Sperm competition and female remating rate in the scorpionfly *Panorpa germanica*, L. (Mecoptera, Panorpidae)

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Dagmar Kock

aus Augsburg

Bonn 2005

Erster Gutachter: Prof. Dr. K. P. Sauer

Zweiter Gutachter: Prof. Dr. T. C. M. Bakker

Tag der Prüfung: 20. September 2005

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit selbst und ohne jede unerlaubte Hilfe angefertigt, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt sowie Zitate kenntlich gemacht habe.

Diese oder eine ähnliche Arbeit habe ich noch keiner anderen Stelle zur Prüfung vorgelegt.

Bonn, den 28. Juli 2005

Table of contents

page

General introduction ______ 6

CHAPTER 1: ______ 10

Patterns of sperm use in the scorpionfly *Panorpa germanica* (Mecoptera, Panorpidae)

Abstract 10

1.1 Introduction 10

1.2 Methods 12

1.2.1 Breeding of *P. germanica* 12

1.2.2 Remating experiments 13

1.2.3 Testing for fertility 14

1.2.4 Microsatellite analysis 14

1.2.5 Statistics 15

1.3 Results 15

1.4 Discussion 18

CHAPTER 2: ______ 22

Partial last male sperm precedence and high intraspecific variation in sperm use in the scorpionfly *Panorpa germanica* (Mecoptera, Panorpidae): potential causes and consequences

Abstract 22

2.1 Introduction 23

2.2 Methods 24

2.2.1 Breeding of *P. germanica* 24

2.2.2 Estimating the lifespan of sperm 24

2.2.3 Remating experiments 25

2.2.4 Determination of sperm transfer rates 25

2.2.5 Statistics 26

2.3 Results 27

2.3.1 Lifespan of sperm 27

2.3.2 Sperm use patterns 29

2.3.3 Sperm transfer rates 31

2.4 Discussion 32

2.4.1 Partial last male sperm precedence 32

2.4.2 High variance in sperm use 33

CHAPTER 3 ______ 37

Remating frequency in wild females of *Panorpa germanica* (Mecoptera, Panorpidae) and its implication for sperm competition

Abstract 37

3.1 Introduction 38

3.2 Methods 39

3.2.1 Determination of number and frequencies of different alleles of the PG2-locus in the field 39

3.2.2 Mating status of females collected at different points of time throughout the flying season 40

3.3 Results 40

3.3.1 Number and frequencies of different alleles of the PG2-locus in the field 40

3.3.2 Mating status of wild-caught females 41

3.4 Discussion 43

General discussion and conclusion ______ 45

Summary of the main findings 45

Discussion 46

Conclusion 49

Summary ______ 50

Acknowledgement ______ 52

References ______ 53

General introduction

According to Darwin (1871) sexual selection can be defined as the non-random differential reproductive success resulting either from competition within one sex for access to members of the opposite sex (intrasexual selection) or from differential choice by one sex for members of the opposite sex (intersexual selection). Strategies for maximisation of reproductive success may differ considerably between males and females due to asymmetric reproductive investment (Bateman 1948; Parker 1970; Parker et al. 1972; Trivers 1972). Since females are limited in the production of their nutrient-rich gametes their reproductive output does in general not increase with mating rate. Females may rather maximise their reproductive success by choosing high quality mates siring high quality offspring. As the production of male sex cells generally involves relatively little metabolic energy investment a male's reproductive success is usually limited by its success in fertilising the females' limited number of eggs rather than its ability to produce sperm cells. Consequently, males can often increase their reproductive output by mating with as many females as possible, causing high levels of competition within males for access to females.

Parker (1970) was the first to point out that this male-male competition may continue after copulation if sperm from two or more males compete inside the female's reproductive tract for fertilisation of the ova, a process he termed sperm competition. Its intensity largely depends on the remating frequency of females (Parker 1970). Sperm competition extends sexual selection beyond the point of copulation and can clearly act as a powerful selective force in the evolution of reproductive strategies and mating systems, favouring male traits that increase and assure paternity (Stockley 1997). For instance, in *Odonata* species males are able to displace rival sperm by means of complex genital structures (Cordoba-Aguilar et al. 2003). It is quite evident that the risk of sperm competition plays a major role in the evolution of many reproductive traits such as testes size, sperm numbers and sperm size, mate guarding, or

frequency and duration of copulations (Smith 1984; Birkhead and Møller 1998). In order to avoid sperm competition males may exhibit mate guarding after copulation for preventing females from remating as it was shown for the dungfly *Scatophaga stercoraria* (Parker 1978). Instead of mate guarding males of some species produce copulatory plugs preventing rival sperm from penetrating the female's reproductive tract (e. g. in the funnel-web spider *Agelena limbata*, Masumoto 1993).

During the last decades a variety of insect species has been studied in terms of sperm competition (reviewed by Gwynne 1984; Birkhead & Hunter 1990; Danielsson 1998; Simmons & Siva-Jothy 1998). In these studies patterns of sperm use are typically reported as the mean species value of P_2, determined as the proportion of offspring sired by the second male to copulate with a doubly mated female (Boorman & Parker 1976). Generally, intermediate values of P_2 are taken as evidence for sperm mixing, whereas extreme values of P_2 indicate sperm precedence, i. e. the non-random utilisation of sperm from one of several males to mate with a female (Simmons & Siva-Jothy 1998). So far, it has turned out that in insects a typical pattern of sperm use in doubly mated females is a majority of fertilisations gained by the last male, i. e. a last male sperm precedence; in other cases both males share paternity, whereas first male sperm precedence seems to be rather scarce (for reviews see Gwynne 1984; Birkhead & Hunter 1990; Danielsson 1998; Simmons & Siva-Jothy 1998).

An extensively studied mating system is that of the scorpionfly *Panorpa vulgaris* (Mecoptera, Panorpidae). In scorpionflies males produce nutritious saliva secretions and offer them to females as nuptial gifts prior to and/or during copulation (e.g. Sindern 1996; Sauer et al. 1998; Sauer 2002). For *P. vulgaris* this saliva secretion has been shown to function as an honest signal for male quality (Sauer 1996; Sindern 1996; Sauer et al. 1998; Kurtz & Sauer 1999) according to the 'good gene' models of sexual selection (Zahavi 1975; Andersson 1982). Females as well as males of *P. vulgaris* exhibit a polygamous mating behaviour (Sauer

et al. 1999), resulting in high levels of sperm competition. The mechanism of sperm competition is well understood in this species (e. g. Sauer et al. 1998; 1999). It could be shown that the duration of a copulation depends on the number of salivary masses a male presents and sperm is transferred continuously throughout copulation (Sauer et al. 1997; 1998). Males that are able to produce many salivary masses therefore also achieve longer copulations and hence transfer more sperm than males with lower saliva secretion. Using polymorphic microsatellite loci Sauer et al. (1999) determined paternity among the offspring of multiply mated females and their results suggest a complete mixing of sperm from different males inside the female's spermatheca – i. e. sperm of different males compete numerically for fertilisation of the female's eggs. Because of this pattern of sperm use female *P. vulgaris* allocate paternity in proportion to male quality, since high quality males achieve longer copulations through a high saliva secretion and hence transfer more sperm than low quality males (Sauer 1996; 2002; Sindern 1996; Sauer et al. 1998).

Until now *P. vulgaris* was the only Mecopteran species that had been studied in terms of sperm competition. Aim of the present study was to elucidate the mechanism of sperm competition and its implications in another *Panorpa* species, *P. germanica*, showing a very different mating system compared to that of *P. vulgaris*. Males of *P. germanica* release pheromones in order to attract females, mating takes place only in the evening and at night, and with a mean duration of 436.9 ± 135.4 minutes copulations are rather long in this species (Gerhards 1999). Another difference to *P. vulgaris* is the remating frequency of females. In laboratory experiments females of *P. germanica* showed a monandrous or slightly polyandrous mating behaviour, whereas males behave polygynous (Gerhards 1999, see also CHAPTER 3). Since some females do mate multiply the question arises how sperm from different males are utilised in these cases.

Primary aim of this study therefore was the investigation of sperm use patterns in doubly mated females of *P. germanica* as presented in CHAPTER 1. Searching for possible causes of the observed patterns I tested the actual lifespan of *P. germanica* sperm and, furthermore, investigated the relevance of differences in sperm transfer rates for the outcome of sperm competition. These experiments are presented in CHAPTER 2 and further potential causes and consequences of the observed patterns of sperm use are discussed. Finally, CHAPTER 3 deals with remating frequencies of wild female *P. germanica* and its implication for the selective force of sperm competition. A general discussion will summarise all findings and conclusions about the role of sperm competition in the mating system of *P. germanica* will be drawn.

As each chapter is intended to be comprehensible in itself some repetition of descriptions and explanations is occasionally inevitable.

CHAPTER 1

Patterns of sperm use in the scorpionfly *Panorpa germanica* (Mecoptera, Panorpidae)

Abstract

Sperm competition can be a powerful selective force in the evolution of reproductive strategies and mating systems. In studies on sperm competition patterns of sperm use are typically reported as the mean species value of P_2, determined as the proportion of offspring sired by the second male to copulate with a doubly mated female. However, the within-species variance in P_2 has mostly been ignored, although taking this variance into account may be crucial for understanding the underlying mechanisms of sperm competition.

Paternity analysis among the offspring of doubly mated females of *Panorpa germanica* (Mecoptera, Panorpidae) revealed a very high intraspecific variance in P_2. The distribution of paternity was found to depend mainly on the proportional duration of both copulations, but with the second male on average having some advantage over the first male. However, for individual males the outcome of sperm competition remains uncertain because variance in P_2 is extremely high in this species, irrespective of copulation durations.

1.1 Introduction

Sperm competition occurs when the sperm from more than one male are present inside the female's reproductive tract at the same time, competing for fertilisation of the ova (Parker

1970). This male-male competition can act as a powerful selective force in the evolution of mating systems shaping many reproductive traits such as testes size, sperm numbers and sperm size, mate guarding, or frequency and duration of copulations (Smith 1984; Birkhead and Møller 1998). In studies on sperm competition patterns of sperm use are often inferred from the mean species value of P_2 (*sensu* Boorman & Parker 1976). Generally, intermediate values of P_2 are believed to be the result of sperm mixing, whereas extreme values of P_2 are taken as evidence for sperm precedence (Parker et al. 1990; Simmons & Siva-Jothy 1998).

Sperm competition has been studied in a variety of insect species (reviewed by Gwynne 1984; Birkhead & Hunter 1990; Danielsson 1998; Simmons & Siva-Jothy 1998). Although most studies revealed a rather considerable intraspecific variation in P_2, this within-species variance has often been ignored (Lewis & Austad 1990; Simmons & Siva-Jothy 1998; Simmons & Achmann 2000). Yet, explaining this within-species variance could help to understand the underlying mechanisms of sperm competition (Lewis & Austad 1990; Simmons & Siva-Jothy 1998; Simmons & Achmann 2000).

