att taggarna inte utvecklats för att skada honorna utan för att fylla någon annan funktion. En trolig förklaring är att de fungerar som ett ankare och förhindrar andra hanar att tränga undan hanar som parar sig. Honor som inte kunde sparka hanarna under parningarna producerade färre ägg under sin livstid än honor som kunde sparka. Det verkar alltså som om sparkandet mildrar skadorna honorna ådrar sig.

Parningsbeteendet hos *C. maculatus* skulle kunna bero på en konflikt över parningslängden. Hanar kan ofta förväntas föredra långa parningar eftersom de då kan föra över större mängder spermier och substanser som gör honorna mindre parningsvilliga eller stimulerar deras äggproduktion. Honor å sin sida kan förväntas föredra kortare parningar som ger dem de spermier de behöver men minimerar kostnaderna av t.ex. skador hanarna eller substanserna de för över åsamkar dem. Vi fann inte något stöd för en sådan konflikt när vi manipulerade parningslängden i uppsats **V**. Tvärtom gynnades honor av långa parningar. Honor som parat sig relativt länge producerade fler avkommor under sin livstid än honor som parat sig relativt kort tid när honorna endast parade sig vid ett tillfälle. Detta berodde inte på att honor med korta parningar led av brist på spermier eftersom hanar vid en parning för över många fler spermier än vad en hona behöver under hela sitt liv. Ejakulaten (sädsvätskan) är mycket stora hos *C. maculatus* och väger upp till 10% av hanens kroppsvikt. Det verkar som om honorna tillgodogör sig vatten och/eller när-

ing från dem vilket kan förklara resultaten i uppsats V eftersom ejakulatstorleken ökade med parningslängden.

I uppsats VI undersökte vi om honor av *C. maculatus* kan undvika inavel genom att avvisa uppvaktande bröder eller genom att para om sig snabbt när de parat sig med bröder. Vi fann att trots att inavel hade en negativ effekt på avkommans överlevnad, parade sig honor lika gärna med bröder som med andra hanar. De förkortade inte heller tiden till nästa parning när de parat sig med bröder. Inavelsundvikande har uppenbarligen inte spelat en avgörande roll i utvecklingen av honornas parningsbeteende hos denna art.

Parningar medför båda påtagliga kostnader i form av fysiska skador och fördelar i form av vatten och/eller näring för honor av *C. maculatus*. Honorna borde följaktligen enbart para sig när fördelarna överväger nackdelarna. Medan skadorna förmodligen alltid är kostsamma, kommer värdet av fördelarna att bero på honornas behov av att få ytterligare vatten eller näring. Det lönar sig inte lika mycket att äta när man är mätt som när man är hungrig. Detta ger hanarna en möjlighet att fördröja honornas nästa parning genom att förse dem med ett stort ejakulat innehållande en stor mängd vatten eller näring. Tidigare studier har visat att stora ejakulat gör att *C. maculatus* honor väntar längre med att para sig igen. Detta behöver dock inte nödvändigtvis bero på resurser honorna får från ejakulaten utan skulle också kunna orsakas av substanser hanarna för över som gör honorna mindre parningsvilliga. För att komma runt det här problemet, undersökte jag i uppsats VII om honor parar sig mindre ofta när de har tillgång till vatten. *C. maculatus* lever i torra områden och hålls normalt sett utan tillgång till vatten i laboratoriet. Vatten kan därför vara en viktig begränsande faktor för honornas livslängd och avkommeproduktion. Jag höll honor med och utan tillgång till vatten och gav dem chansen att para sig en gång varje dag så länge de levde. Det visade sig att honor som hade tillgång till vatten levde längre och producerade fler avkomor än honor som inte hade tillgång till vatten. Trots detta parade de sig färre gånger. Det verkar alltså som om resurserna honorna får från ejakulaten, åtminstone till viss del, består av vatten samt att honornas parningsvillighet påverkas av deras törst. Detta medför att hanarna kan minska honornas parningsvillighet genom att förse dem med en stor mängd vatten.

Sammanfattningsvis kan sägas att denna avhandling visar på betydelsen, för både hanar och honor, av företeelser som äger rum under och efter parningen. Kryptiskt honligt val verkar vara av stor vikt för hanarnas reproduktiva framgång hos både *T. castaneum* och *C. maculatus*.

