Katri Ronkainen

POLYANDRY, MULTIPLE MATING AND SEXUAL CONFLICT IN A WATER STRIDER, AQUARIUS PALUDUM
KATRI RONKAINEN

POLYANDRY, MULTIPLE MATING AND SEXUAL CONFLICT IN A WATER STRIDER, AQUARIUS PALUDUM

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Technology and Natural Sciences of the University of Oulu for public defence in Kuusamonsali (YB210), Linnanmaa, on 27 May 2016, at 12 noon

UNIVERSITY OF OULU, OULU 2016
Abstract

Sexual selection results from competition between individuals over access to gametes of the opposite sex. Starting with anisogamy, with females producing larger and fewer gametes than males, females usually invest more in reproduction and thus have lower potential reproductive rates than males. Therefore males are traditionally suggested to compete over females whereas females are choosy. The different evolutionary interests between the sexes result in different selection regimes considering e.g. mating rates. In some species, this conflict is even seen as apparent struggles between males and females over mating decision and/or duration, resulting in antagonistic coevolution with both sexes evolving adaptations to gain more control over the other sex. In this thesis, I studied the role of *A. paludum* female abdominal spines as an adaptation to reject male mating attempts. I studied both the effect of polyandry and multiple matings on female fecundity to distinguish between the potential material and genetic benefits from mating to females. I also explored the role of various male and female morphological traits on precopulatory sexual selection and the consequent female reproductive output. My results show that female abdominal spines are likely evolved through arms races between the sexes to increase female control over mating decision but according to my studies, female spines are not under active selection. Instead, certain male morphological types were more successful in achieving matings, and mating with these males also increased female fecundity. I found that multiple matings increase female fecundity up to a point, supporting a theory of optimal female mating rate. According to my results, the benefits from multiple mating to *A. paludum* females result from material origin whereas polyandry *per se* is even detrimental to female fecundity.

Keywords: antagonistic coevolution, fecundity, polyandry, sexual conflict, sexual selection
To my family
Acknowledgements

Perhaps I should not confess this, but I spend my first day working with this thesis wondering, how could I ever be accustomed with these animals. I still don’t know if I did, but the fascinating theory of sexual selection has always enthralled me. Finding reasons (and reason) in a complicated world is just rewarding. However, this achievement would not have been possible without help from many friends and colleagues.

First of all, I wish to express my deepest gratitude to Professor Arja Kaitala for the possibility to work under her professional supervision. It took a long time for me to finish this thesis, but you never lost your trust (or at least did not show it!) in me. I really appreciate your continuous support and your encouraging and positive attitude during these years.

I am very grateful to the official examiners, Professor Raine Kortet and Doctor Topi Lehtonen, for accepting the invitation to review my thesis at such a short notice. Their careful review and valuable comments really helped me to improve the manuscript. Anna Vuolteenaho is acknowledged for revising the English language of this thesis.

For scientific collaboration I would like to thank my co-authors: Riikka Huttunen, Sami M. Kivelä and Panu Välimäki. Many other people have helped me on the way, but especially I would like to thank Tomi Kumpulainen for teaching me how to find and capture these animals, and all professionals in the physics workshop who developed many of the equipment needed. This study was supported financially by the Academy of Finland, Finnish Cultural Foundation, Oskar Öflund Foundation, Emil Aaltonen Foundation, and the University of Oulu Faculty of Sciences and the University of Oulu Scholarship Foundation.

I would also like to acknowledge all my colleagues, especially Sami M. Kivelä, Panu Välimäki and Marko Mutanen for their help and guidance. You are all real professionals in the evolutionary ecology and insect behaviour studies, and I am grateful that I had a possibility to turn to you when I had any troubles.

I want to express my deepest thanks to all those who commented my studies or read my manuscripts during these years: Professor Göran Arnegvist, Professor Juha Tuomi, Åsa Borg, Annukka Kaitala, Laura Härkőnen, David Carrasco, Phillip Watts, Juhani Hopkins, Jenni Kesäniemi, Gautier Baudry and Netta Keret.

I am thankful to my mother for all her help but most of all for teaching me not to give up. I also wish to send thanks to my father on the other side, since it was him who gave me the original idea to study biology. I am also very grateful
to my sisters Pia and Riikka for their friendship and support. Many thanks to
Ronkainen family for all their help and support in everyday life. I would also like
to thank my grandparents, Kalle-pappa, Anna-Liisa-mummo, Helvi-mummo and
Veikko-pappa for giving a strong basis for my life.

Finally, I wish to thank my beloved family. Deepest thanks to my husband
Veli-Pekka for the most obvious reasons at all. You were my strength when I was
weak. I would not be in this place today without you. Many, many thanks to my
playful sons Jaakko and Otso for giving a meaning and joy to my life. You are my
little sunshines.

Oulu, April 2016

Katri Ronkainen
List of original articles and contributions

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


# Table of contents

**Abstract**  
9

**Tiivistelmä**  
9

**Acknowledgements**  
11

**List of original articles**  
13

**Table of contents**  
13

1 **Introduction**  
15  
1.1 Sexual selection ....................................................................................... 15  
1.1.1 Male-male competition ........................................................................ 16  
1.1.2 Mate choice ...................................................................................... 16  
1.2 Sexual conflict ........................................................................................ 18  
1.2.1 Sexually antagonistic coevolution ..................................................... 19  
1.2.2 Sexually antagonistic coevolution in insects ................................... 20  
1.3 Polyandry and multiple matings .............................................................. 21  
1.3.1 Material benefits ............................................................................. 21  
1.3.2 Genetic benefits ............................................................................... 22  
1.3.3 Convenience polyandry .................................................................. 22  
1.4 Aim of the study .................................................................................... 23

2 **Materials and methods**  
25  
2.1 Study species ........................................................................................... 25  
2.2 Collection and rearing ............................................................................. 26  
2.3 Sexually antagonistic coevolution (I) ..................................................... 26  
2.4 Polyandry and multiple matings (II) ....................................................... 27  
2.5 Sexual selection on morphological traits (III) ....................................... 27

3 **Results and discussion**  
29  
3.1 Sexual conflict as an evolutionary force ................................................. 29  
3.1.1 The role of abdominal spines in precopulatory struggles ............... 29  
3.1.2 Precopulation selection on male morphological traits................... 31  
3.2 Mating and female fecundity ................................................................. 34  
3.2.1 Multiple mating and polyandry ......................................................... 34  
3.2.2 Male precopulatory success .............................................................. 38  
3.2.3 Morphological traits ...................................................................... 41

4 **Concluding remarks**  
43

**References**  
45

**Original articles**  
53
1 Introduction

1.1 Sexual selection

Sexual selection results from competition between individuals over access to gametes of the opposite sex. It is virtually always present whenever there is sexual reproduction, even when unisexual organisms exchange genetic material, but it has its most prominent evolutionary consequences on population and species level only in the presence of two different sexes with their defined types of gametes, with females producing fewer and bigger gametes than males. This basic asymmetry between the sexes, anisogamy, is believed to be the reason why sexual selection has led to the many behavioural and morphological differences seen between the sexes (see Andersson 1994 for review).