Extensive studies on the mating system of *P. vulgaris* (see Sauer et al. 1998; 1999) have shown that paternity distribution among the offspring of multiply mated females of this species is highly variable, but clearly represents cryptic female choice. Since sperm is transferred continuously throughout copulation (Sauer et al. 1997), males being able to produce many salivary masses transfer more sperm than males with lower salivary secretion, because females adjust copulation duration according to the male's production of salivary masses (Sauer et al. 1998). Through a complete mixing of sperm from different males (Sauer et al. 1999) female *P. vulgaris* allocate paternity in proportion to male quality, since high quality males achieve longer copulations through a high saliva secretion (Sauer 1996; 2002) and hence transfer more sperm than low quality males (Sindern 1996; Sauer et al. 1998). This 'cryptic female choice' (*sensu* Thornhill 1983) based on saliva secretion as an honest signal for male quality (Sauer 1996; Sindern 1996; Sauer et al. 1998; Kurtz & Sauer 1999) is a

secure method to discriminate between males of different qualities. However, in order to do so females of *P. vulgaris* always have to mate multiply.

In contrast to *P. vulgaris* females of *P. germanica* show a monandrous or slightly polyandrous mating behaviour (Gerhards 1999, see also CHAPTER 3). However, since some females do mate multiply the question arises how sperm from different males are utilised in these cases. As female remating frequency is much lower in *P. germanica* than in *P. vulgaris* the selective force of sperm competition should also act less strongly – possibly resulting in different sperm precedence patterns in both species.

In order to investigate patterns of sperm use (i. e. estimating P_2) in the scorpionfly *Panorpa germanica* I determined paternity among the offspring of doubly mated females using a polymorphic microsatellite locus.

1.2 Methods

1.2.1 Breeding of *P. germanica*

For the experiments on sperm use patterns I used F1-offspring from *P. germanica* collected in the field near Freiburg i. Br., Germany – a collection site at which *P. germanica* has two fully developed generations per year. Adults of the first generation emerge at the end of April/beginning of May and those of the second generation in July/August. First experiments were conducted in July/August 2002 with F1-offspring from *P. germanica* collected in May 2002 and further experiments were carried out in May 2003 with offspring of adults collected in August 2002. In order to obtain F1-offspring pairs of wild *P. germanica* were kept in plastic boxes (10 cm x 10 cm x 6 cm) equipped with small petri dishes filled with moist peat for oviposition. Food (segments of last larval instar of *Tenebrio molitor*) was provided ad libidum. Larvae obtained from pairs in the spring generation were reared on an 18 h light/6 h

dark-photoperiod inducing diapause-free development (Sauer 1970; 1977). Reaching the third larval instar larvae were transferred into soil-filled, open-bottomed plastic cylinders (∅ 40 cm, depth 1m) placed outdoors in the ground for finishing development until hatching of adults in summer. Larvae obtained from pairs in the summer generation were reared on a 12 h light/12 h dark-photoperiod inducing diapause. As third larval instar they were again transferred into the outdoor plastic cylinders, but hibernated. Having finished diapause adults hatched the next spring. After emergence females as well as males were kept separately in small plastic cylinders (∅ 3.5 cm, height 8 cm) containing moist tissue for water supply. All adult scorpionflies were provided with 1 segment of a last larval instar of *Tenebrio molitor* at the day of hatching and in the following every fourth day.

1.2.2 Remating experiments

In order to investigate patterns of sperm use female *P. germanica* were each mated to two different males and the offspring was then taken for paternity analysis. Gerken et al. (1998) established a method for extracting DNA from a small amount of insect hemolymph. Taking 1-3 µl hemolymph of an adult *Panorpa* scorpionfly does not harm the animal but is sufficient for DNA extraction. Thus, individuals already genotyped with respect to the PG2-microsatellite-locus, which was used for paternity determination (see chapter *1.2.4 Microsatellite analysis*, pp. 9-10), could be used in the remating experiments. This method allowed arranging mating partners in a way that paternity of the offspring could always be determined unambiguously.

Prior to the experiments all animals were marked individually on their forewings for identification and then were transferred into observation cages (30 cm x 30 cm x 60 cm, 6 males and 6 females per cage). Of all copulations taking place the involved individuals as well as the time of beginning and end of the copulation was recorded. After their first copulation females were allowed to mate a second time, but with a different male.

In the first experiment (August 2002) the durations of the two copulations of a female were manipulated resulting in three distinct groups of females (see Table 1. *P_2-values and proportional copulation durations*, p. 11): in *group 1* the first copulation of the female was terminated after 3 hours, while the second copulation ended naturally (i. e. was long); in *group 2* females had a natural (i. e. long) first copulation but the second copulation was interrupted after 3 hours; and females of *group 3* always had two natural (i. e. long) copulations. In the experiment in May 2003 females always had two natural copulations and hence belong to *group 3*. One female, however, had a very short second copulation (60 min) and was thus assigned to *group 2*. In all experiments the females were allowed to lay eggs after the second copulation. All hatched larvae were transferred into 100% ethanol and kept for paternity analysis. After the experiments males and females were stored at a temperature of -80°C until DNA extraction.

1.2.3 Testing for fertility

Males that were used in the remating experiments were tested for fertility by mating them once more to a virgin female. These females were then kept for egg deposition. If any larvae hatched the according male was taken as being fertile. Thus, if P_2-values of 1.0 or 0.0 occur infertility of the according males can be excluded.

1.2.4 Microsatellite analysis

The DNA of adults and larvae was extracted using the standard protocol of an isolation kit (Nucleo Spin Tissue, Machery & Nagel). Paternity was determined using a polymorphic microsatellite locus, named PG2, which had been identified in collaboration with Prof. Dr. Epplen, University of Bochum, and his research team using standard procedures (e.g. Epplen et al. 1998; Lubjuhn & Sauer 1999). The PG2-locus represents a $(TA)_m$ $(CA)_n$ $(TA)_m$ A $(TA)_m$ repeat and primers used for its amplification were PG 25: 5´ - GGA GAC CAA TGA GTA

TAG TAA ATC - 3´ and PG 23: 5´- ATC GTT AAT TCG TAA TCC GAG GC - 3´. The primer PG 25 was fluorochrome (HEX) labelled for automatic fragment sizing of PCR products on an ABI 377 sequencer, utilising the GeneScan software program. For each DNA sample PCR reactions contained approximately 50-100 ng DNA, 1 x PCR buffer, 200 µM dNTPs, 3 pmol of each primer, 2.3 mM $MgCl_2$, and 0.25 U Taq polymerase (Invitrogen), and were performed in a T1 thermocycler (Biometra). The initial denaturing time was 5 min at 94°C, thereafter 1 min for 6 cycles and 30 seconds for another 25 cycles. Annealing time was 1 min at 68°C at the first cycle, but with a decline of 1°C in each of the following 5 cycles (touchdown PCR) reached 63°C in cycle 6 at which temperature it remained for the last 25 cycles. Elongation lasted 1 min at 70 °C in every cycle and 5 min at 72°C in the final step.

1.2.5 Statistics

All statistics were performed using the SPSS 12.0 software. Quoted significance values are for two-tailed tests. The level of significance was set to $p < 0.05$. Data sets were tested for deviation from normal distribution using the Kolmogorov-Smirnov test with Lolliefors correction. A one-sample-t-test was performed testing for deviations of observed P_2-values from an expected distribution inferred from proportional copulation durations. Spearman rank correlations were applied in order to test for a relationship between proportional copulation duration and proportional paternity.

1.3 Results

In total 1054 larvae obtained from 26 doubly mated females were taken for paternity analysis. Table 1 summarises proportional copulation durations of both males and the pattern

of sperm use found in the three female groups. The mean P_2-value as well as standard deviations and ranges of P_2 are given for each group.

Table 1.
P_2-values and proportional copulation durations in the three female groups.

female group	n =	proportional copulation duration of 2nd male ± SD	mean P_2 ± SD	range of P_2
1 (1st cop. short, 2nd cop. long)	5	0.736 ± 0.026	0.77 ± 0.24	0.51-1.0
2 (1st cop. long, 2nd cop. short)	8	0.229 ± 0.052	0.25 ± 0.26	0.0-0.68
3 (both cop. long)	13	0.457 ± 0.061	0.62 ± 0.28	0.13-1.0

No clear sperm precedence can be detected as P_2-values are intermediate, ranging from 0.25 in *group 2* to 0.77 in *group 1*. The mean P_2-value obtained in *group 3*, 0.62, can be taken as the mean species P_2 as this group best reflects natural conditions with both copulations of the females having ended naturally. The variation of P_2 is rather high in all three groups (see standard deviation and range of P_2). Copulation duration apparently has an effect on paternity, as P_2 is high in *group 1*, in which the second copulation was long and the first copulation short, but low in *group 2*, in which the first copulation was long and second copulation short. In *group 3* consisting of females that had two long copulations we find an intermediate value for P_2, but slightly shifted towards a higher proportion of offspring sired by the second male ($P_2 = 0.62$).

Calculating the proportional copulation duration for both males, a correlation between the proportional copulation duration of the second male and the P_2-value turns out (Spearman rank correlation; $n = 26$; $r_s = 0.641$; $p < 0.001$; Fig. 1). These results suggest mixing of sperm from different males in the female's spermatheca, although variance in P_2 is extremely high. Overall, proportional paternity is slightly shifted towards the second male, as most data points lie above the bisecting line (indicated in the diagram, Fig. 1). Assuming complete mixing of

sperm according to the 'fair raffle' model of Parker et al. (1990) P_2-values can be predicted by calculating the proportional copulation durations of both males. The mean difference between observed and expected P_2 was found to be 0.085 ± 0.050 (n = 26). A one-sample-t-test revealed no significant deviation from the expected distribution of P_2, only a tendency towards the second male turned out ($t_{25} = 1.71$; p = 0,100).

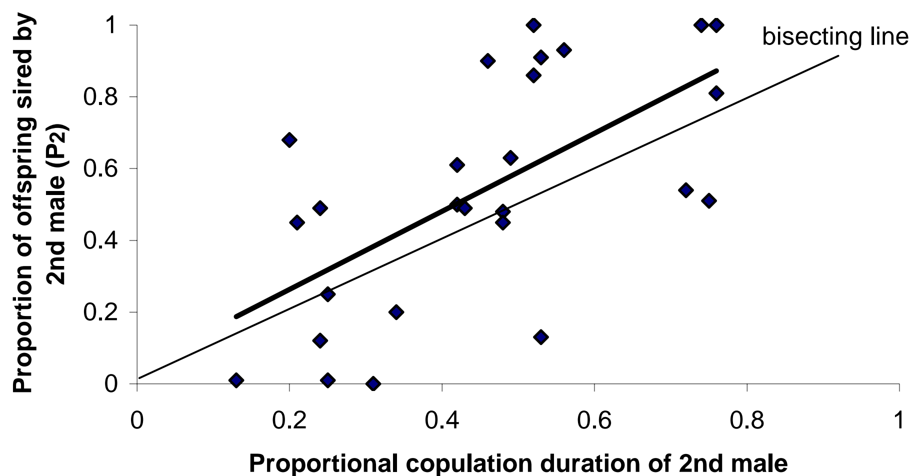

Figure 1.
Correlation between P_2 and the proportional copulation duration of 2nd male (all female groups): Spearman rank correlation; n = 26; $r_s = 0.64$; $p < 0.001$.

