

Males of a Neotropical spider adjust prey-gift construction but not mate search in response to sperm competition

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Abstract

In scramble competition mating systems, males do not guard females and females usually mate polyandrously, leading to high levels of sperm competition. Mathematical models predict that males facing sperm competition can either decrease or increase their reproductive effort. To test how male reproductive responses vary according to sperm competition risk, we ran two experiments using the polyandrous gift-giving spider *Paratrechalea ornata*. First, we tested whether males prefer substrates containing silk of unmated females than substrates containing silk of once-mated females, competitor males or without silk. Males spent more time on substrates containing silk of females but showed no preference for unmated females, which indicates that males may optimize mate search by prioritizing substrates containing cues of potential mating partners. The lack of preference for unmated females suggests that males cannot detect female mating status from silk or that the costs of searching preferentially for unmated females are high in the field. Second, we tested whether males adjust prey-gift construction when facing sperm competition risk. Experimental groups (substrate containing silk of an unmated female and silk of another male vs. substrate containing only silk of an unmated female) did not differ in the frequency of gift construction, latency to gift construction and number of flies added to the gift. However, males facing sperm competition risk constructed gifts with less silk, suggesting the occurrence of a less evident form of male mate choice based on differential allocation of resources to prey-gift. Additionally, given the trade-off between investing in prey-gifts quantity or quality, a lower silk investment should enable males to increase the number of gifts constructed and the number of courted females. Taken together, our results indicate that males detect female and male pheromones on substrate and adjust the construction of prey-gifts in response to apparent sperm competition risk.

KEYWORDS

chemical cues, male mate choice, nuptial gift, polyandry, reproductive investment, scramble competition

1 | INTRODUCTION

Some animal mating systems are characterized by individuals (usually males) that defend the access to mating opportunities, either by directly guarding sexual partners or by defending a resource used

by potential sexual partners (Emlen & Oring, 1977). In opposition, in a mating system named scramble competition, such attempts to defend the access to mating opportunities are not feasible (Emlen & Oring, 1977). Under scramble competition, males are not able to directly or indirectly defend mating opportunities because

receptive females are synchronously available over a short time-frame, females show a wide spatial dispersion (Thornhill & Alcock, 1983), and/or the population presents an extreme density of males (Schwagmeyer & Woontner, 1986). Consequently, in scramble competition mating systems, agonistic interactions among males are not common, and male reproductive success seems to be mainly influenced by males' ability to find and mate with as many receptive females as possible (Herberstein et al., 2017).

Given that in scramble competition mating systems male reproductive effort mainly consists in the search for mating partners rather than mate monopolization (Herberstein et al., 2017), females are not prevented from mating multiple times. High levels of polyandry should lead to increased levels of sperm competition when compared with mating systems in which females are guarded by the males (Holwell et al., 2016; Kappeler, 1997). Under sperm competition, males can increase the quantity and/or the quality of their sperm, which should enhance their fertilization success per mating event (e.g. DelBarco-Trillo, 2011; Simmons et al., 2007). Accordingly, a higher level of sperm competition correlates with a higher investment in post-mating competition (e.g. expenditure in sperm quantity or sperm quality per ejaculate), as suggested by mathematical modelling (Parker et al., 2013) and empirical evidence (e.g. Katsuki et al., 2013; Kelly et al., 2010). However, depending on the level of sperm competition, male responses per mating opportunity vary. At first, as the level of sperm competition increases, males are predicted to increase the investment per ejaculate, but once sperm competition reaches higher levels, male investment per ejaculate should start to decrease as the fitness payoff of ejaculatory expenditure is reduced (Parker et al., 2013). This decrease in the fitness payoff of investing in each ejaculate may be the outcome of a trade-off between investing in pre- and post-mating competition. The higher is the investment in ejaculates, the lower will be the investment in securing mating opportunities (e.g. Dines et al., 2015; Dunn et al., 2015). Thus, depending on the level of sperm competition, males may increase or decrease the investment in post-mating competition per mating opportunity.

