



Original Article

Male spiders reduce pre- and postmating sexual investment in response to sperm competition risk

Cristina Tuni,^a Sabrina Weber,^a Trine Bilde,^b and Gabriele Uhl^c

^aDepartment of Biology, Ludwig Maximilian University of Munich, Grosshaderner Str. 2, 82152 Planegg-Martinsried, Germany, ^bDepartment of Bioscience, Aarhus University, Ny Munkegade 116, DK-8000 Aarhus C, Denmark, and ^cDepartment of General and Systematic Zoology, Ernst Moritz Arndt University of Greifswald, Zoological Institute and Museum, Anklamer Str.20, D-17489 Greifswald, Germany

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The interplay between pre- and postmating responses to intrasexual competition remains enigmatic. Sperm competition models often assume a trade-off between pre- and postmating traits that enhance mate acquisition and fertilization success, respectively. However, when males court females through food donations (i.e., nuptial gifts), pre- and postmating responses may be aligned, as nuptial gifts have the dual function of facilitating both mate acquisition and sperm transfer. In the spider *Pisaura mirabilis*, nuptial gifts consist of silk-wrapped prey. We tested whether males respond to a competitor by altering: 1) premating investment in the gift, 2) postmating sperm investment, and 3) whether pre- and postmating responses are coupled and respond to competition in the same direction or not. Under competition risk males silk-wrapped their gifts for significantly shorter time and transferred less sperm to females, pointing to a reduction of both pre- and postmating responses. Because silk is not a target of female choice, reducing gift construction may speed up mate acquisition. In accordance with models of sperm allocation, perceived high levels of competition and/or sperm priority patterns may explain the reduced patterns of sperm transfer found in our study. Overall, our findings suggest that in competitive environments pre- and postmating traits are coupled and respond in the same direction.

Key words: nuptial gifts, sexual selection, sperm competition.

INTRODUCTION

The sociosexual environment is characterized by fluctuations in sex ratios, encounter rates between the sexes and hence variation in the levels of intrasexual competition. To optimize reproductive success in response to changes in sexual competition, males are known to perform fast adjustments of courtship, mating and ejaculatory strategies (Bretman et al. 2011; Kelly and Jennions 2011; Weir et al. 2011). Competition with other males should promote male premating strategies that increase mating success through enhanced mate searching, courtship or by outcompeting rivals aggressively (Andersson and Simmons 2006). In species in which females mate with more than one male, producing larger ejaculates can function to outcompete sperm of competitors and thus promote relative fertilization success (Parker 1970; Simmons 2001). The interplay between pre- and postmating

responses to different levels of male–male competition, however, remains enigmatic. Because male reproductive success will depend on both the number of mating partners achieved (i.e., investment in premating traits) and the fertilization outcome of each mating (i.e., investment in postmating traits), male responses to competition will likely be shaped by the relative lifetime fitness gain from expenditure in pre- and postmating strategies (Parker et al. 2013). However, while notable effort has been made to understand how males respond to competitors by investing in mate acquisition or to competitors' ejaculates by enhancing fertilization success, the 2 processes are rarely addressed simultaneously (Evans and Garcia-Gonzalez 2016).

Theoretical models on postmating responses to competition predict that males should produce ejaculates of greater sperm numbers when facing the risk of competing against the sperm of a single rival male (“risk models”) (Parker 1990). Interestingly, when the number of rivals increases and males face high sperm competition intensity, the opposite prediction can arise, suggesting that males should reduce sperm investment (“intensity models”) (Parker and

Address correspondence to C. Tuni. E-mail: cristina.tuni@bio.lmu.de. T.B. and G.U. share equal contribution.