Acknowledgements

First and foremost, I would like to thank my supervisor Göran Arnqvist for giving me the opportunity to work on a great project in a fascinating field and for sending me down the road of insect sex and leg ablation with good advice such as “there are certain things you just can’t do with goshawks”. Thanks also for all the valuable discussions, comments and suggestions!

My other collaborators have also all had a positive influence on this thesis. Thanks to Tom Tregenza, Rolando Rodríguez-Muñoz and David “la Maquina” Canal. Thanks also to Bo Stenerlöv and Karin Karlsson for practical help with irradiation of beetles.

Thanks to the “deputy” supervisors, Barbara Giles, Göran Englund and Christian Otto in Umeå and Mats Björklund in Uppsala for encouragement and advice.

Thanks to all past and present members of our group for practical help and intellectual input! Urban Friberg for many stimulating discussions over the years and for thinking just like me but in a different way. Tina Nilsson and Claudia Fricke for being fellow flour beetle pioneers up in Umeå. Johanna Rönn for sharing your expertise in male genitalia with great enthusiasm. José Andrés for sharing your expertise in real food with great enthusiasm. Ted Morrow for knowing when not to use the word “bring”. Mari Katvala for being really cool in that Finnish down-to-earth way. Alexei Maklakov for revealing the true nature of “Tjeborasjka i Gena”. Damian Dowling for teaching me some useful dirty footy tricks.

I have had the privilege of spending time at three great universities during my PhD. Hence, quite a few people have been forced to share offices with me. Thanks to Jens Andersson and Urban Friberg; Tomas Brodin; Amanda Bretman, Clara Saldamando, Kirsten Klappert, Pedro Pedro et al.; Marnie Demandt. You have all been excellent office mates.

Thanks to Maano Aunapuu, Johannes Bergsten, Urban Friberg, Barbara Giles, Micael Jonsson, Johan Lövgren, Anders Nilsson, Tina Nilsson, Bo W Svensson, and others for pleasant teaching collaborations.

A big thanks to Tom Tregenza for inviting me to spend some time in the second best county of England and for, together with Nina Wedell, Roger Butlin and others, being very welcoming hosts at the School of Biology at the University of Leeds.

Administrators make the world go around. Thanks to Sussi Mikaelsson and Ylva Linghult in Umeå and Ingela Ericsson in Uppsala for making my life easier.

Thanks to all the great people who happened to be at EG in Umeå, the School of Biology in Leeds and Animal Ecology in Uppsala when I was also there!

Thanks to my parents for commenting on the Swedish summary and for stimulating my early interest in the natural world. Thanks to my mother for having the patience to supervise me during long hours while I was conducting extensive studies of ducks back when I was about the size of a mallard myself. Thanks to my father for taking me on countless birding excursions. Thanks also to my sister for conceding that looking for phalaropes in the mountains is actually a meaningful way of spending a family vacation.

Thanks to Fleur for commenting on an earlier draft of this summary and for being wonderful. The Day is almost here now.

Finally, I would like to acknowledge the financial support of The Swedish Foundation for International Cooperation in Research and Higher Education (STINT), Stiftelsen för Zoologisk Forskning and The Foundation of Knut and Alice Wallenberg.