If only those females that had two long copulations (i. e. females of *group 3*) are considered a correlation between proportional copulation duration of the 2nd male and P_2 cannot be detected any longer (Spearman rank correlation; n = 13; $r_s = 0.442$; p = 0.130; Fig. 2). In this group the proportional copulation duration of the second male is always close to 0.5 (i. e. both copulations were similarly long), but a very high variance in P_2 remains. However, the overall proportional paternity is even more shifted towards the second male than it was considering all four groups together (n = 13; mean diff. = 0.147 ± 0.071; one-sample-t-test: $t_{12} = 2.07$; p = 0.061).

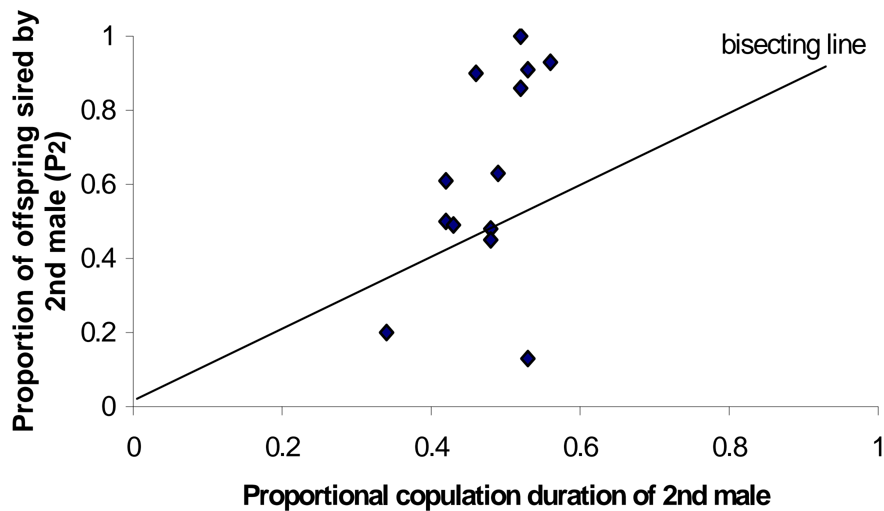

Figure 2.
Correlation between P_2 and the proportional copulation duration of 2nd male (only female group 3): Spearman rank correlation; $n = 13$; $r_s = 0.442$; $p = 0.130$.

1.4 Discussion

Paternity analysis among the offspring of 26 doubly mated females of *P. germanica* revealed a correlation between the proportional copulation duration of an individual male and the proportion of the female's offspring this male will sire. If the durations of two copulations of a female are very disproportionate, the male achieving the longer copulation will also sire a larger proportion of the female's offspring. Since sperm is transferred continuously throughout the copulation (Gerhards 1999) the mating duration determines the number of sperm each male contributes. The observed correlation between proportional copulation duration and paternity thus suggests mixing of sperm from both males inside the female's spermatheca. However, this mixing of sperm appears to be somewhat incomplete, as paternity is overall slightly shifted towards the second male fathering a higher percentage of the female's offspring than its proportional copulation duration would predict in case of complete

sperm mixing. It has been suggested that high values of P_2 could result from the depletion or death of first male sperm (Tsubaki & Yamagishi 1991), rather than being attributable to an incomplete mixing of sperm. In scorpionflies no evidence for the depletion of sperm has been observed (Sauer, personal communication). I furthermore assume that death of first male sperm is rather unlikely to be the cause of high P_2-values, as in my experiments the time span between first and second copulation of the females was usually only about 1-2 days, rarely exceeding 5 days. Nevertheless, I tested the actual lifespan of *P. germanica* sperm in a subsequent experiment. Results will be presented in CHAPTER 2 and the relevance of the lifespan of sperm for the outcome of sperm competition will be discussed.

In my experiments short copulations (i.e. less than 4 hrs) were - apart from a single case - only obtained by interrupting copulating pairs. Assuming that such short copulations seldom occur under natural conditions the proportion of copulation duration of the two males of a doubly mated female will often be close to 0.5. In this case of two similarly long matings the second male will on average have some advantage over the first male, inseminating a higher proportion of the female's eggs. Males should therefore aim to either be the only or the last mate of a female. Interestingly, Gerhards (1999) found in laboratory experiments that the remating frequency of female *P. germanica* depends on the duration of the first mating: the longer the first copulation of a female, the less likely she will mate again. Consequently, males should always aim to achieve copulations that are as long as possible. In case a male is the first male to mate with a female a long copulation will reduce the probability that the female will mate again, in which case this male will fertilise all of the female's eggs – i. e. long copulation durations reduce the risk of sperm competition. If, on the other hand, the female has already mated a further male again should aim to achieve a long copulation as this is likely to increase its proportional paternity.

However, for individual males the outcome of sperm competition remains uncertain because variance in P_2 was found to be extremely high in this species, irrespective of copulation duration.

Intraspecific variation in sperm precedence is often believed to represent the outcome of conflicts between male and female interests, either driven through male adaptations (e. g. increased rates of sperm transfer; Simmons and Parker 1992), female adaptations (e. g. cryptic female choice; Eberhard 1996) or an interaction of both (Stockley 1997; Birkhead and Møller 1998). Assuming sperm mixing an expected variance in P_2 can be predicted if the variation in the number of sperm transferred by different mates of a female can be estimated. In order to reveal if the paternity distribution observed reflects differences in sperm transfer rates I designed a further experiment in which the number of sperm different males transferred in remating experiments was estimated. The results of these experiments will be presented and discussed in CHAPTER 2.

It has also been proposed that infertile matings caused by male sterility or insemination failures might be responsible for high intraspecific variances in sperm precedence patterns (García-González 2004). In the experiments presented in this chapter, however, fertility of the involved males was tested and paternity analysis was only conducted in those families of which both males were found to be fertile. Yet, sperm viability may have an influence on paternity distribution (Hunter & Birkhead 2002; García-González & Simmons 2005), but could not be measured in my experiments.

Harvey & Parker (2000) demonstrated that high variances in P_2 could also simply be explained by a random mixing of discrete ‘sperm packets’ from different males, referred to as ‘sloppy sperm mixing’. Assuming sperm competition to begin with ejaculates forming discrete packages of sperm from different males, sperm mixing can only occur if these packages break up into smaller ‘packets’. Depending on size and number of packets formed, a random mixing of sperm packets can result in a variety of P_2 distribution patterns. These

models clearly help to understand the possible proximate causes of P_2 distribution patterns, the selective forces behind these processes, however, often remain obscure.

Potential causes as well as consequences of the observed patterns of sperm use in *P. germanica* will be the focus of CHAPTER 2.

CHAPTER 2

Partial last male sperm precedence and high intraspecific variation in sperm use in the scorpionfly *Panorpa germanica* (Mecoptera, Panorpidae): potential causes and consequences

Abstract

In the last decades many insect species have been studied in terms of sperm competition. Patterns of sperm use are often inferred from the mean species value of P_2, defined as the mean proportion of offspring sired by the second male in double-mating trials. In the present study double-mating trials with female scorpionflies, *Panorpa germanica* (Mecoptera, Panorpidae), were conducted controlling for copulation durations. Despite of identical copulation durations the variance in P_2 turned out to be extremely high and paternity was overall shifted towards the second male on average siring a higher percentage of offspring than the first male. Testing the actual lifespan of sperm I conclude that this partial last male sperm precedence is not caused by death of first male sperm. Estimating sperm transfer rates of both mates of a female it can furthermore be concluded that the high intraspecific variance in P_2 cannot be explained by variances in sperm transfer rates among *P. germanica* males. Other factors possibly causing the observed patterns as well as male and female strategies for fitness maximisation are discussed.

2.1 Introduction

Sperm competition has been studied in a variety of insect species (reviewed by Gwynne 1984; Birkhead & Hunter 1990; Danielsson 1998; Simmons & Siva-Jothy 1998). In these studies patterns of sperm use are usually inferred from the mean species value of P_2 (*sensu* Boorman & Parker 1976). Intermediate values of P_2 are believed to be the result of sperm mixing, whereas extreme values of P_2 indicate sperm precedence (Parker et al. 1990; Simmons & Siva-Jothy 1998). Although most studies revealed a rather considerable intraspecific variation in P_2, this within-species variance has often been ignored (Lewis & Austad 1990; Simmons & Siva-Jothy 1998; Simmons & Achmann 2000). Yet, explaining this within-species variance could be crucial for understanding the underlying mechanisms of sperm competition (Lewis & Austad 1990; Simmons & Siva-Jothy 1998; Simmons & Achmann 2000).

The experiments on sperm use patterns in the scorpionfly *Panorpa germanica* presented in CHAPTER 1 revealed a very high intraspecific variance in P_2. The distribution of paternity was found to depend mainly on the proportional duration of both copulations, but with the second male on average having some advantage over the first male (mean P_2: 0.62 ± 0.28). It has been suggested that high values of P_2 could be the result of depletion or death of first male sperm (Tsubaki & Yamagishi 1991). In scorpionflies no evidence for the depletion of sperm has been observed (Sauer, personal communication). In order to reveal if the shift in paternity distribution towards the second male could be caused by death of first male sperm the actual lifespan of sperm of *P. germanica* was now tested.

Apart from paternity distribution being shifted towards the second male the intraspecific variance in P_2 was found to be extremely high in this species. An expected variance in P_2 can be predicted if the variation in the number of sperm transferred by different mates of a female can be estimated. In order to reveal if the observed variation in P_2 simply reflects variation in

sperm transfer rates between *P. germanica* males, I designed an experiment in which females got mated equally long to two different males of which the sperm transfer rates got determined. Paternity analysis among the offspring of these doubly mated females were carried out in order to reveal if paternity depends on differences in sperm transfer rates.

2.2 Methods

2.2.1 Breeding of *P. germanica*

Experiments on the life span of sperm were carried out in July/August 2003 with F1-offspring from *P. germanica* collected near Freiburg i. Br., Germany, in May 2003 and remating experiments (including estimation of sperm transfer rates) were conducted in May 2004 with offspring from adults collected in August 2003. F1-offspring was obtained by breeding *P. germanica* in the laboratory as described in *chapter 1.2.1*, pp. 7-8 (see also Sauer 1970; 1977).

2.2.2 Estimating the lifespan of sperm

In order to estimate the lifespan of sperm of *P. germanica* adult females were each mated to a single male. Some females were allowed to lay eggs from the day of copulation on. These females were kept in plastic boxes (10 cm x 10 cm x 6 cm) equipped with small petri dishes filled with moist peat for egg deposition. Food (segments of last larval instar of *Tenebrio molitor*) was provided ad libidum. Some females were prevented from egg deposition for 5 days after copulation by keeping them in small plastic cylinders (∅ 3.5 cm, height 8 cm) containing moist tissue but lacking adequate equipment for oviposition (i. e. peat-filled petri dishes). Furthermore, only a minimum amount of food was provided (1 segment of a last larval instar of *Tenebrio molitor* every sixth day). After 5 days these females were transferred

into plastic boxes and kept for egg deposition as described above. Other females were prevented from egg deposition for 10 days after copulation, others for 15 days, and some for 20 days. Since not all females immediately laid eggs at the day they were allowed to, this experimental design resulted in first clutches obtained between 1 day up to 30 days after copulation. Eggs were always transferred into small petri dishes (∅ 5 cm) equipped with moist tissue for further development. At a temperature of 16-18°C larvae hatched after 5-7 days. As the number of eggs had been recorded hatching rates could be determined. Declining hatching rates were taken as evidence for increasing death rates of sperm.