Pizzari 2010). The “risk model” has received compelling empirical evidence (delBarco-Trillo 2011; Kelly and Jennions 2011) and has been used to develop general predictions on male premating expenditure in reproduction, which generally imply trade-offs between premating and postmating responses. Increased investment in winning postmating competitive fertilizations through larger ejaculates is indeed expected to occur at the expense of premating investment in mate acquisition and this is most likely in systems where access to females occurs by scramble competition (i.e., mate search effort) (Danielsson 2001; Parker et al. 2013). On the contrary, in systems where males compete through contest competition, because of the high reproductive payoffs, males may invest disproportionately in premating traits such as weaponry (i.e., horns, antlers) (Fitzpatrick et al. 2012; Simmons and Buzatto 2014) or strategies to secure social dominance (Rudolfson et al. 2006; Pizzari et al. 2007; Tuni et al. 2016) at the expense of ejaculate size. Selection might, however, also favor increased expenditure in both pre- and postmating sexual traits generating positive covariance between them (Sheldon 1994; Hosken et al. 2008). This may occur in systems where males do not monopolize females through male–male contest and competition for mating partners is equally important during pre- and postmating episodes of selection (Lupold et al. 2014). “Intensity models” have received less empirical support (delBarco-Trillo 2011; Kelly and Jennions 2011), with the general expectation that along with reduced investment in ejaculates, males should consistently reduce premating responses such as aggressiveness and courtship due to the low expected fitness reward derived by high levels of competition (Parker and Pizzari 2010; Bretman et al. 2011; Weir et al. 2011).

The spider *Pisauwa mirabilis* (Pisauridae) provides a useful system to investigate how males respond to different competitive scenarios pre- and postmating. Males and females of this species mate multiply (Tuni et al. 2013). Males court females by donating a nuptial gift consisting of an insect prey wrapped in silk, which is offered to the female through a series of courtship displays (Bristowe 1958). The transfer of a nuptial gift is sexually selected as males without donations suffer from reduced mating success (Stålhandske 2001). The gift, however, also plays a crucial role postmating by enhancing fertilization success, because male sperm transfer occurs concurrent to female consumption of the donated gift (Drengsgaard and Toft 1999; Stålhandske 2001). Gift size correlates with mating duration and longer copulations lead to more sperm transferred to the female’s reproductive tract and results in higher fertilization success (Albo et al. 2011, 2013). Males may enhance postmating competitiveness by prolonging copulation and sperm transfer, facilitated by increased premating investment in the gift. By wrapping the gift in additional silk layers, males produce larger nuptial gifts that ultimately prolong female consumption time and thereby mating duration (Lang 1996). Furthermore, males have evolved a behavioral death-feigning strategy (thanatosis), where the male holds onto the gift in response to mating interruptions by females. By grasping the gift with their fangs and remaining attached in a motionless collapsed posture, death feigning allows the male to readily resume the mating position at a later stage ultimately prolonging both mating success and copulation duration (Bilde et al. 2006; Hansen et al. 2008).

Spiders transfer sperm to the female sperm storage organ with their copulatory organs (pedipalps), that, upon reaching sexual maturity, they fill with sperm produced in their reproductive tract (Foelix 2010). This peculiar mode of insemination allows addressing male investment in sperm transfer in separation from sperm production. Owing to the dual function of the nuptial gift in

facilitating mate acquisition and sperm transfer, *P. mirabilis* males should increase allocation to nuptial gifts in response to competition, as these in return confer competitive fertilization advantages. However, if males are able to enhance sperm transfer through strategies other than nuptial gifts, for example, by prolonging overall mating duration through more frequent use of thanatosis; they may rather trade allocation to gifts for ejaculate investment. We examined these hypotheses by exposing males to a potential competitor during courtship and mating and measuring male performance in: 1) premating investment in silk-wrapping of the nuptial gift and courtship effort, 2) behaviors at mating, such as copulation duration and thanatosis, and 3) postmating investment in sperm transfer to the female storage organ. We compared data on these traits to those of males without rival male exposure. If male sperm allocation is controlled prevalently by gift properties, those facing a competitor should optimize their reproductive success by increasing gift allocation (i.e., silk-wrapping) in order to ultimately achieve longer copulations and higher rates of sperm transfer. However, males have means other than gifts to control sperm transfer, for example, through higher rates of thanatosis; hence when facing a rival, they may alternatively enhance the amount of sperm transferred, while reducing expenditure on costly gift-wrapping.

