

Insemination does not affect female mate choice in a nuptial feeding spider

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Abstract

Nuptial feeding is an important part of mating in several arthropods including some spiders. A male can contribute via the nuptial gift to his own offspring (paternal investment) and/or the nuptial gift may allow him to better transfer sperm or to make the female more willing to copulate (mating effort). From the female's perspective, however, weighing a male's quality according to the presence of a gift should coincide with her mating status, because male rejection by the virgin female may decrease her fitness by increasing the likelihood of remaining unmated. Therefore, female mate choice would depend on her mating status and virgin females are expected to be less choosy than mated females. I examined this hypothesis in the nursery web spider, *Pisaura mirabilis*, in which females are polyandrous and mate both with males with and without a gift. I found no support for my hypothesis; instead the presence of a gift was crucial in accomplishing successful copulation regardless of female mating status. Interestingly, sexual size dimorphism was found to be a significant predictor for duration of copulation.

Keywords: Female mate choice, *Pisaura mirabilis*, nuptial gift, sexual size dimorphism

Introduction

Females invest more energy and resources into reproduction and are traditionally considered the choosier sex than males. The majority of animals mate multiply, i.e. they have more than one sexual partner. Polyandrous females may obtain either direct benefits, which increase female survival and/or fecundity or indirect, genetic benefits that increase offspring fitness (see Kokko et al. 2003 for a review). In some nuptial feeding arthropods, the female may obtain a direct benefit from the male's nutritional contribution and/or the nuptial gift has a function as mating effort (i.e. attract female, maximize ejaculate transfer, etc.; see Vahed 1998 for a review). Selective pressures toward the presence and/or size of a nuptial gift should coincide with the female's reproductive status and/or her future mating opportunities, because virgin females at the end of the mating season are expected to be less selective toward courting males (Newman & Elgar 1991).

Moreover, mated females become choosier toward additional males (Gabor & Halliday 1997) and/or are less willing to copulate again (Andrade & Banta 2002; Maklakov et al. 2003; Aisenberg & Costa 2005).

Mated female spiders are generally considered to be less sexually receptive than virgin spiders (Austad 1984). Recently, Aisenberg & Costa (2005) showed that the presence of sperm in the female receptacles affects female re-mating reluctance. Females that were mated with spermless males were more willing to copulate again, in contrast to females that received a normal copulation. Females of the nursery web spider, *Pisaura mirabilis* (Clerck, 1757), are polyandrous (Austad & Thornhill 1986) and copulate both with male with or without a nuptial gift (Stålhandske 2001a). The nuptial gift consists of a dead prey the male has caught and wrapped up in silk (Nitzsche 1988). However, the probability of accomplishing copulation is twice as high when the gift is present (Stålhandske 2001a). Here I

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manipulate the male quality and the mating status of female nursery web spiders to examine whether the presence of sperm in a female's reproductive tract affects her selectivity towards males of different quality. I predict that unmated females should be less selective towards courting males of low quality. In contrast, mated females should be more likely to reject low quality males, because additional mating is not necessary.

Materials and methods

Juvenile and subadult spiders were collected in April and early May 2005 in various grasslands and small woods in western Slovakia. The spiders were individually kept in a ventilated 0.33 l glass jars at room temperature (ca. 20°C) and at natural photoperiod. They were fed with flies (*Musca domestica*) and cricket nymphs (*Acheta domestica*) *ad libitum* approximately three times per week. Water was sprayed directly on the spiders every day to provide them with drinking water. Mature spiders were fed with prey equivalent to three or four houseflies per week. After adulthood was reached, the spiders were randomly assigned to two experimental and one control treatment: HL ($n=21$), NL ($n=22$) and control (C) ($n=10$). Females from HL treatment copulated first with the high quality male (i.e. with a nuptial gift) and then with low the quality male (i.e. without a nuptial gift). Females from NL treatment were first exposed to the male of high quality, but after accepting a nuptial gift just before copulation the male was removed by cautiously aborting mating using soft forceps. The female was allowed to eat the nuptial gift in order to standardize differences between treatments. Females from NL treatment thus experienced a normal courtship, but they were not inseminated. Females from the control group (C) were allowed to mate with high quality males for the two times. Females from all treatments were allowed to copulate (NL females just courted without mating) for the first time after reaching sexual receptivity (6–7 days after final moulting) and for the second time just before the production of the egg sac (13–14 days old). Females that did not copulate on the day of the experiment were allowed to copulate on the following day. If copulation still did not take place, the female was rejected from further experiments.

