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Determinants of sperm transfer in the scorpionfly *Panorpa cognata*: male variation, female condition and copulation duration

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strategic mating effort.

Abstract

Recent studies suggest that sperm production and transfer may have significant costs to males. Male sperm investment into a current copulation may therefore influence resources available for future matings, which selects for male strategic mating investment. In addition, females may also benefit from actively or passively altering the number of sperm transferred by males. In the scorpionfly *Panorpa cognata*, the number of sperm transferred during copulation depended on copulation duration and males in good condition (residual weight) copulated longer and also transferred more sperm. Moreover, sperm transferred and stored per unit time was higher in copulations with females in good condition than in copulations with females in poor condition. Males varied greatly and consistently in their sperm transfer rate, indicative of costs associated with this trait. The duration of the pairing prelude also varied between males and correlated negatively with the male's sperm transfer rate, but no other male character correlated significantly with male sperm transfer rate. The results are consistent with strategic mating effort but sperm transfer could also be facilitated by the physical size of females and/or females in good condition may be more cooperative during sperm transfer.

Introduction

Since Parker's (1970) influential review on sperm competition, i.e. the competition between the sperm from two or more males for the fertilization of a given set of ova (Parker, 1998), this field of research has attracted considerable interest both empirically and theoretically (e.g. Smith, 1984; Birkhead & Møller, 1998). Sperm competition theory predicts that in species where female multiple mating is frequent, male sperm expenditure will be higher than in species with relatively low levels of sperm competition (Parker *et al.*, 1996, 1997), a prediction that is well supported empirically (e.g. Harcourt *et al.*, 1981; Svärd & Wiklund, 1989; Gage, 1994). Consequently, ejaculate costs for males in species with high levels of sperm competition may be substantial (Dewsbury, 1982). For instance, sperm production causes nontrivial costs to male adders (Olsson *et al.*, 1997), ejaculate size or sperm numbers are often continuously reduced in consecutive matings in a wide variety of species (e.g. Nakatsuru & Kramer, 1982; Svärd & Wiklund, 1986; Pitnick & Markow, 1994b; Cook & Gage, 1995), and sperm number has been shown to be reduced under nutritional stress (Gage & Cook, 1994; Pitnick & Markow, 1994a).

Because of the different parental investment of the sexes, the traditional view is that males will mate indiscriminately with any female (Trivers, 1972), but if sperm is costly, males should be selected to allocate sperm cautiously (Dewsbury, 1982; Parker, 1998). It has been shown that males benefit from higher investment when sperm competition risk is high (Gage, 1991; Simmons *et al.*, 1993; Cook & Gage, 1995; Parker *et al.*, 1997; Wedell & Cook, 1999b), and when sperm competition intensity is low (Parker *et al.*, 1996; Simmons & Kvarnemo, 1997; Pilastro *et al.*, 2002).

A special case of variation in sperm competition intensity occurs when females differ in fecundity. Everything else being equal, sperm competition intensity per egg will be lower when there are more eggs to be fertilized (Parker, 1998; Reinhold *et al.*, 2002).

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Consequently, if copulations cause costs, we may expect males to have evolved sensitivity regarding female fecundity. It should be noted that this form of cryptic male choice (cryptic *sensu* Thornhill, 1983) need not be limited to sperm *per se*. Any costly mating activity that can increase the sperm competitive ability of males may be a potential candidate for male discriminatory behaviour. For instance, if the cost of mating is associated with copulation duration and sperm is transferred continuously during copulation, males should copulate longer with more fecund females (Sauer, 1996; Sauer *et al.*, 1998; Parker *et al.*, 1999; Bonduriansky, 2001; Engqvist & Sauer, 2001).

Only a few studies have examined effects of female fecundity, despite its obvious importance, on male ejaculatory decisions. In the Indian meal moth *Plodia interpunctella*, ejaculates contain more sperm if males copulate with heavier females that have increased fecundity (Gage, 1998). Similarly, virgin males of *Pieris rapae* provide heavier females with more sperm (Wedell & Cook, 1999a), and male *Gryllodes supplicans* increase sperm number when mating with larger females that are likely to be more fecund (Gage & Barnard, 1996). Analogously, males of the dung fly *Scatophaga stercoraria* copulate longer and, hence, displace more rival sperm in copulations with females containing more eggs (Parker *et al.*, 1999), and males of the scorpionfly *Panorpa vulgaris* are more likely to offer costly nuptial gifts and consequently increase copulation duration and sperm transfer in copulations with high quality females (Sauer, 1996; Sauer *et al.*, 1997, 1998; see also Engqvist & Sauer, 2001). However, some of these studies may be interpreted as male responses to increased sperm competition risk, as heavier females are likely to remate sooner (Gage, 1998; Wedell & Cook, 1999a).

