

Influence of Nutrition on Courtship and Mating in the Scorpionfly *Panorpa cognata* (Mecoptera, Insecta)

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Abstract

Scorpionflies have been used as model organisms for the study of alternative male mating tactics as well as sexual conflict and coercive mating. Here we describe the courtship and mating behaviour of the scorpionfly *Panorpa cognata* at different levels of nutrition. Alternative mating tactics in scorpionflies involve nuptial food gifts, and we expected an effect of nutrient availability and male individual condition on the relative frequency of these mating tactics. Subsequent to female attraction by means of male pheromonal emission (calling) and a conspicuous pairing prelude, the majority of matings were initiated by male secretion of one relatively large salivary mass on which females feed during copulation. Usually, males produced only a single salivary mass per mating, and the copulation was terminated after the female had consumed the salivary mass. Alternatively, in 40% of the copulations, males offered females a dead arthropod as nuptial gift. However, these matings were neither preceded by male calling nor by the pairing prelude. Copulations with no gifts were extremely rare, and forced copulations were absent. The manipulation of the clamp-like notal organ used by male scorpionflies in coercive matings had no effect on the duration of copulation, suggesting that *P. cognata* males are not able to enforce longer matings. Copulations involving salivary mass gifts were significantly longer than copulations with prey provided as gifts. Although contrary to our expectations, nutrition had no effect on the relative frequency of the different male mating tactics, it had several effects on courtship and mating. First, well-fed individuals copulated significantly more often, both with prey and salivary secretions, than individuals with limited nutrient resources available. This was true for both sexes, although the effect was stronger for males. Higher availability of nutrients decreased the time until male and female sexual maturity and increased male calling duration per day. Furthermore, high nutrient availability decreased the duration of the pairing prelude, and consequently pairs started copulating earlier at night in the high nutrient treatment.

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Introduction

The mating systems of different scorpionfly species of the genus *Panorpa* (Mecoptera, Insecta) have been extensively studied (e.g. Thornhill 1981; Byers & Thornhill 1983; Bockwinkel & Sauer 1994; Sauer et al. 1998, 1999; Gerhards 1999; Aumann 2000). Conspicuous and well-known features of scorpionfly mating systems include nuptial gifts that are provided by the male during mating (e.g. Thornhill 1981; Byers & Thornhill 1983; Sauer et al. 1998), the adoption of alternative male mating tactics (Thornhill 1981; Bockwinkel & Sauer 1994; Sauer et al. 1998) and cryptic female choice of males, which deliver large or numerous food gifts (Thornhill & Sauer 1992; Sauer et al. 1998). In addition, some scorpionfly species are characterized by male emission of pheromones in order to attract females (Thornhill 1979; Rathmann-Schmitz 2000), sexual conflict and coercive mating (Thornhill 1984; Thornhill & Sauer 1991), as well as crepuscular nocturnal mating activities (Thornhill 1981; Byers & Thornhill 1983). However, within the genus *Panorpa*, there seem to be large interspecific differences in virtually all the characteristics associated with their reproductive behaviour; traits are differently expressed in different species and sometimes absent. For instance, females of several scorpionfly species mate promiscuously (e.g. Sauer et al. 1999), whereas those of other species have been shown to be almost monandric (Gerhards 1999). Likewise, according to Thornhill (1980, 1981, 1984) forced copulations are a common feature in the mating behaviour of several North American species, but this behaviour has never been observed in European species (see e.g. Bockwinkel 1990). Moreover, males of different species differ in their ability to forcefully prolong copulation duration, and this ability seems to covary with the size of the males' notal organ (cf. Bockwinkel 1990; Thornhill & Sauer 1991; Gerhards 1999; Aumann 2000). This is a clamp-like structure positioned dorsally on the males' abdomen, in which the anterior edge of one of the female's forewing is secured during mating, and which is a male adaptation to enforce longer copulations (Thornhill & Sauer 1991).

All these traits are, however, not universal within the genus *Panorpa*. Furthermore, the absence of a phylogeny has so far prevented a comparative analysis of the evolution of scorpionfly mating systems. As a result, the evolutionary origins of these traits are not entirely understood. Recently, however, Misof et al. (2000) presented the first molecular phylogeny of panorpid scorpionflies, enabling a comparative analysis. Nevertheless, to fully comprehend the evolution of mating systems within the genus *Panorpa*, behavioural data from more species are needed. Reliable behavioural data are lacking from the scorpionfly *Panorpa cognata* Ramb., and one of the aims of this study was to examine the courtship and mating behaviour of this species. A noticeable morphological character of *P. cognata*, which has potential influences on the

mating system, is the small size of the males' notal organ. Therefore, in addition to behavioural observations, we tested by experimental manipulation whether males can enforce longer copulations.

In general, male scorpionflies may obtain matings in three different ways, two of which involve nuptial feeding (Thornhill 1981; Byers & Thornhill 1983; Sauer et al. 1998). Males may either secrete a salivary mass, or defend a food item, usually a dead arthropod. There is also the possibility to mate without nuptial gift. As different males often seem to adopt different tactics (Thornhill 1981; Bockwinkel & Sauer 1994), they are often referred to as alternative mating strategies (see also Gross 1996). Previous studies have shown that there is usually a large influence of nutrient resource availability on the mating system, and on courting and mating behaviour in scorpionflies (Bockwinkel & Sauer 1994; Sauer et al. 1998; Gerhards 1999; Aumann 2000; Engqvist & Sauer 2001, 2002). Particularly in *P. vulgaris*, this is most obviously observed in the frequency of the alternative mating tactics used by males (Bockwinkel & Sauer 1994; Sauer et al. 1998). When nutrient availability is low, males are usually less able to use the mating tactic involving the offering of salivary secretions, which require the highest mating investment (Bockwinkel & Sauer 1994; Sauer et al. 1998; Gerhards 1999; Aumann 2000). Therefore, under resource-limitation the low-cost mating tactics involving the offering of an arthropod carrion (prey), or even no gift, are more frequent (e.g. Bockwinkel & Sauer 1994; Aumann 2000).