METHODS

Study organisms

Approximately 100 juvenile *P. mirabilis* were collected in grasslands surrounding Lodi (Italy) between September and October 2014 and were brought to the laboratory at the Ludwig Maximilians University of Munich (Germany) where they were raised under standard laboratory conditions. Spiders were kept individually in vials (5 cm diameter, 10 cm height) topped with foam lids and supplied with a substrate of freshly collected moss (*Sphagnum sp.*). During the initial 5–6 weeks, they were kept at room temperature (approximately 22 °C) and natural photoperiod. Subsequently, they were placed under light bulbs 3.5 h per day (12:00–15:30), which increased the temperature to approximately 27 °C. This was done to increase growth rate throughout the winter, as spiders naturally diapause during this time of the year. Spiders were fed one fly (*Lucilia sericata*) at either larval or adult stage or one cricket nymph (*Gryllus bimaculatus*) twice a week and every 2–3 days moss was sprayed with water. Vials were checked daily for the presence of moulted exuvia in order to determine spider maturation to adulthood. Adult spiders (a total of 47 males and 44 females) were used in our experiments 2 weeks after reaching adulthood. All animals were unmated to control for previous reproductive and social experience.

Experimental design

Male spiders were randomly assigned to one of the 2 experimental treatments: “competition risk” males ($n = 25$) were exposed to a rival male before and during mating and “control” males ($n = 22$) were paired to females with no rival male exposure. Prior to experiments, 45 males and 39 females were weighed to the nearest 0.01 g using a digital scale (KERN PKT, KERN & SOHN GmbH, Balingen, Germany), ensuring that animals assigned to each treatment did not differ in their average body mass (male weight [g] \pm SE, competition risk 0.122 ± 0.003 , $n = 24$ and control 0.117 ± 0.004 , $n = 21$, t -test, $t = -1.16$, $df = 43$, $P = 0.25$; female weight [g] \pm SE, competition risk 0.185 ± 0.008 , $n = 20$ and

control 0.178 ± 0.008 , $n = 19$; t -test, $t = -1.61$, $df = 37$, $P = 0.11$). All males were further marked with a dot of acrylic paint (Idena, Iden Berlin, Berlin, Germany) on their abdomen to allow individual identification. Whereas all focal males had no previous social experience, rival males consisted of previously mated focal males. Each rival male was randomly assigned to control or competition risk treatments and used for a maximum of 4 times.

Experiments were conducted in a transparent plastic terrarium ($23 \times 15 \times 14$ cm) provided with a bottom sheet of absorbent paper. The female assigned to the trial was placed in the terrarium for 30 min in order to leave draglines, which are known to elicit male sexual arousal and gift-construction (Lang 1996). In the competition risk treatment, upon removal of the female, a rival male was placed in the terrarium for 5 min before being confined under a net dome consisting of a plastic cup cut open and enclosed with a layer of mesh netting (Figure 1), which was located in a standardized position in the terrarium. The focal male was subsequently added and after 5 min of habituation, the rival male was freed and the 2 were allowed to establish physical contact. To avoid injuries or death, males were rapidly separated using a paintbrush when engaging in aggressive physical interactions (i.e., biting). Following contact, the rival male was reconfined under the net dome. This design allowed males to exchange chemical and visual cues throughout the trial. In control trials, before introducing the focal male to the terrarium, an empty net dome was placed in the arena at the same position. Domes were rinsed with water and dried using towels after each trial in order to remove potential cues.

Premating investment in silk wrapping of the gift and courtship

Once the rival male was confined under the net, focal males were given a cricket nymph to wrap in silk (cricket body mass 0.02–0.04 g). During the breeding season, males of this species are commonly found carrying silk-wrapped gifts in their mouthparts while seeking for females (Ghislandi et al. 2017), indicating that gift construction occurs prior to female encounters. Therefore, silk wrapping of the gift was tested in the absence of a female. Upon male

acceptance of the prey, we measured total silk-wrapping duration (seconds) as the sum of the durations of all wrapping sequences. Duration in time of all behaviors described in the study was measured using a digital stopwatch (Conrad G-501). During silk wrapping, the male moves in rounds, keeping its silk-spinning organs located at the ventral rear of the abdomen in contact with the prey that is attached to the ground by silk threads. This process is not continuous and can be shortly interrupted and hence fragmented into several silk-wrapping sequences. Silk wrapping was considered completed when males had stopped wrapping for 10 consecutive minutes. Males that did not accept or wrap the prey within 60 min were returned to their vials and tested on the following day using the same procedure and assigning them to the same treatment.