The scorpionfly *Panorpa cognata* Ramb. (Insecta: Mecoptera) offers various opportunities for testing hypotheses concerning optimal male mating investment (see also Engqvist & Sauer, 2001). Females of this species are polyandrous, creating high levels of sperm competition. Simultaneously, male mating investment is high. Three potentially costly male mating behaviours can be identified. Prior to mating, pairs engage in pairing preludes of considerable duration (Engqvist & Sauer, 2002). Secondly, males provide females with a nuptial gift, a salivary secretion, on which they feed during copulation (Engqvist & Sauer, 2001). Finally, as this study indicates, sperm transfer during copulation may be associated with significant costs. Female sperm storage organs consist of a long, narrow spermathecal duct, which is followed by a single spermatheca, where all sperm is stored. Males inject sperm into the females' spermatheca by the contractions of a sperm pump (cf. Grell, 1942; Sauer *et al.*, 1997). To this pump, two muscles of substantial size are connected (Grell, 1942) indicating that sperm pumping in scorpionflies need considerable force.

Studies on sperm competition, particularly in insects, have often revealed high variance in paternity success between males (e.g. Lewis & Austad, 1990; review in Simmons & Siva-Jothy, 1998). Males may differ in their sperm competitive ability and females may also benefit from manipulating sperm transfer, storage or usage if this enables them to bias the paternity of offspring towards males of high quality (Thornhill, 1983; Eberhard, 1996; Sauer *et al.*, 1998; Edvardsson & Arnqvist, 2000). In *P. cognata* it has been shown that males in good condition copulate longer, as they are able to provide females with a larger nuptial gift (Engqvist & Sauer, 2001). Therefore, the first aim of the present study was to examine if these longer copulations of males in good condition lead to a greater amount of stored sperm, i.e. to investigate the causal relationship between copulation duration and the amount of sperm transferred during copulation. We also investigated if males, independent of copulation duration, differ in the amount of sperm that are transferred and stored by females during copulation. Inspired by our results, we subsequently performed a more thorough investigation in order to study the effect of female fecundity and male variation in sperm transfer rate. Using a standardized mating procedure, males were allowed to copulate with two females, differing in condition and, hence, fecundity. We tested if (1) the rate of sperm transfer is influenced by female condition, consistent with the hypothesis of strategic mating investment, (2) sperm transfer rates differ between males, and (3) male sperm transfer rate correlates with any male characteristics such as male size, condition and courtship duration.

Materials and methods

Breeding of *P. cognata*

At our collection site near Freiburg in Br., in southwestern Germany, *P. cognata* has two generations a year; adults of the first generation emerge in May/June, the second generation in July/August. Here results from two experiments are presented. The first experiment (effects of copulation duration on sperm transfer) was carried out in August 1998 and the second experiment (effects of female condition on sperm transfer) was conducted as two replicates, carried out in November 1998 and in May 1999.

For experiment 1, adults were collected in May 1998, and the larvae were reared on a 18L : 6D photoperiod enabling diapause-free development (Sauer, 1970). Larvae reaching the third larval instar were transferred to soil-filled, open bottomed plastic cylinders (∅40 cm, depth 1 m) placed outdoors in the ground. Animals were collected at emergence (22 July–14 August).

The parents of the animals used in experiment 2 were collected in August 1998. Animals used in the first replicate were reared on a 18L : 6D photoperiod but were not placed in the outdoor cylinders; instead

they finished their larval development in the laboratory. Larvae were held in the petri dishes until they stopped feeding (~25 days after egg hatch) and placed in small peat filled plastic beakers (∅6.0 cm, depth 7.5 cm). Adults emerged after approximately 6 weeks. Larvae for the second replicate were reared on a 12L : 12D photoperiod and, as third larval instars, transferred to the outdoor cylinders, where they overwintered. Adults were collected at the day of emergence (30 April–13 May). For details of breeding protocols see Sauer (1970, 1977) and Thornhill & Sauer (1992).