The ‘Darwin-Bateman paradigm’ suggests that starting with anisogamy, females usually invest more in any given reproductive event and thus have lower potential reproductive rates than males, leading to more males being sexually active than females (a male-biased operational sex ratio) (see e.g. Kvarnemo & Simmons 2013). Therefore males have to compete in order for their sperm to reach a female’s egg before the sperm of their rivals while females are more choosy, resulting in stronger sexual selection acting on males than females (Bateman 1948, Trivers 1972). However, the study of female polyandry may have revolutionary consequences for our understanding of how sexual selection works (Pizzari & Wedell 2013) and even generate debates about sexual roles on cultural level (Parker & Birkhead 2013). When the early taxonomists focused on male sexually selected traits (such as antlers and horns), the modern sexual selection and polyandry studies have revealed less obvious forms of sexual selection, such as cryptic female choice and sperm competition, and also less obvious sexually selected female traits, such as sensory perception. At the same time, these findings have extended the definition of sexual selection beyond mating to the point of fertilization (Kvarnemo & Simmons 2013).

Animal sex roles are defined as ‘conventional’ when male competition for females is strongest and ‘reversed’ when female competition for males is strongest (Vincent et al. 1992). For simplicity, I will not discuss sexually role-reversed species here (see Andersson 1994 for review), although this classification may sometimes even be altogether misleading (Forsgren et al. 2004).
1.1.1 Male-male competition

Male-male competition (i.e., intrasexual selection) refers to the competition between males to fertilize female ova. This competition may favour actual fights or fighting displays between males to gain matings, as in contest competition, or the ability to remain reproductively active during a large part of the season, termed endurance rivalry. The ability to find females before the rivals do may also be selected in a scramble type of competition (Andersson 1994, Hunt et al. 2009).

Sperm competition can be seen as the postcopulatory equivalent of male-male competition (Miller & Svensson 2014). Sperm competition occurs when the semen of one male interacts directly with the semen of another male (Eberhard 2009) as a distinction from cryptic female choice (see below). The level of sperm competition varies according to the rate of female polyandry (Eberhard 2009, Parker & Pizzari 2010, Kvarnemo & Simmons 2013). The mechanisms by which sperm of different males compete may vary from diluting the sperm of previous males by increasing the allocation of own ejaculate when each sperm has equal probability of fertilization (“fair raffles”) to directly or indirectly displacing the sperm of previous males (Eberhard 2009, Ingleby et al. 2010, Parker & Pizzari 2010).

The features of the sperm itself may also contribute to the sperm competition, such as sperm quality and survival in the female reproductive duct or the degree of sperm mixing (Parker & Pizzari 2010). Moreover, several seminal products other than sperm may have important effects on the outcome of the competition (Chapman 2001, House & Simmons 2006, Yamane et al. 2015). Males may also enhance their success in sperm competition behaviourally, for example by postcopulatory guarding or “retaliatory” copulations when a paired female exhibits extra-pair copulations (Eberhard 2009). However, maintaining both precopulatory (e.g. armaments) and postcopulatory (e.g. ejaculates) sexual traits can be energetically demanding, forcing males to trade off between gaining matings versus gaining fertilizations (Lüpold et al. 2014, Fitzpatrick & Lüpold 2015).

1.1.2 Mate choice

Mate choice (i.e., intersexual selection) includes mating preference, i.e., a tendency to mate with a certain male phenotype, and choosiness, i.e., the extent of mate sampling effort before mating (e.g. Kokko et al. 2006). The evolution of
mate choice remains a subject of debate in spite of an extensive advancement in this study area (Kokko et al. 2003, Andersson & Simmons 2006, Jones & Ratterman 2009). In the evolutionary context, female preference may have evolved directly or indirectly (Chapman et al. 2003, Kokko et al. 2006). In direct models, female choice itself is favoured by natural selection when females prefer males who provide them with material advantages, such as nuptial gifts, high-quality territory or better parental care (Andersson & Simmons 2006).

Indirect models can be divided in two different theories. According to these theories, female preference is evolved either through a Fisherian sexy sons process, where females with a preference will produce attractive sons, or through good-genes (or indicator) processes, where attractive male traits indicate male genetic quality or compatibility with female egg’s genotype and thus mating with attractive males results in high-viability offspring (Arnqvist & Rowe 2005, Andersson & Simmons 2006). Male ornaments may also signal resistance against parasites (Hamilton & Zuk 1982, Kortet et al. 2004). In addition to direct and indirect benefit hypotheses, there is also another explanation for the evolution of female mate choice which suggests that a male display trait incidentally exploited a pre-existing female sensory bias initially related for example to foraging (e.g. Andersson & Simmons 2006, Jones & Ratterman 2009).

Females may also have postcopulatory mechanisms that bias the paternity of the offspring (Eberhard 1996, Eberhard 2009, Parker & Pizzari 2010), thus being active participants of the many evolutionary processes of reproduction (Zeh & Zeh 2003). Females often perform cryptic choice by differentially killing or storing sperm of different males, thus selectively fertilizing eggs with sperm of higher quality or from genetically more compatible males (Andersson & Simmons 2006, Parker & Pizzari 2010, Eberhard 2015), but there is still a lack of knowledge of the mechanisms behind cryptic female choice and the costs involved (Parker & Pizzari 2010). Cryptic female choice can also act through females allocating more resources to offspring when breeding with an attractive mate (‘differential allocation’, Burley 1986, Sheldon 2000). The assumptions of differential allocation are based on life-history theory; to begin with, that parents always have to trade off between their current and future reproduction. Mate attractiveness, when related to higher genetic quality, some material advantages etc., is a factor essentially affecting this trade-off (Sheldon 2000, Ratikainen & Kokko 2010).

Females may allocate their resources to eggs differentially based on the quality of their mate, for example by laying more eggs when mated with an
attractive male (Balzer & Williams 1998) or by increasing egg size or quality (Cunningham & Russell 2000, Osorno et al. 2006). However, increased female investment may also be a result of male manipulation (Cunningham & Russell 2000, Colegrave 2001, Gil & Graves 2001). In addition, more studies are needed to be able to understand the evolutionary interactions between the postcopulatory mechanism by males and females and the effect of precopulatory events on these (Parker & Pizzari 2010). The studies conducted so far have yielded mixed results as to the relative importance of pre- and postcopulatory events on sexual selection (Devigili et al. 2015).

1.2 Sexual conflict

Sexual conflict occurs when the interests of males and females differ. Even though the sexes share the same goal of producing offspring, their evolutionary interests generally differ thereafter (Arnqvist & Rowe 2005). Sexual conflict is thus very common and occurs any time a female is courted by a male but does not permit him to fertilize all of her eggs (Kokko & Jennions 2014, Eberhard 2015). Sexual conflict is not an alternative to sexual selection, although it may extend beyond the traits under sexual selection; sexual selection is rather a source for sexual conflict (Kokko & Jennions 2014).

On a genetic level sexual conflict may occur between the same allelic variations in a phenotypic trait expressed in both sexes if the optimal level of expression is different between the sexes (see e.g. Pischeda and Chippindale 2006, Bonduriansky and Chenoweth 2009, Cox and Calsbeek 2009). In this kind of intralocus sexual conflict, selection favours different values for the trait in males and females, with neither sex reaching its adaptive optimum but more like an evolutionary compromise (Arnqvist & Rowe 2005, Pennell et al. 2016).