The time of first mating was similar as in other studies on this species (Drengsgaard & Toft 1999; Stålhandske 2001b). The time of the second mating was selected because about 14-day-old females were observed to lay egg sacs in the laboratory (see

references above). Moreover, such 'old' females are expected to have fewer mating opportunities, because males have a relative short life span in comparison with females (males vs. females, 49 vs. 119 days after final moult; Drengsgaard & Toft 1999) and females do not copulate after carrying egg sacs, which take about 3 weeks (Austad & Thornhill 1986; Stålhandske 2001b). Females in this experiment produced an egg sac at 15.2 ± 0.2 days after final moult. This period was similar across three treatments (ANOVA, $F_{2,50}=0.15$, $P=0.86$). The males were used for mating five days after maturing at the earliest.

On the day of the experiment, all individuals were anaesthetized with CO₂, and body mass (to the nearest 0.001 g) and prosoma width (at the broadest point) were measured, because it is reasonable indicator of body size in spiders (Foelix 1996). Spiders interact with each other during courtship with their fore legs, so the length of tibia–patella of the first right leg was also measured. Male and female condition was measured as residuals of the regression between size and weight. Sexual size dimorphism (SSD) of wild-caught individuals (measured as prosoma width) is generally female-biased (Stålhandske 2001b) although Lang and Klarenberg (1995 cited by Stålhandske 2001b) did not find a similar result for a laboratory-reared population. In my experiment, the females were significantly heavier (0.13 ± 0.003 vs 0.075 ± 0.02 g, $t_{60}=2.59$, $n_1=n_2=31$, $P<0.001$) with a larger prosoma width (3.54 ± 0.04 vs 3.35 ± 0.04 mm, $t_{60}=2.32$, $n_1=n_2=31$, $P=0.02$). Only the tibia–patella length did not differ between sexes (6.19 ± 0.07 vs 6.22 ± 0.07 mm, $t_{60}=-0.15$, $n_1=n_2=31$, $P=0.88$). In several cases, however, the SSD in the tibia–patella length was conspicuously large. I therefore used SSD (female/male ratio; Johnson 2005a) of body mass, prosoma width and tibia–patella length as potential predictors of mating behaviour.

All matings took place in open $30 \times 20 \times 20$ cm glass terraria. The inside bottom of each box was covered with paper towels. Each female was individually placed inside the terrarium approximately 30 min. before the mating experiment. After the male was introduced into the mating arena, he touched the drag-lines made by the female, and showed 'sexual excitement' that included trembling of the palps and abdomen, jerking of the body, moving in jerks and rapid rubbing of the legs (Lang 1996). When the male exhibited these behaviours I gave him one dead laboratory-reared cricket (*A. domestica*) of equal size as a prey (15 ± 0.3 mg). The male immediately paralysed the prey and started to wrap it up. These males can be considered as high

quality males, because offering a nuptial gift increases the likelihood of successful mating (Stålhandske 2001a). Then I recorded the time until female acceptance of the gift, and the time until copulation took place and copulation duration. Spiders were observed continuously, so all measurements were done in accuracy to 1 min.

Females that produced an egg sac prior second mating ($n=3$, one per treatment) were excluded from the experiment. Values are presented as means \pm SE.

Results

Mating behaviour

Neither body mass, prosoma width, nor tibia–patella length (two-way ANOVA, interaction between treatment \times sex, $F_{2,102}=0.22, 0.37$ and $0.49, P=0.8, 0.69$ and 0.62 , respectively) of spiders differed between the three treatments.

Time to acceptance of gift and following time to start of copulation was also similar across treatments (Table I). Copulation duration could be compared only for HL and C treatment (see below), where no significant difference was found (median test, $\chi^2_1=1.99, P=0.3$). I therefore pooled data from these two treatments and analysed them together.

A stepwise multiple regression with male and female condition, SSD in body mass, prosoma width and tibia–patella length revealed that SSD estimated via prosoma width significantly and positively correlated with copulation duration ($R^2=0.168, F_{1,29}=5.85, P=0.022$; Figure 1).

