



Copulation interruption decreases female reproductive success in a false widow spider

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Abstract

Copulation is essential for transferring sperm from males to females in most animals. During copulation, males and females are often static and thus prone to predation or other threats. Its duration should therefore be reduced to minimize costs but sufficient to ensure the fertilization of eggs. Here, we investigated reproductive behavior and success in the false widow spider, *Steatoda grossa* (Araneae: Theridiidae), when copulation was interrupted after 1, 3, 5, 10, or 20 min or was not experimentally interrupted (control). Copulation duration in this study is defined as the total duration of insertions of male pedipalps into a female's copulatory openings. In *S. grossa*, uninterrupted copulations typically last 40 to 60 min. We found that within the first 5 min, copulation interruption negatively affected reproductive success (i.e., number of egg sacs, their total mass, and number of spiderlings), and delayed production of the first egg sac within the first 10 min (in some extreme instances by over 200 days). However, when copulation duration was 10 min or longer, reproductive outcome was unaffected. In the 1-min treatment, the number of egg sacs and their mass varied greatly, which indicates considerable variation among males with respect to the speed and efficiency of sperm transfer. We discuss the costs and benefits of extended copulation duration on female reproduction and on male and female fitness.

Significance statement

Interrupted copulation had a significant negative effect on reproductive success in the false widow spider, *Steatoda grossa*, but only if mating was terminated within the first 10 min of copulation. This implies that early during the copulation sequence sufficient amounts of sperm are transferred to fertilize most of the female's eggs. When copulation duration is less than 10 min, female spiders delayed the time to first reproduction. Prolonging the latency of reproduction can significantly affect female fitness. The role of extended mating in arthropods has focused on its effects on mate guarding, male-male competition for access to females and decreasing a female's attractiveness to other males. Preliminary data suggest that extended copulation does not reduce receptivity of *S. grossa* females to other males. Prolonged mating in *S. grossa* may play a more important role in reproductive success of the males.

Keywords Copulation duration · Copulation interruption · Egg sac · Offspring · *Steatoda grossa* · *Theridiidae*

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Introduction

One of the most significant transitions in evolution was the shift from asexual to sexual reproduction (Crow 1994; Smith and Szathmari 1997). This transition was enormously successful, as most eukaryotic organisms in nature now reproduce sexually (Lively and Morran 2014). Sexual reproduction in animals involves a remarkable spectrum of behaviors, such as courtship rituals and copulation duration, that profoundly impact reproductive success (Anholt et al. 2020). Copulation duration usually refers to the total duration of insertions of the male's genital organs into that of the female. The primary function of copulation is sperm transfer from the male to the female and its duration varies greatly among sexually reproducing animals with internal fertilization. In many animals, copulation is relatively short in duration. For example, many bird and rodent species copulate for only a few seconds or less (Birkhead et al. 1993; Dewsbury 1972). At the other end of the extreme are animals that copulate for several hours, such as in some dasyurid marsupials in the genus *Antechinus* (up to 18 h; Taggart et al. 1998). In arthropods, such as insects, copulation duration is extremely variable both intra- and inter-specifically (Sears et al. 2020). For example, it can be as short as a few seconds (e.g., 13 s in the yellow fever mosquito, *Aedes aegypti*; Spielman 1964), up to several hours (e.g., 5 h in the damselfly *Ischnura graellsii*; Cordero 1990), or even longer (e.g., 12 h to 7 days in fire bug *Pyrrhocoris apterus*; Schöfl and Taborsky 2002). This variation in copulation duration suggests that there are costs and benefits associated with the process, and that it can be influenced by natural or sexual selection (Daly 1978).

Copulation can be energetically and ecologically costly, thus selection for reduced copulation duration is expected. Time spent *in copula* cannot be used for other activities such as foraging or searching for other potential mates, and it can increase the incidence of disease transmission and enhance the risk of predation (Daly 1978; Sheldon 1997). Extended copulation, on the other hand, can increase reproductive success, when prolonged copulation enhances fertilization success (Andrés and Cordero Rivera 2000; Parker 1970; Wang et al. 2008). When more sperm are transferred during extended copulation, this can influence the outcome of sperm competition when females mate with multiple males (Parker and Pizzari 2010; Wedell et al. 2002). Prolonged copulation can also prevent females from mating with other males (i.e., mate guarding) (Kelly and Jennions 2011; Pérez-Staples and Abraham 2023; Prenter et al. 2006) or allow a male to remove the sperm of rivals (i.e., sperm displacement) (Michiels 1992; Siva-Jothy 1987). In addition to sperm, other substances can be transferred during copulation that can also reduce female receptiveness to other