In this study we manipulated resource levels and recorded the effects of nutrition on mating success, choice of mating tactic, and mating behaviour in males of *P. cognata*. In addition, we recorded the effects of resource manipulation on female mating frequency and fecundity. Furthermore, we examined if the male's choice of tactic depends on some aspect of its individual status, especially condition and size and, hence, if the different mating behaviours in *P. cognata* are truly alternative mating strategies (see Gross 1996). We expected that at low nutrient (LN) availability, prey gifts or no gifts will be more frequent than at high nutrient (HN) availability, and salivary secretions will be more frequent when nutrient availability is high. Furthermore, within each treatment, we expected that well-fed males in good condition would better be able to produce saliva, and therefore predominantly use the mating tactic involving the offering of salivary secretions, which at least in *P. vulgaris* gives the highest pay-off in terms of copulation duration and success in sperm competition (Sauer et al. 1998, 1999), whereas males in poor condition will be forced to use the low-cost tactics with prey gift or no gift.

Materials and Methods

At our sample site (near Freiburg i. Br., in south-western Germany), *P. cognata* has two discrete generations per year. Here we present results from observations that were made in enclosure populations during the second generation 1998 and the first generation 1999. In both generations (replicates),

we used F_1 offspring from field-caught adults, that were bred using standard breeding protocols (see Sauer 1970, 1977; Thornhill & Sauer 1992). In brief, the standard breeding protocol used was as follows: field-caught adults were kept pairwise in plastic boxes (10 cm \times 10 cm \times 7 cm) containing moist paper tissues and peat-filled Petri dishes for egg-laying. Larvae were reared at 18°C on moist paper tissues with food ad libitum at a maximum density of 20 individuals per 12 cm diameter Petri dish. To induce diapause (adults observed during the first generation 1999), larvae were reared on a 12L:12D (light:dark) photoperiod, whereas on an 18L:6D photoperiod all larvae showed diapause-free development (adults observed during the second generation 1998). Larvae reaching the third larval instar were transferred to soil-filled, open-bottomed plastic cylinders (diameter 40 cm, depth 1 m) placed outdoors in the ground. Animals were collected at emergence.

In both replicates, two enclosure populations were observed. Throughout the experiment, the two populations were held at $18 \pm 1^\circ\text{C}$ in two large enclosures (150 cm \times 70 cm \times 70 cm) containing cut leaves and stems from stinging nettle (*Urtica dioica*). Each population consisted of 30 individuals: 15 females and 15 males. All animals were individually marked to facilitate identification, and enable the detection of sudden interaction changes. In both replicates, the two populations were assigned to one of two feeding regimes. Populations were daily provided with either 10 (HN) or five (LN) one-segment pieces of last instar mealworms (*Tenebrio molitor*). Observations were made every day until the end of the mating season (second generation 1998: 47 d; first generation 1999: 42 d). Each day we started observations 5 h before the onset of the scotophase, the dark phase. The photoperiod was 18L:6D, with half an hour of 'dawn': a period with reduced light intensity at the beginning and end of each light period. Observations were made until the beginning of the next light phase, or when all pairs had stopped copulating or courting.

Mate Attraction and Copulation

The pheromonal gland of male scorpionflies is positioned on the genitalic bulb, and males emit pheromones by everting the gland (Thornhill 1979; Gerhards 1999). Thus, pheromonal emission (calling) is easily observable. Pairs and individual scorpionflies were observed at least every 5–10 min. We defined pairs to be associated, and courtship to have begun, when females were standing closer than 5 cm of the male. Males then almost invariably stop calling by reverting the pheromonal gland.

We recorded the beginning and end of male calling behaviour as well as behavioural data of female–male interactions including time of female attraction, time of salivary mass secretion, start of copulation and copulation duration. Correspondingly, we observed interactions around all mealworm segments (prey items). To determine the timing of pheromone emission, we additionally made observations through six 24-h periods.

The Notal Organ

To investigate the influence of the notal organ on the copulation duration, 50 males were assigned to one of two treatments: males with and without functional notal organs. Notal organs were made non-functional by covering them with a thin layer of the composite adhesive Protemp II. The males assigned to the functional notal organ treatment were pseudomanipulated by putting a droplet of the composite adhesive on the abdominal segment anterior to the notal organ. All males used were on a low nutrient regime.

Statistical Analysis

Statistical analyses were performed using SPSS 9.0 and JMP 3.2.2 (Power statistics) software. Mean values are given as $\bar{x} \pm \text{SD}$ unless otherwise specified. In all analyses, we used the mean value for each individual if more than one data point were obtained for each individual.

We used the mean length of the left and right forewings as a measure of body size. Measurements were made to the nearest 0.1 mm with a dissecting microscope at 10 \times magnification. Every day before the start of the observations, animals were weighed to the nearest 0.1 mg. As an index of individual nutritional condition, we used the residuals from the regression of mean lifetime body weight on body size (wing length). Neither sex and treatment nor replicate had any significant effect on the slope of this regression (maximum F for all interactions: $F_{1,100} = 0.757$, $p > 0.3$). Therefore, we used the pooled regression coefficient, i.e. the weighted average of the regression coefficients for each group, to calculate the standardized residuals within each of the eight groups (same sex, same treatment and same replicate).