In 12 out of 31 cases in HL and C treatment the female ‘snatched’ the gift and ran away. In most of these cases the male fought with the female for the gift, but did not win. Forward stepwise multiple logistic regression with SSD in body mass, prosoma width and tibia–patella length showed that SSD estimated via tibia–patella length can explain the likelihood of the female successful ‘snatching’ the gift (only SSD in tibia–patella length remained in the model, Wald’s $\chi^2=4.06, P=0.043, n=31$ trials). If

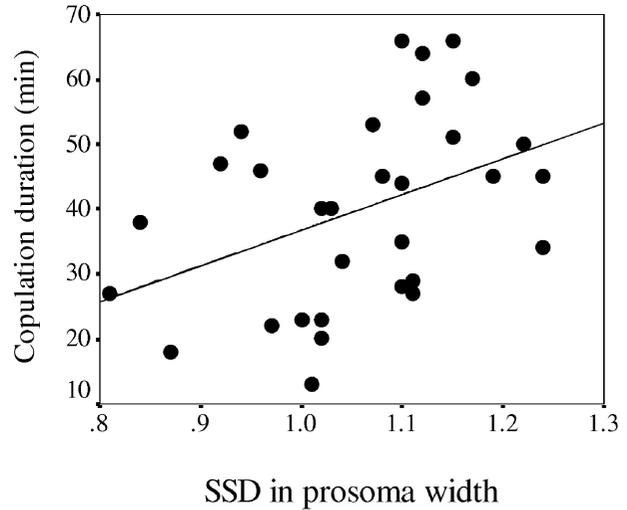


Figure 1. Significant relationship between copulation duration (min.) and SSD (estimated via prosoma width) extracted from multiple regression model ($\beta=0.41, P=0.022, n=31$). Untransformed data are shown.

the female had longer a tibia–patella, she ‘snatched’ the gift more successfully.

Female mate choice

None of the 20 females in HL (one female that produced an egg sac was excluded) accepted a male without a gift. Similarly, only 2 out of 21 females in NL (one female was excluded as in the previous case) accepted the male without a nuptial gift (Figure 2). However, copulations without a gift were

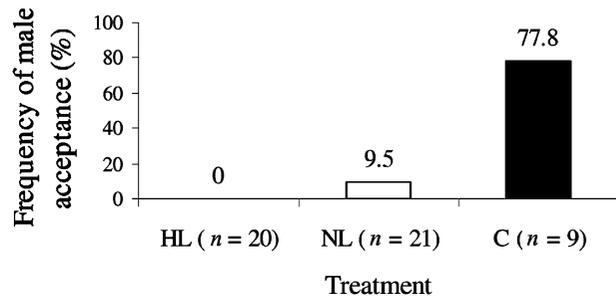


Figure 2. Frequency of acceptance of low quality males (HL and NL treatments) and high quality males (black bar) by non-virgin females.

Table I. Descriptive characteristic of observed stages of mating in *Pisaura mirabilis*. Values are in minutes and standard errors (SE) are in parentheses.

	Treatment			ANOVA	
	HL ($n=21$)	NL ($n=22$)	C ($n=10$)	$F_{2,50}$	P
Time to female acceptance of gift	13.09 (4.4)	10.0 (1.7)	5.2 (1.7)	1.10	0.34
Time to start of copulation	10.0 (2.6)	15.8 (2.1)	10.5 (3.4)	1.67	0.20
Copulation duration	41.4 (3.2)	–	37.0 (5)	–	–

extremely short: one male started to copulate, but after 4 min was killed by the female. It was also the only case of sexual cannibalism. In another case, the male copulated without a gift for only 1 min and then the female escaped. Even after accepting these two cases in NL treatment as successful copulation, there was significant difference in the likelihood of accepting a male with (treatment C) and without a gift (pooled data from HL and NL treatment) between treatments, because almost all females (7 of 9) in C did re-mate with the male offering a gift (2/41 vs. 7/9: Fisher's exact test, $P < 0.001$). Thus, females generally rejected males without a gift despite still being able to copulate. Copulation duration of doubly mated females ($n=7$) in C treatment was similar between first and second mating (mean = 36.7 ± 5.7 vs. 43.6 ± 7.6 ; Wilcoxon signed ranks test, $z = -1.01$, $P = 0.31$).