mating partners (i.e., physiological mate guarding) and increase male fertilization success (Avila et al. 2011; Poiani 2006; Vahed 1998). In addition to substances that benefit fertilization success of the current male, 'nuptial gifts' may benefit both parents, either directly, e.g., by providing nutrients or carbohydrates that enhance fecundity, or indirectly, e.g., through chemicals that protect eggs against predation (South and Lewis 2011). In the European nursery web spider *Pisaura mirabilis*, for example, males facultatively provide females with a nuptial gift (i.e., prey wrapped in silk) during mating. This gift-giving extends copulation duration in this species and females that had mated with gift-giving males stored more sperm and experienced greater hatching success of their offspring compared to females that mated without a nuptial gift (Albo et al. 2013). Similar results were found for gift-giving male scorpionflies (*Panorpa cognata*; Engqvist and Sauer 2001). Thus, copulation duration can influence reproductive success of both males and females.

The risk of early termination of copulation by external factors is higher in animals with relatively long copulation durations. A number of factors can affect copulation duration (Birkhead et al. 1987; Corbet 1980). Environmental conditions, such as physical disturbance (i.e., resulting from wind and rain), the presence of predators or rival males, and sex-related variation in mating behaviors (in particular, behavior that does not benefit both sexes to the same extent in terms of reproductive success), can reduce copulation duration (Pellegrino et al. 2013; Schaefer and Uhl 2003; Schneider et al. 2006). The consequences of interrupted copulation on reproduction can be significant, in particular, when sperm transfer continues throughout copulation and thus early termination of copulation results in fewer sperm being transferred. This phenomenon has been described in a range of insect taxa such as flies and beetles (Lorch et al. 1993; Pérez-Staples and Abraham 2023; Qazi et al. 1996; Takami 2002). In the bruchid beetle *Callosobruchus maculatus*, copulation duration had a significant positive effect on lifetime offspring production (Edvardsson and Canal 2006). However, in many species, copulation often lasts longer than is necessary for the transfer of sufficient amounts of sperm to fertilize all eggs (Linn et al. 2007; Sears et al. 2020; Snow and Andrade 2004). This suggests that prolonged copulation occurs for reasons other than direct sperm transfer, and is most likely related to sperm competition and enhancement of fertilization success of the male (Herberstein et al. 2011a; Schneider et al. 2006).

Copulation duration also varies within and across spider species in different families (from less than 1 min in many web-building spiders to 15 h in salticid *Pseudicius* sp.; Elgar 1995), and many spiders copulate longer than required for successful insemination of females (Stratton et al. 1996; Linn et al. 2007). Male spiders do not have a

primary sex organ, but use modified tips of their pedipalps to inseminate a female. These are loaded with sperm by dipping them into a sperm web on which a drop of sperm has been deposited. Courtship in many spider families consists of species-specific ritualized behavioral sequences, which play an important role in mate assessment and ensure that the male is recognized as a conspecific mating partner and not as prey (Robinson 1982). This is followed by a single or alternate insertions of the tip of the pedipalps(s) into the female copulatory organ (Elgar 1998; Foelix 2011). Studies that examined copulation duration in spiders have focused on the effect of copulation duration on sperm transfer and/or intra-sexual competition. For example, in the orb-weaver spider *Trichonephila clavipes*, copulation duration did not influence sperm number transferred to females (Linn et al. 2007). In the wolf spider *Pardosa agrestis*, interrupted copulation lowered paternity of the first male when the female was allowed to mate with a second male (Kiss et al. 2019). For some spider species, it has been shown that most sperm are transferred early during copulation (Snow and Andrade 2004; Szirányi et al. 2005). How copulation duration affects life-time reproductive outcome of both males and females, however, has been little studied. For *P. agrestis*, which usually produces a single egg sac after copulation, it was found that copulation duration did not affect the number and size of the offspring (Szirányi et al. 2005). However, the number of egg sacs that were abandoned prematurely, supposedly due to sterility, was significantly higher in *P. agrestis* females with shorter copulation durations (Szirányi et al. 2005). Thus, when mating disturbance reduces sperm transfer, this can have more profound consequences on reproductive outcome in spider species that produce multiple egg sacs following mating.