All data were analysed using a General Linear Model (GLM) with treatment (nutrient regime), replicate, individual condition and individual size as independent variables potentially influencing each response variable. Treatment and replicate were categorical, size and condition continuous variables. Before the analyses, we examined if the residuals of our models were normally distributed. In two response variables we found deviations from the normal distribution (Lilliefors-test, $p < 0.2$). Whereas, the mean copulation duration of prey copulations conformed with normality following log-transformation (Lilliefors-test, $d_{\text{max}} = 0.084$, $df = 44$, $p > 0.2$), the distribution of mean copulation duration of matings with salivary secretions was slightly leptokurtic but not skewed. As only very skewed distributions have a marked effect on the significance level (Sokal & Rohlf 1995, p. 407), we left this variable untransformed. Thereafter we tested for heteroscedasticity in our data set and, finally, we made sure that the effects of size and condition were similar in each replicate and treatment, i.e. that there were no significant size \times treatment/replicate or condition \times treatment/replicate interaction terms. In none of the analyses did we find a significant deviation from homogeneity of slopes. Replicate was entered as factor in all analyses, but is only reported when significant.

To remove the possible confounding effect of lifespan in the analysis of individual mating frequency/success, we used mean matings per day as a measure of individual mating frequency. Throughout, we only used data from the 10th day onwards, as animals take a minimum of 9 d to mature. This also applies to the assessment of mean lifetime body weight and, hence, condition.

Results

Mating Behaviour and Phenology

Males were distinctly crepuscular in their calling activities. The first males started calling approximately 5 h before the beginning of the scotophase. The activity peaked at about half an hour before and abruptly stopped at the onset of scotophase (Fig. 1). The 24-h observations confirmed that males call exclusively at the end of the day.

When calling, males usually curved their abdomen upwards, and occasionally rhythmical wing movements could be observed. Females actively searched for calling males. Of totally 1081 male–female interactions, the female approached the male in 945 (87.1%) cases, 817 (86.4%) of which entailed a calling male.

Males almost invariably stopped calling as soon as a female approached them. At this point males began displaying by moving their abdomen up and down in a pulsating, rhythmical fashion, accompanied by vibrating wing movements. Subsequently, females oriented themselves, standing either parallel or antiparallel, towards the males. Consequently, the wings of the male and female overlap, so that the female's forewings are touched by the male's vibrating

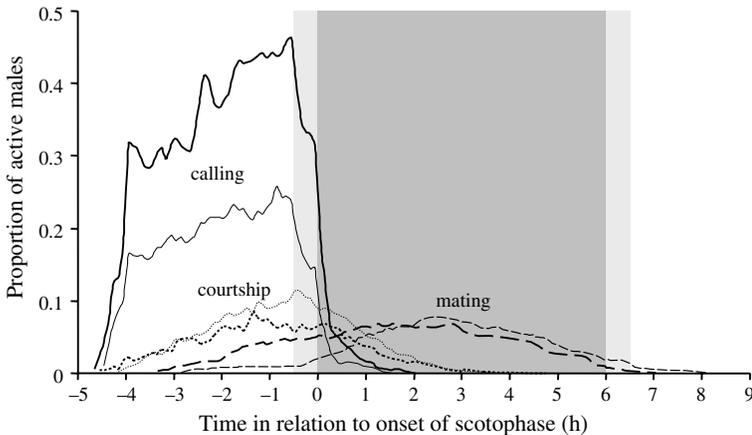


Fig. 1: Temporal order of calling, courtship and mating activity in the first generation 1999 (bold lines: high nutrient availability, thin lines: low nutrient availability; solid lines: calling, dotted lines: courtship; broken lines: mating). Values are averages from the total mating period. The dark area illustrates the scotophase and the shaded area the dawn periods

wing movements. This movement pattern was sporadically interrupted by genitalic contact of short duration (approx. 5 s). However, the disconnection of genitalia did not in any way appear to be associated with a conflict between males and females. The duration of this premating prelude was highly variable and ranged from a few minutes to almost 7 h (see also Engqvist & Sauer 2002). Eventually, in one-third of these interactions (see also Engqvist & Sauer 2002), males initiated the copulation by producing a salivary mass, which was glued on to the substrate (usually a leaf). The time interval between secretion of the salivary mass and onset of copulation was 5–10 min. Immediately before copulation, males vehemently fluttered their wings, complemented by vigorous abdominal movements. The males guided the female to find the salivary mass, and genitalia were not connected until the female had started consuming the salivary mass. Thereby pairs stood in the V-formation typical to *Panorpa* scorpionflies.

Of the 276 copulations initiated by the offering of a salivary mass, all were terminated almost immediately after the females had consumed the salivary mass. The salivary mass remained glued to the substrate throughout the copulation. In most cases, the pairs dissociated after the end of copulation. However, in 11 cases, pairs remained together and the male produced another salivary mass after a mean interval of 66 min (range: 10–150 min). Furthermore, in six cases the male used one salivary mass to copulate with two females.

Copulations also occurred with an arthropod carcass (prey) as nuptial gift. However, these copulations were usually not preceded by male calling and the complex courtship behaviour. In only one of the 185 copulations involving a carcass, the male also called. In all other cases, the females approached non-calling males feeding on the prey, presumably attracted by the scent of the prey itself, or males approached feeding females. Fights over the prey item almost always preceded copulations with prey. Males are usually dominant: of 377 observed fights, males won 345 (91.5%). If a female won the fight, no copulation occurred. If a male won, it either chose to offer the prey item to the female in exchange for a copulation [185 of 345 (53.6%) interactions], or fed itself on the carcass. Similar to copulations involving salivary secretions, females start feeding before males were allowed to connect the genitalia. Forced copulations were never observed.