2.2.3 Remating experiments

Female *P. germanica* were each mated to two different males and paternity of the offspring was determined using a polymorphic microsatellite locus (see *chapter 2.2.4 Microsatellite analysis*, pp. 9-10). For identification all animals were marked individually on their forewings prior to the experiments and were then transferred into observation cages (30 cm x 30 cm x 60 cm, 6 males and 6 females per cage). Of all copulations taking place the involved individuals and the time of beginning of the copulation was recorded. In order to control for copulation duration all copulations were interrupted after 180 minutes. After a first copulation of 180 minutes females were allowed to mate again for 180 minutes, but with a different male. After the second copulation females were kept for oviposition as described above. All hatched larvae were transferred into 100% ethanol and were kept for paternity analysis. Males and females used in the experiments were stored at a temperature of -80°C until DNA extraction.

2.2.4 Determination of sperm transfer rates

In order to reveal if paternity distribution reflects differences in sperm transfer rates the number of sperm different males transferred in the remating experiments was estimated. In

order to do so both mates of some females used in the remating experiments were each mated once more to a virgin female. Again the copulation was interrupted after 180 minutes for determining the number of sperm a given male transfers during this period of time. For staining and counting of sperm females were anesthetised using CO_2 and the spermatheca was dissected in a petri dish containing Ringer's solution. The spermatheca was then placed on a glass slide in a drop of a DNA-specific fluorochrome dye (DAPI = 4´, 6-diamidino-2-phenylindole; Cal Biochem GmbH, Frankfurt, Germany; concentration 5 g/ml 0.1 molar, pH7 Trisbuffer). Rupturing the spermatheca with needles and dispersing the contained sperm in the DAPI-solution the DNA-carrying regions of the spermatozoa got fluorochrome labelled. After adding a drop of glycerine and a cover slip spermatozoa were counted using an Orthoplan-Fluorescence Microscope (magnification 200-400 x).

2.2.5 Statistics

All statistics were performed using the SPSS 12.0 software. Quoted significance values are for two-tailed tests. The level of significance was set to $p < 0.05$. Data sets were tested for deviation from normal distribution using the Kolmogorov-Smirnov test with Lolliefors correction. A one-sample-t-test was performed testing for deviations of observed P_2-values from an expected distribution inferred from proportional copulation durations. The Spearman rank correlation was applied in order to test for a correlation between hatching rate and time of oviposition, and again to test for a correlation between proportional paternity and proportional sperm numbers.

2.3 Results

2.3.1 Lifespan of sperm

In total hatching rates of clutches from 117 different females obtained between 1 and 30 days after copulation were determined in order to estimate the lifespan of sperm. Of each female only the first clutch was included in this analysis, since lower hatching rates in subsequent clutches could occur as sperm gets used up. Figure 3 shows the relationship between hatching rate and period of time having passed between copulation and egg deposition. Hatching rates decline significantly with number of days after copulation (Spearman rank correlation; $n = 117$; $r_s = -0.525$; $p < 0.001$). A considerable variance in hatching success can be detected over the whole period of time. The analysis was redone including only clutches containing more than 10 eggs, since smaller clutches often show no hatching success at all (personal observation). Again, hatching rates decline significantly, but even stronger with time after copulation (Spearman rank correlation; $n = 92$; $r_s = -0.735$; $p < 0.001$, Fig. 4). However, in contrast to the first analysis hatching rates are always rather high during the first week after copulation, if clutches smaller than 10 eggs are excluded. I therefore conclude the lifespan of sperm of *P. germanica* to be at least 7 or 8 days.

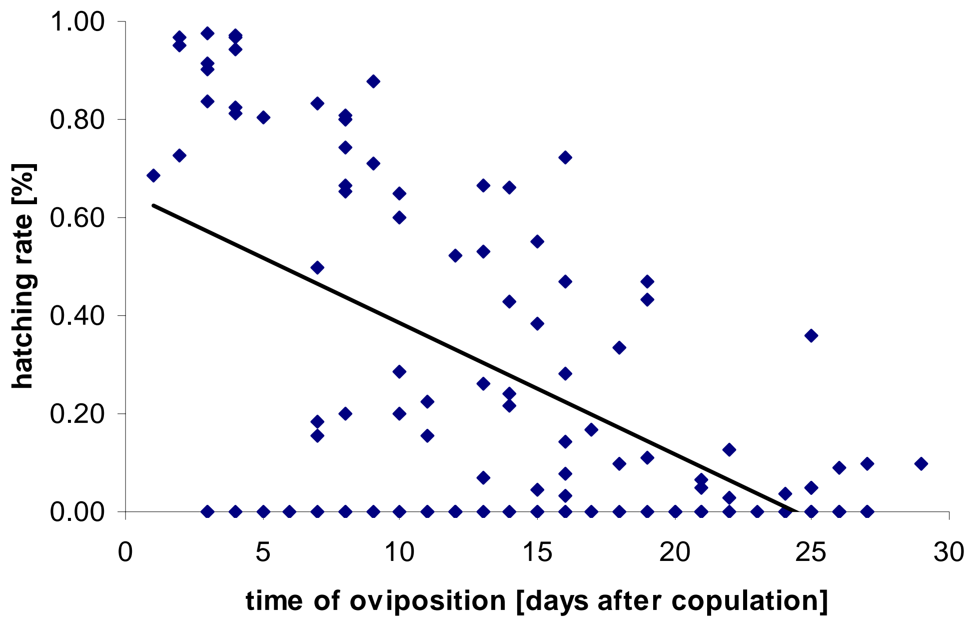

Figure 3.
Correlation between hatching rate and time of oviposition (all first clutches of females included)): Spearman rank correlation; n = 117; r_s *= -0.525; p < 0.001.*

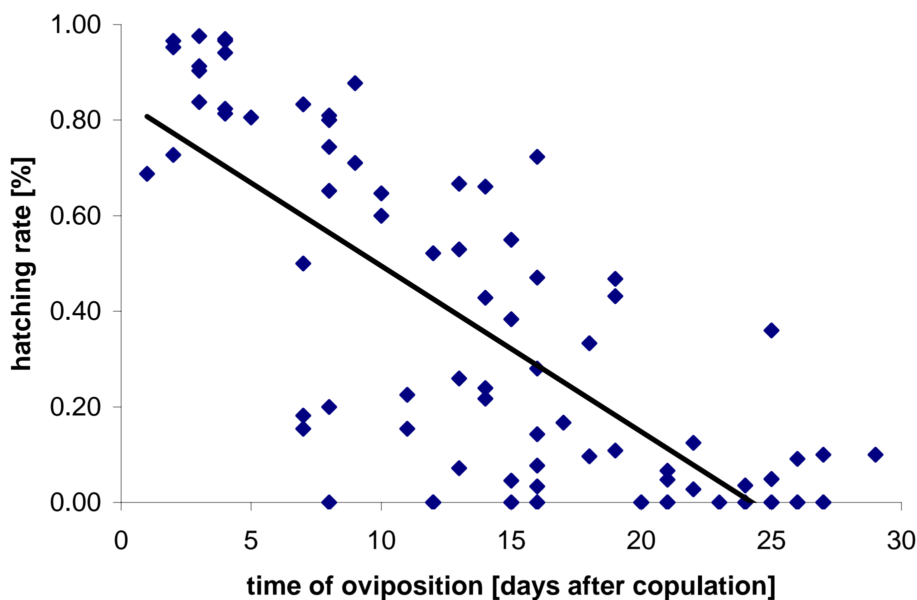

Figure 4.
Correlation between hatching rate and time of oviposition (only first clutches of females > 10 eggs included): Spearman rank correlation; n = 92; r_s *= -0.735; p < 0.001.*

2.3.2 Sperm use patterns

Paternity analysis among the offspring of 21 females having been mated equally long to two different males revealed some last male sperm precedence, as the mean P_2-value was found to be 0.66 ± 0.22. Figure 5 shows all P_2-values obtained for these 21 families. A P_2 of 0.5 can be expected if assuming complete mixing of sperm according to the 'fair raffle' model of Parker et al. (1990), since copulation duration of both males was equal. A one-sample-t-test revealed a significant shift towards a higher proportion of offspring being sired by the second male, as most data points lie above the expected value of 0.5 (n = 21; mean diff. = 0.155 ± 0.218; one-sample-t-test: $t_{20} = 3.256$; $p < 0.01$). The variance in P_2, however, turned out to be rather high (standard deviation = 0.22; range: 0.15-0.90).

Since experiments on the lifespan of sperm showed a decline in hatching rates with time after copulation this analysis was carried out once more including only those clutches that had been obtained during the first 8 days after the copulation with the first male. In these cases we can be rather sure that the sperm of both males was fully viable at time of insemination. The pattern concerning paternity distribution does not change compared to the analysis including all clutches. The mean P_2-value was 0.62 (n = 12), again with the same high variance (standard deviation = 0.27; range: 0.10-0.92). Figure 6 shows the distribution of P_2 obtained for the 12 families, in which eggs were obtained during the first 8 days after copulation. In this case, however, the shift towards a higher proportion of offspring being sired by the second male was not significant (n = 12; mean diff. = 0.119 ± 0.271; one-sample-t-test: $t_{11} = 1.525$; $p = 0.155$), possibly due to the much lower sample size.

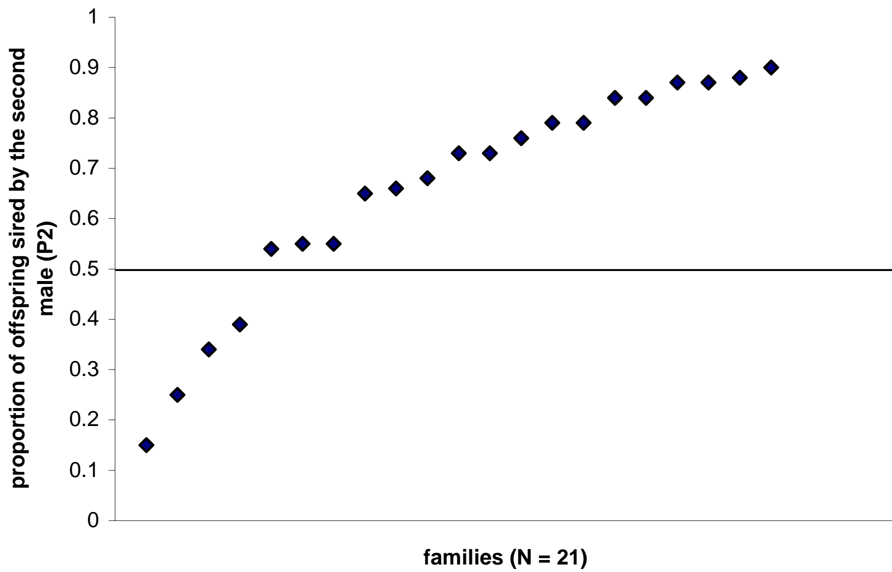

Figure 5.
Paternity distribution among the offspring of females with two identically long copulations. N = 21 families, mean $P_2 = 0.66 \pm 0.22$, range of P_2: 0.15-0.90.