Once gift-construction was completed a female was added to the arena with the focal male and the rival still confined under the net dome and upon establishment of physical contact with the focal male, durations of courtship behaviors were scored. Courtship in *P. mirabilis* consists of a repeated series of stereotyped male displays such as rapid “rubbing” movements of the first and second pair of legs, vertical stretching of the first pair of legs (hereafter, vertical legs display) and gift offering consisting of a hyperflexed posture with males bending backwards raising the first pair of legs and spreading their pedipalps (sperm transfer organs) apart (Nitzsche 2011). These behaviors do not necessarily occur in the described order. We measured the duration of any of the above-mentioned behaviors (vertical legs display, leg rubbing, and gift offering) until females grasped the gift in their mouthparts. If males did not perform any courtship behavior within 30 min the trial was terminated. If the females did not accept a courting male within 60 min the trial was terminated.

Behaviors at mating: copulation duration and thanatosis

Once the female holds the gift in her mouthparts, the male moves underneath in an antiparallel position and reaches for the female epigyne (the genital area with opening of the female reproductive tract) with one of his 2 pedipalps and transfers sperm. We considered a mating to be successful if the male coupled a pedipalp on

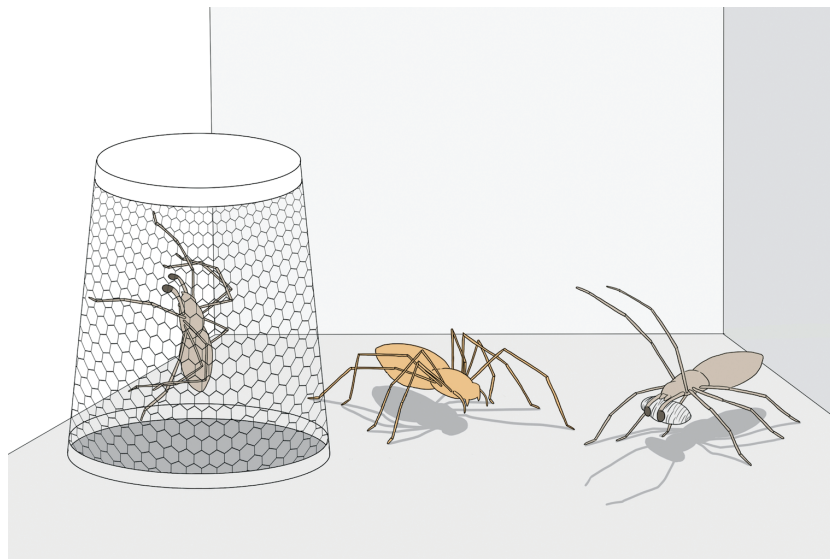


Figure 1

Graphic representation of the experimental set up showing the net dome used to delimit the rival male during the mating trials in the competition risk treatment. An empty dome was added to the mating arena in control trials.

the epigyne. Interruptions of sperm transfer may occur as males often decouple and switch to the other pedipalp. We noted for how long each palp (right or left) was being used and measured total copulation duration as the sum of all pedipalp insertion durations. In cases where females move, copulations are also interrupted leading to male thanatosis behavior. We measured the total duration in time (as the sum of all durations) males spent in the death feigning posture.

If the female lost the gift to the male, the male was allowed to reoffer the gift for a period of 20 min. If the male lost the gift to the female, the mating trial was terminated as males without a gift are unlikely to remate (Albo et al. 2011).