References

- Andersson, M. 1994 *Sexual Selection*. Princeton University Press.
- Arnqvist, G. and Nilsson, T. 2000 The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* **60**, 145-164.
- Ben-Ari, E.T. 1999 Paternity battles. How males compete for fatherhood via sperm competition. *BioScience* **49**, 951-956.
- Ben-Ari, E.T. 2000 Choosy females. Exploring the role of cryptic female choice in sexual selection and battles over paternity. *BioScience* **50**, 7-12.
- Bernasconi, G., Ashman, T.-L., Birkhead, T.R., Bishop, J.D.D., Grossniklaus, U., Kubli, E., Marshall, D.L., Schmid, D., Skogsmyr, I., Snook, R.R., Taylor, D., Till-Bottraud, I., Ward, P.I., Zeh, D.W. and Hellriegel, B. 2004 Evolutionary ecology of the prezygotic stage. *Science* **303**, 971-975.
- Bernasconi, G. and Keller, L. 2001 Femaly polyandry affects their sons' reproductive success in the red flour beetle *Tribolium castaneum*. *J. Evol. Biol.* **14**, 186-193.
- Birkhead, T.R. 1998 Cryptic female choice: Criteria for establishing female sperm choice. *Evolution* **52**, 1212-1218.
- Birkhead, T.R. and Møller, A.P. 1998 *Sperm Competition and Sexual Selection*. Academic Press, London.
- Birkhead, T.R. and Pizzari, T. 2002 Postcopulatory sexual selection. *Nature Rev. Genet.* **3**, 262-273.
- Bishop, J.D.D. 1996 Female control of paternity in the internally fertilizing ascidian *Diplosoma listerianum*. I. Autoradiographic investigation of sperm movements in the female reproductive tract. *Proc. R. Soc. Lond. B* **263**, 369-376.
- Blanckenhorn, W.U., Hosken, D.J., Martin, O.Y., Reim, C., Teuschl, Y. and Ward, P.I. 2002 The costs of copulating in the dung fly *Sepsis cynipsea*. *Behav. Ecol.* **13**, 353-358.
- Bloch Qazi, M.C., Aprille, J.R. and Lewis, S.M. 1998 Female role in sperm storage in the red flour beetle, *Tribolium castaneum*. *Comp. Biochem. Physiol.* **120**, 641-647.
- Bloch Qazi, M.C., Herbeck, J.T. and Lewis, S.M. 1996 Mechanisms of sperm transfer and storage in the red flour beetle (Coleoptera: Tenebrionidae). *Ann. Entomol. Soc. Am.* **89**, 892-897.
- Boorman, E. and Parker, G.A. 1976 Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol. Entomol.* **1**, 145-155.
- Bretman, A., Wedell, N. and Tregenza, T. 2004 Molecular evidence of postcopulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. *Proc. R. Soc. Lond. B.* **271**, 159-164.

- Burley, N. 1986 Sexual selection for aesthetic traits in species with biparental care. *Am. Nat.* **127**, 415-445.
- Charnov, E.L. 1982 *The theory of sex allocation*. Princeton University Press.
- Chapman, T., Arnqvist, G., Bangham, J. and Rowe, L. 2003 Sexual conflict. *Trends Ecol. Evol.* **18**, 41-47.
- Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F. and Partridge, L. 1995 Cost of mating in *Drosophila melanogaster* is mediated by male accessory gland products. *Nature* **373**, 241-244.
- Christy, J.H. 1995 Mimicry, mate choice, and the sensory trap hypothesis. *Am. Nat.* **146**, 171-181.
- Constantz, G.D. 1984. Sperm competition in Poeciliid fishes. In: *Sperm Competition and the Evolution of Animal Mating Systems*. Smith, R.L. ed. Academic Press, London.
- Cordero, C. 1996 On the evolutionary origin of nuptial seminal gifts in insects. *J. Insect Behav.* **9**, 969-974.
- Córdoba-Aguilar, A. 1999 Male copulatory sensory stimulation induces female ejection of rival sperm in a damselfly. *Proc. R. Soc. Lond. B* **266**, 779-784.
- Crudgington, H.S. and Siva-Jothy, M.T. 2000 Genital damage, kicking and early death. *Nature* **407**, 855-856.
- Dick, K.M. and Credland, P.F. 1984 Egg production and development of three strains of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. stored Prod. Res.* **20**, 221-227.
- Eady, P.E. 1991 Sperm competition in *Callosobruchus maculatus*: a comparison of two methods used to estimate paternity. *Ecol. Entomol.* **16**, 45-53.
- Eady, P.E. 1995 Why do male *Callosobruchus maculatus* inseminate so many sperm? *Behav. Ecol. Sociobiol.* **36**, 25-32.
- Eberhard, W.G. 1985 *Sexual selection and animal genitalia*. Harvard University Press.
- Eberhard, W.G. 1991 Copulatory courtship in insects. *Biol. Rev.* **66**, 1-31.
- Eberhard, W.G. 1994 Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution* **48**, 711-733.
- Eberhard, W.G. 1996 *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press.
- Ellegren, H., Gustafsson, L. and Sheldon, B.C. 1996 Sex ratio adjustment in relation to paternal attractiveness in a wild bird population. *Proc. Natl. Acad. Sci. USA* **93**, 11723-11728.
- Falconer, D.S. and Mackay, T.F.C. 1996 *Introduction to Quantitative Genetics*. 4th edn. Harlow: Longman.
- Faustini, D.L., Burkholder, W.E. and Laub, R.J. 1981 Sexually dimorphic setiferous sex patch in the male red flour beetle, *Tribolium castaneum* (Hebst) (Coleoptera: Tenebrionidae): site of aggregation pheromone production. *J. Chem. Ecol.* **7**, 465-480.
- Fowler, K. and Partridge, L. 1989 A cost of mating in female fruitflies. *Nature* **338**, 760-761.
- Fox, C.W. 1993 Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Funct. Ecol.* **7**, 203-208.

