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High variation in sperm precedence and last male advantage in the scorpionfly *Panorpa germanica* L. (Mecoptera, Panorpidae): Possible causes and consequences

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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 *Panorpa germanica* (Mecoptera, Panorpidae), P_2 largely depends on relative copulation durations of both males, but with the second male on average having some advantage over the first male. Estimating the presence of fertile sperm inside the female's reproductive tract in relation to time after copulation we conclude this partial last male sperm precedence not to be caused by natural death, loss, or depletion of first male sperm. Estimating sperm transfer rates of both mates of a female we, furthermore, found that the high intraspecific variance in P_2 that can be observed cannot solely be explained by variances in sperm transfer rates among *P. germanica* males. Other factors possibly causing the observed patterns of paternity success are discussed.

Keywords: Sperm competition; Sperm viability; Sperm transfer; Intraspecific variance; Mate choice

1. Introduction

Sperm competition occurs when the sperm from two or more males are present inside the female's reproductive tract at the same time, competing for fertilization of the ova (Parker, 1970). In the last decades, sperm competition has received a great deal of attention, revealing its major role 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; Stockley, 1997; Birkhead and Møller, 1998). A considerable number of insect species has been studied in terms of sperm competition (for reviews see Gwynne, 1984; Birkhead and Hunter, 1990; Danielsson, 1998; Simmons and Siva-Jothy, 1998); in these studies patterns of sperm use are usually reported as the species' mean value of P_2, i.e. the proportion of offspring that is sired by the second male in double-mating trials (Boorman and Parker, 1976). Underlying mechanisms are also often inferred from this mean species P_2. In general, intermediate values are believed to be the result of sperm mixing, whereas extreme values of P_2 are often taken as evidence for sperm precedence, i.e. the non-random utilization of sperm from one of several mates of a female (Simmons and Siva-Jothy, 1998). Yet, high values of P_2 can also result from sperm displacement (Parker, 1970) or the loss, depletion or death of first male sperm (Tsubaki and Yamagishi, 1991; Yamagishi et al., 1992). An advantage of the first male, on the other hand, can for instance occur if males apply mating plugs, thereby preventing sperm from subsequent males from entering the female's reproductive tract (Parker, 1970; Boorman and Parker, 1976; Aiken, 1992). Strictly speaking, sperm competition is not involved in these cases. Furthermore, in many studies a considerable intraspecific variation in P_2 has been detected, in which case mean values will tell little about underlying processes (Lewis and Austad, 1990; Simmons and Siva-Jothy, 1998; Simmons and Achmann, 2000). Male adaptations to sperm competition, such as strategic ejaculation (e.g. Parker, 1990;

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Gage and Barnard, 1996; Wedell and Cook, 1999; Engqvist and Sauer, 2001, 2003) or differences in sperm quality (Hunter and Birkhead, 2002; García-González and Simmons, 2005), may cause considerable variation in paternity outcome. In addition, female-mediated processes, such as cryptic female choice, will also lead to differences in fertilization success between males (Eberhard, 1996; Cook et al., 1997). Taking within-species variances in P_2 into account can thus be crucial to understand underlying selective forces and resulting mechanisms of sperm competition.

In the scorpionfly *Panorpa germanica* (Mecoptera, Panorpidae), paternity analyses among the offspring of doubly mated females revealed a high intraspecific variance in P_2 (Kock et al., 2006). A positive correlation between the proportional copulation duration of a male and its relative paternity suggests mixing of sperm from different males inside the female's spermatheca. However, this mixing appears to be incomplete as the second male on average sires a larger proportion of offspring than its relative copulation duration would predict in case of complete sperm mixing. To our knowledge, removal of rival sperm has not been observed in any scorpionfly species and hence is very unlikely to account for the advantage of the second male. Other possible causes could be the loss, the depletion or death of first male sperm (Tsubaki and Yamagishi, 1991; Yamagishi et al., 1992) or simply stratification effects (i.e. last-in-first-out-mechanisms; Birkhead and Hunter, 1990).

Despite the relationship between copulation duration and paternity and the general advantage of the second male, the outcome of sperm competition remains unpredictable for individual males, as P_2 may range from about 0.2 to 0.8 irrespective of mating order or copulation durations (Kock et al., 2006). Assuming continuous sperm transfer and sperm mixing P_2 can be predicted if differences in sperm transfer rates between different mates of a female can be estimated.

In the present study, we test if the variation in P_2 that was found in *P. germanica* could reflect variation in sperm transfer rates. Furthermore, we investigate if in *P. germanica* high values of P_2 can be attributable to the depletion, loss or natural death of first male sperm by investigating for how long sperm is present and fully fertile in the female's reproductive tract after a single copulation.

2. Materials and methods

2.1. Breeding and keeping *P. germanica* in the laboratory

Adult *P. germanica* were collected in the field near Freiburg i. Br., Germany, and then were bred in the laboratory for obtaining F1 offspring (for breeding conditions see Sauer, 1970, 1977; Kock et al., 2006). Experiments on the presence of fertile sperm in relation to time after copulation were carried out in July/August 2003 with offspring from *P. germanica* collected in May of that year. Double-mating trials, together with estimations of sperm transfer rates, were conducted in May 2004 with offspring from adults collected in August the previous year. After hatching imagos were kept separately in plastic cylinders (diameter 3.5 cm, height 8 cm) containing moist tissue paper until used in the experiments. Adult scorpionflies were each provided with one segment of a last larval instar of *Tenebrio molitor* at the day of hatching and every fourth day thereafter. Only females that were used in the experiment on the presence of fertile sperm were kept on a different diet (see below).

2.2. Estimating the presence of fertile sperm in relation to time after copulation

In order to estimate for how long sperm remains fully fertile inside the female's reproductive tract, a total number of 257 females were each mated to a single male. Some females were allowed to lay eggs from the day of copulation on; others were prevented from oviposition for 5, 10, 15 or 20 days. Each female having copulated successfully was randomly assigned to one of these five groups. We prevented females from laying eggs by keeping them in small plastic cylinders (diameter 3.5 cm, height 8 cm) containing moist tissue paper but lacking adequate substrate for oviposition (i.e. peat-filled Petri dishes). Furthermore, only a minimum amount of food was provided (one segment of a last larval instar of *T. molitor* every sixth day) for restricting energy resources females need for egg production. Preventing females from egg production was necessary, as they sometimes start ovipositing even without adequate substrate (personal observations). For oviposition, females were transferred into the plastic boxes (10 × 10 × 6 cm) containing peat-filled Petri dishes and food *ad libitum*. Since not all females immediately laid eggs at the day they were allowed to, this experimental design resulted in first clutches obtained between one and 30 days after copulation. Eggs were always transferred into small Petri dishes (diameter 5 cm) equipped with moist tissue paper 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 decreasing numbers of fertile sperm, either due to the death or loss of sperm.

Probably due to the low food supply a number of females died before being transferred into the plastic boxes. In total, 157 females survived to that point, but of these some did not lay any eggs or died before oviposition, respectively. Thus, eggs were obtained from 117 different females only. Hatching rates were determined exclusively for the first clutch of each female, since lower hatching rates in subsequent clutches could occur as sperm gets used up. Furthermore, we dropped clutches consisting of less than 10 eggs from our analysis, as these very small clutches often have no hatching success at all (personal observation; threshold arbitrarily chosen), leaving us with a sample size of 92 clutches.