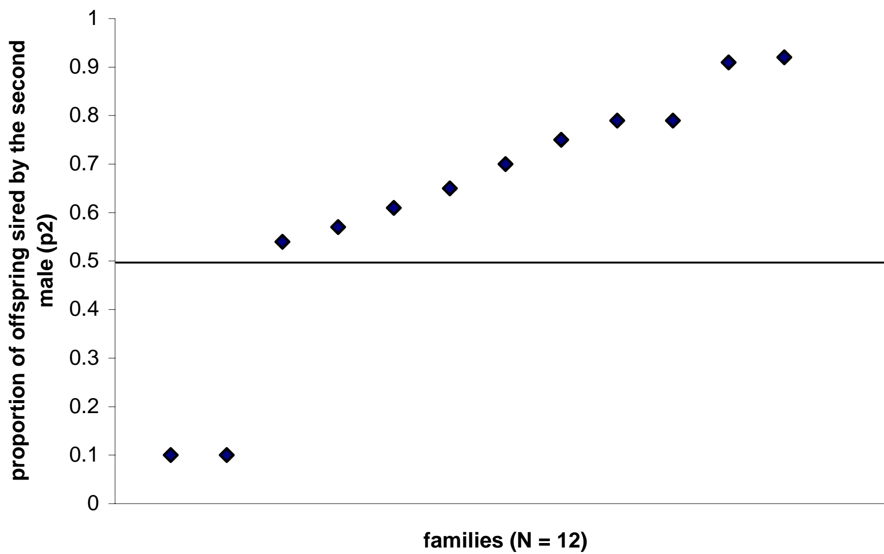

Figure 6.
Paternity distribution among the offspring of females with two identically long copulations: only early clutches (up to 8 days after first copulation) included. N = 12 families, mean $P_2 = 0.62 \pm 0.27$, range of P_2: 0.10-0.92.

2.3.3 Sperm transfer rates

During a copulation of 180 minutes males on average transferred 616 ± 153.1 spermatozoa (n = 20). For 8 females from the remating experiments sperm transfer rates of both mates could be estimated. For these 8 families the mean P_2 was 0.60 ± 0.28 (range: 0.15-0.87). The proportional number of sperm transferred by the second male (S_2) on average was 0.47 with a much lower variance than found for the paternity distribution (standard deviation = 0.08; range: 0.39-0.61). Testing for correlation no relationship between the proportion of offspring sired by the second male (P_2) and the proportional number of sperm of the second male (S_2) was found (Spearman rank correlation; n = 8; rs = 0.531; p = 0.176; Fig. 5). Although proportional sperm numbers were always close to 0.5, proportional paternity was highly variable with the second male on average having some advantage over the first male.

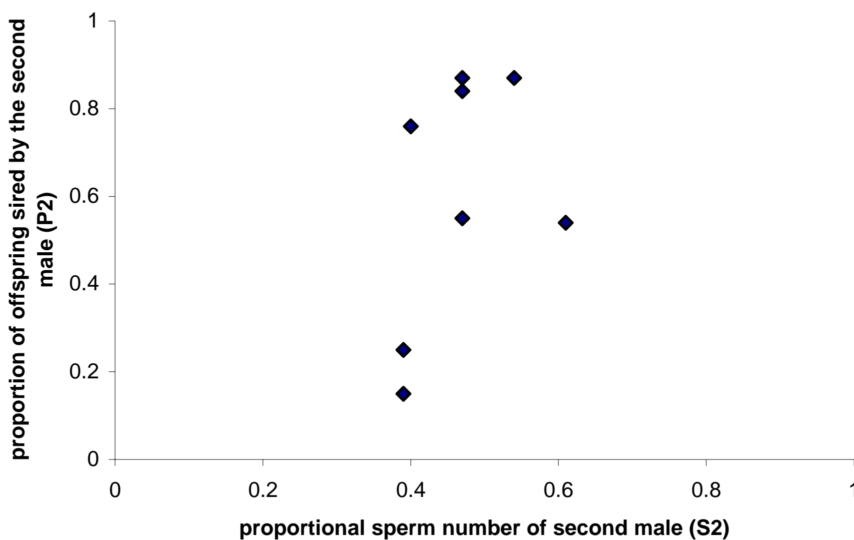

Figure 5.
Relationship between proportion of offspring sired by the second male (P_2) and its proportional sperm number (S_2). Spearman rank correlations: $r_S = 0.531$, $N = 8$ families, $p = 0.176$.

2.4 Discussion

Studies on sperm use patterns in the scorpionfly *Panorpa germanica* (Mecoptera, Panorpidae) have revealed that in double mating trials paternity distribution largely depends on the proportional duration of both copulations, but with the second male on average having some advantage over the first male (see CHAPTER 1). Overall, the intraspecific variance in P_2 was found to be extremely high in this species.

In the experiment presented in this chapter I controlled for copulation duration by interrupting all copulations after 180 minutes. Assuming equal sperm transfer rates for all males and a complete mixing of sperm according to the 'fair raffle' model of Parker et al. (1990) the predicted P_2-value in our experiment is 0.5 for all families because of identical copulation durations of both males. Still, the variance in P_2 turned out to be extremely high and paternity was again shifted towards the second male siring a higher percentage of offspring than the first male despite of identical copulation durations.

2.4.1 Partial last male sperm precedence

The correlation between proportional copulation duration and paternity that was found in the first experiment presented in CHAPTER 1 suggests some mixing of sperm from both males inside the female's spermatheca. Yet, this mixing of sperm appears to be somewhat incomplete, since in all experiments the second male on average sired a higher percentage of the female's offspring than its proportional copulation duration would predict. Testing the actual lifespan of sperm it can now be concluded that this partial last male sperm precedence is not caused by death of first male sperm, since patterns do not change if only early clutches (i. e. sperm of both males still fully viable) are considered. Furthermore, the two copulations of the females were in most cases no more than 2 days (66.7%) and in a wide majority of

cases no more than 5 days (85.7%) apart. Accordingly, sperm of both males was in general similarly old at time of insemination.

Because of a low remating frequency of female *P. germanica* (Gerhards 1999; and CHAPTER 3) the intensity of sperm competition can be presumed to also be low in this species and consequently there is hardly any selection pressure favouring complete sperm mixing. The advantage of the second male could then simply be due to some sperm stratification effects or sperm displacement, respectively. As the experiment on the lifespan of sperm has shown sperm displacement could in some cases even constitute a direct benefit for females as hatching rates decline with time after copulation. If a rather long time has passed between first and second copulation a complete mixing of sperm would be detrimental because of high death rates of first male sperm. If on the other hand both copulations are temporally close together – as it was mostly the case in the present experiment – sperm displacement is still of no disadvantage, hence there is no selection pressure acting against it. I conclude that the observed partial last male sperm precedence in *P. germanica* could well be due to sperm displacement; the exact underlying processes, however, remain unknown.

2.4.2 High variance in sperm use

Apart from paternity distribution being shifted towards the second male the intraspecific variance in P_2 was striking in the present as well as in the previous experiment presented in CHAPTER 1. Assuming sperm mixing an expected variance in P_2 can be predicted if the variation in the number of sperm transferred by different mates of a female can be estimated. In order to reveal if the paternity distribution observed reflects differences in sperm transfer rates the number of sperm different males transferred in the remating experiments was estimated. For 8 females sperm transfer rates of both mates could be determined. Although proportional sperm numbers were always close to 0.5, proportional paternity was highly

variable and no relationship between the proportion of offspring sired by the second male (P_2) and the proportional number of sperm of the second male (S_2) was found. I therefore conclude that the high intraspecific variance in P_2 that was found in double mating trials of female *P. germanica* cannot be explained by variances in sperm transfer rates among *P. germanica* males.

A variety of P_2 distribution patterns including high intraspecific variances can be explained by assuming a random mixing of discrete 'sperm packets' from different males, which form as the initially discrete sperm packages from both males each break up into smaller packets (Harvey & Parker 2000). Such a process referred to as 'sloppy sperm mixing' (Harvey & Parker 2000) could cause the observed patterns on the proximate level, possible selective forces behind these processes, however, remain unidentified. Cook et al. (1997) suggested that any mismatch between the observed and predicted P_2 distribution could reflect cryptic female choice (sensu Thornhill 1983). If females are able to discriminate between sperm from different males paternity should be allocated according to male quality. In scorpionflies males produce nutritious saliva secretions and offer them to females as nuptial gifts prior to and/or during copulation (e.g. Sindern 1996; Sauer et al. 1998; Sauer 2002). For *P. vulgaris* this saliva secretion has been shown to function as an honest signal for male quality (Sauer 1996; Sindern 1996; Sauer et al. 1998; Kurtz & Sauer 1999) according to the 'good gene' models of sexual selection (Zahavi 1975; Andersson 1982). In *P. germanica* the ability of salivary secretion also plays an important role in the reproductive success of males, since females reject potential mates if no salivary mass is offered (Gerhards 1999; and personal observation). Furthermore, a relationship between the number of salivary masses provided during copulation and the copulation duration a male achieves has been observed (Gerhards 1999). Achieving long copulations is extremely important for a male's reproductive success. In doubly mated females in the case of two copulations with very disproportionate durations the male that achieves the longer copulation will also sire a higher proportion of the female's

offspring (see CHAPTER 1). In the case of two similarly long copulations, however, the second male will on average have some advantage over the first male. Yet, the probability that a female will get into a second copulation depends on the duration of its first copulation: the longer the first copulation the less likely this female will mate again (Gerhards 1999). Consequently, males should always aim to achieve copulations that are as long as possible. In case a male is the first male to mate with a female a long copulation will reduce the probability that the female will mate again, i. e. a long copulation reduces the risk of sperm competition. If, on the other hand, the female has already mated a further male again should aim to achieve a long copulation, as this is likely to increase its proportional paternity. Since copulation duration is linked to the number of salivary masses a male provides, the ability of saliva secretion largely influences the reproductive success of male *P. germanica*. However, the ability to produce salivary masses is not the only quality trait determining male reproductive success. It has become evident that the pheromones male *P. germanica* release in order to attract females also function as a signal for male quality as the number of copulation a male gains throughout life not only depends on lifetime ‘calling time’ (time of courtship display with extended pheromone gland; Gerhards 1999) but also on the pheromone’s concentration of a certain substance (tricosen-9; Sauer, unpublished data). I therefore assume females to be able to assess a male’s quality on the basis of its pheromones and females will thus behave choosy already prior to copulation, resulting in low remating frequencies. In my study no data on substances in the pheromones or on the males’ saliva secretion were recorded, hence I cannot test for a relationship between paternity and male quality. Nevertheless, cryptic female choice could well be an important factor influencing paternity distribution thereby causing high intraspecific variances in P_2.