Postmating investment in sperm transfer

At the end of the mating trial, females ($n = 36$) that had mated successfully were immediately placed in small plastic zipper bags (6×8 cm) with individual IDs and frozen at -80°C . This timely procedure was used to limit any further cryptic manipulation of stored sperm from females. Samples were transferred on ice packs to University of Greifswald (Germany). We conducted sperm counts from dissected female sperm storage organs (spermatheca) using a standard protocol for this species (Gabel and Uhl 2013). Briefly, immediately after excision with razor blades under a stereomicroscope, the female spermatheca was transferred to $20\ \mu\text{l}$ of CASY@ton (Schärfe System, Reutlingen, Germany) and ruptured with fine forceps to release sperm. Ultrasonic treatment for homogenization was conducted (twice for 30 s at 50% power with a 30-s break to avoid overheating of the samples) and processed by using a cup booster for small volumes of an ultrasonic homogenizer (Bandelin Sonoplus HD 2070) to avoid sample loss. Samples were centrifuged at $5000 \times g$ and vortexed for 60 s before transferring $10\ \mu\text{l}$ to the counting chamber of C-Chip disposable hemocytometers (Neubauer improved, NanoEnTek). We counted sperm cells in 16 small squares (i.e., one L-square) under an Olympus light microscope and used the mean of 4 L-squares to calculate the total number of sperm cells in our sample. Sperm counts were repeated twice per sample. First and second measures were highly correlated (linear regression, $R^2 = 0.98$, $n = 36$, $F = 1875.7$, $P < 0.0001^*$).

Statistical analysis

We tested the effect of treatment (competition risk and control) on male behaviors using t -test for independent data when analyzing

continuous response variables (duration of silk-wrapping, mating, and thanatosis) and courtship effort that was calculated by dividing the total duration of courtship by the total duration of the courtship trial (which ended until mate acceptance or until trial was considered ended). When needed, variables were log-transformed or arcsine-transformed to meet assumptions of normal distribution of residuals. Chi-square test was used when analyzing binary data (occurrence of silk wrapping, mating and thanatosis) across treatments. Difference in sperm numbers transferred to females between treatments was tested using nonparametric median test. All statistical analyses were performed using the software JMP 13.0 (SAS Institute, Cary, NC). Means are reported \pm SE.

RESULTS

Premating investment in silk wrapping of the gift and courtship

Males from both treatments were equally likely to accept a cricket nymph and wrap it in silk: Of the total males tested, 22 out of 25 of the competition risk treatment and 20 out of 22 from the control constructed a gift (chi-square test, $\chi^2 = 0.43$, $P = 0.51$). In 5 trials, duration of silk wrapping of the gift was not measured, leaving us with a sample of 37. We found that males experiencing competition risk wrapped their gifts for significantly shorter time compared to those not experiencing a rival (Table 1; Figure 2). Among those that constructed a gift, 2 males did not court and data on courtship displays were missing from 5 mating trials, allowing us to measure courtship effort in 35 trials. We found that male courtship effort did not differ between treatment groups (Table 1).

Behaviors at mating: copulation and thanatosis

Male mating success was not affected by treatment, with males mating in 95.5% (21/22) of the competition risk and in 90% (18/20) of the control trials (chi-square test, $\chi^2 = 0.46$, $df = 1$, $P = 0.49$). We found no significant difference in total mating duration, measured in 36 trials, between males experiencing a rival and controls (Table 1). No evidence was detected for a difference in the likelihood for males to perform thanatosis in competition risk (94%, $n = 18$) and control (82%, $n = 14$) treatments (chi square, $\chi^2 = 1.18$, $df = 1$, $P = 0.27$), with similar thanatosis duration across treatments (Table 1).