- Frank, S.A. 1990 Sex allocation theory for birds and mammals. *Annu. Rev. Ecol. Syst.* **21**, 13-55.
- Gavrilets, S., Arnqvist, G. and Friberg, U. 2000 The evolution of female mate choice by sexual conflict. *Proc. R. Soc. Lond. B.*
- Giga, D.P. and Smith, R.H. 1983 Comparative life history studies of four *Callosobruchus* species infesting cowpeas with special reference to *Callosobruchus rhodesianus* (Pic.) (Coleoptera: Bruchidae). *J. stored Prod. Res.* **4**, 189-198.
- Gwynne, D.T. 1997 The evolution of edible 'sperm sacs' and other forms of courtship feeding in crickets, katydids and their kin (Orthoptera: Ensifera). In *The Evolution of Mating Systems in Insects and Arachnids*. Choe, J.C. and Crespi, B.J. eds. Cambridge University Press.
- Hellriegel, B. and Ward, P.I. 1998 Complex female reproductive tract morphology: its possible use in postcopulatory female choice. *J. theor. Biol.* **190**, 179-186.
- Holland, B. and Rice, W.R. 1998 Perspective: Chase away sexual selection: Antagonistic seduction versus resistance. *Evolution* **52**, 1-7
- Hosken, D.J., Martin, O.Y., Born, J. and Huber, F. 2003 Sexual conflict in *Sepsis cynipsea*: female reluctance, fertility and mate choice. *J. Evol. Biol.* **16**, 485-490.
- Houle, D. and Kondrashov, A.S. 2002 Coevolution of costly mate choice and condition-dependent display of good genes. *Proc. R. Soc. Lond. B* **269**, 97-104.
- Jennions, M.D. and Petrie, M. 2000 Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**, 21-64.
- Johnson, J.C., Ivy, T.M. and Sakaluk, S.K. 1999 Female remating propensity contingent on sexual cannibalism in sagebush crickets, *Cyphoderris strepitans*: a mechanism of cryptic female choice. *Behav. Ecol.* **10**, 227-233.
- Johnstone, R.A. and Keller, L. 2000 How males can gain by harming their mates: Sexual conflict, seminal toxins, and the cost of mating. *Am. Nat.* **156**, 368-377.
- Labeyrie, V. 1981 *The ecology of bruchids attacking legumes (pulses)*. Dr W. Junk Publishers, The Hague.
- Lessells, C.M. 1999 Sexual conflict in animals. In: *Levels of selection in evolution*. Keller, L. ed. Princeton University Press.
- Lessells, C.M. 2005 Why are males bad for females? Models for the evolution of damaging male mating behavior. *Am. Nat.* In Press.
- Lewis, S.M. and Austad, S.N. 1994 Sexual selection in flour beetles: the relationship between sperm precedence and male olfactory attractiveness. *Behav. Ecol.* 219-224.
- Macías Garcia, C. and Ramirez, E. 2005 Evidence that sensory traps can evolve into honest signals. *Nature* **434**, 501-505.
- Mack, P.D., Hammock, B.A. and Promislow, D.E.L. 2002 Sperm competitive ability and genetic relatedness in *Drosophila melanogaster*: similarity breeds contempt. *Evolution* **56**, 1789-1795.
- Michiels, N.K. 1998 Mating conflicts and sperm competition in simultaneous hermaphrodites. In: *Sperm competition and sexual selection*. Birkhead, T.R. and Møller, A.P. eds. Academic Press, London.
- Morrow, E.H., Arnqvist, G. and Pitnick, S. 2003 Adaptation versus pleiotropy: Why do males harm their mates? *Behav. Ecol.* **14**, 802-806.