Experiment 1: Effects of copulation duration on sperm transfer

On the day following emergence, the male scorpionflies used in the experiment were given an individual label on one of their forewings. Throughout the experiment, males were held in two enclosures (30 × 30 × 60 cm) containing cut stinging nettle (*Urtica dioica*) stems and leaves at 18 ± 1 °C on a 18L : 6D photoperiod. Each enclosure contained 12 males. Males were given water *ad libitum* and four one-segment pieces of last instar mealworms (*Tenebrio molitor*) per day and enclosure. Only virgin females were used, and these were held individually in small (8 × 3.5 cm) plastic tubes. They were supplied with water *ad libitum* and a one-segment piece of last instar mealworm every third day. Mating trials were performed as described for experiment c in Engqvist & Sauer (2001). In order to experimentally manipulate copulation duration, 10 randomly selected copulations were prematurely terminated at varying times. All other pairs were allowed to mate undisturbed. Mated females were killed under CO_2-anaesthesia and dissected within 10 h after the termination of copulation. Dissections and sperm counts were performed using standard protocols (cf. Sauer *et al.*, 1997). All males were allowed to mate again the following day.

As a measure of body size, the mean length of the left and right forewing was used. Measurements were made to the nearest 0.1 mm with a dissecting microscope at 10× magnification. Before each mating trial, males and females were weighed to the nearest 0.1 mg. The residuals from the regression of body size on body weight were used as an index of condition. Previous experiments have shown that this measurement correlates strongly with male fitness, as it influences male mating success (Engqvist & Sauer, in press) and male ability to produce large quantities of saliva for their nuptial gifts (Engqvist & Sauer, 2001). Furthermore, condition has been proven to be a good estimator of female fecundity, whereas female size is not (Engqvist & Sauer, in press). The measures of size and condition were used as correlates in the analyses of sperm amount transferred.

Experiment 2: Effects of female condition on sperm transfer

The scorpionflies were treated as in experiment 1, but this time the males were assigned to one of two treatments. In the high nutrient treatment (HN), an enclosure with 12 males was supplied with four one-segment pieces of last instar mealworms every day. The males in the low nutrient treatment (LN) were given only two one-segment pieces of last instar mealworms per enclosure and day. In each replicate a total of 24 males was used. Similarly, the females assigned to the HN-treatment were given a one-segment piece of last instar mealworms every third day, those in the LN-treatment one segment every sixth day. The females in the HN-treatment were only used if their body weight exceeded 48 mg. Accordingly, the maximum body weight of the females in the LN-treatment was set to 43 mg. As the individuals in the second replicate were slightly larger, the corresponding weights were set to 50 mg for the HN-treatment and 45 mg for the LN-treatment, respectively. However, only one LN-female in replicate 1 had to be omitted as a consequence of this procedure. Our manipulation of female condition resulted in a mean weight difference between the two female treatments of about 10 mg (replicate 1: HN 50.8 ± 0.84 mg, LN 41.7 ± 0.82 mg, $t_{24} = 7.76$, $P < 0.001$; replicate 2: HN 53.0 ± 0.32 mg, LN 42.6 ± 0.44 mg, $t_{42} = 19.2$, $P < 0.001$), though there was no difference in female size (i.e. wing length) between the two female treatments (repeated measures ANOVA: $F_{1,34} = 0.091$, ns). The minimum weight difference between females mated to the same male was 6.8 mg, whereas the maximum difference was 18.7 mg. In replicate 2, we also counted the number of eggs at dissection. The treatment had a highly significant effect on egg number (HN: 62.4 ± 2.0, LN: 16.7 ± 2.3, $t_{21} = 13.9$, $P < 0.001$). Moreover, the distributions did not overlap, and the minimum difference between females mated to the same male amounted to 15 eggs.