In this study, we investigated the effect of copulation interruption on reproductive success in the false widow spider, *Steatoda grossa* Koch (Araneae; Theridiidae), a synanthropic species that likely originated in Eurasia but which is now widely introduced across the world. When mating is not interrupted, copulation duration usually lasts about 40–60 min (Dong et al. 2023) and females can produce 10 or more egg sacs from a single copulation event (Khandelwal and Sharma 2014). This suggests that when mating is not interrupted females do not suffer from sperm limitation. Biotic and abiotic factors may interfere with mating behavior and reduce copulation duration. For example, a previous study showed that male-male competition leads to significantly reduced copulation duration in *S. grossa* (Dong et al. 2023). Reduced copulation duration may have consequences for reproductive success. In another Theridiidae species, the Australian redback spider (*Latrodectus hasselti*), most sperm are transferred within the first 5 min of copulation (Snow and Andrade 2004). Therefore, we hypothesize that

copulation duration in *S. grossa* is positively correlated with offspring production (total number of offspring and/or number of egg sacs) because it might influence the amount of sperm transfer, at least when interruption occurs early during copulation. To investigate the effect of copulation interruption on reproduction, we paired unmated virgin males and females and either artificially ended copulation prematurely after 1, 3, 5, 10, or 20 min, or allowed mating to terminate naturally (control). We, then, determined life-time offspring production in the females.

Materials and methods

Animal collection and maintenance

A laboratory population of *S. grossa* was established from several hundred individuals and 20–30 egg sacs collected from several sites in Gelderland, the Netherlands between 2019 and 2021. All sites were spatially separated and located within 10–20 km of each other. Egg sacs were placed in Petri dishes (8 cm in diameter) until the spiderlings hatched. Spiderlings were reared individually in Petri dishes in a climate room at 22 ± 2 °C, 16:8 h light: dark cycle, and 50% relative humidity. They were fed on a standardized diet of fruit flies (*Drosophila spp.*) and house cricket nymphs (*Acheta domesticus*) obtained from commercial providers. Males mature after 5–7 molts while females have 1–2 additional molts, resulting in maturation times of approximately 6 months for males and 10–14 months for females. At adulthood, females were transferred and placed individually in plastic boxes (11 × 11 × 6 cm) with two crossed wooden sticks. For two weeks, females were allowed to build a web for prey capture and to release sex pheromones that stimulate attraction of males (Fischer et al. 2022; Scott et al. 2018). Males remained in Petri dishes until mating because they don't make elaborate webs. Males and females used in experiments had molted into adults over the previous 2–4 weeks. For the mating experiment, virgin adult females and males produced by 16 different unrelated mothers were selected. Mating pairs were unrelated and only one male and female produced by the same mother were assigned to the same treatment.

Experimental design

We assigned spiders to one of the following five copulation interruption treatments, i.e., interruption after 1 ($n=15$), 3 ($n=16$), 5 ($n=16$), 10 ($n=15$), or 20 min ($n=16$), and a control treatment (with no interruption, $n=16$). One unrelated male was introduced into the box with a female. The pair of spiders was observed during the entire copulation trial. It

was visually observed when copulation was initiated, i.e., when the male successfully inserted one of the pedipalps into the female copulation organ. Typically, when mating is not interrupted, males alternate pedipalp insertions three of four times (Dong et al. 2023). Individual palp insertions last 15 to 30 min (*personal observation*). In the mating interruption trials that lasted 10 min or less, males only inserted one palp into the female's reproductive organ, whereas both palps were inserted or alternated palp insertions occurred in the 20-min and control trials. All mating pairs copulated. Between palp insertions, mating pairs separated and additional palp insertion were preceded by renewed courtship behavior. Mating was terminated by tapping the box manually or by gently touching the web around the in-copula pair using forceps. In a control group, mating was allowed to proceed until it was terminated naturally by either the male or the female. After mating, males were removed from the boxes, while females were left undisturbed and were subsequently provided with one house cricket nymph (*A. domesticus*, 6th instar) as food per week until the end of the experiment.

Boxes with mated females were monitored daily for egg sac production until the female died or no more egg sacs were produced within 6-months since the last egg sac was produced. Egg sacs were removed from the boxes by gently cutting the silk threads with micro-scissors, weighed on a Mettler-Toledo Microbalance MT5 (accuracy 0.001 mg, Columbus, Ohio, USA), and placed individually in Petri dishes (8 cm diameter). The dishes were maintained in a climate chamber set at $22 \pm 2^\circ\text{C}$ during daytime and $12 \pm 2^\circ\text{C}$ during nighttime, a relative humidity of 60%, and a 16:8 light-dark photoperiod. Females occasionally lay sterile egg sacs. If egg sacs were viable, the number of spiderlings that hatched from them was counted and spiderling counts were summed across all egg sacs to give total viable offspring production per female. In addition, the time interval between mating and production of the first egg sac was recorded.