- Møller, A.P. and Alatalo, R.V. 1999 Good-genes effects in sexual selection. *Proc. Roy. Soc. Lond. B* **266**, 85-91.
- Nilsson, T., Fricke, C. and Arnqvist G. 2002 Patterns of divergence in the effects of mating on female reproductive performance in flour beetles. *Evolution* **56**, 111-120.
- Ofuya, T.I. 1995 Multiple mating and its consequences in males of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. stored Prod. Res.* **31**, 71-75.
- Olsson, M., Madsen, T., Ujvari, Beata. and Wapstra, Erik. 2004 Fecundity and MHC affects ejaculation tactics and paternity bias in sand lizards. *Evolution* **58**, 906-909.
- Olsson, M., Shine, R., Madsen, T., Gullberg, A. and Tegelström, H. 1996 Sperm selection by females. *Nature* **383**, 585.
- Parker, G. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525-567.
- Parker, G.A. 1974 Courtship persistence and female-guarding as male time investment strategies. *Behaviour* **48**, 157-184.
- Parker, G.A. 1979 Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects*. Blum, M.S. and Blum, N.A. eds. Academic Press, New York.
- Partridge, L. and Hurst, L.D. 1998 Sex and conflict. *Science* **281**, 2003-2008.
- Pai, A. and Yan, G.Y. 2002 Polyandry produces sexy sons at the cost of daughters in red flour beetles. *Proc. R. Soc. Lond. B* **269**, 361-368.
- Pai, A. and Yan, G.Y. 2003 Effects of tapeworm infection on male reproductive success and mating vigor in the red flour beetle, *Tribolium castaneum*. *J. Parasit.* **89**, 516-521.
- Pilastro, A., Simonato, M., Bisazza, A. and Evans, J.P. 2004 Cryptic female preference for colorful males in guppies. *Evolution* **58**, 665-669.
- Pizzari, T. and Birkhead, T.R. 2000 Female fowl eject sperm of subordinate males. *Nature* **405**, 787-789.
- Pizzari, T., Løvlie, H. and Cornwallis, C.K. 2004 Sex-specific, counteracting responses to inbreeding in a bird. *Proc. R. Soc. Lond. B* **271**, 2115-2121.
- Pusey, A. and Wolf, M. 1996 Inbreeding avoidance in animals. *Trends Ecol. Evol.* **11**, 201-206.
- Qi, Y. and Burkholder, W. E. 1982 Sex pheromone biology and behavior of the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J. Chem. Ecol.* **8**, 527-534.
- Rowe, L. and Houle, D. 1996 The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**, 1415-1421.
- Rup, P.J. 1986 Mating and its attendant behaviour in *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. stored Prod. Res.* **22**, 77-79.
- Ryan, M.J. 1998 Sexual selection, receiver biases, and the evolution of sex differences. *Science* **281**, 1999-2003.
- Sakaluk, S.K. 2000 Sensory exploitation as an evolutionary origin to nuptial food gifts in insects. *Proc. R. Soc. Lond. B* **267**, 339-343.
- Savalli, U.M. and Fox, C.W. 1998 Genetic variation in paternal investment in a seed beetle. *Anim. Behav.* **56**, 953-961.
- Savalli, U.M. and Fox, C.W. 1999 The effect of male mating history on paternal investment, fecundity and female remating in the seed beetle *Callosobruchus maculatus*. *Funct. Ecol.* **13**, 169-177.