To understand how sperm competition in a scramble competition system affects male reproductive decisions, we used the gift-giving spider *Paratrechalea ornata* (Trechaleidae) as study system. This spider presents a clear example of scramble competition: females are scattered along rivers' margins (Spindler, 2015), which prevents males from defending the access to mating opportunities (Costa-Schmidt et al., 2008; Klein et al., 2014), and males wander around in active search for mating partners (Costa-Schmidt et al., 2008). Males court females by offering a prey-gift wrapped in silk, which is consumed by the females during copulation (Costa-Schmidt et al., 2008). The duration of each copulation positively correlates with prey-gift size (Klein et al., 2014), potentially allowing the male to transfer more sperm to the female. This higher investment in sperm transfer should increase male fitness in a context of sperm competition, as females can mate multiply (Pandulli-Alonso et al., 2017). Therefore, while the prey-gift is mainly a mating effort (Albo &

Costa, 2010), it also influences how much a male will succeed in post-mating competition.

The main question of this study is: how does sperm competition shape the mating behaviour of *P. ornata* males? To answer this question, we focused on two phases of male mating behaviour, namely mate searching and prey-gift construction. First, we tested male preferences when searching for females. Mate search in wandering spiders may be based on silk draglines deposited on the substrate by conspecifics, because chemicals contained in these draglines may inform the sex of the individual who deposited them (e.g. Albo et al., 2009; Roberts & Uetz, 2005; see also Beyer et al., 2018 for an example in another gift-giving spider), and also female mating status (e.g. Roberts & Uetz, 2005). Given that unmated females represent no immediate prospect of sperm competition while mated females do, males should prefer substrates containing chemical cues of the presence of unmated females than substrates containing no cues or containing chemical cues of the presence of mated females or competitor males. This hypothesis assumes that chemical cues in the draglines vary according to female mating status. To test our first hypothesis, we registered male residency time on substrates containing chemical cues deposited by unmated females, once-mated females, competitor males or no cues.

Second, we tested whether males adjust the timing and frequency of prey-gift construction, as well as the quality of the gift according to chemical cues of the presence of male competitors and the perceived risk of sperm competition. Because *P. ornata* females are polyandrous (Pandulli-Alonso et al., 2017), sperm competition is probably an important factor determining male reproductive success. Thus, we hypothesized that males should increase their reproductive effort as they perceive the risk of sperm competition (following the model by Parker et al., 2013). To increase the chance of mating and egg fertilization, males should begin gift construction sooner, aggregate more flies and silk to the gift, and perform more prey wrappings when they perceive the risk of sperm competition. To test our second hypothesis, we assessed prey-gift construction investment in two contrasting conditions: (a) when males have no cue of the presence of competitor males, i.e. the perceived risk of sperm competition is zero and (b) when males have cues of the presence of a competitor male, i.e. the perceived risk of sperm competition changes from zero to one.

2 | MATERIAL AND METHODS

2.1 | Animal collection and housing

We collected individuals of *P. ornata* at Pedra de Amolar River (29°32'02"S; 50°14'47"W), located in the Tramandaí River basin, municipality of Maquiné, state of Rio Grande do Sul, Brazil. We kept the individuals under laboratory conditions in individual plastic vials (8 cm high, 6 cm in diameter) containing water at the bottom and covered with a textile net. We fed the individuals with fruit

flies (*Drosophila* spp.) and small cockroach nymphs twice a week. The average (\pm SD) temperature and humidity in the laboratory were $25 \pm 3^\circ\text{C}$ and $80\% \pm 3\%$, respectively, and the photoperiod was 12 h of light per day. We conducted all experiments between 18 h and 23 h, which corresponds to the period of most intense *P. ornata* activity in the field (L. E. Costa-Schmidt, pers. obs.).

2.2 | Experiment 1: Chemical cues and male preference

We first tested the males' ability to assess information from chemical cues available on the substrate, which may help them during mate search. In this experiment, the chemical cues were represented by the silk draglines (and also any other body material, such as cuticular chemicals or faeces) deposited on the substrate by both females and males while they move. By performing multiple-choice trials and quantifying male residency time on silk, we tested whether males can assess not only the sex of the individual that deposited the draglines but also female mating status.