When there is a conflict over the outcome of the interaction between males and females, it involves different genes in each sex and is called interlocus conflict. This kind of conflict can occur in virtually all forms of interaction, such as mating decision, parental care, fertilization success and female reproductive rate (Arnqvist & Rowe 2005, Pischeda & Chippindale 2006). The resulting antagonistic coevolution is regarded as an ever-continuing chase between the sexes, with both evolving adaptations to bias the outcome of the conflict towards their own optimum (Parker 1979, Rice & Holland 1997, Gavrilets et al. 2001, Arnqvist & Rowe 2005). It is important to note that sexual selection may exist without ensuing sexually antagonistic coevolution and that also other forms of
pre- and postcopulatory events involve sexual conflict, such as mate choice, sperm competition and cryptic female choice (Eberhard 2015).

1.2.1 Sexually antagonistic coevolution

Sexually antagonistic coevolution occurs when sexual selection favours the male’s ability to manipulate or influence the female during reproduction in ways that are advantageous for the male but disadvantageous for the female. The disadvantages to the female involve traits that are under natural rather than sexual selection (Eberhard 2015). For example, males may physically or chemically coerce females to increase the use of their own sperm and promote female’s egg laying rate immediately after copulation whereas if these manipulations were harmful to females, natural selection would work to increase their resistance to male manipulation (for review, see e.g. Eberhard 2009).

Conflict over mating decision can be seen on a behavioural level as females’ avoidance or resistance to matings and males’ eagerness, harassment and even coercion (Arnqvist & Rowe 2005, Head & Brooks 2006). This kind of conflict may result in an antagonistic male-female coevolution, with sexual selection favouring male traits designed to overcome female resistance and natural selection favouring females’ counter-adaptations that help them to reject males (Gavrilets et al. 2001, Chapman et al. 2003, Kokko et al. 2006, Pischeda & Chippindale 2006, Wang et al. 2015). As well as female preference, also female resistance generates mating biases (Andersson & Iwasa 1996, Kokko et al. 2003, Hunt et al. 2009) and may be favoured by natural selection, since superfluous matings may decrease female longevity and males may vary in the costs that they impose on females (reviewed by Kokko et al. 2003). The “benefit” the female gains by avoidance is the reduced costs of matings (e.g. Gavrilets et al. 2001).

However, when coercion is costly to females, harassment may lead to increased costs of rejecting males (Thornhill & Alcock 1983). If there are no benefits associated with matings, female mating biases, i.e., resistance, becomes selected against or towards a condition-dependent expression by natural selection (reviewed by Kokko et al. 2003). As a consequence, females may accept matings for reasons of convenience to avoid further harassment, especially when male harassment rate is high (Thornhill & Alcock 1983) or when some males are more aggressive than others (Eldakar et al. 2009), which may decrease the opportunity for female choice (Head & Brooks 2006).
1.2.2 Sexually antagonistic coevolution in insects

Males of many insects have adaptations that serve as grasping devices prior or during the mating. These are usually modifications in the male pregenital or genital segments but even in the legs and antennae (Eberhard 1985, Eberhard 1996, Arnqvist 1997, Arnqvist & Rowe 2002b, Rowe & Arnvist 2005, Eberhard 2006, Rönn et al. 2007, Hoyle & Gilburn 2010, Rowe & Arnvist 2012). Females have evolved anti-grasping traits, for example in water striders (see below) and diving beetles (Arnqvist & Rowe 2005). In support of the theory of sexually antagonistic coevolution, Arnqvist & Rowe (2002b) found that in a glade of 15 water strider species, male and female armaments were closely correlated, with male grasping traits most evolved in species where female adaptations were best designed to reject them and vice versa.

Besides grasping and anti-grasping traits, there are other behavioural, morphological and physiological male and female traits across species that have the potential to coevolve in an antagonistic way (for review, see Arnqvist & Rowe 2005). It has been suggested that antagonistic coevolution is a continuous evolutionary chase, either an endless ‘arms race’ or a ‘stable limit cycle’ with no single equilibrium point (Gavrilets et al. 2001, Gavrilets & Hayashi 2005, Rowe et al. 2005, Lessells 2006, Pennell et al. 2016) and thus a key force behind the evolution of animal genitals, evolving rapidly and reaching such diversity that many closely related species can only be distinguished by their genitalia (Hosken & Stockley 2004, Arnqvist & Rowe 2005, Rönn et al. 2007).

Antagonistic coevolution due to sexual conflict may even affect population fitness and the rates of speciation and extinction (Arnqvist et al. 2000, Gavrilets & Hayashi 2005, Rowe et al. 2005, Lessells 2006, Arbuthnott et al. 2013). However, in other insect species than water striders, there is far less evidence of antagonistic male and female genital evolution fuelled by sexual conflict (Hosken & Stockley 2003, Eberhard 2006, Hoyle & Gilburn 2010). Instead, evidence suggests that cryptic female choice has played a more important role in genital evolution (Eberhard 2009, Eberhard 2010). It has also been argued that stable equilibria might be common instead of continuous chases or co-evolutionary cycles, which would make it unlikely that sexual selection promotes speciation (Hoyle & Gilburn 2010).
1.3 Polyandry and multiple matings

Polyandry is a taxonomically widespread female mating strategy (Thornhill & Alcock 1983, Eberhard 1996, Birkhead & Møller 1998, Jennions & Petrie 2000, Zeh & Zeh 2001, Birkhead et al. 2009). Bateman (1948) suggested that polyandry represents a way by which males increase their reproductive success, whereas one or a few matings would be sufficient to maximize female lifetime fitness. However, it is now clear that also female reproductive success can depend on the number of mates (Kvarnemo & Simmons 2013). Since studies had shown that females may suffer remarkable costs associated with mating, including e.g. the time and energy spent on mating or rejecting unwanted suitors, increased risk of predation, parasites, diseases or even injuries during searching for a mate or the actual mating (Daly 1978, Arnqvist 1989, Crudgington & Siva-Jothy 2000, Fedorka et al. 2004, McNamara et al. 2008), hypotheses were needed to provide explanations for the evolution of polyandry. Three main adaptive explanations for polyandry that are not mutually exclusive have been suggested (Slatyer et al. 2012).

1.3.1 Material benefits

First, polyandry or multiple matings may be favoured by natural selection if females gain some direct material benefits from mating. The material benefits of mating to females may refer to any resources transferred by the male prior to, during or after the copulation. These benefits may result from male-donated nutrients (for review, see Vahed 1998), fertilization assurance or sufficient supply of viable sperm (Hasson & Stone 2009, Parker & Pizzari 2010), ejaculate defensive compounds (Gonzalez et al. 1999) or paternal care (Jennions & Petrie 2000).

Increased fecundity may also result from substances that the males of some insects secrete from their accessory reproductive glands. The accessory substances are typically various nutritious or hormone-like proteins produced in the male accessory glands or glandular cells in testes or the ejaculatory duct (Arnqvist & Nilsson 2000, Gillott 2003, Yamane et al. 2015). Seminal substances may stimulate female egg laying and egg development rates, modulate ovulation and/or oviposition and render the female unwilling or unable to further matings for some time (reviewed e.g. by Eberhard & Cordero 1995, Gillott 2003). However, increased costs of reproduction may lead to decreased female life span.
Some substances may affect sperm competition, storage and utilization inside the female and thus have a role in paternity assurance (reviewed by Arnqvist & Nilsson 2000, Yamane et al. 2015). However, these substances may also have toxic side effects to females (Siva-Jothy et al. 1998).