Comparison of Nutritional Regimes

The mean lifetime body weight when controlling for variation in body size were significantly higher for the individuals in the HN-treatment than for those in the LN-treatment (ANCOVA: treatment, $F_{1,107} = 20.9$, $p < 0.001$; body size, $F_{1,107} = 43.7$, $p < 0.001$). Thus, treatment had an effect on individual body condition. The effect of nutritional treatment on condition was on average 3.51 ± 0.77 (SE) mg and was significant for both sexes (ANCOVA: males: treatment, $F_{1,54} = 7.37$, $p < 0.01$; body size, $F_{1,54} = 23.7$, $p < 0.001$; females: treatment, $F_{1,52} = 13.2$, $p = 0.001$; body size, $F_{1,54} = 19.6$, $p < 0.001$) and not significantly different (ANCOVA: treatment \times sex, $F_{1,107} = 1.08$, $p > 0.3$).

Calling Patterns

On average, males started calling at 16.1 ± 5.0 d of age. Males in the HN-treatment started calling at a significantly younger age than those in the LN-treatment (Table 1). Furthermore, within each population, males in good condition started calling at a significantly younger age compared with those in poor condition (Table 1). Males in the HN-treatment also spent on average more time per day calling (Table 1), and similarly; within each population, we found a positive correlation between male condition and the average calling duration per day. To calculate the mean calling duration per day, we only considered the time from the males' first calling activities, in order to exclude the effect of time until sexual maturity. Male size had no influence on either of the two calling parameters (Table 1).

Copulations

A total of 462 copulations were observed in the two replicates. Of these, 185 (40.0%) were copulations involving prey offer and 276 (59.7%) copulations with salivary secretions. In only one copulation (45 min), no food offering was involved.

Copulations with salivary secretions lasted significantly longer than those involving prey (189.4 ± 73.5 min vs. 36.9 ± 31.1 min; Wilcoxon-test, paired comparison between mean values for each male: $Z = -5.71$, $p < 0.001$; Table 1). Neither of the two mating strategies differed significantly in copulation duration between the two nutrient availability treatments (Table 1). Similarly, neither condition nor size had any significant influence on the duration of either type of copulation (Table 1).

Copulations with a salivary mass offered began on average 2.1 ± 112.5 min after the beginning of scotophase (Fig. 2a). In the HN-treatment, copulations began significantly earlier than in the LN-treatment (Table 1). Males in good condition started copulations earlier than males in poor condition, but this correlation was not significant (Table 1). Copulations with prey offer began almost exclusively in the light phase ($\bar{x} \pm SD$: 129.0 ± 88.9 min before the onset of scotophase; Fig. 2b). The mean time of copulation begin did not differ significantly between treatments (Table 1; ANOVA: treatment, $F_{1,41} = 0.08$, $p > 0.7$; replicate, $F_{1,41} = 9.0$, $p = 0.004$; treatment \times replicate, $F_{1,41} = 0.73$, $p > 0.3$). Male condition had no influence, but prey copulations involving large males began earlier in the evening than copulations with small males (Table 1).

Male Mating Success and Alternative Mating Tactics

As the sex ratio in all enclosure population was 0.5, the mean number of lifetime copulation were identical for males and females and equalled 7.7 ± 5.3 for males and 7.7 ± 4.9 for females. The variance in mating frequency was not consistently larger for males than for females (cf. Table 1), and there was no

Table 1: Mean values and standard deviations of male and female mating traits in the two replicates and the different nutrient availability treatments

Males	Low nutrient availability			High nutrient availability			Treatment			Condition			Size			
	2nd generation		1st generation	2nd generation		1st generation	F		p	F		β	F		β	p
	1998	1999	1999	1998	1999	1999	df	F	p	F	β	F	β	F	β	p
Male age at first calling (days)	17.5 ± 5.7 (n = 14)	19.9 ± 4.5 (n = 14)	14.0 ± 3.8 (n = 14)	12.8 ± 2.3 (n = 14)	22.4 < 0.001	7.18 -0.29	1,50	22.4	< 0.001	0.01	0.01	0.01	0.01	0.01	0.01	> 0.9
Male calling per day (min)	101.2 ± 34.5 (n = 14)	88.0 ± 33.2 (n = 14)	119.3 ± 29.4 (n = 14)	115.7 ± 27.5 (n = 14)	3.94 0.05	6.16 0.30	1,52	3.94	0.05	0.016	0.04	0.016	0.04	-0.04	0.04	> 0.8
Mean male copulation duration with salivary mass (min)	203.3 ± 52.3 (n = 14)	206.4 ± 63.4 (n = 11)	173.5 ± 23.1 (n = 14)	211.4 ± 44.0 (n = 14)	0.94 > 0.3	1.51 0.17	1,46	0.94	> 0.3	0.25	0.11	0.25	0.11	0.25	0.11	> 0.6
Mean male copulation duration with prey (min)	40.3 ± 21.9 (n = 9)	34.3 ± 23.3 (n = 9)	30.5 ± 20.2 (n = 13)	41.2 ± 21.5 (n = 14)	0.06 > 0.8	0.08 0.04	1,39	0.06	> 0.8	0.7	0.30	0.08	0.04	0.7	0.30	> 0.2
Mean male copulation begin with salivary mass (min after beginning of scotophase)	7.5 ± 81.6 (n = 14)	60.9 ± 47.8 (n = 11)	-13.0 ± 59.0 (n = 14)	3.1 ± 76.6 (n = 14)	4.55 0.038	2.42 -0.21	1,46	4.55	0.038	0.32	-0.12	2.42	-0.21	0.32	-0.12	> 0.5
Mean male copulation begin with prey	-78.5 ± 41.4 (n = 9)	-130.8 ± 53.0 (n = 9)	-86.3 ± 36.4 (n = 13)	-115.5 ± 47.4 (n = 14)	0.03 > 0.8	0.6 -0.11	1,39	0.03	> 0.8	0.4	-0.43	0.6	-0.11	0.4	-0.43	0.05
Mean male mating success	6.9 ± 3.9 (n = 15)	5.1 ± 4.5 (n = 15)	10.9 ± 5.3 (n = 15)	8.0 ± 6.1 (n = 15)	13.1 0.001	32.3 0.57	1,53	13.1	0.001	0.38	0.09	32.3	0.57	0.38	0.09	> 0.5
Females																
Mean female mating frequency	6.9 ± 4.5 (n = 15)	5.1 ± 2.5 (n = 15)	10.9 ± 6.2 (n = 15)	8.0 ± 4.6 (n = 15)	19.9 < 0.001	10.2 0.35	1,51	19.9	< 0.001	4.69	0.37	10.2	0.35	4.69	0.37	0.04
Female fecundity	28.5 ± 40.7 (n = 15)	60.9 ± 57.5 (n = 15)	120.7 ± 89.7 (n = 15)	112.6 ± 86.2 (n = 15)	21.7 < 0.001	16.0 0.24	1,51	21.7	< 0.001	0.44	0.01	16.0	0.24	0.44	0.01	> 0.5
No. of batches	1.1 ± 1.2 (n = 15)	1.5 ± 1.4 (n = 15)	3.7 ± 2.4 (n = 15)	2.9 ± 2.3 (n = 15)	21.1 < 0.001	5.86 0.10	1,51	21.1	< 0.001	0.59	0.01	5.86	0.10	0.59	0.01	> 0.4
Mean batchsize	26.5 ± 15.4 (n = 8)	40.4 ± 14.0 (n = 10)	31.6 ± 10.8 (n = 12)	39.5 ± 12.2 (n = 12)	0.66 > 0.4	14.1 0.29	1,35	0.66	> 0.4	1.13	0.03	14.1	0.29	1.13	0.03	> 0.2