Discussion

Contrary to general expectation, the female *Pisaura* do not distinguish between males with or without a gift based on her mating status. Surprisingly, even after a relatively high risk of being unmated, almost no female accepted a male without a gift. Females of several species of hangingflies (Mecoptera, Bittacidae) also sometimes refuse to copulate with males that donate small or unpalatable prey (reviewed by Vahed 1998). Neither field (Austad & Thornhill 1986) nor laboratory observations (Lang 1996) of *Pisaura* provide empirical evidence of a female mating with a male without a nuptial gift. Stålhandske (2001a), in her experiment where she manipulated the presence and absence of a nuptial gift, showed that in 40% of cases a female accepted a courting male even without a nuptial gift. In contrast, the present study shows that female *Pisaura* are not willing to copulate with a male without a gift, regardless of their mating status. This was not affected by monogamy, because the majority of females re-mated when the gift was present.

Only a few females accepted a male without a gift, but these copulations were very short. Why such strong differences in comparison with the study of Stålhandske (2001a) occurred remains unclear. Some male spiders transfer most of their sperm within the first 5 min after copulation started (Snow & Andrade 2004). Copulation duration in *Pisaura* has been found to be positively correlated with the proportion of eggs sired by a particular male (Drengsgaard & Toft 1999; Stålhandske 2001a). Thus, mating attempts without a gift decrease male mating success (Stålhandske 2001a).

Why do female *Pisaura* not copulate even after absence of sperm in spermatheca and/or higher risk of being unmated? Current investigations on the function of nuptial feeding in this species provided support only for the mating effort hypothesis (Stålhandske 2001a, 2001b, 2002). This means that there is no evidence of a higher number of offspring and/or offspring viability as a consequence of consumption of a nuptial gift by the female *Pisaura* exist. In contrast, I show that the presence of a gift during courtship plays a crucial role in accomplishing copulation. At least three explanations could be applied to this reason. First, *Pisaura* would simply behave stereotypically and the absence of a gift assuming the mating position impossible. If so, it is questionable why no similar patterns were found for the population in Denmark (Stålhandske 2001a). Second, although spiders were reared individually, they were captured mostly in sites where they occurred in high densities. Thus, females in juvenile stages had probably experienced in cohabitation with males, which can affect their future mating behaviour (Hebets 2003; Johnson 2005b). Third, females in the present study were fed less in comparison with Stålhandske (2001a). They could therefore be more selective towards low quality males. The latter explanation is, however, in conflict with the 'sensory trap' hypothesis which predicts that males exploit the maternal care instinct by producing a nuptial gift that closely resembles the female egg sac (Stålhandske 2002). In other words, this hypothesis does not predict that starved females should be more selective towards males with nuptial gifts. Current data, however, do not allow me confirm any of these explanations.

Another interesting finding of this study is the positive relationship between copulation duration and SSD in prosoma width. Numerous selective pressures can account for SSD (Andersson 1994; Fairbairn 1997; Moya-Laraño et al. 2002; Foellmer & Fairbairn 2005). Female *Pisaura* allowed relatively smaller males to copulate for a longer time, which probably increases his reproductive success (Drengsgaard & Toft 1999; Stålhandske 2001a). Recent evidence shows that smaller male orb-web spiders have advantages in prolonged copulation and fathering more offspring over larger ones (Schneider et al. 2000), or the female prolongs copulation with a smaller male by sexual cannibalism (Elgar et al. 2000). The gravity hypothesis proposes that smaller males are more successful in competition when they climb for a female (Moya-Laraño et al. 2002). Female *Pisaura*, however, do not prefer smaller males *per se*, but only males that are smaller relative to female. These patterns may explain why

female-biased SSD is significant, but weak in this species. The explanation of this phenomenon may lie in the potential threat when the male is relative large: sexual conflict in spiders may result in intersexual fights which are dangerous to females mostly when SSD is weak (Schneider & Lubin 1997). Austad & Thornhill (1986) provided evidences when a female *Pisaura* was killed by the male. I also observed one male to female fatal attack and continuous consumption of the female by the male *Pisaura* (P. Prokop, personal observation). Thus, female *Pisaura* favour relative smaller and probably less dangerous male through sexual selection to improve sexual conflict for her own benefit.

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