The experimental arena consisted of a circular container (20 cm in diameter), with a bottom of polystyrene and sides of an opaque card material (2.5 cm high). We divided the bottom into four sections (quadrants), each covered by a piece of filter paper with the same area and shape. In each quadrant, we provided one of the following cues: (a) draglines deposited by an unmated female; (b) draglines deposited by a once-mated female; (c) draglines deposited by an unmated male; and (d) no dragline (control). The procedure to obtain the silk for quadrants (a), (b) and (c) was the same. We kept the silk donor spider on a filter paper for 24 h, and then, we immediately transferred the filter papers to the experimental arena to initiate the test. We used each silk donor only once, and we did not test males against their own draglines. Tested males were not used as mating partners of once-mated females. Each once-mated female ($n = 18$) mated with a different adult male, and we used the silk of once-mated females in the experiment 4.67 ± 1.24 (mean \pm SD) days after they mated. The relative position of each treatment in the experimental arena was randomized for each new trial. We started each trial ($n = 18$) by caging an unmated adult male inside a small plastic cylinder (6 cm diameter) at the centre of the arena for an acclimation period of 10 min. Then, we released the male to explore the arena for 40 min. We recorded each trial with a digital camcorder. After each trial, we cleaned the entire arena with ethanol 70% and replaced the filter papers with new ones before a new trial. From the records, we quantified the time spent by the male on each quadrant (i.e. residency time).

For the statistical analysis, we normalized data distribution of the time spent in each quadrant. We also removed the detected heteroscedasticity by performing a Box-Cox transformation (Box & Cox, 1964), using the *BoxCoxTrans* function from the *caret* package (Kuhn, 2008). Then, using the *lm* function in the software RStudio© (R Core Team, 2017), we ran a linear model with time spent in each quadrant as the response variable and quadrant type as the predictor variable.

2.3 | Experiment 2: Prey-gift construction and sperm competition risk

In the second experiment, we tested whether males adjust their readiness to construct a gift, the frequency of gift construction and the size of the gift when they are exposed to chemical cues that indicate sperm competition risk. We performed the experiment in a Petri dish (9 cm in diameter) with the bottom covered with a filter paper. We created two independent treatments: arenas containing the draglines of both an unmated female and a conspecific male (sperm competition treatment; $n = 18$) and arenas containing only the draglines of an unmated female (no sperm competition treatment; $n = 18$). For the sperm competition treatment, we alternated the order of dragline deposition between donors (an unmated competitor male followed by an unmated female, and an unmated female followed by an unmated competitor male). Each dragline donor was allowed to deposit its draglines for 12 h when the other dragline donor replaced it for another 12 h. For the no sperm competition treatment, we left unmated females in the arena for 12 h. For half of these tests, we kept the unmated females occupying the arena during the 12 h that preceded the test; for the other half, we kept the unmated females in the arena for 12 h and then removed them 12 h before the beginning of the test. We adopted this procedure because it is possible that the detectability of the chemical cues contained in the silk diminishes after some period. Therefore, across trials, the mean time between silk deposition and the beginning of the trial had to be equally distributed among treatments. There was no need for a control group (i.e. a clean substrate) in this experiment because males tend to consume the prey rather than construct a gift in the absence of draglines deposited by females (Albo et al., 2009).

Each test consisted of releasing an unmated male in the centre of the arena, freely to explore it. The trials started after an acclimation period of 15 min when we released six live fruit flies (*Drosophila* spp.) inside the arena as a prey source for the construction of the gifts. All males were maintained under a high-quality feeding regime before the experiment to minimize the chance that they would feed on the flies instead of constructing a gift. All males were tested once, and experimental groups did not share individuals. We recorded each trial with a digital camcorder for 2 h (excluding the acclimation period). From each video, we registered the occurrence of prey-gift construction (yes or no), the latency to start prey-gift construction and three proxies for prey-gift quality: the number of flies added to the gift, the amount of time spent depositing silk around the captured flies and the number of wrapping bouts. Regarding the time spent in silk deposition, we decomposed it into two complementary stages: deposition of silk only on the substrate around the prey (Stage 1) and silk deposition directly on the prey (Stage 2). After performing Stages 1 and 2 at least once, males lift the gift from the substrate and use their pedipalps and/or legs III to involve the prey with silk, a behaviour known as prey wrapping (Costa-Schmidt et al., 2008). Some of the silk deposited during Stage 1 is usually left on the substrate during the wrapping procedure, which means that the gift evaluated by females during

pre- and post-mating choice is mainly wrapped with silk deposited during Stage 2. However, given that the Stage 1 is necessary for gift construction, it also provides information on male investment in prey-gift construction. For each male, we quantified the following: the time spent performing Stage 1, the time spent performing Stage 2 and the total time spent depositing silk (the sum of Stages 1 and 2). Males can engage in Stages 1 and 2 more than once per prey-wrapping event and can perform several prey wrappings in 2 h (see Results and Supplementary Material).