Test statistics refer to the results of each General Linear Model. The standardized regression coefficient is given by β . The degrees of freedom refer to all F-values. Condition and size refers to the overall within population effect of these factors.

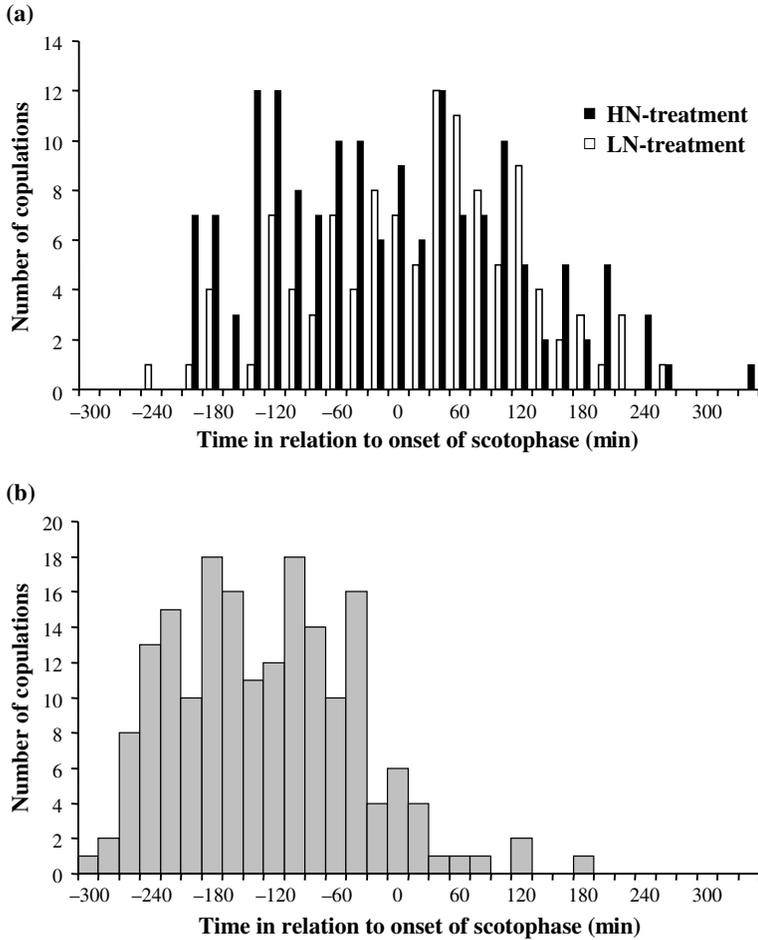


Fig. 2: Time of copulation beginning at (a) salivary mass copulations (solid bars: high nutrient availability; open bars: low nutrient availability), and (b) prey copulations

indication, that the variance in male mating success is influenced by nutrient availability (Table 1). Males in the HN-treatment copulated more often than males in the LN-treatment (Table 1). This difference in total number of copulations resulted from both a difference in number of salivary mass copulations [HN-treatment: 5.4 ± 0.68 (SE) copulations, LN-treatment: 3.8 ± 0.45 copulations; GLM: $F_{1,53} = 6.70$, $p = 0.012$], and an equally large difference in number of prey copulations between treatments [HN-treatment: 3.9 ± 0.54 (SE) copulations, LN-treatment: 2.2 ± 0.45 copulations; GLM: $F_{1,53} = 11.5$, $p = 0.001$].

There was no influence of nutrient availability on the frequency of the mating tactics used [HN-treatment: saliva tactic 162 of 282 copulations (57.4%), prey

tactic 119 of 282 copulations (42.1%); LN-treatment: saliva tactic 114 of 180 copulations (63.3 %), prey tactic 66 of 180 copulations (36.6 %); $\chi^2_1 = 1.47$, $p > 0.2$].