Each male was allowed to mate twice, once with a female from the HN-treatment and once with a female from the LN-treatment. Half of the males in each treatment were allowed to mate first with a female in good condition; the other half mated first with a female in poor condition. Mating trials and dissections were performed as in experiment 1, but this time the duration of the pairing prelude was recorded, and all copulations were interrupted after 120 min by gently touching the pairs. After the first mating, males were supplied with half a segment of a mealworm in order to recover from the mass loss because of the saliva mass secretion. Males were allowed to mate again the next day. If this mating trial was unsuccessful, it was repeated the next day but only within 6 days of the first copulation.

To investigate if female condition treatment had an effect on female genital morphology, which could

influence sperm transfer mechanics, the basal width and maximum width of the spermatheca were measured from females that were not used in the mating trials. As the flow per unit time into a vessel will primarily be limited by its radius, these measures are likely to be important when considering the rate of sperm entering the spermatheca. The spermatheca of dissected females were placed in a droplet of glycerine on a glass slide and mounted with coverslips. The maximum width of the spermatheca was measured using a microscope at 100× magnification and the basal width at 400× magnification.

Statistical analysis

Parametric statistics were used throughout the analysis. After log-transformation of the duration of the pairing prelude, all dependent variables conformed to normality (Lilliefors, ns). Statistical analyses were performed using SPSS 9.0 software (SPSS Inc., Chicago, IL, USA). Values are given as mean ± SE, unless specified otherwise. As the results from experiment 1 revealed high variance between males in their sperm transfer rate, a paired design was used in experiment 2 when analysing female effects on sperm transfer rate, enabling us to reduce variance because of male effects. For the analysis of male effects in experiment 2, mean values from the two copulations were used for sperm transfer rate, male condition and pairing prelude duration. In addition, data from seven males that only copulated once were used.

Results

Experiment 1: Effects of copulation duration on sperm transfer

A total of 55 females mated with 22 males. Thirteen males mated with more than one female. The mean ± SD copulation duration of undisturbed copulations was 196 ± 57 min, and the mean ± SD number of sperm transferred per copulation amounted to 603 ± 376 (Fig. 1). Copulation duration had a significant effect on number of sperm transferred ($y = 3.35x - 47.9$, $r^2 = 0.309$, $F_{1,53} = 23.7$, $P < 0.001$, Fig. 1). The intercept of the linear regression was not significantly different from zero ($F_{1,53} = 0.13$, ns). Neither slopes (interrupted matings: d.f. = 9, $\beta = 3.10 \pm 0.990$; noninterrupted matings: d.f. = 44, $\beta = 3.44 \pm 0.869$, $t_{51} = 0.177$, ns), nor mean number of sperm transferred per unit time (ANCOVA: copulation interruption as factor: $F_{1,52} = 0.027$, ns; copulation duration as covariate: $F_{1,52} = 21.5$, $P < 0.001$) differed between interrupted and noninterrupted matings. The effect of copulation duration on number of sperm remained significant when only the males' first copulations were taken into account ($r = 0.566$, $F_{1,20} = 9.41$, $P = 0.006$). A model using a quadratic equation to fit the data ($y = 0.0048x^2 + 1.49x + 114.7$, $r^2 = 0.312$) was not significantly better than the linear regression ($F_{1,52} = 0.272$, ns). Thus, mean sperm transfer rate did not change significantly during copulation.

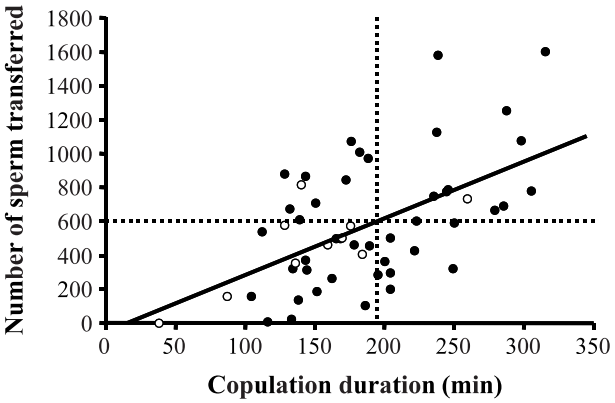

Fig. 1 Relationship between copulation duration and the number of sperm transferred during copulation (solid marks: undisturbed copulations; open marks: interrupted copulations). Dashed lines indicate mean copulation duration and mean number of sperm transferred in undisturbed copulations. Solid line is from the least-square regression.