For the statistical analyses, we applied the same procedures performed for the first experiment to data normalization and control of heteroscedasticity. We tested the effect of the treatments (i.e. sperm competition vs. no sperm competition) on the frequency of prey-gift construction using Fisher's exact test. For each of the remaining response variables, we ran a linear model containing the treatments as a predictor variable. For the model on the latency to gift construction, we excluded individuals that failed to start gift construction during the trial ($n = 2$). All individuals were included in the other models. We ran all models using the *lm* function in the software RStudio© (R Core Team, 2017).

3 | RESULTS

3.1 | Experiment 1: Chemical cues and male preference

Males of *P. ornata* spent more time on quadrants of the arena containing silk of females than on quadrants containing silk of a male competitor or without silk; the time spent on the control quadrant and on the quadrant containing silk of a male competitor were similarly low (control_(intercept): $t = 13.91$, $df = 68$; male competitor: $t = 13.11$, $p = .426$; once-mated female: $t = 18.05$, $p < .0001$; unmated female: $t = 18.51$, $p < .0001$; Figure 1; Table S1). There was no difference in the time spent by males between the quadrants with silk of unmated females and with silk of once-mated females ($t = -0.456$; $df = 68$; $p = .650$).

3.2 | Experiment 2: Prey-gift construction and sperm competition risk

In the sperm competition group, 16 out of 18 males constructed a prey-gift (one individual started to construct a prey-gift but did not finish it before the end of the trial). In the no sperm competition group, 17 out of 18 males constructed a prey-gift. There was no difference in the frequency of prey-gift construction between treatments (Fisher's exact test: $p = 1.000$, odds ratio = 0.480). There was also no difference between treatments in the mean number of flies captured nor the latency to gift construction (Figure 2a,b; Table 1 and S2). Males in the sperm competition group invested less in prey wrapping than males in the no sperm competition group, both in the amount of silk deposited (Stage 1 alone, Stage 2 alone and

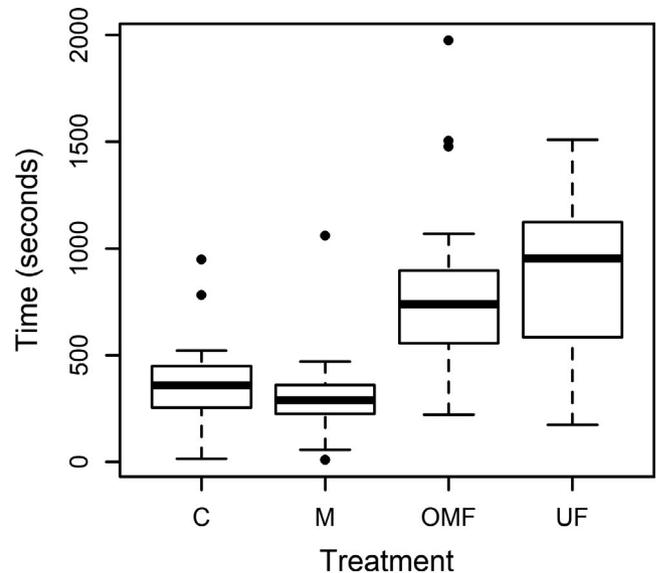


FIGURE 1 Time spent by *Paratrechalea ornata* males on each quadrant of the experimental arena. Control (C): quadrant without silk; male competitor (M): quadrant with silk of a competitor male; once-mated female (OMF): quadrant with silk of a once-mated female; unmated female (UF): quadrant with silk of an unmated female. Bold horizontal line: median; box: superior limit of the first quartile and inferior limit of the fourth quartile; whiskers: values range; black dots: outliers

Stages 1 and 2 combined) and in the number of prey-wrapping bouts (Figure 2c,f; Table 1 and S2).