It has also been suggested that sperm quality could play an important role in sperm competition (Hunter & Birkhead 2002; García-González & Simmons 2005). For vertebrates there is evidence that sperm motility for instance matters in insemination success (e.g.

Birkhead et al. 1999; Donoghue et al. 1999). In *Panorpa*-scorpionflies spermatozoans are immobile (personal observation), but sperm viability, measured as the proportion of live sperm in an ejaculate, has been shown to influence paternity in insects (García-González & Simmons 2005). In this study data on sperm quality could not be recorded, but variances in sperm viability could possibly account for some variance in sperm precedence patterns.

Nevertheless, it has become evident that in *P. germanica* females can maximise their reproductive success largely by choosing high quality males prior to copulation rather than by discriminating between sperm from different males. Males on the other hand can maximise their reproductive output by mating with as many females as possible, always aiming to achieve rather long copulation durations. Sperm quality could be of major importance but could not be investigated in this study.

CHAPTER 3

Remating frequency in wild females of *P. germanica* (Mecoptera, Panorpidae) and its implication for sperm competition[1]

Abstract

Theory predicts that because production of sperm involves little metabolic energy investment a male's reproductive success generally increases with mating rate, whereas females are limited in their production of eggs, so that one or a few matings are sufficient for the maximisation of reproductive output. Yet, females of many animal species mate multiply. Female remating frequency obviously plays an important role in many evolutionary processes; the intensity of sperm competition in a species, for instance, largely depends on the remating rate of females. In order to better understand the selective forces acting in the mating system of *P. germanica*, e. g. in terms of sperm competition and female choice, I aimed to estimate female remating rates in natural populations in the field. In order to do so female *P. germanica* were collected at different points of time during the flying season and the number of matings these females have had before having been captured were inferred from mother-offspring analysis of a polymorphic microsatellite locus. It turned out that females of *P. germanica* often mate only once, sometimes twice during their entire life. Hence, intensity of sperm competition is also rather low in this species.

[1] The study presented in this chapter was conducted in collaboration with the undergraduate student Alexander Weiss within the scope of his Diploma project. Methods and results thus comply with sections of his thesis.

3.1 Introduction

Theory predicts that the reproductive success of males generally increases with mating rate as the production of sperm cells does not involve any major metabolic costs, whereas females are limited in their production of eggs so that one or a few matings are usually sufficient for maximisation of the reproductive output (Bateman 1948; Parker 1970; Parker et al. 1972; Trivers 1972). However, in contrast to these predictions, females of many animal species behave polyandrous (Birkhead & Møller 1998). Various explanations for why females mate multiply have been suggested, e. g. females may simply ensure receipt of sufficient sperm (Walker 1980), but they may also gain direct benefits such as nuptial gifts (Gwynne 1984) or indirect, genetic benefits (Yasui 1998). Although there is no general explanation for the evolution of polyandry, it is clear that female remating frequency plays an important role in many evolutionary processes such as sperm competition or cryptic female choice (Ridley 1988; Arnqvist & Nilsson 2000). The intensity of sperm competition in a species for instance largely depends on the remating rate of females (Parker 1970).

In *P. germanica* males release pheromones in order to attract females (Gerhards 1999) and it has become evident that these pheromones may also function as male quality traits (see CHAPTER 2). If females can estimate a male's quality on the basis of substances in its pheromones, females may well maximise their reproductive success by choosing high quality males prior to copulation rather than discriminating between sperm from different males, possibly resulting in low remating rates. Consequently, intensity of sperm competition should also be rather low in this species.

In laboratory experiments on life history traits of *P. germanica* a mean number of 2-3 matings per female during lifetime has been observed (Gerhards 1999). However, for wild populations an even lower remating frequency of females can be assumed, as the density of individuals is generally much higher in laboratory experiments than under natural conditions (personal

observation), resulting in increased encountering probabilities and high concentrations of the males' pheromones.

In order to better understand the selective forces acting in the mating system of *P. germanica* e. g. in terms of sperm competition and female choice I aimed to estimate female remating rates in natural populations in the field. In order to do so female *P. germanica* were collected at different points of time during the flying season and the number of matings these females have had before having been captured were inferred from mother-offspring analysis of a polymorphic microsatellite locus (PG2 locus, see *chapter 1.2.4 Microsatellite analysis*, pp. 9-10). Screening the offspring of a female for paternal alleles allows the determination of a minimum number of males involved. Furthermore, frequency distributions of the different alleles were determined in order to estimate the explanatory power of each, since alleles occurring very frequently in the population are less suitable for the inference of the number of involved males than those that are less common.

3.2 Methods

3.2.1 Determination of number and frequencies of different alleles of the PG2-locus in the field

In order to determine different alleles of the PG2 microsatellite locus along with their frequency distribution adult males and females of *P. germanica* were collected in the field in spring 2003 as well as in spring 2004 at a collection site near Bonn, Germany. Collecting took place every 4-5 days throughout the whole flying season. Males were stored at a temperature of –80 °C for subsequent genetic analysis. In order to determine the current mating status females were kept in small plastic boxes for oviposition (see *chapter 3.2.2*). After having obtained a sufficient number of larvae or if females died they were also stored at –80 °C until

DNA-extraction. Microsatellite analysis was conducted applying the methods described in *chapter 1.2.4 Microsatellite analysis*, pp. 9-10.

3.2.2 Mating status of females collected at different points of time throughout the flying season

The females collected in spring 2003 and in spring 2004 (see *chapter 3.2.1*) were kept in the laboratory in plastic boxes (10 cm x 10 cm x 6 cm) equipped with moist tissue and small petri dishes (Ø 5 cm) filled with moist peat for egg deposition. Eggs were transferred into small petri dishes (Ø 5 cm) containing moist tissue for further development. All hatched larvae were transferred into 100% ethanol until used for microsatellite analysis. Dead females were stored at -80°C until DNA-extraction. The offspring of the wild-caught females was screened for paternal alleles with respect to the PG2-microsatellite locus. For methods applied see *chapter 1.2.4 Microsatellite analysis*, pp. 9-10. Twenty larvae of each female were taken for this analysis. Having genotyped mothers and offspring with respect to the PG2-microsatellite locus, a minimum number of fathers can be inferred from the number of different alleles found among the offspring.

3.3 Results

3.3.1 Number and frequencies of different alleles of the PG2-locus in the field

In total 206 individuals collected in the field near Bonn, Germany, were genotyped with respect to the PG2-microsatellite locus: 36 females and 41 males collected in spring 2003, and 73 females and 56 males collected in spring 2004. Seven different alleles of the PG2-locus could be detected with fragment length ranging from 116 to 134 base pairs. Table 2 lists the

frequencies of alleles found among males and females within the two years. The identification numbers of alleles refer to fragment length given as number of base pairs (=bp).

Table 2.
Alleles of the PG2-microsatellite locus found among males and females collected in spring 2003 and spring 2004 and their frequency distribution (bp = base pairs).

allele (length of fragment [bp])	**♀2003**	**♂2003**	**♀2004**	**♂2004**	**total**
116	0	3	1	1	5
118	7	12	36	19	74
124	1	2	2	6	11
128	0	0	2	1	3
130	22	19	36	39	116
132	13	14	21	12	60
134	3	1	4	3	11

The allele 130 bp turned out to be the most common, followed by 118 bp and 132 bp. The alleles 124 bp and 134 bp were always found to be present in low quantities, whereas the alleles 116 bp and 128 bp were detected only sporadically.

Out of 206 individuals 74 (= 35.9 %) turned out to be heterozygous, whereas for the remaining 132 individuals only one allele could be amplified. However, these individuals can either be homozygous for the detected allele or carry a null-allele, i. e. an allele that cannot be amplified. The existence of null-alleles in the PG2-system is known from the mother-offspring analyses (see *chapter 3.3.2*), as they can be detected if larvae lack maternal alleles. In this analysis however, null-alleles cannot be distinguished from the homozygous state, hence the exact rate of heterozygosis in the wild population cannot be determined.

3.3.2 Mating status of wild caught females

In total 482 larvae obtained from 27 different wild caught-females were screened for paternal alleles. Table 3 shows the genotype of each female with respect to the PG2-microsatellite locus and the different alleles found among their offspring. Again, the identification numbers

of alleles refer to fragment length given as number of base pairs (bp). Alleles noted as 0 bp (null alleles) are fragments that could not be amplified.

Table 4.
Genotypes of females, alleles among their offspring and minimum number of involved fathers

female	**date of capture**	**female genotype (length of fragments [bp])**	**alleles among the offspring (length of fragments [bp])**	**minimum number of fathers**
1	04/05/2003	130-0	118, 124, 130, 132, 0	2
2	08/05/2003	130	130	1
3	12/05/2003	134-0	118, 132, 134, 0	1
4	12/05/2003	130	130	1
5	18/05/2003	132	118, 128, 130, 132	2
6	14/05/2004	130-0	118, 130,132, 0	1
7	18/05/2004	118-0	118, 130,132, 0	1
8	18/05/2004	118-0	118, 130, 0	1
9	18/05/2004	130	118, 130,132	1
10	18/05/2004	130	118, 128, 130	1
11	18/05/2004	132-0	118, 132, 0	1
12	18/05/2004	130	128, 130	1
13	18/05/2004	132-0	118, 132, 134, 0	1
14	18/05/2004	130	118, 128, 130, 132	2
15	22/05/2004	118-0	118, 130, 0	1
16	25/05/2004	118-0	118, 130, 132, 0	1
17	25/05/2004	132, 0	130, 132, 0	1
18	25/05/2004	118	116, 118, 130	1
19	25/05/2004	118-0	118, 124, 130, 132, 0	2
20	30/05/2004	118-0	118, 130, 0	1
21	30/05/2004	118-0	118, 124,130, 134, 0	2
22	30/05/2004	130-132	130, 132, 0	1
23	30/05/2004	118-0	118, 130, 132, 0	1
24	02/06/2004	132-0	118, 132, 0	1
25	07/06/2004	118-130	118, 130	1
26	14/06/2004	132-0	118, 130, 132, 0	1
27	14/06/2004	124-130	118, 124, 130	1

In 22 out of 27 cases it turned out that one male possibly fathered all offspring. Solely 5 cases could be determined in which at least two males were involved. A minimum number of more than 2 mates could not be found. Still, the exact number of fathers cannot be determined as some males are likely to share the same alleles and hence cannot be discriminated. This could especially be the case for the most common alleles 130 bp, 118 bp, and 132 bp (see *chapter*

3.3.1). The 5 cases of two or more fathers are evenly distributed over the whole period of collecting, hence, the mating status of females does not relate to age.