In all four enclosure populations male mating success (mean number of matings per day) increased significantly with male condition (Table 1), but not with male size (Table 1). The overall effect size (η^2_p : equivalent to the r^2 in regressions) of male condition on mating success was estimated to 0.379. Furthermore, the effect of condition on male mating success was not significantly different between treatments and replicates (GLM: all interactions $F_{1,48} < 1.08$, $p > 0.3$). Male condition increased both number of matings involving salivary secretions (GLM: $\eta^2_p = 0.367$, $F_{1,53} = 30.7$, $p < 0.001$; Fig. 3) and the number of copulations with prey as nuptial gift (GLM: $\eta^2_p = 0.226$, $F_{1,51} = 15.5$, $p < 0.001$; Fig. 3). The strength of this effect was similar for both mating tactics (Fig. 3), and the two slopes were not significantly different in any of the four enclosure populations (Fig. 3; LN-treatment, second generation 1998: $\beta_{\text{saliva}} - \beta_{\text{prey}} = \Delta\beta = -0.0030 \pm 0.0065$ (SE), $t_{26} = -0.47$, $p > 0.6$; first generation 1999: $\Delta\beta = 0.0053 \pm 0.0069$, $t_{26} = 0.76$, $p > 0.4$; HN-treatment, second generation 1998: $\Delta\beta = 0.0063 \pm 0.0052$, $t_{26} = 1.21$, $p > 0.2$; first generation 1999: $\Delta\beta = 0.0025 \pm 0.010$, $t_{26} = 0.26$, $p > 0.7$).

To conclude, in our analyses both nutrient availability and male condition influence male mating success, but their effect on the both mating tactics are equally strong and therefore do not influence the relative frequency of copulations with prey and salivary secretions.

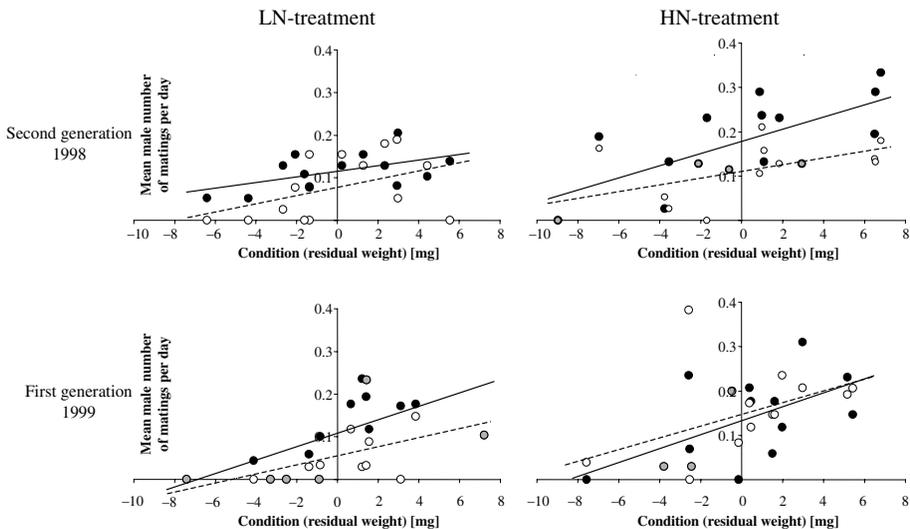


Fig. 3: The correlation between male condition and mean number of matings per day in the four experimental populations (solid marks and lines: salivary mass copulations, open marks and dashed lines: prey copulations). Note that each male is represented by two data points, one for each mating tactic. When these exactly overlap, this is depicted by shaded marks

The effect of condition on male mating frequency was twofold: First, males in good condition were significantly younger at their first copulation (GLM: $F_{1,46} = 7.12$, $p = 0.01$; all interactions $F_{1,46} < 0.8$, $p > 0.3$), and secondly, they mated significantly more frequently thereafter (GLM: $F_{1,46} = 7.65$, $p < 0.01$; all interactions $F_{1,46} < 2.7$, $p > 0.1$).

There were significantly more male–female interactions per day around prey in the HN-treatment than in the LN-treatment, and within populations males in good condition were significantly more often involved (GLM: treatment $F_{1,53} = 10.1$, $p = 0.002$; condition $F_{1,53} = 16.8$, $p < 0.001$; size $F_{1,53} = 0.79$, $p > 0.3$). Furthermore, within populations, the proportion of interactions that led to copulation was significantly higher for males in good condition than for males in poor condition and also higher, though not significantly so, in the HN-treatment compared with the LN-treatment (GLM: treatment $F_{1,46} = 2.46$, $p = 0.12$; condition $F_{1,46} = 6.1$, $p < 0.01$; size $F_{1,46} = 0.73$, $p > 0.3$).

Female Mating and Reproduction

Female fecundity was significantly higher in the HN-treatment than in the LN-treatment (Table 1). This result was primarily due to a difference in the number of egg batches between treatments (Table 1), but not due to differences in mean egg batch size between treatments (Table 1). Within populations, females in good condition laid both larger and more egg batches than those in poor condition (Table 1). Female size had no significant effect on fecundity (Table 1).

In all enclosure populations, female mating frequency increased with female condition, and the overall effect was significant (Table 1). The effect of condition on mating frequency was stronger for males than for females (GLM: sex \times condition $F_{1,100} = 5.96$, $p = 0.016$). In contrast to what was found for males, larger females mated significantly more frequently than small females (Table 1).