- Sheldon, B.C. 2000 Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* **15**, 397-402.
- Shu, S., Koepnick, W. L., Mbata, G. N., Cork, A. and Ramaswamy, S. N. 1996 Sex pheromone production in *Callosobruchus maculatus* (Coleoptera: Bruchidae): electroantennographic and behavioral responses. *J. stored Prod. Res.* **32**, 21-30.
- Simmons, L.W. 2001 *Sperm Competition and its Evolutionary Consequences in the Insects*. Princeton University Press.
- Simmons, L.W. and Parker, G.A. 1989 Nuptial feeding in insects: mating effort versus paternal investment. *Ethology* **81**, 332-343.
- Smith, R.L. 1984 *Sperm Competition and the Evolution of Animal Mating Systems*. Academic Press, London.
- Sokoloff, A. 1972 *The biology of Tribolium with special emphasis on genetic aspects*, vol. 1. Oxford University Press.
- Sokoloff, A. 1974 *The biology of Tribolium with special emphasis on genetic aspects*, vol. 2. Oxford University Press.
- Sokoloff, A., Slatis, H. M. and Stanley, J. 1960 The black mutation in *Tribolium castaneum*. *J. Hered.* **51**, 131-135.
- Southgate, B.J. 1979 Biology of the Bruchidae. *Ann. Rev. Entomol.* **24**, 449-473.
- Stockley, P. 1999 Sperm selection and genetic incompatibility: does relatedness of mates affect male success in sperm competition? *Proc. R. Soc. Lond. B* **266**, 1663-1669.
- Tallamy, D.W., Powell, B.E. and McClafferty, J.A. 2002 Male traits under cryptic female choice in the spotted cucumber beetle (Coleoptera: Chrysomelidae). *Behav. Ecol.* **13**, 511-518.
- Tallamy, D.W., Burton Darlington, M., Pesek, J.D. and Powell, B.E. 2003 Copulatory courtship signals male genetic quality in cucumber beetles. *Proc. R. Soc. Lond. B* **270**, 77-82.
- Tatar, M. and Carey, J.R. 1995 Nutrition mediates reproductive trade-offs with age specific mortality in the beetle *Callosobruchus maculatus*. *Ecology* **76**, 2066-2073.
- Thornhill, N.W. 1993 *The natural history of inbreeding and outbreeding*. The University of Chicago Press.
- Thornhill, R. 1976 Sexual selection and nuptial feeding behaviour in *Bititacus apicalis* (Insecta: Mecoptera). *Am. Nat.* **110**, 529-548.
- Thornhill, R. 1983 Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am. Nat.* **122**, 765-788.
- Thornhill, R. and Alcock, J. 1983 *The evolution of insect mating systems*. Harvard University Press.
- Tregenza, T. and Wedell, N. 2002 Polyandrous females avoid costs of inbreeding. *Nature*, **415**, 71-73.
- Trivers, R.L. and Willard, D.E. 1973 Natural selection of parental ability to vary the sex ratio of offspring. *Science* **179**, 90-92.
- Vahed, K. 1998 The function of nuptial feeding in insects: a review of empirical studies. *Biol. Rev.* **73**, 43-78.
- Ward, P.I. 1998 A possible explanation for cryptic female choice in the yellow dung fly, *Scathophaga stercoraria* (L.). *Ethology* **104**, 97-110.
- Ward, P.I. 2000 Cryptic female choice in the yellow dung fly *Scathophaga stercoraria* (L.). *Evolution* **54**, 1680-1686.

- Ward, P.I. Vonwil, J., Scholte, E.-J. and Knop, E. 2002 Field experiments on the distributions of eggs of different phosphoglucomutase (PGM) genotypes in the yellow dung fly *Scathophaga stercoraria* (L.). *Mol. Ecol.* **11**, 1781-1785.
- Watson, P.J., Arnqvist, G. and Stallmann, R.R. 1998 Sexual conflict and the energetic costs of mating and mate choice in water striders. *Am. Nat.* **151**, 46-58.
- Weddle, C.B. and Sakaluk, S.K. 2003 Ingestion of male haemolymph and mating propensity of female sagebush crickets: no evidence of male-derived antiaphrodisiac. *Anim. Behav.* **65**, 83-88.
- Wedell, N. 1991 Sperm competition selects for nuptial feeding in a bushcricket. *Evolution* **45**, 1975-1978.
- Wedell, N. and Arak, A. 1989 The wartbiter spermatophore and its effect on female reproductive output (Orthoptera: Tettigoniidae, *Decticus verrucivorus*). *Behav. Ecol. Sociobiol.* **24**, 117-125.
- West-Eberhard, M.J. 1984 Sexual selection, competitive communication and species-specific signals in insects. In *Insect communication*. Lewis, T. ed. Academic press, New York.
- Wickler, W. 1985 Stepfathers and their pseudo-parental investment. *Z. Tierpsychol.* **69**, 72-78.
- Wirtz, P. 1997 Sperm selection by females. *Trends Ecol. Evol.* **12**, 172-173.
- Wojcik, D.P. 1969 Mating behavior of eight stored-product beetles (Coleoptera: Dermestidae, Tenebrionidae, Cucujidae and Curculionidae). *Fla. Entomol.* **52**, 171-197.

Acta Universitatis Upsaliensis

*Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Science and Technology 43*

Editor: The Dean of the Faculty of Science and Technology

A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-5753



ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2005