Male effects

Male condition had a significant positive effect on copulation duration (first noninterrupted mating: $r = 0.466$, $F_{1,19} = 5.27$, $P = 0.03$) and on number of sperm in the spermatheca (first noninterrupted mating: $r = 0.438$, $F_{1,19} = 4.50$, $P < 0.05$).

To analyse if individual males differ in the number of sperm transferred per unit time an ANCOVA model with copulation duration as covariate was performed. In addition, we also used female size, condition (residual weight) and age as covariates to control for possible effects of these traits on sperm transfer. This analysis revealed that males differed significantly in the number of sperm transferred per unit time (Table 1). Besides copulation duration, female condition correlated positively with number of sperm, whereas female size and age had no effect (Table 1). Thus, sperm is transferred faster in copulations with females in good condition. When controlling for copulation duration and female condition, male size correlated negatively with number of sperm transferred in the male's first mating but no effect of male condition on sperm transfer rate was found (multiple regression, male size: $r = -0.664$, $F_{1,19} = 15.1$, $P = 0.001$; male condition: $r = 0.055$, $F_{1,19} = 0.11$, ns).

Experiment 2: Effects of female condition on sperm transfer

Thirteen of the 24 males successfully mated with two females in replicate 1. Seven additional males mated with one female, but failed to mate with the second female

Source of variation	d.f.	Mean SS	F	$\beta \pm$ SE	η_p^2	P-value
Male	21	1.51×10^5	5.49	–	0.799	<0.001
Covariates						
Copulation duration	1	13.3×10^5	48.3	4.03 ± 0.58	0.625	<0.001
Female condition	1	3.3×10^5	12.2	24.8 ± 7.09	0.296	0.002
Female size	1	0.17×10^5	0.61	51.4 ± 66.0	0.021	>0.4
Female age	1	0.16×10^5	0.57	–5.76 ± 7.64	0.019	>0.4
Error	29	0.27×10^5				

Table 1 ANCOVA table for the variation between males in number of sperm transferred during copulation, controlling for variation because of copulation duration and different female traits (model: $F_{25,29} = 8.96$; adjusted $r^2 = 0.787$). The effect size is given by η_p^2.

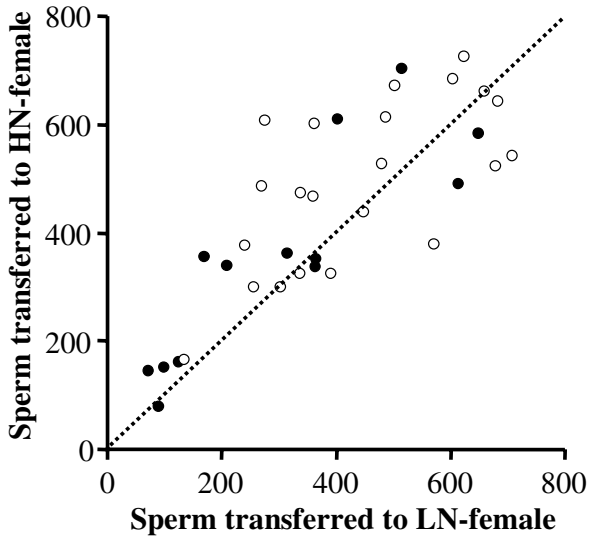

Fig. 2 Comparison between the number of sperm transferred in copulations with females from the high nutrient treatment and copulations with females from the low nutrient treatment. Each data point represents one male that mated twice (replicate 1: solid marks; replicate 2: open marks). Dashed line depicts expected values assuming no difference between treatments. The data from the two different male nutrient treatments were pooled to obtain this figure.

within the time limit or were not able to copulate for 120 min. In replicate 2, 22 of the 24 males mated twice.

Significantly more sperm were transferred in matings with females in good condition than in matings with females in poor condition (repeated measures ANOVA: $F_{1,31} = 5.77$, $P = 0.01$, one-tailed test; Fig. 2). We found no significant interaction between replicate or mating order (i.e. if males mated first with a HN- or a LN-female) and the effect of female condition (repeated measures ANOVA: female condition × replicate: $F_{1,31} = 0.04$, ns; female condition × mating order: $F_{1,31} = 0.16$, ns; female condition × replicate × mating order: $F_{1,31} = 0.63$, ns). Thus, the effects of female condition on sperm transfer were consistent between experimental blocks. Females in the HN-treatment received on average 443 sperm per 2-h mating, females in the LN-treatment received only 391 sperm (mean difference in sperm number: 51.4 ± 20.4). There was no effect of time between male matings on this difference ($r = 0.035$, $F_{1,33} = 0.041$, ns).