4 | DISCUSSION

Here, we show that males of *P. ornata* respond to chemical cues left on the substrate by conspecifics of both sexes and, more importantly, we show that males modulate their reproductive investment in prey-gift construction depending on the chemical cues deposited on the substrate. In our first experiment, males spent more time on substrates containing silk of females instead of substrates containing no silk or silk of a competitor male, suggesting that males prefer places where the chances of finding a female are high. However, males did not prefer substrates with cues of unmated females in comparison to substrates with cues of mated females. This result not only contrasts with the evidence from other wandering spider species, in which males prefer unmated females (e.g. Baruffaldi & Costa, 2010; Rypstra et al., 2003), but also refutes our prediction that males would prefer unmated females as a way to avoid sperm competition risk. In our second experiment, males' propensity and readiness to construct a gift, as well as the investment in gift size, were not modulated by the perceived risk of sperm competition. However, when facing sperm competition risk, males deposited less silk during gift construction and performed less prey-wrapping bouts, indicating that they decrease their reproductive effort in response to sperm competition risk. A similar response was found in the European gift-giving spider *Pisaura mirabilis*, but in this case, males were tested in

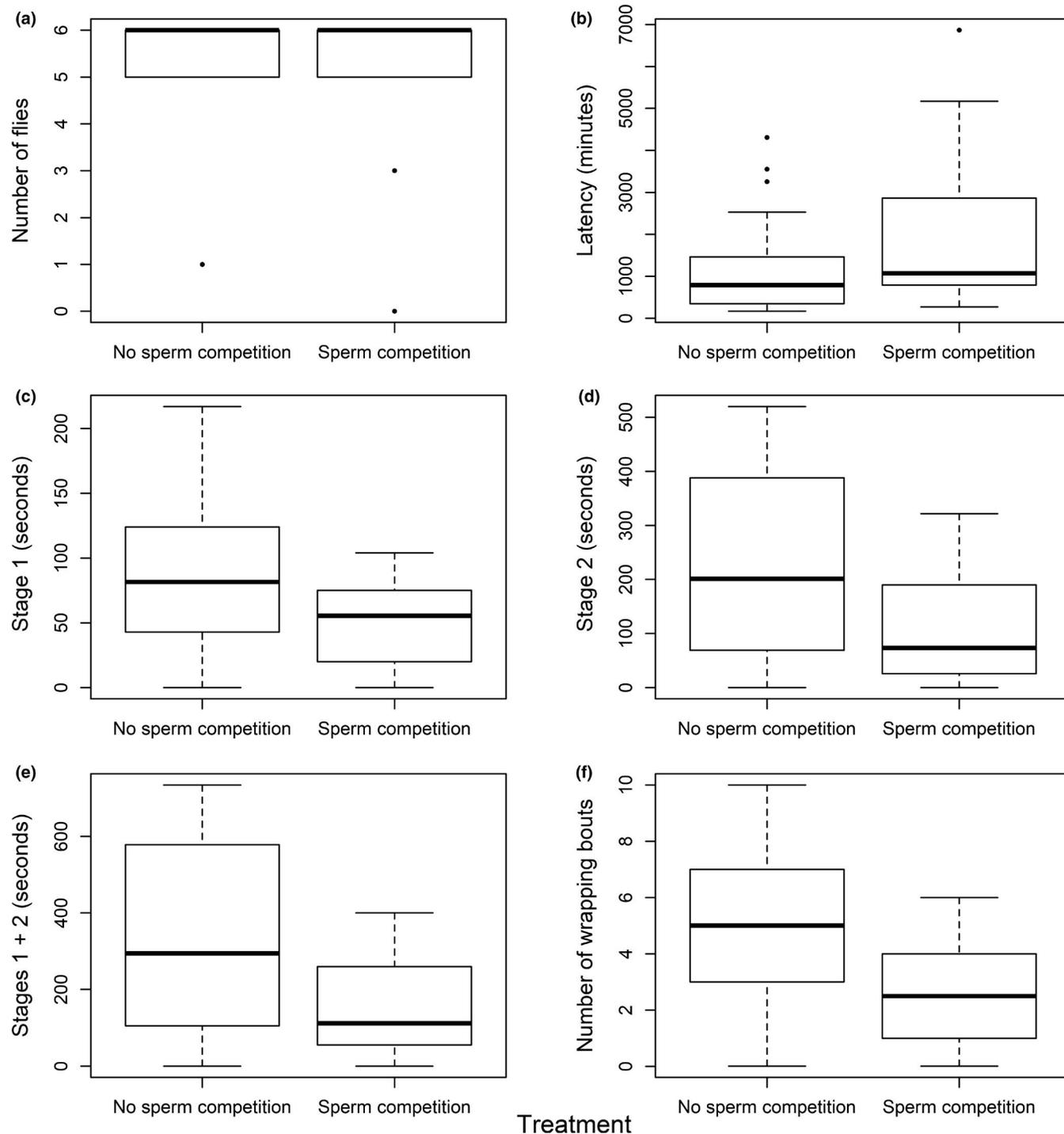


FIGURE 2 Male reproductive effort in the gift-giving spider *Paratrechalea ornata* according to the perceived sperm competition risk. "Sperm competition": arenas containing silk of both an unmated female and a competitor male; "No sperm competition": arenas containing only silk of an unmated female. We ran one model for each of the six response variables (see results in Table 1): (a) number of flies captured by the male and added to gift; (b) latency to start gift construction; (c) total time spent in Stage 1 of gift construction (i.e. deposition of silk on the substrate around the flies); (d) total time spent in Stage 2 of gift construction (i.e. deposition of silk directly on the flies); (e) total time spent depositing silk on the gift (Stages 1 + 2); and (f) number of wrapping bouts. Bold horizontal line: median; box: superior limit of the first quartile and inferior limit of the fourth quartile; whiskers: values range; black dots: outliers

the presence of a rival so that they could interact aggressively before gift construction (Tuni et al., 2017).

The prediction that males should efficiently detect potential sexual partners in species with scramble competition polygyny

(Herberstein et al., 2017; Schwagmeyer & Woontner, 1986; Thornhill & Alcock, 1983) was corroborated in Experiment 1 because males spent more time on substrates containing cues of females. However, males did not prefer substrates containing cues of unmated females.

TABLE 1 Results of the linear models contrasting the reproductive effort of *Paratrechalea ornata* males belonging to two treatments: (a) sperm competition (model intercept): arenas containing silk of both an unmated female and a male competitor; and (b) no sperm competition: arenas containing only silk of an unmated female

Response variable	t value	df	p value
Number of flies captured	0.242	34	.810