1.3.2 Genetic benefits

The second explanation suggests that polyandry is favoured by indirect selection since mating with several mates confers genetic benefits that increase offspring fitness (see e.g. Slatyer et al. 2012 for review). Genetic benefits may arise if polyandry increases genetic diversity among offspring (Loman et al. 1988, Yasui 1998, Garcia-Gonzales et al. 2014) or enables inbreeding avoidance (Stockley et al. 1993). Furthermore, females from socially monogamous species may engage in extrapair copulations with males of higher genetic quality than their social mates (Kempenaers et al. 1992).

Polyandry may also enable sperm competition or female choice of sperm, both of these increasing the probability that eggs are fertilized by sperm of high-quality males (Madsen et al. 1992, Birkhead et al. 1993, Tomkins et al. 2010), or sperm that is genetically compatible with the egg’s genotype (Zeh & Zeh 1996, Zeh & Zeh 1997, Tregenza et al. 2000, Puurtinen et al. 2009). The difference between the genetic and nongenetic benefits is that nongenetic benefits may also arise by mating repeatedly with the same partner, whereas genetic benefits arise only by mating with two or more different males (Tregenza & Wedell 1998, Worden & Parker 2001).

1.3.3 Convenience polyandry

The third explanation for polyandry predicts that rather than gaining benefits from mating, the female mating rate is mediated by sexual conflict over mating rate. According to this convenience polyandry hypothesis, males have the upper hand in mating decisions due to adaptations that overcome female resistance (Holland & Rice 1998). Females thus mate several times to avoid the costs of resisting male mating attempts (Thornhill & Alcock 1983, Arnqvist & Rowe 2005). Males may gain an edge over females in the conflict if the costs of mating for females are low compared to the cost of rejecting male mating attempts (Gavrilets et al. ...
Otherwise, female counter-adaptations are expected to evolve in order to bring mating rates toward their optimum (Parker 1979).

It is hypothesized that there should be an optimal mating rate for a female that maximizes her fitness (Arnqvist & Nilsson 2000). The optimum is a trade-off between the various costs and benefits of mating to females. According to Arnqvist and Nilsson (2000), the fundamental question is not the maintenance of polyandry itself but the factors that determine the optimal female mating rate and whether the actual mating rates in nature diverge from that optimum.

1.4 Aim of the study

The objective of this thesis was to study sexual conflict and the consequent arms race between the sexes in the water strider *A. paludum*. Specifically, I wanted to explore female ability to control mating decision and perform mate choice. Water striders are widely used as model species in sexual conflict and consequent antagonistic coevolution studies (see 2.1 for more details). However, most studies concern *Gerris* water striders that inhabit small ponds where females may not be able to escape male harassment. Instead, *A. paludum* exists in lakes with varying density and females able to choose to avoid males, so the selection pressure acting on each sex may also differ from other water strider species. *A. paludum* females have abdominal spines that in other species are believed to have evolved as female counteradaptation to male grasping traits. Interestingly, multiple matings seem not to be as harmful to *A. paludum* females as observed in other species.

During the course of the study it became necessary to examine more closely the role of polyandry and multiple mating in female fecundity. My aim was to investigate whether females benefit from multiple matings and whether they get material or genetic benefits from polyandry. Finally, I studied whether females’ resistance biases paternity in favour of certain male types advantageous to female fecundity. I claim that water strider females are active participants in the evolutionary processes involving mate choice instead of only being reactive to male adaptations and attempting to minimize the costs of multiple matings.
2 Materials and methods

2.1 Study species

Water striders (Heteroptera: Gerridae) are semiaquatic bugs that are well suited as models in sexual conflict studies since they can be easily studied in their natural environment and also reared in laboratory (Rowe et al. 1994, Arnqvist 1997). Water striders inhabit surfaces of many kinds of natural waters, feeding mainly by preying or scavenging arthropods dropped at the water surface. The mating period lasts from one to three months and is characterized in most species by an apparent conflict between the sexes over both mating decision and mating duration (Arnqvist 1997). My study species *A. paludum* is less studied, yet a species with apparent sexual conflict and high level of female polyandry, which makes it an interesting study object.

Water strider males frequently harass single females, attempting to mate. Females usually try to escape male harassment but if the male succeeds to contact a female, he tries to grasp her anteriorly with his forelegs and posteriorly with his genitalia, which he bends downwards to grasp the tip of the female abdomen. The female usually responds with resistance, trying to dislodge the male in a precopulatory struggle (Rowe et al. 1994, Arnqvist & Rowe 1995, Arnqvist 1997).

Multiple matings are considered to be costly to water strider females due to reduced mobility, increased predation risk and increased energy consumption (Arnqvist 1989, Fairbairn 1993, Rowe et al. 1994, Watson et al. 1998). Only a few direct benefits of mating to females are found (Arnqvist 1997). Yet females of most water strider species copulate much more often than is necessary for acquiring fertile sperm (Arnqvist 1997). This is thought to result from convenience polyandry since it is also energetically costly to resist male mating attempts. In fact, mating may provide protection against further harassment (Rowe et al. 1994, Rowe & Arnqvist 2002, Arnqvist 1997). Since the male harassment rate and thus the costs of resisting male mating attempts are higher in male-biased populations, females may adjust their resistance to mate in a condition-dependent manner to balance the costs imposed by males (Arnqvist 1992a, Rowe 1992, Devost & Turgeon 2016).

The outcome of premating struggles depends on male ability to grasp the female tightly or female ability to dislodge the male (Arnqvist & Rowe 2002a,
Thus male grasping traits and female antigrasping traits are likely to evolve in an antagonistic manner (for reviews, see Arnqvist 1997, Arnqvist & Rowe 2002, Rowe & Arnqvist 2002). In males, large front femurs may increase the ability to grip the female anteriorly. Flattened distal part of the male abdomen as well as the length and certain modifications of the terminal abdominal segments and non-intromittent genitalia are likely related to his ability to achieve a tight posterior grip of a female (Andersen 1997, Arnqvist 1997, Arnqvist & Rowe 2002b, Rowe & Arnqvist 2012). In contrast, the female genital opening is often protected by lobes or spines (Andersen 1997, Arnqvist & Rowe 2002b), which increase female ability to reject male mating attempts (Arnqvist & Rowe 1995, Perry & Rowe 2011). Also long genitalia bent downward, large fore femurs and relatively larger female size may increase female ability to resist courting males (Arnqvist & Rowe 2002b, Gagnon & Turgeon 2011). In *A. paludum*, abdominal spines are clearly visible in both sexes, but the role of these spines had not been previously studied in this species.

### 2.2 Collection and rearing

I collected overwintered, reproductively active water striders in early spring and kept them in the laboratory with a simulated natural light regime and at a constant temperature at 20 ± 2°C. While in captivity, the waters striders were fed *ad libitum* with frozen fruit flies and blowflies and provided with pieces of Styrofoam as egg laying and resting sites. Prior to experiments, I kept the water striders in aerated aquariums (100 cm × 60 cm × 80 cm, water depth 10 cm), females isolated from males.