The spermatheca of 50 females were successfully measured: 25 in poor condition and 25 in good condition. The mean ± SD basal width of the spermatheca was 62.7 ± 4.90 μm, the mean maximum width 506 ± 29.3 μm. There was no significant difference between the two female treatments in the size of the spermatheca (basal width: LN-treatment 62.6 ± 0.87 μm, HN-treatment 62.8 ± 1.10 μm, $t_{48} = 0.07$, ns; maximum width: LN-treatment 508 ± 5.37 μm, HN-treatment 504 ± 6.41 μm, $t_{48} = -0.464$, ns).

Male effects

Males differed significantly in their sperm transfer rate (ANOVA: coefficient of intraclass variation, $r_i = 0.758$, $F_{34,35} = 7.26$, $P < 0.001$, Fig. 2). Whereas there was no difference between male treatments, replicate had a significant effect (ANOVA: treatment $F_{1,31} = 0.237$, ns; replicate $F_{1,31} = 4.82$, $P = 0.036$; interaction $F_{1,31} = 0.127$, ns, Figs 2, 3), so the variance among males may be slightly overestimated because of differences between replicates. Males in replicate 2 transferred more sperm per copulation than males in replicate 1 (replicate 1: 332 ± 52.8, $n = 13$; replicate 2: 467 ± 31.2, $n = 22$). In replicate 1, the repeatability of male sperm transfer rate was high (ANOVA: $r_i = 0.842$, $F_{12,13} = 11.66$, $P < 0.001$). The variance between males in replicate 2 was lower but still remarkably high (ANOVA: $r_i = 0.634$, $F_{21,22} = 4.46$, $P < 0.001$).

As sperm transfer rate differed between replicates, the effects of male size and condition were analysed separately for the two replicates. Multiple regression revealed no significant correlation ($\alpha = 0.025$ after Bonferroni correction) between sperm transfer rate and male condition (replicate 1: $r = 0.461$, $F_{1,17} = 4.66$, $P = 0.046$; replicate 2: $r = 0.009$, $F_{1,19} = 0.002$, ns) nor between sperm transfer rate and male size (replicate 1: $r = 0.144$, $F_{1,17} = 0.453$, ns; replicate 2: $r = 0.414$, $F_{1,19} = 3.93$, $P = 0.062$).

Similar to sperm transfer rate, the duration of the pairing prelude varied between males (see also Engqvist & Sauer, 2002), causing a moderate repeatability (repeated measures ANOVA: replicate 1 $r_i = 0.479$, $F_{12,12} = 2.84$, $P < 0.05$; replicate 2 $r_i = 0.470$, $F_{21,21} = 2.77$, $P < 0.01$). Female treatment had no influence on

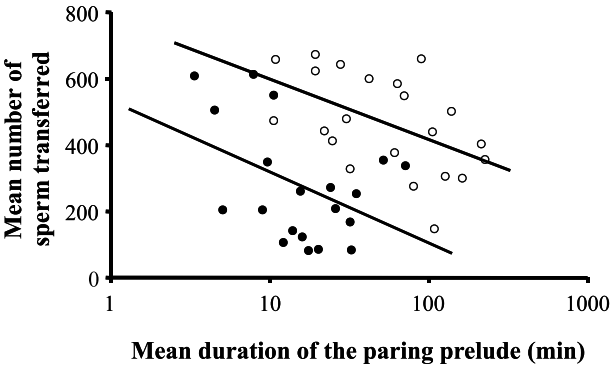

Fig. 3 Correlation between duration of the paring prelude and sperm transfer rate of males (replicate 1: solid marks; replicate 2: open marks). The lines indicate the least-square regressions for the two replicates. The data from the two different male nutrient treatments were pooled to obtain this figure. Note the logarithmic scale of the *x*-axis.