Latency to gift construction (time)	-1.564	32	.128
Silk deposition: Stage 1 (time)	2.060	34	.047
Silk deposition: Stage 2 (time)	2.226	34	.033
Silk deposition: Stages 1 + 2 (time)	2.160	34	.038
Number of prey wrappings	2.054	34	.048

Note: We ran one model for each response variable. For further details on the results of the models, see Table S2.

We identify three possible explanations for this unexpected result. First, males may be unable to recognize the mating status of females based on their draglines. Given that mating polyandrously may result in both direct benefits (i.e. nutritious free-meals from gift-offering) and indirect benefits (e.g. a more genetically diverse offspring) (Arnqvist & Nilsson, 2000; Slatyer et al., 2012), females benefit from not signalling their mating status. Second, males may recognize that the substrate contains silk from both unmated and mated females, but the presence of silk from a competitor male in a nearby quadrant interferes with the patterns of preference because of the sperm competition risk. In the field, males probably receive chemical cues from both females and males simultaneously, and thus, our experimental arena resembles natural conditions faced by the males. Finally, the costs of searching mainly for unmated females in the field may be high enough to prevent males from doing it. These costs may exist because females achieve maturity asynchronously (L. E. Costa-Schmidt, pers. obs.), biasing the operational sex ratio towards males and increasing pre-mating male-male competition (Kvarnemo & Ahnesjö, 1996; see also Albo et al., 2019 for an example in a gift-giving spider). Moreover, the costs of searching preferentially for unmated females can be even higher if the male already constructed a gift, since holding a gift imposes a 37% increase in male metabolic rates, as reported for the spider *Pi. mirabilis* (Prokop & Okrouhlik, 2021). Thus, evolution should not favour preference based on female mating status because the chances of finding unmated females are low and the costs of this preference are high.

The absence of male response to sperm competition risk is recognized when analysing some (but not all) proxies of male reproductive effort measured in Experiment 2. Prey-gift construction frequency and latency did not differ according to sperm competition risk, indicating that males promptly court females regardless of intrasexual competition levels. As males in scramble competition mating systems are under a selective pressure to find mates as fast as possible (Herberstein et al., 2017), and as the possession of a prey-gift has a positive effect on the initial steps of female choice (Brum et al., 2012), males may gain little or no benefits from

avoiding gift construction in non-ideal contexts (i.e. when a male competitor is nearby). Additionally, the number of flies added to the gift did not differ according to sperm competition risk. As all experimental males were well fed, the rearing protocol may have attenuated the males' trade-off between somatic investment (i.e. consumption of prey to self-maintenance) and reproductive investment (i.e. construction of the prey-gift) (Macedo-Rego et al., 2016). Therefore, the costs of adding many flies to the gift even under sperm competition risk may have been relatively low in our experiment, explaining why males from both treatments added almost all offered flies to their gifts.