3.4 Discussion

Mother-offspring microsatellite analyses suggest a very low remating rate in wild females of *P. germanica*: only 5 out of 27 females were found to have had a minimum number of two different mates, whereas the majority of females had offspring showing a number of paternal alleles that could be attributable to a single male. However, more than one male could be involved in these cases, since males carrying the same alleles cannot be discriminated. Yet, other studies support the finding of a low remating rate of female *P. germanica*. In laboratory experiments females had a mean number of 2-3 matings during their lifetime (Gerhards 1999). As the density of individuals in these laboratory experiments was much higher than in the field (personal observation), I assume the observed mating rate to be overestimated with respect to natural populations, caused by higher encountering probabilities and high concentrations of the males' pheromones in the laboratory. A previous study on the remating rate of wild females of *P. germanica* strongly supports the assumption of only 1-2 matings per female during lifetime (Sauer, unpublished). In this study the mating status of females collected at different points of time during the season was estimated on the basis of sperm numbers present inside the females' spermatheca. Adult females of *P. germanica* were collected in spring 1998 at our collection site near Bonn every 2-5 days throughout the whole flying season. As a measure for the number of copulations these females have already had at that point of time the number of sperm present inside their spermatheca was determined (for methods see *chapter 2.2.5*, pp. 20-21) and divided by the mean number of sperm an average

wild-caught male transfers during a natural copulation. This mean number of sperm transferred during copulation was obtained by collecting male *P. germanica* again at our collection site near Bonn and mating them in the laboratory to virgin females, which had been obtained by breeding *P. germanica* (see *chapter 1.2.1*, pp. 7-8, and Sauer 1970; 1977 for details). After having copulated the number of sperm transferred was determined applying the methods described in *chapter 2.2.5*, pp. 20-21. Knowing the mean number of sperm an average wild male transfers during a copulation the mating status of a wild-caught female can be inferred from the number of sperm present in the spermatheca. This study revealed a mean mating rate of 1.01 for all females ranging from 0.45 to 2.73. Taking this result together with results obtained through the mother-offspring microsatellite analyses clearly indicates a low remating frequency of female *P. germanica* in the field.

It is quite evident that female remating frequency plays an important role in many evolutionary processes such as sperm competition or cryptic female choice (Ridley 1988; Arnqvist & Nilsson 2000). The intensity of sperm competition in a species for instance largely depends on the remating rate of females (Parker 1970). Accordingly, the low remating rate of females of *P. germanica* has rather strong implications for the relevance of sperm competition in the mating system of this species. Along with the low mating frequency of females the intensity of sperm competition can be presumed to also act rather weakly in this species. These findings could well help to explain the observed patterns of sperm use presented in CHAPTER 1 and CHAPTER 2. Apparently, due to low remating rates of females the risk of sperm competition is rather low in this species, hence there is hardly any selection pressure favouring any complex mechanism of sperm competition. I assume the low remating rate of females to be attributable to the females' ability to assess a male's quality on the basis of its pheromones. If females are able to estimate a male's quality based on substances in its pheromones, females may well maximise their reproductive success by choosing high quality males prior to copulation rather than discriminating between sperm from different males.

General discussion and conclusion

In this study the mechanism of sperm competition in the scorpionfly *Panorpa germanica* (Mecoptera, Panorpidae) was investigated, elucidating possible evolutionary causes and consequences of the observed patterns of sperm use.

Summary of the main findings

Patterns of sperm use in doubly mated females of *P. germanica* were investigated. As presented in CHAPTER 1 paternity analysis among the offspring of doubly mated females revealed a correlation between the proportional copulation duration of an individual male and the proportion of the female's offspring this male will sire. This correlation between proportional copulation duration and paternity suggests mixing of sperm from both males inside the female's spermatheca, since mating duration determines the number of sperm each male contributes. Yet, this mixing of sperm appears to be somewhat incomplete, as paternity was overall shifted towards the second male fathering a higher percentage of the female's offspring than its proportional copulation duration would predict in case of complete sperm mixing. Overall, the intraspecific variance in P_2 was found to be extremely high in this species.

CHAPTER 2 focuses on possible causes and consequences of the observed patterns of sperm use. Double-mating trials in female *P. germanica* were conducted controlling for copulation duration. Despite of identical copulation durations the variance in P_2 remained extremely high and paternity was still shifted towards the second male. Testing the actual lifespan of sperm I conclude that this partial last male sperm precedence is not caused by death of first male sperm. Estimating sperm transfer rates of both mates of a female it can furthermore be concluded that the high intraspecific variance in P_2 cannot be explained by variances in sperm transfer rates among *P. germanica* males.

CHAPTER 3 deals with the mating frequency of female *P. germanica* and its implication for sperm competition. It turned out that in mutual elucidation with previous investigations (Gerhards 1999; and Sauer, unpublished) a low remating rate of wild female *P. germanica* can be presumed. Consequently, sperm competition should also act rather weakly in this species.

Discussion

The correlation between proportional copulation duration and paternity that was found in the first experiment suggests some mixing of sperm from both males inside the female's spermatheca, yet appearing to be somewhat incomplete as a partial last male sperm precedence turned out in all experiments. Testing the actual lifespan of sperm it can now be concluded that this partial last male sperm precedence is not caused by death of first male sperm. Because of a low remating frequency of female *P. germanica* the intensity of sperm competition can be presumed to also be low in this species and consequently there is hardly any selection pressure favouring complete sperm mixing. The advantage of the second male could then simply be due to some sperm stratification effects or sperm displacement, respectively. The exact underlying processes, however, remain unknown.

Apart from paternity distribution being shifted towards the second male the intraspecific variance in P_2 was striking in all experiments. Estimating sperm transfer rates of both mates of a female it turned out that the high intraspecific variance in P_2 cannot be explained by variances in sperm transfer rates among *P. germanica* males.

A variety of P_2 distribution patterns including high intraspecific variances can be explained by some 'sloppy sperm mixing' (Harvey & Parker 2000), assuming a random mixing of discrete 'sperm packets' from different males, which form as the initially discrete sperm packages from both males each break up into smaller packets. Depending on size and number of packets formed, a variety of P_2 distribution patterns may result. These models can clearly help

to understand the possible proximate causes of P_2 distribution patterns, the selective forces behind these processes, however, often remain obscure. Cook et al. (1997) suggested that any mismatch between observed and predicted P_2 distributions could reflect cryptic female choice (*sensu* Thornhill 1983). If females are able to discriminate between sperm from different males paternity should be allocated according to male quality.

Extensive studies on the mating system of *P. vulgaris* (see Sauer et al. 1998; 1999) have shown that paternity distribution among the offspring of multiply mated females of this species clearly represents cryptic female choice. It could be shown that copulation duration increases with the number of salivary masses transferred and sperm is transferred continuously throughout the copulation (Sindern 1996; Sauer et al. 1997; 1998). Through a complete mixing of sperm from different males (Sauer et al. 1999) female *P. vulgaris* allocate paternity in proportion to male quality, since high quality males achieve longer copulations through high saliva secretions and hence transfer more sperm than low quality males. This 'cryptic female choice' based on saliva secretion as an honest signal of male quality (Sauer 1996; Sindern 1996; Sauer et al. 1998; Kurtz & Sauer 1999) is a secure method to discriminate between males of different qualities. However, in order to do so females of *P. vulgaris* always have to mate multiply.

In *P. germanica* the ability of salivary secretion also plays an important role in the reproductive success of males, since females reject potential mates if no salivary mass is offered (Gerhards 1999; and personal observation). Furthermore, copulation duration increases with the number of salivary masses provided (Gerhards 1999). Achieving long copulations is extremely important for a male's reproductive success: in doubly mated females in the case of two copulations with very disproportionate durations the male that achieves the longer copulation will also sire a higher proportion of the female's offspring; in the case of two similarly long copulations, however, the second male will on average have some advantage over the first male. Furthermore, the probability that a female will get into a

second copulation depends on the duration of its first copulation: the longer the first copulation the less likely this female will mate again (Gerhards 1999). Consequently, males should always aim to achieve copulations that are as long as possible: in case a male is the first male to mate with a female a long copulation will reduce the probability that the female will mate again – i. e. a long copulation will reduce the risk of sperm competition; if, on the other hand, the female has already mated a further male again should aim to achieve a long copulation as this is likely to increase its proportional paternity. Since copulation duration is linked to the number of salivary masses a male provides, the ability of saliva secretion largely influences the reproductive success of male *P. germanica*. However, the ability to produce salivary masses is not the only quality trait determining male reproductive success. It has become evident that the pheromones male *P. germanica* release in order to attract females also function as a signal for male quality. If females are able to assess a male's quality on the basis of its pheromones mate choice will already take place prior to copulation, resulting in low remating frequencies. In my study no data on substances in the pheromones or on the males' saliva secretion were recorded, hence I cannot test for relationship between paternity and male quality. Nevertheless, cryptic female choice could well be an important factor influencing paternity distribution.

It has also been suggested that sperm quality could play an important role in sperm competition (Hunter & Birkhead 2002; García-González & Simmons 2005). For instance in some vertebrates it has been observed that sperm motility matters in insemination success (e.g. Birkhead et al. 1999; Donoghue et al. 1999). In *Panorpa*-scorpionflies spermatozoans are immobile (personal observation), but sperm viability, measured as the proportion of live sperm in an ejaculate, has been shown to influence paternity in insects (García-González & Simmons 2005). In my study data on sperm quality could not be recorded, but variances in sperm viability could well be a factor accounting for some intraspecific variances in sperm precedence patterns.

Conclusion

It has become evident that in *P. germanica* females can maximise their reproductive success largely by choosing high quality males prior to copulation rather than by discriminating between sperm from different males. Males on the other hand can maximise their reproductive output by mating with as many females as possible, always aiming to achieve rather long copulation durations, thereby reducing the risk of sperm competition. Sperm quality could be of major importance but could not be investigated.

Summary

Sperm competition can be a powerful selective force in the evolution of reproductive strategies and mating systems. In the last decades many insect species have been studied in terms of sperm competition (for reviews see Gwynne 1984; Birkhead & Hunter 1990; Danielsson 1998; Simmons & Siva-Jothy 1998). Patterns of sperm use are often inferred from the mean species value of P_2, defined as the mean proportion of offspring sired by the second male in double-mating trials (Boorman & Parker 1976). The within-species variance in P_2, however, has mostly been ignored, although explaining this intraspecific variance may be crucial for understanding the underlying mechanisms of sperm competition (Lewis & Austad 1990; Simmons & Siva-Jothy 1998; Simmons & Achmann 2000).

Aim of the present study was to elucidate patterns of sperm use and possible evolutionary causes and consequences in the scorpionfly *Panorpa germanica* (Mecoptera, Panorpidae). Paternity analysis among the offspring of doubly mated females of *P. germanica* revealed a very high intraspecific variance in P_2. A correlation between proportional copulation duration and paternity was found if females had two copulations of disproportionate duration. These results suggest some mixing of sperm from both males inside the female's spermatheca. Yet, this mixing of sperm appears to be somewhat incomplete, as the second male on average sired a higher percentage of the female's offspring than its proportional copulation duration would predict. Testing the actual lifespan of sperm it can be concluded that this partial last male sperm precedence is not caused by death of first male sperm. Estimating sperm transfer rates of both mates of a female and controlling for mating durations by interrupting all copulations after a given period of time it can furthermore be concluded that the high intraspecific variance in P_2 that was found in all experiments cannot be explained by variances in sperm transfer rates among *P. germanica* males. Other factors possibly causing the observed patterns of sperm use are discussed.