premating duration, whereas in replicate 1, there was a significant effect of mating number on pairing prelude duration, which was shorter in males' second matings (repeated measures ANOVA: replicate 1: female treatment $F_{1,11} = 0.122$, ns; mating number $F_{1,11} = 12.1$, $P = 0.005$; replicate 2: female treatment $F_{1,20} = 0.026$, ns; mating number $F_{1,20} = 0.453$, ns). In both replicates, there was a negative correlation between the mean time males spent in premating and mean number of sperm transferred (Fig. 3). This correlation was significant and, again, replicate had a significant effect on sperm transfer rate (ANCOVA: covariable premating duration: $r = -0.486$, $F_{1,39} = 10.7$, $P = 0.002$; factor replicate: $F_{1,39} = 28.8$, $P < 0.001$).

Discussion

Copulation duration

During copulation sperm amount increased linearly and the average rate of sperm increase per time unit was more or less even (Fig. 1). These results are consistent with studies of the scorpionfly *P. vulgaris* (Sauer *et al.*, 1997, 1998), and strongly imply a continuous sperm transfer in *P. cognata*. By interrupting copulations prematurely, we also confirmed that number of sperm transferred do depend on the duration of the copulation. There was also no evidence that the females' spermathecae are filled during these copulations of naturally occurring duration, as the rate of sperm increase did not decrease with increasing copulation duration (cf. Fig. 1). Copulation duration is affected by the size of the nuptial gift presented by males (Engqvist & Sauer, 2001). The present study confirms that males in good condition, that are able to produce large salivary secretions (Engqvist & Sauer, 2001), will also copulate longer and as a consequence transfer more sperm during copulation.

Female condition

Our results are consistent with a form of cryptic male choice: males invest more effort and more sperm in copulations with females that will produce more eggs. Relatively few studies have examined the influence of female fecundity on male mating decisions (e.g. Gage & Barnard, 1996; Sauer, 1996; Sauer *et al.*, 1998; Gage, 1998; Parker *et al.*, 1999; Wedell & Cook, 1999a; Engqvist & Sauer, 2001). Our investigation further increases the indications that female fecundity influences mating decisions made by males. Additionally, this is to the authors' knowledge the first study suggesting that males may be constrained in their ability of fast sperm transfer, and that males invest more in fast sperm transfer in matings with high quality females.

Could there be other explanations than cryptic male choice for the influence of female fecundity on sperm transfer rate? A general problem with cryptic choice, cryptic male choice as well as cryptic female choice, is that it is difficult to rule out the direct influence from the opposite sex (but see Edvardsson & Arnqvist, 2000; Pitnick & Brown, 2000). Thus, an alternative explanation of our findings could be that it is easier for males to inseminate females in better condition. This could be achieved by two possible mechanisms. Sperm transfer could be constrained by the physical size of females. However, the size of female genitalia is determined during larval development. As we did not manipulate females until after the adult metamorphosis, where the definite size is already fixed, and measurements of the spermatheca confirmed that our treatment had no visible influence on spermathecal size, the physical size of females is unlikely to have confounded our results. A second alternative – highly fecund females may, if sperm is limited, permit males to transfer at a faster rate. This argument seems plausible at first glance but has one logical weakness. If it is in the interest of males to transfer as many sperm as possible regardless of female quality, reduction of sperm transfer rate by females in poor condition implies resistance costs. However, there are no obvious compensatory benefits for females that reduce sperm transfer in relation to their own condition. This behaviour would not give females the potential benefit of cryptic choice between males, since the proportion of sperm from each male would remain the same. Benefits of reducing toxic ejaculate substances are imaginable (e.g. Rice, 1996). However, if toxic substances were important and females have control of sperm transfer, copulation duration dependent sperm transfer (cf. Fig. 1) should be disadvantageous. However, to exclude the possibility of a confounding effect of female condition or fecundity itself on the outcome, one must experimentally manipulate male perception of female quality without

changing female quality *per se* (see also Pitnick & Brown, 2000). At this point we do not know how male *P. cognata* perceive female quality. Consequently, we cannot manipulate male perception.