Table 1

Statistical comparisons of male i) premating investment in silk-wrapping of the nuptial gift and courtship effort, ii) behaviors at mating, such as copulation duration and thanatosis, and iii) postmating investment in sperm transfer to the female storage organ, measured with and without exposure to rival male (respectively, competition risk and control treatment)

	Treatment		Statistics
	Competition risk	Control	
i) Premating investment			
Silk wrapping of gift (minutes) ^a	2.67 \pm 0.35 (19)	3.86 \pm 0.40 (18)	t -test $t = 2.34$, $df = 35$, $P = \mathbf{0.025}$
Courtship effort (courtship/total trial duration) ^b	0.27 \pm 0.06 (17)	0.22 \pm 0.05 (18)	$t = 0.71$, $df = 33$, $P = 0.48$
ii) Behaviors at mating			
Copulation duration (minutes)	66.68 \pm 6.01 (19)	52.74 \pm 8.25 (17)	t -test $t = 1.36$, $df = 34$, $P = 0.18$
Thanatosis duration (minutes) ^a	5.86 \pm 1.15 (17)	6.98 \pm 2.69 (14)	$t = 0.35$, $df = 30$, $P = 0.72$
iii) Postmating investment			
Sperm in female storage organ (numbers)	13 571.25 \pm 2965.44 (20)	15876.56 \pm 2537.48 (16)	Median test $\zeta = 1.98$, $df = 1$, $P = \mathbf{0.047}$

Data is given in mean \pm SE and sample size (N). Bolded values denote statistical significance ($P < 0.05$).

^aLog transformation and ^barcsine transformation of data.

Postmating investment in sperm transfer

Females mated to competition risk males had a significantly lower number of sperm in their storage organs than those mated to controls (Table 1; Figure 3).

DISCUSSION

Our study revealed that males in a competitive environment respond with reduced investment in premating and postmating traits. When facing sperm competition risk, males wrapped their prey in silk for a significantly shorter time compared to those not experiencing a rival, suggesting an overall reduction in gift investment. Reduced premating trait expression was followed by slightly prolonged matings; these, however, did not lead to increased sperm transfer. On the contrary, in competitive settings, males transferred fewer sperm to their mates, pointing to a strategy of concordant reduction in both pre- and postmating investment, rather than an opposite allocation of resources under competition risks.

Most studies investigating courtship in the presence of a rival find a reduction in male courtship behaviors (Bretman et al. 2011). This response is triggered by cues of rivals, as described for male newts (*Lissotriton boscai*) that reduce duration and number of courtship displays when chemical stimuli from another male are present in the water (Aragón 2009), and/or rival male's presence, as

for garter snakes (*Thamnophis sirtalis*) that are known to reduce the vigor of courtship when they are in larger courting groups (Shine et al. 2003). Many studies suggest that such alteration is triggered by the fact that males spend more time being vigilant and attending to the other male, than courting the female. In other words, these males face a decision-making trade-off between courting a potential mate and defending their resources (e.g., mate, territory) against a competing male. Such risk-dependent modulation was reported for example in the spider *Pholcus phalangioides*, where males reduced courtship in the presence of a rival, while copulating for longer (Schaefer and Uhl 2003). Our results only partially conform to this scenario, as males reduced gift construction, which is fundamental aspect of male premating courtship but not courtship displays per se. There may be possible alternative explanations for the finding that the rival male presence had no effect on courtship effort. Courtship displays may be honest signals of male quality and therefore males may not be able to change the intensity of their displays based on competition risks (Doutrelant et al. 2001). Or conversely, displays may not be determinant for male reproductive success (e.g., not a target of female mate choice) and therefore there may be weak selection for behavioral tailoring in response to social environmental conditions. Improving our understanding on condition-dependence and the resulting effects of courtship displays on male reproductive fitness in *P. mirabilis* represent interesting venues for future research.