In females, there was only an effect of condition on time until first copulation (GLM: $F_{1,45} = 8.20$, $p < 0.01$; all interactions $F_{1,46} < 1.0$, $p > 0.3$), but no significant effect of condition on copulations per day after becoming sexually active (GLM: $F_{1,45} = 0.80$, $p > 0.3$; all interactions $F_{1,46} < 0.4$, $p > 0.3$), in contrast to what was found for males.

The Notal Organ

The $\bar{x} \pm \text{SD}$ copulation duration was 200.7 ± 56.2 min. There was no significant difference in copulation duration between males with functional and non-functional notal organs (functional: 201.8 ± 10.3 min, non-functional: 199.5 ± 12.3 ; $t_{48} = 0.144$, $p > 0.8$). Our test had a power of 0.95 to reveal a copulation duration difference ($\alpha = 0.05$) between treatments of 29.5 min. Therefore, the power of our experiment to reveal a difference ($\alpha = 0.05$) as large as in *P. germanica* (268 min) (Gerhards 1999), *P. communis* (74 min) (Aumann 2000), and *P. vulgaris* (33 min) (Thornhill & Sauer 1991) in equivalent experiments was larger than 0.95 in all three cases.

Discussion

In this study, we have described the reproductive behaviour of *P. cognata*. We found no evidence that nutrition had any effect on the choice or frequency of different male mating tactics, but found several other effects of nutrition on different components of courtship and copulation. Principally, higher nutrient availability increases sexual activity. Compared with individuals in poor condition, well-fed males and females became reproductively active at a younger age. Males in good condition were younger when they started calling and they also spent more time per day calling (Table 1). Furthermore, nutrient availability and individual nutritional condition increased mating frequency and female reproductive output (Table 1). The two different mating tactics, prey offering and salivary secretion offering, resulted in copulations of considerably different duration (Table 1), copulations with salivary secretions lasted roughly five times longer than prey copulations. Finally, we found no evidence that male *P. cognata* use their notal organ to enforce longer copulations, as has been described for other scorpionfly species (e.g. Thornhill & Sauer 1991).

Males in good condition achieved more copulations; this was evident from both the between treatment comparison (Table 1) and the within population correlation (Fig. 3). However, in disagreement with our hypothesis, we found no effect of nutrition or male condition on the relative frequency of mating tactics. Instead, copulations with both prey gifts and salivary masses increased equally in both treatments (Table 1), and there was no difference in the effect of male condition on the frequency of prey copulations and salivary mass copulations (Fig. 3). Hence, males in better condition employed and were more successful with both mating tactics. Therefore, in *P. cognata* these mating tactics do not seem to be alternative mating strategies (see Gross 1996) in the sense that only some males are able to use the most favourable tactic, and others have to be content with other possibilities as described for other scorpionflies (Thornhill 1981; Bockwinkel & Sauer 1994). Instead, both alternatives seem to be constrained by male condition.

In the HN-treatment, males had more access to prey items, and this is presumably one reason why prey copulations were more frequent in this treatment. Nevertheless, within each population, males in better condition achieved more prey copulations than males in poor condition (Fig. 3). First, these males are more likely to have found more prey items, therefore they encountered more females around these prey items, and thus had more opportunities to use this tactic. In addition, these encounters were more likely to result in a copulation if males were in good condition than if males were in poor condition. Perhaps, as a consequence of their own food shortage, males in poor condition may be more unwilling to offer prey as nuptial gift. But both prey and salivary mass copulations may also have been influenced directly by female choice: females may have been more likely to accept nuptial gifts from males in good condition.

Salivary gland development is closely connected with individual nutritional condition (Engqvist & Sauer 2001). Therefore, the observed effect of condition on

the number of salivary mass copulations was expected. Furthermore, male calling is almost exclusively connected with salivary mass copulations. Presumably, males in poor condition therefore showed less calling activity, as these males will need more time to recover saliva supply and it will be meaningless for a male to call unless it has the ability to produce a salivary mass. Unexpectedly, though, resource availability and condition had no significant effect on copulation duration in this study. Other experiments performed, looking more in detail on this relationship (Engqvist & Sauer 2001), have indeed revealed only a weak effect of male condition on salivary mass size and copulation duration.

Condition also had an effect on female mating frequency. As both female and male nutritional condition differed between the two treatments, the observed difference in mating frequency between treatments did probably not depend solely on male condition differences. However, the GLM showed that the effect of condition on mating frequency was stronger for males than for females. Furthermore, in contrast to males, female condition did not affect mating frequency per se. Instead, the observed effect was only due to the fact that females in better condition developed faster and reached sexual maturity sooner. They were therefore also able to lay more batches of eggs.

In our analysis concerning effects of nutrient availability on different courtship and mating traits, there is a potential problem, as the behaviour of individual scorpionflies may have been non-independent of the behaviour of other individuals in the same treatment. This problem is most critical regarding the differences in male calling behaviour and number of matings between treatments. In our experiment, this is unlikely to have generated the observed differences between treatments. The two partially perforated cages with the different nutrient availability treatments were positioned next to each other in the same laboratory. Therefore, if males call for longer or start calling earlier if they sense the presence of other male callers, this would have affected the individuals in the other treatment as well, as the pheromones are relatively long-ranging (see also Thornhill 1979). Similarly, as our populations were relatively small, the probability of further interactions or copulations will decline for each beginning female–male interaction or copulation. However, this acts conservatively on our analysis of differences in mating frequency and these statistically significant differences would perhaps have been even larger using larger populations or a different design. The solidity of these results is further underlined by the consistently, significantly positive correlations within each population between condition and mating success.