Male variation

We observed two sources for male variation in number of sperm transferred during copulation. First, males in better condition copulated longer and therefore transferred more sperm. Secondly, our study revealed differences in males' ability of fast sperm transfer. The magnitude of this variance is especially astonishing, as characters like sperm transfer rate are likely to be under strong directional sexual selection in this scorpionfly. As copulation duration is determined by the size of the offered nuptial gift (Engqvist & Sauer, 2001), the total number of sperm transferred to the females' spermatheca will also be influenced by the rate of sperm transfer. Males cannot as e.g. in dung flies compensate a low sperm transfer rate by copulating longer (Simmons *et al.*, 1996). As a result, sperm transfer rate is presumably a trait closely related to fitness. When sperm transfer is costly, some of this variability between males may be explained by the ability of males to pay these costs. It is also imaginable that females exercise cryptic choice (Eberhard, 1996), through modifiable resistance dependent, not on their own quality, but on the quality of males. Females could decrease sperm transfer rate for certain males and increase it for others. Alternatively, females may use resistance as a test of male vigour, a form of passive female choice (Otronen & Siva-Jothy, 1991; Alexander *et al.*, 1997). However, although it is difficult to distinguish whether the variance in sperm transfer rate is caused by variance in male ability or by female control (Simmons *et al.*, 1996; Simmons & Siva-Jothy, 1998; Pitnick & Brown, 2000), both possibilities are perfectly compatible with the hypothesis concerning cryptic male choice. In addition, our results concerning continuous sperm transfer can be interpreted as cryptic female choice in a different context (see Eberhard, 1996; Sakaluk & Eggert, 1996; Sauer *et al.*, 1998, 1999): because of the constant and continuous sperm transfer rate, longer copulations lead to a larger number of sperm transferred. *Panorpa cognata* females consent to longer copulations with males which deliver a larger salivary mass (Engqvist & Sauer, 2001). The combined effect is obviously that males offering more and/or larger nuptial gifts (i.e. males in good condition) also transfer more sperm (see also Sauer *et al.*, 1997, 1998) and will presumably sire more offspring (cf. Sauer *et al.*, 1998, 1999).

Given the large variability between males, further questions immediately arise. Is male sperm transfer rate correlated with any attribute of male quality? For instance, in other species male size (Simmons & Parker, 1992; Simmons *et al.*, 1996) or nutritional condition (Taylor & Yuval, 1999) have been shown to influence sperm transfer. In our study, the effect of male size on sperm transfer rate was inconsistent: we observed a strong significant negative effect of male size on sperm transfer rate in experiment 1 and a weak positive effect in the second replicate of experiment 2. Furthermore, as sperm transfer appears to be costly, it might be suggested that males in good condition transfer sperm faster than males in poor condition, but our results did not confirm this hypothesis. Male condition correlated poorly with sperm transfer rate in *P. cognata*. However, the negative correlation between the duration of the pairing prelude and sperm transfer rate (Fig. 3) may be interpreted as support for a resource-constrained sperm transfer. Obviously, this correlation is not a causal relationship: males do not decide to negate their previous investment in premating by transferring less sperm. The correlation is rather an indication of a common cause. Males with limited resources have been shown to be more prudent and wait longer before they offer females their costly nuptial gift (Engqvist & Sauer, 2002). A negative correlation between premating duration and sperm transfer rate may thus be obtained, when sperm transfer rate of males is also constrained by the available resources.

Conclusions

Three major conclusions can be drawn from this study concerning sperm transfer in *P. cognata* scorpionflies. First, sperm amount increases continuously during copulation (Fig. 1). Accordingly, there are most likely great benefits for males able to produce large nuptial gifts and thus permitted to copulate long (see also Engqvist & Sauer, 2001). Secondly, and more importantly, sperm transfer per time unit is different depending on female condition: when mating with females in good condition, hence highly fecund females, males transfer sperm at a faster rate than in matings with females in poor condition (Fig. 2). This is consistent with hypotheses concerning strategic sperm allocation. However, differential sperm transfer rate between females may also be the result of an effect from female condition itself. Finally, sperm transfer rate varied highly between males (Table 1, Fig. 2), indicating costs associated with this trait. These three factors, copulation duration, female condition and male individual, explain a very large amount of the variance in sperm number (see Table 1). Future experiments are planned in order to investigate the causes for the observed male variation in sperm transfer; these include experimental manipulation of conditions during larval development as well as analyses of genetic variance.

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