As the intensity of sperm competition largely depends on the remating frequency of females I also aimed to estimate female remating rates in natural populations in the field. In mutual elucidation with previous investigations (Gerhards 1999; and Sauer, unpublished) it turned out that females of *P. germanica* usually mate only once, sometimes twice during their entire life. I therefore assume the selective force of sperm competition to act rather weakly in this species. Consequently, there is hardly any selection pressure favouring any complex mechanism of sperm competition.

Discussing the observed patterns it becomes evident that in *P. germanica* females can maximise their reproductive success largely by choosing high quality males prior to copulation rather than by discriminating between sperm from different males. Males on the other hand can maximise their reproductive output by mating with as many females as possible, always aiming to achieve rather long copulation durations, thereby reducing the risk of sperm competition.

Acknowledgements

During my PhD project I have received help, advice, and encouragement from a number of people.

First, I would like to thank Prof. Dr. K. P. Sauer for enabling and supervising my PhD project. His excellent guidance and permanent support have strongly contributed to its success. Numerous discussions going far beyond the scope of this thesis have in addition greatly expanded my understanding of evolution.

I am grateful to Prof. Dr. T. Bakker for contributing as the second reviewer of this thesis.

I would like to express my thanks to Julia Leven, Christiane Wallnisch, Sabrina Bleidissel, and Maria Orland for helping me with the genetic analyses.
I thank Barbara Siegmund, Philipp Jeitz, and Alexander Weiss for their assistance and appreciated company during the experiments.
I would also like to thank my 'room-mates' Andreas Vermeulen and Leif Engqvist for the pleasant atmosphere in our office and many fruitful discussions and conversations. Leif Engqvist also helped me with statistical analyses.
I also thank my colleagues Carsten Pollmann, Merle Missoweit, Gerhild Bornemann, and especially Sierk Engels for all kinds of social occasions, many fruitful discussions and a good cooperation with respect to any aspects of my work.
I also thank all other colleagues at the Institute for Evolutionary Biology and Ecology for discussions and for creating such a friendly environment.

I am deeply grateful to my parents Christel Memering and Dr. Heinz Kock for supporting me at all times, giving me the opportunity to pursue my interests. I would also like to thank my sisters Anke Kock and Caren Bettels for their persistent encouragements.

Finally, I thank the 'Studienstiftung des deutschen Volkes' for providing a full Ph.D. scholarship.

References

Andersson, M. 1982 Sexual selection, natural selection and quality advertisement. *Biol. J. Linn. Soc.* **17**, 375-393.

Arnqvist, G. & Nilsson, T. 2000 The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Beh.* **60**, 145-164.

Bateman, A. J. 1948 Intrasexual selection in *Drosophila*. *Heredity* **2**, 349-368.

Birkhead, T. R. & Hunter, F. M. 1990 Mechanisms of sperm competition. *Trends Ecol. Evol.* **5**, 48-52.

Birkhead, T. R. & Møller, A. P. 1998 *Sperm competition and sexual selection*. San Diego, Calif: Academic.

Birkhead, T. R., Martinez, J. G., Burke, T. & Froman, D. P. 1999 Sperm mobility determines the outcome of sperm competition in the domestic fowl. *Proc. R. Soc. Lond. B* **266**, 1759-1764.

Boorman, E. & 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.

Cook, P. A., Harvey, I. F. & Parker, G. A. 1997 Predicting variation in sperm precedence. *Phil. Trans. R. Soc. Lond.* B **352**, 771-780.

Cordoba-Aguilar, A., Uhia, E. & Rivera, A. C. 2003 Sperm competition on Odonata (Insecta): the evolution of female sperm storage and rivals' sperm displacement. *J. Zool. Lond.* **261**, 381-398.

Danielsson, I. 1998 Mechanisms of sperm competition in insects. *Ann. Zool. Fennici* **35**, 241-257.

Darwin, C. 1871 *The descent of man, and selection in relation to sex*. London: Murray.

Donoghue, A. M., Sonstegard, T. S., King, L. M., Smith, E. J. & Burt, D. W. 1999 Turkey sperm mobility influences paternity in the context of competitive fertilization. *Biol. Reprod.* **61**, 422-427.

Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton: Princeton University Press.

Epplen, C. O., Lubjuhn, T. Epplen, J. T. & Sauer, K. P. 1998 Genetic distinction of scorpionflies (*Panorpa vulgaris*) by highly informative microsatellites. *Mol. Ecol.* **7**, 1256-1258.

García-González, F. 2004 Infertile matings and sperm competition: the effect of 'nonsperm representation' on intraspecific variation in sperm precedence patterns. *Am. Nat.* **164**, 457-472.

García-González, F. & Simmons, L. W. 2005 Sperm viability matters in insect sperm competition. *Curr. Biol.* **15**, 271-275.

Gerhards, U. 1999 Paarungssystem und Spermienkonkurrenzmechanismus der Skorpionsfliege *Panorpa germanica* (Insecta, Mecoptera). *Thesis*. University of Bonn.

Gerken, T., Kurtz, J., Sauer, K. P. & Lubjuhn, T. 1998 DNA preparation and efficient microsatellite analysis from insect hemolymph. *Electrophoresis* **19**, 3069-3070.

Gwynne, D. T. 1984 Male mating effort, confidence of paternity, and insect sperm competition. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 117-149, Orlando: Academic.

Harvey, I. F. & Parker, G. A. 2000 'Sloppy' sperm mixing and intraspecific variation in sperm precedence (P2) patterns. *Proc. R. Soc. Lond.* B **267**, 2537-2542.

Hunter, F. M. & Birkhead, T. R. 2002 Sperm viability and sperm competition in insects. *Curr. Biol.* **12**, 121-123.

Kurtz, J. & Sauer, K. P. 1999 The immunocompetence handicap hypothesis: testing the genetic predictions. *Proc. R. Soc. Lond. B* **266**, 2515-1522.

Lewis, S. M. & Austad, S. N. 1990 Sources of intraspecific variation in sperm precedence in red flour beetles. *Am. Nat.* **135**, 351-359.

Lubjuhn, T. & Sauer, K. P. 1999 DNA fingerprinting and profiling in behavioural ecology. In *DNA profiling and DNA fingerprinting* (ed. J. T. Epplen & T. Lubjuhn), pp. 39-52, Basel, Boston, Berlin: Birkhäuser.

Masumoto, T. 1993 The effect of the copulatory plug in the funnel web spider, *Agelena limbata* (Araneae, Agelinidae). *J. Arachnol.* **21**, 55-59.

Parker, G. A. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525-567.

Parker, G. A. 1978 Searching for mates. In *Behavioural ecology: an evolutionary approach* (ed. J. R. Krebs & N. B. Davies), pp. 214-244, Oxford: Blackwell Scientific Publications.

Parker, G. A., Baker, R. R. & Smith, V. C. F. 1972 The origin and evolution of gamete dimorphism and the male-female phenomenon. *J. theor. Biol.* **36**, 529-553.

Parker, G. A., Simmons, L. W. & Kirk, H. 1990 Analysing sperm competition data: simple models for predicting mechanisms. *Behav. Sociobiol.* **27**, 55-65.

Ridley, M. 1988 Mating frequency and fecundity in insects. *Biol. Rev.* **63**, 509-549.

Sauer, K. P. 1970 Zur Monotopbindung einheimischer Arten der Gattung *Panorpa* (Mecoptera) nach Untersuchungen im Freiland und im Labor. *Zool. Jb. Syst. Ökol.* **97**, 169-207.

Sauer, K. P. 1977 The adaptive significance of genetic variability of photoperiodic response in *Panorpa vulgaris*. *Zool. Jahrb. Syst.* **104**, 489-538.

Sauer, K. P. 1996. Sexual selection and ecological differentiation. *J. Zool. Syst. Evol. Res.* **34**, 235-249.

Sauer, K. P. 2002 Natürliche und sexuelle Selektion und die Evolution des Paarungssystems der Skorpionsfliegen. *Jahrb. Deutsch. Akad. Naturf. Leopoldina* (R3) **47**, 521-547.

Sauer, K. P., Sindern, J. & Kall, N. 1997 Nutritional status of males and sperm transfer in the scorpionfly *Panorpa vulgaris*. *Entomol. Gen.* **21**, 189-204.

Sauer, K. P., Lubjuhn, T., Sindern, J., Kullmann, H. & Kurtz, J. 1998 Mating system and sexual selection in the scorpionfly *Panorpa vulgaris* (Mecoptera: Panorpidae). *Naturwissenschaften* **85**, 219-228.

Sauer, K. P., Epplen, C., Over, I., Lubjuhn, T., Schmidt, A., Gerken, T. & Epplen, J. T. 1999 Molecular genetic analysis of remating frequencies and sperm competition in the scorpionfly *Panorpa vulgaris* (Imhoff and Labram). *Behaviour* **136**, 1107-1121.

Simmons, L. W. & Achmann, R. 2000 Microsatellite analysis of sperm-use patterns in the bushcricket *Requena verticalis*. *Evolution* **54**, 942-952.

Simmons, L. W. & Parker, G. A. 1992 Individual variation in sperm competition success of yellow dung flies, *Scatophaga stercoraria*. *Evolution* **46**, 366-375.

Simmons, L. W. & Siva-Jothy, M. J. 1998 Sperm competition in insects: mechanisms and the potential for selection. In *Sperm competition and sexual selection* (ed. T. R. Birkhead & A. P. Møller), pp. 341-434, London: Academic.

Sindern, J. 1996 Einfluß der Nahrungsdichte auf die Lebensgeschichte und Fitneß von Individuen der Skorpionsfliege *Panorpa vulgaris* (Mecoptera; Insecta). *Thesis*. University of Bonn.

Smith, R. L. 1984 *Sperm competition and the evolution of animal mating systems.* London: Academic.

Stockley, P. 1997 Sexual conflict resulting from adaptations to sperm competition. *Trends Ecol. Evol.* **12**, 154-159.

Thornhill R. 1983 Cryptic female choice in the scorpionfly *Hylobittacus apicalis* (Mecoptera) and its implications. *Am. Zool.* **24**, 367-383.

Trivers, R. L. 1972 Parental investment and sexual selection. In *Sexual selection and the descent of man* (ed. B. Campbell), pp. 136-179, Chicago: Aldine.

Tsubaki, Y. & Yamagishi, M. 1991 'Longevity' of sperm within the female of the melon fly, *Dacus cucurbitae* (Diptera: Tephritidae), and its relevance to sperm competition. *J. Insect Beh.* **4**, 243-250.

Walker, W. F. 1980 Sperm utilization strategies in non-social insects. *Am. Nat.* **115**, 780-799.

Yasui, Y. 1998 The 'genetic benefits' of female multiple mating reconsidered. *Trends Ecol. Evol.* **13**, 246-250.

Zahavi, A. 1975 Mate selection – a selection for a handicap. *J. theor. Biol.* **53**, 205-21.