All the experiments in this thesis were carried out in the same laboratory with the same environmental conditions. The experiments were performed in plastic jars (17 × 17 × 11 cm, water depth 3 cm) with one female per jar. The water and food items in these jars were changed daily.

### 2.3 Sexually antagonistic coevolution (I)

I explored the role of *A. paludum* female abdominal (or connexival) spines in precopulatory struggles between the sexes. I hypothesized that abdominal spines increase female ability to resist male mating attempts and thus reduce the costs of excessive matings. To test this hypothesis, I carried out two experiments where I manipulated the length of the female abdominal spines and observed the resulting
mating frequency and egg laying (I). For the first experiment, I divided females into four experimental groups: 1) intact spines and a male present, 2) removed spines and a male present, 3) intact spines and no male, 4) removed spines and no male. The males used in the experiment were chosen randomly. During the 6-day experiment, I enclosed males with females in groups 1 and 2 daily and observed all copulation attempts, female rejections and matings. Each day, I counted the number of eggs laid per female.

For the second experiment, females were divided into 3 groups with intact, shortened or removed spines. During 27 days, I enclosed the females daily for half an hour with a randomly chosen male (the same male with the same female every day). I observed the number of copulations during this time. Every third day I counted the eggs the females had laid and replaced the pieces of Styrofoam.

2.4 Polyandry and multiple matings (II)

I studied both the effect of multiple mating (with the same partner) and the effect of polyandry (with different partners) on female fecundity. In the first study, I controlled the female mating rate with the same male by controlling the time the females were accompanied by the males. I enclosed the females for 5 hours with one randomly chosen male every day, every second day, every third day or every fourth day. The same male accompanied the same female every time during the study. I observed all matings and counted the eggs daily.

In the second study, I controlled the number of partners per female. I randomly divided the females into three different groups. In the first group, females mated four times with a single male, whereas in the second group the females mated twice with both of two males and in the third group once with each of four males. After the four matings, each female was allowed to lay eggs over a week. I then counted the number of eggs per female and let them hatch to measure egg-hatching success.

2.5 Sexual selection on morphological traits (III)

I studied the role of multiple male and female morphological traits on the outcome of precopulatory male-male and male-female interactions. I also explored the effect of these morphological traits on various fitness factors, which were egg number, fertilization success and hatching probability and offspring survival. To rank males according to their success in precopulatory sexual
selection, I performed a two-step study. First, I studied male success to gain mating in the presence of another male. This was done by enclosing two randomly chosen males with one female and then observing which male would copulate with the female. The animals were not allowed to mate during this trial but were gently disengaged when about to copulate.

Secondly, I ranked the males according to female preference measured as the duration of premating struggle. The same two males used in previous trial were consequently enclosed with the same female, one male at a time, both of the males twice in a randomized order. I observed the time to the first mating attempt, the duration of the precopulatory struggle and whether the female would accept copulation. No matings were allowed in this trial, either. I concluded that the female preferred the male she resisted less, i.e., the male with whom she had a shorter average duration of precopulatory struggle before copulation would have started. If the female dislodged one of the males but not the other, I decided that she had chosen the one she had not rejected.

I divided the females into two groups. The females in the first group were allowed to mate four times with the males they preferred while the females in the other group mated four times with the males they rejected or had longer duration of precopulatory struggle with. Then I observed the females’ egg number, fertilization and hatching success and offspring survival. Finally, to get morphological measurements each male and female was frozen and photographed with Olympus SZX-12 stereomicroscope with SIS CC-12 colour camera. The 20-day-old offspring were also frozen and photographed to measure their average tibia length.
3 Results and discussion

My results show that *A. paludum* females benefit from multiple matings whereas polyandry *per se* may even decrease female fecundity (I, II). The abdominal spines of female *A. paludum* give them more control over mating decisions and may thus have evolved through sexually antagonistic coevolution (I). During precopulatory struggles, females exhibit less resistance towards males with certain morphological types, and mating with these males leads to higher female fecundity (III).

3.1 Sexual conflict as an evolutionary force

3.1.1 The role of abdominal spines in precopulatory struggles

Shortening or removing *A. paludum* female abdominal spines resulted in increasing mating numbers and decreasing the proportion of rejected male mating attempts by females (I). However, statistically significant differences arise only when cumulative mating numbers are compared between the spine treatment groups (Fig. 1). The duration of precopulatory struggles was not affected by natural variation in *A. paludum* female spine length or by any other female morphological trait I measured (III).

I expected that the costs of mating and rejecting males would be reflected in the female egg number, so that the more costs a male imposed on the female the less eggs she would lay. However, I found no support for this hypothesis, since the females in my experiment with removed or shortened spines that mated most also laid the most eggs whereas females with intact spines that mated least also laid the least eggs. My results also show that mating rate and mere male presence affects female fecundity more than abdominal spine treatment (I).

My results (I) support the hypothesis that abdominal spines increase water strider females’ ability to reject harassing males and have thus evolved via arms races between the sexes (Arnqvist & Rowe 1995, Arnqvist & Rowe 2002b, Perry & Rowe 2012). However, due to dissimilarities between *A. paludum* and the study species of Arnqvist and Rowe (1995), *G. incognitus*, the results cannot be directly compared.
Fig. 1. Cumulative mean number of matings per female in three-day periods with removed (■), shortened (□) and intact spines (▲). Notice that matings were recorded only for half an hour per day (I).

Whereas in *G. incognitus* female abdominal spines are notably longer than male spines, in *A. paludum* males have even longer spines than females. However, *A. paludum* male spines differ in shape from female spines that recurve over genital segments whereas male spines are almost straight (I). Interestingly, the function of *A. paludum* male abdominal spines is unknown. The experimental effect reported in Arnqvist and Rowe (1995) was between females with lengthened and intact, not between intact and shortened spines, whereas I did not have spine-lengthening manipulation.

My results suggest that female morphological traits are not under active sexual selection and the role of abdominal spines thus only emerges when the length is manipulated. The evolution of sexual traits is, however, determined by complex interaction between environmental heterogeneity and phenotypic plasticity causing the selection regime to fluctuate in a temporal and spatial
manner (Cornwallis & Uller 2010). This applies to water striders as well since the females adjust their resistance according to environmental conditions (mainly male harassment rate) (Arnqvist 1992a, Rowe 1992, Devost & Turgeon 2016). Thereby the selection pressure for female abdominal spines may be manifested in other environmental conditions than the laboratory.

Arnqvist and Rowe (1995) also suggested that by increasing female ability to thwart a harassing male, abdominal spines reduce the overall costs of mating interactions to females. This was not the case in my study where the number of eggs laid increased with the number of matings. This finding suggests that *A. paludum* females somehow benefit from mating despite the apparent conflict between the sexes over both mating decision and duration. However, the hypothesis that high mating rates are beneficial for water strider males but costly for females may be wrong altogether. In a recent study, Devost and Turgeon (2016) observed that contrary to this prediction, in *G. buenoi* the number of matings had no effect on either female or male reproductive success. The relationship between mating number and reproductive success was insignificant even when surveying these variables over individuals’ lifetime (Devost & Turgeon 2016).