As expected, we found many traits typical for scorpionfly mating behaviour. The provision of nuptial gifts seems to be universal within the genus *Panorpa*. Furthermore, male emission of pheromones in combination with crepuscular/nocturnal mating activities are both common scorpionfly traits (Byers & Thornhill 1983), although recently the traditional view that most scorpionfly species mate at twilight has been challenged, as many species have been shown to mate exclusively during the day (Byers 1963; Gassner 1963; Sauer et al. 1998; Aumann 2000; K. P. Sauer, unpubl. data). However, *P. cognata*

display some behavioural traits, which seem highly derived. The provision of one relatively large salivary mass in combination with the functional loss of the notal organ in this species are presumably unique to *P. cognata*. Studies on the function of the notal organ of North American *Panorpa* species have indicated that this clamp-like structure can serve as a grasping device, helping to secure matings with unwilling females by force (Thornhill 1980, 1984). The hitherto studied European species, however, do not exhibit forced copulations (cf. Thornhill & Sauer 1991). Nevertheless, as the females' wings are held by the notal organ during mating, males are able to enforce longer copulations. This study demonstrated that with the reduction of the notal organ, males of *P. cognata* also lost the ability to forcefully prolong mating duration. Males with an intact notal organ were not able to copulate any longer than males with their notal organs covered by an adhesive. Our results support the assumption that, in an interspecific comparison, the size of the notal organ reflects the males' ability to enforce longer copulations (see also Bockwinkel 1990; Thornhill & Sauer 1991; Gerhards 1999; Aumann 2000). Nonetheless, we are currently unable to provide a satisfactory explanation to why the notal organ has been reduced in *P. cognata*. An elevated predation risk is possible, but further research is needed.

In *P. cognata*, the size of the salivary mass was conspicuously larger than the salivary masses of related species. Because of the small notal organ, males have difficulties in retaining hold of the female. Copulations are therefore interrupted soon after the female has consumed the nuptial gift. Owing to their reduced ability to manoeuvre females, males will have greater difficulties to prolong copulations by offering additional salivary masses. As sperm transfer is continuous in *P. cognata* (Engqvist & Sauer 2003), as in many other scorpionflies (see e.g. Sauer et al. 1997), males have an interest in long copulations, as this allows them to transfer more sperm (Sauer et al. 1998; Engqvist & Sauer 2003). Consequently, males who initially invest in one large salivary mass will copulate longer and transfer more sperm than males with smaller salivary masses.

We also have reason to believe that the third novel trait in the courtship and copulation behaviour of *P. cognata*, the long pairing prelude, is associated with the loss of the notal organ, and the consequential large salivary mass. This association is further discussed by Engqvist & Sauer (2002). As a consequence of this long prelude, copulations in *P. cognata* usually begin considerably later, typically in complete darkness (cf. Figs 1 and 2), than in other species with similar crepuscular calling activity, e.g. *P. germanica* (cf. Gerhards 1999).

A dead arthropod was offered as nuptial gift in approximately 40% of the copulations. However, these copulations were of considerably shorter duration than the copulations with a salivary mass. If similar sperm transfer rates of prey and salivary mass copulations are assumed, on average about 90% of the total sperm in a female's spermatheca will stem from salivary mass copulations. It should be noted, however, that the duration of prey copulations will most probably vary with the size of prey (see also Engqvist & Sauer 2001). Thus, the duration of 'natural' prey copulations will vary with the 'natural' size distribution

of prey. The difference in copulation duration between prey and salivary mass copulations is not, however, only a result of size differences. Quite oppositely, the segments of a mealworm used in this study weighed in the order of five times more than the average salivary mass. Rather, it seems easier for the scorpionflies to handle and digest these dead arthropods, which resembles their primary food (cf. Byers & Thornhill 1983; Bockwinkel & Sauer 1993), so that the observed difference in copulation duration reflects a difference in digestibility. An intriguing possibility is that the salivary secretions may be 'designed' to be hard to digest, in order for the males to copulate as long as possible.

Our study also suggests that males of *P. cognata* primarily view dead arthropods as food for themselves and secondarily as nuptial gifts, in contradiction to what is found for North American *Panorpa* species (Thornhill 1981); after obtaining a dead arthropod, males rarely attract a female by emitting pheromones. Instead, males feed on the prey. The female-male encounters that precede and eventually lead to copulations involving arthropod prey gifts only occur when an individual by chance locates a prey already occupied by an individual of the opposite sex.

In addition, the difference in the timing of prey vs. salivary mass copulations is notable (Fig. 2). Pheromones that are emitted at dawn only play a role in salivary mass copulations. In contrast, copulations with prey occur almost exclusively during the light phase. In darkness, the search activity abruptly stops. Therefore, although many prey items remain unexploited, new food items are rarely found and copulations rarely begin at night, when most copulations with salivary secretions begin. It seems reasonable to assume that prey copulations will be randomly spread over the time when individuals search for prey.

The use of scorpionflies as model organisms has in the last decades considerably contributed in increasing our understanding of the evolution of mating systems and the adoption of different male mating tactics (e.g. Thornhill 1981; Byers & Thornhill 1983; Bockwinkel & Sauer 1994; Sauer et al. 1998, 1999; Engqvist & Sauer 2001). Although this study concord largely with previous studies, it is also clear that scorpionfly mating behaviour is highly diverse and it may sometimes be difficult to generalize results. For instance, the observation that different male phenotypes employ different repertoires of alternative behaviours (Thornhill 1981), could not be confirmed by this study. Neither could forced copulations be observed. It should also be noted that these differences subsist, although different species seem to correspond largely in their general ecology and distribution (Sauer 1970; Thornhill 1981; Byers & Thornhill 1983). Thus, ecology alone seems to only insufficiently help us understand the diversity of reproductive behaviour (see Wanntorp et al. 1990; Martins 1996).

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