It is instead well-known that achieving success in mating in this species critically depends on the presence of a nuptial gift, which males carry during mate searching in order to promptly court females upon encounter (Stålhandske 2001). Despite silk being an honest indicator of male quality with males in better body condition releasing more silk (Albo et al. 2011), the amount of silk covering nuptial gifts is not a predictor for male attractiveness or mating success (Bilde et al. 2007; Albo et al. 2012). Hence, by investing less in silk-wrapping males do not undermine their ability to attract females and mate. On the contrary, by spending less time on gift construction males may be able to start searching for and courting females sooner in time (i.e., gift is promptly ready). They may also minimize the likelihood of the nearby competitor succeeding in mating with the female during the time males engaged in silk wrapping. Although not quantified, males were also often observed engaging in physical contact with rivals through the separating net, further suggesting that they allocated time to defensive male–male interactions. By reducing silk wrapping, males may further reduce some of the energetic costs associated with silk proteins (Craig et al. 1999; Nentwig and Kuhn-Nentwig 2013) that could potentially be allocated elsewhere, such as solving intrasexual competitive interactions (Scharf et al. 2013). Experimental laboratory studies indeed reveal males frequently interrupting courtship of other males and agonistic behavior, with males aggressively assaulting and chasing away each other during interactions over a single female (Nitzsche 2011). Competition over mating partners and rival male interference are also likely to occur in the field where a high number of sexually mature individuals of both sexes are often found in relatively small areas (Tuni and Uhl, personal observations).

The evolutionary significance of silk wrapping has been thoroughly investigated in *P. mirabilis*. Silk wrapping of the gift is known to ease male handling ability when carrying it and control of the gift during mating (Andersen et al. 2008; Hansen et al. 2008). To this end, the benefits of faster gift construction should increase the risks of losing control of poorly wrapped gifts in competitive social environments. Silk has also been suggested to play a crucial role in

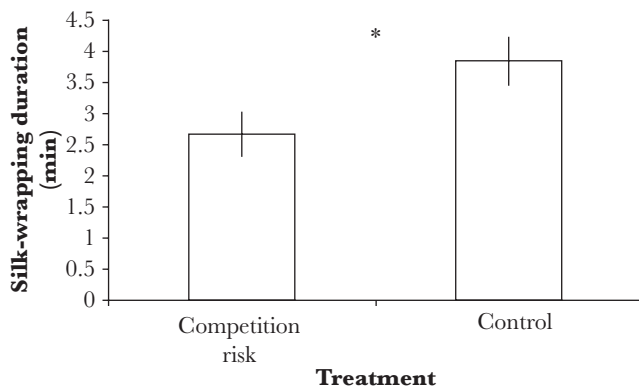


Figure 2

Mean duration of silk wrapping (seconds) of the nuptial gift performed by males experiencing competition risk (exposure to a rival male) and controls (no rival male exposure). * Denotes statistical significance.

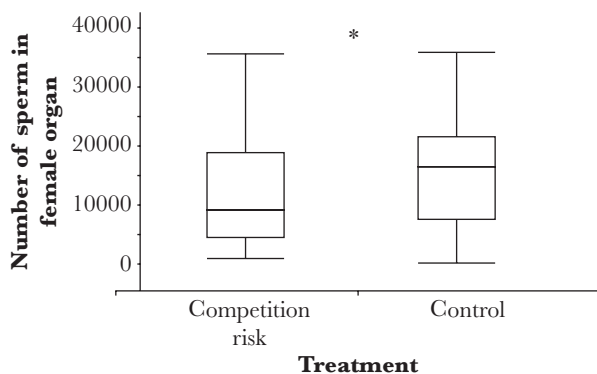


Figure 3

Number of sperm transferred to the female storage organ by males experiencing competition risk (exposure to a rival male) and controls (no rival male exposure). *Denotes statistical significance.

prolonging mating with females that spend more time feeding on gifts covered by larger silk amounts (Lang 1996) and longer matings ultimately enhance male sperm transfer and hatching success of the female's eggs (Albo et al. 2013). In the present study, despite more silk being deposited on gifts of control males, copulation duration did not differ between treatment groups. This result strongly suggests that sperm transfer is not entirely embedded in nuptial gift properties but rather an active strategy under male control.