It was once thought that females would ‘need’ to mate only to get their eggs fertilized (Bateman 1948). In many water strider species females are able to store viable sperm for up to weeks (Arnqvist 1997), so e.g. in *A. remigis* even one mating every 3 weeks would be enough to fertilize all female eggs (Rubenstein 1989). Neither do *A. paludum* females easily suffer from sperm depletion since females enclosed without males do not show an increase in oviposition rate after being allowed to mate (I). Today, there is growing evidence that also in water striders cryptic female choice and sperm competition readily play an essential role in reproduction (Campbell & Fairbairn 2001, Devost & Turgeon 2016), and polyandry certainly increases the possibilities for these postcopulatory sexual selection processes.

### 3.1.2 Precopulation selection on male morphological traits

*A. paludum* males resisted less by females in precopulatory struggles (i.e., “preferred” by females) are also successful in gaining matings in the presence of another male (III). My results did not reveal any certain male trait to be under sexual selection; rather I found a more complex pattern with allometric relationships between different traits. Females thus seem to favour three types of males: 1) short, successful males with a long thorax, 2) long, successful males
with a short thorax and 3) unsuccessful males with wide 7th abdominal segment and a long pygophore (III, Fig. 2). Success refers here to the male performance in the presence of another male. Instead, female morphological characteristics have no effect on the duration of precopulatory struggles between males and females.

While in many animal species females prefer males with some ornaments or other cues indicating male quality (Andersson & Iwasa 1996, Kokko et al. 2006), in water striders female variation in resistance to mate may represent choice (Sih et al. 2002). In line with my study (III) also other studies in water striders have found male small size to be favoured by females, probably because small males are relatively easy to carry yet they protect females from harassment by other males (Sih et al. 2002). Short *A. paludum* males with a long thorax are also successful in gaining matings in the presence of another male (III). In *A. remigis* male-male competition has been found to favour long males (Kaitala & Dingle 1993, Sih et al. 2002). Precopulatory sexual selection may, however, favour small male size (Bertin & Fairbairn 2005) as small males require less energy for movement and maintenance under natural conditions, thus being able to outcompete larger males in scrambles (Krupa & Sih 1993, Blanckenhorn et al. 1995, Bertin & Fairbairn 2005).

![Fig. 2. Fitted regression lines for the effects of (a) the width of the 7th abdominal segment and (b) the length of pygophore in males on the duration of precopulatory struggles between males and females in relation to success in the presence of another male (III).](image-url)
Previously, a relatively long thorax has been found to correlate positively with male mating success in western harvester ants and suggested to give males an advantage in combat or in maintaining a hold on a female during mating (Abell et al. 1999). The male ability to grasp a female tightly during the precopulatory struggle is probably of profound importance in *A. paludum*. In several other water strider species, increasing fore femur width increases male mating success by strengthening the anterior grip (Kaitala & Dingle 1993, Arnoqvist & Rowe 2002b).

Selection favouring male traits involved in achieving and/or maintaining intromission may be common in insects and thus have a profound role in male genital evolution (Bertin & Fairbairn 2005). Many water strider studies have found a positive correlation between male genitalia length and mating success, which increase male ability to overcome female resistance (Arnoqvist 1992b, Ferguson & Fairbairn 2000, Sih et al. 2002, Bertin & Fairbairn 2005) or facilitate the intromission in spite of female resistance (Fairbairn et al. 2003). Since *A. paludum* females cease precopulatory struggles immediately when the male succeeds in extending and bending his genitalia beyond the tip of the female’s abdomen and is about to intromit (own observations), males prompt in reaching the female abdomen tip before being dislodged may have a selection advantage. My studies show that females’ resistance is indeed of shorter duration against long males and males with a wide 7th abdominal segment and a long pygophore (III). Instead, in *G. incognitus*, precopulatory sexual selection favours a short rather than long external 1st genital segment because such male morphology scopes better with erected female abdominal spines in that species (Arnoqvist et al. 1997). Interestingly, *G. incognitus* is another species besides *A. paludum* in which abdominal spines in the female have so far been found to increase her control over copulation (Arnoqvist & Rowe 1995). A wide 7th abdominal segment and a long pygophore might also be *A. paludum* males’ counteradaptation to female abdominal spines (III).

Male-male competition can also drive the evolution of male genitalia if genital traits enable males to achieve intromission in a scramble type of competition or prevent other males’ take-over in a contest competition (Simmons 2001). Male genitalia associated with intromission and insemination might be common in insects and more likely selected by postmating processes affecting fertilization success than by arms races between the sexes (Eberhard 2004, Eberhard 2009, Eberhard 2010).
3.2 Mating and female fecundity

3.2.1 Multiple mating and polyandry

Multiple matings with the same male increase female egg number in *A. paludum* up to a point beyond which the egg number declines. Female maximum egg production is reached at the level of about 1.4 matings per day (II, Fig. 3).

On the contrary, polyandry (i.e., different mating partners) *per se* decreases female fecundity in *A. paludum*. Females that mated four times with one male laid the most eggs and had the highest egg hatching percentage whereas females that mated four times with four different males laid the least eggs and had the lowest egg hatching success (II, Fig. 4).

![Fig. 3. The number of eggs laid per female in four groups. In the first group a female was accompanied by a male every day, in the second group a male was present every second day, in the third group every third day, and in the fourth group every fourth day (II).](image)

The result that female egg number increases with mating number suggests that females gain some benefits from mating that are converted to egg production. However, since matings at the same time bear costs to females, the balance between these costs and benefits determines females’ fecundity. When the female mating number is low, the benefits from mating exceed the costs whereas in the case of higher mating numbers the costs exceed the benefits. This finding
supports the hypothesis that there is an optimal mating rate for females that maximizes their fitness (Arnqvist & Nilsson 2000).

Fig. 4. The mean of female a) egg number (+ 1 S.E.) and b) egg hatching success (+ 1 S.E.) when mated with a single or two males or four different males (II).
It remains to be tested what the role of convenience polyandry in *A. paludum* mating behaviour is in natural conditions where male harassment rates may be much higher than in my study. On the other hand, *A. paludum* inhabits quite big water areas (mostly lakes) where the local densities may be highly varying (own observations). Females may thus be better able to behaviourally avoid males than in *Gerris* species inhabiting smaller ponds. These kinds of differences in the intensity of sexual selection may help to explain why females’ ability to reject harassing males varies between water strider species (Arnqvist 1997). For example, whereas in *G. buenoi* 92% of precopulatory struggles do not lead to copulation (Devost & Turgeon 2016) *A. paludum* females succeed to reject males only in about 50% of struggles (I).

**Benefits from mating – material or genetic?**

The difference between the material and genetic benefits from mating to females is that material benefits can be gained by mating repeatedly with the same male whereas genetic benefits only arise when mating with different males. Thus standardizing female mating frequency and varying the number of partners could distinguish material from genetic benefits if male mating history does not vary among females (Tregenza & Wedell 1998, Worden & Parker 2001). My results (II) thus suggest that the benefits *A. paludum* females gain from matings are not genetic but material.