However, while males did not adjust prey-gift quality through the number of flies added to the gift, males facing sperm competition risk deposited less silk and performed fewer prey-wrapping bouts. As silk production in spiders is costly (Craig, 2003), there is a possible trade-off between the number of gifts constructed (i.e. the number of females courted) and the quality (i.e. amount of silk used in prey wrapping) of each gift, which in *Pi. mirabilis* is positively correlated with copulation duration and probably with the quantity of sperm transferred to the female (Lang, 1996). In the model developed by Parker et al. (2013) on male reproductive effort in scramble competition mating systems, sperm competition levels influence the fitness output of investing in traits involved in pre-mating competition or traits involved in post-mating competition. According to the model, males are expected to invest more in the option that maximizes their fitness benefits. Following this, our results indicate that *P. ornata* males facing risk of sperm competition reduced the quality of each gift, which should enable them to increase the number of gifts constructed and, consequently, the number of courted females during the breeding season. An opposite result was reported for the springtail *Orchesella cincta*, in which males deposit spermatophores on the substrate irrespective of the presence of females—a highly unusual case of scramble competition. Males exposed to olfactory cues of competitor males decrease the number of spermatophores deposited when compared to males not exposed to olfactory cues of other males (Zizzari et al., 2013). Therefore, in *P. ornata* and *O. cincta*, males facing sperm competition risk adjust the reproductive investment per ejaculate in different ways to maximize their reproductive success: while the former decrease the investment per mating opportunity, the latter increased it probably because the costs of producing various spermatophores are high.

Three non-exclusive hypotheses can explain why *P. ornata* males may increase their reproductive success by depositing more silk on gifts when they face no immediate sperm competition risk. First, the increase in silk investment may occur because male silk contains chemicals that induce females to accept the gift (Brum et al., 2012) and the fitness return of being accepted by an unmated female tends to be higher than the fitness return of being accepted by a once-mated female. Second, a well-wrapped gift possibly extends copulation duration, which may allow the male to transfer more sperm and achieve higher reproductive success, especially when mating with an unmated female (see Lang, 1996). Every time a male faces

sperm competition, there is a chance that he will lose some paternity. However, being the sole sexual partner of a given female does not guarantee that the male will fertilize all the female eggs because the quantity of sperm transferred may not suffice to accomplish this task (Andrade & Banta, 2002 and reference therein). Therefore, a male may maximize his reproductive success by transferring more sperm to an (until that time) unmated female. A similar pattern of higher investment in male ejaculate in response to high-quality females has already been recorded for various animal species (Kelly & Jennions, 2011). Third, given the higher investment towards higher quality females and the possibility that well-wrapped gifts extend copulation duration, long-lasting copulation with an unmated female can improve males' fertilization success, even if the female later accepts other males. This may happen because males with high-quality gifts have more time to court the partner and their sperm can be favoured by cryptic female choice (Albo & Peretti, 2015).

Finally, a broader picture emerges from simultaneously analysing both of our experiments. The fact that males in Experiment 1 preferred substrates containing silk of females does not suffice to demonstrate that *P. ornata* males can identify draglines of other males, mainly because the time spent on the male quadrant was similar to the time spent on the control quadrant. However, the fact that tested males in Experiment 2 reduced their reproductive effort when finding cues of another male's presence demonstrates that *P. ornata* males can detect the sex of the individuals that released each silk dragline. Still comparing the experiments, males in Experiment 1 did not spend more time on substrates containing silk of unmated females, which could lead to the conclusion that there is no pre-copulatory male mate choice in *P. ornata*. However, Experiment 2 shows that males invested less in prey-gift construction when facing sperm competition risk, which demonstrates the occurrence of a less evident form of male mate choice based on differential allocation of resources to prey-gift. This finding corroborates the idea that male mate choice can evolve even in polygynous mating systems with no male parental care (Edward & Chapman, 2011), such as the scramble competition mating system of *P. ornata*. Taken together, our results indicate that males are able to identify the sex of the individual who deposited each silk dragline and demonstrate that males adjust their effort on prey-gift construction in response to chemical cues left by conspecifics that indicate the sperm competition risk.

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CONFLICT OF INTEREST

There is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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