The expected increase in allocation to sperm transfer, following reduction in premating traits, was not observed. Two independent meta-analysis show compelling evidence across taxa for males producing larger ejaculates in the presence of a rival than without (delBarco-Trillo 2011; Kelly and Jennions 2011), although empirical studies show also the opposite pattern. For example, house mice (*Mus musculus domesticus*) facing sperm competition are known to deliver fewer sperm to their mates in order to achieve more rapid sperm transfer (Ramm and Stockley 2007) and in the frog *Crinia Georgiana* males maintain constant ejaculate sizes irrespective of rival males' presence (Byrne 2004). A reduction in sperm transfer may be expected at intense levels of sperm competition, where paternity gains achieved from mating with a potentially promiscuous female do not overcome the costs of sperm production (Parker 1970; Parker and Pizzari 2010). The latter may explain our observed (reduced) patterns of sperm transfer if males perceived the presence of the competitor as an indicator of intense sperm competition over fertilizations. In fact, the information on the presence or absence of a rival nearby may not necessarily coincide with males having information on the number of competing ejaculates (Engqvist and Reinhold 2005). Moreover, under natural conditions, males commonly face intense sperm competition as *P. mirabilis* females are highly promiscuous and frequently mate with at least 4 different males (Bilde, unpublished data), suggesting selection for sperm reduction, as the sperm competition intensity is generally high. The reduction in sperm transfer under competition may also be explained by sperm priority patterns. Sperm from different males do not have the same probability of fertilizing the females' eggs in this species (Drengsgaard and Toft 1999), likely due to position effects in the female sperm storage organ (i.e., distance to the fertilization site). When females mate with 2 mating partners the first male to mate fathers on average 70–75% of the female's offspring. However, this first male advantage turns into a last male advantage when females mate with 4 mating partners (Drengsgaard and Toft 1999). Hence, in multiple mated females, being the last to mate becomes more valuable to males as these achieve a fixed proportion of fertilizations that override first male's priority. Theoretical “risk” models of sperm competition that take into account the “unfairness” of the raffle in terms of effects of sperm position and mating sequence (loaded raffles) predict a decrease in sperm allocation in response to competition in males occupying favored mating positions, as by investing the least these males will still benefit from highest fitness return (Parker 1990; Parker and Pizzari 2010). Under sperm competition risks, *P. mirabilis* males should therefore decrease sperm transfer when being the first to mate, as in our study, or the last to mate with multiple mated females. Our findings would be in accordance with the latter scenario if the presence of a competitor signals the female (erroneously) to be already mated and hence, the male likely to be the last to mate. Indeed, there is no indication that female mating status alone elicits changes in male mating behaviors or mating duration in this species (Tuni and Bilde 2010).

Finally, female *P. mirabilis* may have also played an active role in receiving and retaining less sperm in their storage organs in the presence of a competitor because they are able to exert cryptic mate choice through control over sperm stores (Albo et al. 2013). Because females were exposed to both, focal and rival males, it may have been in their best interest to store less sperm from their current mating partner in order to ensure sperm transfer from future matings. Polyandrous females derive indirect fitness benefits in terms of increased egg-hatching success from mating with multiple partners (Tuni et al. 2013). Alternatively, females may have exerted cryptic preference based on gift quality assessed via silk quantity, retaining more sperm from the better-wrapped gifts of control males. Although premating females do not select mating partners based on silk wrapping of nuptial gifts (Albo et al. 2012), we cannot fully exclude that such condition dependent trait is selected postmating. Despite the latter being plausible explanations, our experimental design aimed at limiting female cryptic manipulation of sperm by storing females immediately after mating so that they would have had very limited opportunity to exercise cryptic female choice.

In conclusion, we show that males respond to cues of rival males by reducing pre- and postmating investment. Because access to females is not limited by a reduction in gift quality (silk-wrapping), in response to mating competition selection may lead to plasticity in male behavior to reduce investment in premating gift-construction in order to secure matings at lower costs and faster rates. At the post-mating level, males respond to competition risk depending on how they perceive their chances in competitive fertilizations: High levels of sperm competition and position in mating order that determines their fertilization success may explain the reduction in sperm allocation found in our study. Overall, we reported a 15% decrease in sperm transfer from males experiencing a competitive environment, which is shown to be not trivial in terms of reproductive output (Albo et al. 2013). It is likely that males have evolved specific mating strategies in response to social conditions that enhance their lifetime reproductive success by reduced effort in mate acquisition (i.e., gift quality) and in winning competitive fertilizations (i.e., sperm).

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Tuni et al. (2017).

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