Since water strider males do not provide females with any food items, spermatophores or other direct material advantages during matings (see Arnvqvist 1997), only the ejaculate compounds or other factors affecting postcopulatory sexual selection could explain my results. In water striders, the role of male seminal fluid in postcopulatory processes has been ignored as it has been thought to contain nothing but sperm (Arnvqvist 1997), but the recent advancements in this study area imply that this view should be updated. Besides accessory substances (Thornhill & Alcock 1983, Zeh & Smith 1985, Andersson 1994, Arnvqvist & Nilsson 2000) also other components of ejaculate, such as water, parasperm and macromolecules are now recognized to have a role in postcopulatory sexual selection and conflict (Perry *et al.* 2013, Yamane *et al.* 2015). If the seminal fluid of *A. paludum* has some accessory substances playing a role in sperm competition but having toxic side effects on females, this could explain why polyandrous females in my study (II) had lower fecundity than monandrous females. *A. paludum* males do not have specialized collateral glands (Andersen 1982), and
little is known about the existence of glandular cells in testes or the ejaculatory duct. No definitive conclusions about the role of accessory substances in the *A. paludum* mating system can thus be drawn as yet.

It is also possible that the males in my study (II) mating repeatedly with the same female may have invested more in their ejaculates when more certain of their paternity, as found in some species (Thomas & Simmons 2007, Bonilla *et al.* 2011). This hypothesis, however, remains to be directly tested. Although increasing female instant fecundity, male substances in many insect species decrease her lifespan (reviewed by Arnqvist & Nilsson 2000).

Alternatively, my results could arise due to female-related postcopulatory processes. Water strider females are able to store sperm from different males in their spermatheca (Andersen 1993) and the complex and unique morphology of their reproductive organs is likely to provide them with some control over sperm usage (Devost & Turgeon 2016). Cryptic female choice is usually suggested to increase female fecundity by allowing them to choose to fertilize their eggs with the ‘right’ sperm (Andersson & Simmons 2006, Parker & Pizzari 2010, Eberhard 2015), but in my study the polyandrous females had the lowest fecundity, leaving the role of cryptic female choice unclear.

Importantly, the fitness benefits that females obtain from polyandry have differed even within species in different studies (Ivy & Sakaluk 2005), which suggests that besides mating pattern, several other factors combine to determine female fitness (Tregenza *et al.* 2003). For example, due to genotype–environment interactions the genetic benefits of polyandry may appear only in certain environmental conditions (Hosken & Stockley 2003, Maklakov & Lubin 2006). Polyandry may also function as a risk-spreading strategy (bet-hedging) over several generations in changing environmental conditions (Garcia-Gonzales *et al.* 2014), which my study design was not able to test. There are also life history and population differences that may pertain to the costs and benefits females obtain from polyandry (Shuker *et al.* 2006).

A number of other studies using the same experimental design as my study II have reported increases in different female fitness components as a result of polyandry. These include increased fecundity, egg hatching success, offspring survival, offspring size, and sons’ reproductive success (Jennions *et al.* 2007). Polyandry reduced female fitness in only a few studies (Jennions *et al.* 2007), for example by reducing her life span (Fedorka & Mousseau 2002, Dunn *et al.* 2005). However, some studies with different designs have reported reduced female lifetime fecundity resulting from polyandry as found in my experiment (Jennions
et al. 2007). Interestingly, in *G. buenoi* female reproductive success increases with the level of offspring sired by different males. However, the number of different mating partners *per se* does not affect female fecundity, indicating that rather than precopulatory differences in male mating success, postcopulatory differences in male fertilization success were significant in terms of female fecundity (Devost & Turgeon 2016).

### 3.2.2 Male precopulatory success

I found that *A. paludum* males successful in precopulation selection are also beneficial for female fecundity. Females mating with males successful in gaining matings in the presence of another male or alone with a female have higher egg fertilization and hatching probability and offspring survival (III, Table 1). My results also show a correlation between the duration of precopulatory struggle and egg fertility; a short precopulatory struggle strongly increases egg fertility (III, Table 1).

My results imply either that females show preference for certain males and precopulatory struggles thus serve as tools to assess male quality, or that males successful in overcoming females’ resistance have better fecundity as well. Some studies have shown that preferred males bring fitness costs to females (Friberg & Arnqvist 2003) while others have found the opposite tendency (Cunningham & Russell 2000, Drickamer *et al.* 2000, Drickamer *et al.* 2003, Anderson *et al.* 2007).

Contrary to my results (III), in the water strider species *A. remigis*, the duration of both precopulatory struggle and mating correlates positively with mature eggs carried by females (Weigensberg & Fairbairn 1996). In *A. remigis*, mating success is associated with paternity assurance as well (Vermette & Fairbairn 2002) while other studies have found a positive correlation between mating duration and fertilization success (Rubenstein 1989, Arnqvist & Danielsson 1999a, Arnqvist & Danielsson 1999b), perhaps due to increased sperm transfer (Campbell & Fairbairn 2001). In *A. paludum*, the benefits of mating possibly arise from specific ejaculate components, while differential paternal allocation might have a role as well (II). Contrary to previous hypotheses, precopulatory struggles also seem to be costly to water strider males, perhaps because males engaging in more struggles transfer smaller and lower quality ejaculates leading to lower fertilization success (Devost & Turgeon 2016). It is thus possible that in my study, longer precopulatory struggles decreased females’ fecundity also due to energetic costs of struggling to males.
Females may also allocate resources to eggs differentially based on the quality of their mate, for example by laying more eggs or by increasing egg size or quality when mated with an attractive male (see Eberhard 2015 for review). Increased female investment may also reflect male ability to manipulate e.g. female sperm use or ovipositioning (Gillott 2003). The role of parental allocation in female fecundity remains to be tested in *A. paludum.*
Table 1. Model-averaged fixed effects of male and female characters and their relative weights of generalized mixed-effects models explaining variation in (a) realized female fecundity, (b) fertility, (c) egg hatching success, (d) survival of the nymphs, and (e) juvenile survival until the 20th day of nymphal development (II).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Source of variation</th>
<th>z</th>
<th>p</th>
<th>Rel. weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Fecundity</td>
<td>(Intercept)</td>
<td>0.302</td>
<td>0.763</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female abdomen width</td>
<td>2.995</td>
<td>0.0028</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Male abdomen width</td>
<td>2.85</td>
<td>0.0044</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Male success (winner)</td>
<td>1.34</td>
<td>0.1802</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Male preference status (preferred)</td>
<td>14.51</td>
<td>0.357</td>
<td>0.20</td>
</tr>
<tr>
<td>(b) Fertility</td>
<td>(Intercept)</td>
<td>0.586</td>
<td>0.5576</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female abdomen width</td>
<td>4.22</td>
<td>2.44×10^{-5}</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male preference status (preferred)</td>
<td>2.165</td>
<td>0.0304</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male success (winner)</td>
<td>2.222</td>
<td>0.0263</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male abdomen width</td>
<td>2.203</td>
<td>0.0276</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Precopulatory struggle duration</td>
<td>5.326</td>
<td>1.0×10^{-7}</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male preference status (preferred) × Male success</td>
<td>1.489</td>
<td>0.1365</td>
<td>0.42</td>
</tr>
<tr>
<td>(c) Egg hatching success</td>
<td>(Intercept)</td>
<td>5.677</td>
<td>2.0×10^{-16}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female abdomen width</td>
<td>3.387</td>
<td>0.0007</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male preference status (preferred)</td>
<td>2.271</td>
<td>0.0231</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male success (winner)</td>
<td>0.047</td>
<td>0.9628</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male abdomen width</td>
<td>5.235</td>
<td>2.0×10^{-7}</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Precopulatory struggle duration</td>
<td>2.309</td>
<td>0.021</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Male preference status × Male success</td>
<td>2.432</td>
<td>0.015</td>
<td>0.65</td>
</tr>
<tr>
<td>(d) Nymph survival</td>
<td>(Intercept)</td>
<td>2.711</td>
<td>0.0067</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female abdomen width</td>
<td>2.244</td>
<td>0.0248</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male abdomen width</td>
<td>2.406</td>
<td>0.0161</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male success (winner)</td>
<td>0.928</td>
<td>0.3534</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Male preference status (preferred)</td>
<td>0.81</td>
<td>0.4177</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Precopulatory struggle duration</td>
<td>0.79</td>
<td>0.4297</td>
<td>0.18</td>
</tr>
<tr>
<td>(e) Juvenile survival</td>
<td>(Intercept)</td>
<td>6.005</td>
<td>2.0×10^{-16}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female abdomen width</td>
<td>5.142</td>
<td>3.0×10^{-7}</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male preference status (preferred)</td>
<td>0.196</td>
<td>0.8446</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male success (winner)</td>
<td>1.024</td>
<td>0.3058</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male abdomen width</td>
<td>3.325</td>
<td>0.0009</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Precopulatory struggle duration</td>
<td>4.045</td>
<td>5.23×10^{-5}</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Male preference status (preferred) × Male success</td>
<td>2.123</td>
<td>0.0338</td>
<td>0.72</td>
</tr>
</tbody>
</table>
3.2.3 Morphological traits

When measured as total body length, *A. paludum* female size does not correlate with fecundity (II). However, when the size is measured as abdomen height, both male and female body sizes affect female fecundity significantly (III, Table 1). Females with higher abdomen have better egg fertilization rates as well as realized fecundity, whereas male size correlates negatively with both of these parameters. However, both male and female size has a positive effect on egg hatching success as well as offspring survival (III, Table 1).

In many insect species, female body size is positively correlated with fecundity (Honěk 1993), whereas male body size may correlate positively (Savalli & Fox 1998) or negatively with female fecundity (Pitnick & Garcia-Gonzalez 2002). Female abdomen width is known to indicate her gravidity fluctuating over her reproductive cycle with ‘fat females’ carrying more mature eggs (Bonduriansky 2001). It is thus reasonable to conclude that in *A. paludum*, female abdomen height acts as this kind of indicator.

Interestingly, in my study (III) larger male size increased female egg hatching success and offspring survival but decreased egg fertilization rate and female realized fecundity. Since large males are likely to be able to produce larger ejaculates (see Savalli & Fox 1998), my results might be explained by ejaculate compounds or nutrients increasing female egg production. However, if the amount of sperm does not vary much according to ejaculate size, or if males trade off between the sperm and other ejaculate compounds, all produced eggs are not inseminated. Notably, since the females in experiment III only mated with one male, cryptic female choice or sperm competition were not involved in these results.
4 Concluding remarks

In this thesis, I investigated sexual conflict, polyandry and the ensuing precopulatory selection pressure and fecundity effects in a water strider, *A. paludum*. Water striders are quite often used as study models in sexual conflict studies due to their mating system with a high level of polyandry and apparent conflict between the sexes with several antagonistically evolving traits (Rowe *et al.* 1994, Arnqvist 1997, Arnqvist & Rowe 2002).

The mating system of *A. paludum* is characterized by several factors influencing the outcome of mating and fecundity. I have shown that female abdominal spines serve as a tool to increase their control over copulation decision (I) as previously shown in *G. incognitus* (Arnqvist & Rowe 1995). However, the duration of precopulatory struggle is independent of the natural variation in female spine length in *A. paludum* indicating quite low current selection pressure for this female trait. The duration of precopulatory struggle was not affected by any other female morphological trait, either (III). Instead, female resistance seems to result in selecting certain male types that can be divided into three groups: 1) short, successful males with a long thorax, 2) long, successful males with a short thorax and 3) unsuccessful males with a wide 7th abdominal segment and a long pygophore (III). Male success refers to his performance in the presence of another male. Female resistance acting as a selection force for male traits that increase his ability to grasp the female during precopulatory struggle or his success in scramble-type competition between males has been shown before in several water strider species (see e.g. Arnqvist & Rowe 2002a, Sih *et al.* 2002, Bertin & Fairbairn 2005).

Contrary to predictions I found female *A. paludum* to benefit from multiple matings with the same male while polyandry decreased female fecundity (I, II), which in turn suggests that females gain some material benefits from matings (see Tregenza & Wedell 1998). When mating repeatedly with the same male, female egg number was maximized approximately with 1.4 matings per day whereas both higher and lower mating rates resulted in lower egg production (II). This supports the hypothesis that there is an optimal mating rate for females that maximizes their fitness (see Arnqvist & Nilsson 2000 for review). The possible material benefits *A. paludum* females may gain from matings are discussed.

Interestingly, males successful in precopulatory struggles and in gaining matings in the presence of another male are also beneficial for female fecundity in terms of egg fertilization success, egg hatching probability and offspring survival.
(III). This may reflect either that females prefer certain males and precopulatory struggles thus serve as tools to assess male quality, or that males successful in overcoming females resistance have better fecundity as well.
References


Original articles


Reprinted with permission from Springer (I, II).

Original papers are not included in the electronic version of the dissertation.


663. Pakanen, Minna (2015) Visual design examples in the evaluation of anticipated user experience at the early phases of research and development

664. Hyry, Jaakko (2015) Designing projected user interfaces as assistive technology for the elderly


666. Luukkanen, Tero (2016) New adsorption and oxidation-based approaches for water and wastewater treatment : studies regarding organic peracids, boiler-water treatments, and geopolymers

667. Tolkkinen, Mari (2016) Multi-stressor effects in boreal streams : disentangling the roles of natural and land use disturbance to stream communities

668. Kaakinen, Juhani (2016) Öljyllä ja raskasmetalleilla pilantuentea maita koskevan ympäristölänsäätiön ja lupamenetelystä edistäminen kemiallisella tutkimuksella


670. Rönkä, Nelli (2016) Phylogeography and conservation genetics of waders

671. Fucci, Davide (2016) The role of process conformance and developers’ skills in the context of test-driven development

672. Manninen, Outi (2016) The resilience of understorey vegetation and soil to increasing nitrogen and disturbances in boreal forests and the subarctic ecosystem

673. Pentinsaari, Mikko (2016) Utility of DNA barcodes in identification and delimitation of beetle species, with insights into COI protein structure across the animal kingdom

674. Lassila, Toni (2016) In vitro methods in the study of reactive drug metabolites with liquid chromatography / mass spectrometry

675. Koskimäki, Janne (2016) The interaction between the intracellular endophytic bacterium, Methylobacterium extorquens DSM13060, and Scots pine (Pinus sylvestris L.)

Book orders:
Granum: Virtual book store
http://granum.uta.fi/granum/
Katri Ronkainen

POLYANDRY, MULTIPLE MATING AND SEXUAL CONFLICT IN A WATER STRIDER, AQUIARUS PALUDUM