Eight females in the 1-min-copulation treatment produced only few and 6 females produced no egg sacs at all, whereas the other copulation-interruption treatments had no or only marginal effects on reproduction. To test whether reduced egg sac production was caused by sperm limitation, we conducted a small additional experiment (we had only few females available to test this). Females from the 1 min copulation treatment were allowed to remate with a virgin mature male after more than 6 months of reproductive inactivity and were monitored for egg sac production for an additional 3 months. Females from the control treatment which produced multiple egg sacs and did not produce a new egg sac for at least 6 months (5 out of 16 in total) were also allowed to remate. In addition, we tested whether short copulation and uninterrupted mating affected receptivity to

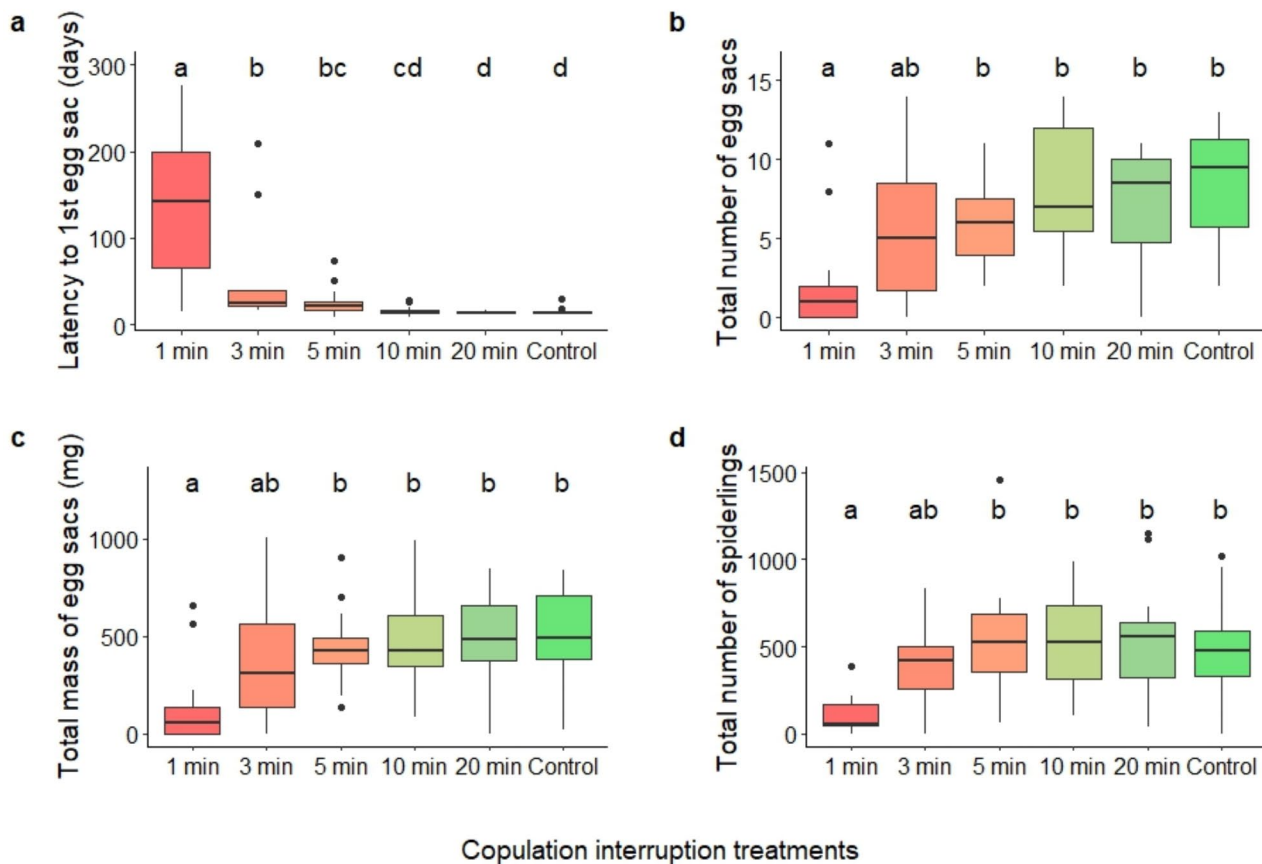
a second male. Young virgin females taken from the rearing were allowed to copulate for 10 min ($n=5$), as described previously, or were allowed to copulate without interruption ($n=6$). Females from both groups were allowed to remate the following day with another virgin male. As females in both treatments readily remated, this experiment was not repeated further.

Statistical analysis

All analyses were carried out using R 4.1.3 (R Core Team 2023). We tested the effect of copulation duration treatments on several response variables associated with female reproductive success. These variables included the time interval between mating and production of the first egg sac, total number of egg sacs, cumulative mass of all egg sacs, and total number of spiderlings. We used a general linear model (GLM) to investigate the effect of copulation interruption treatment (factor with 6 levels) on the various response variables. If the effect of copulation interruption was significant, Tukey post-hoc tests were conducted to reveal differences between means. As assumptions of normality and homoscedasticity were violated for time lapse until first egg sac production, the nonparametric Kruskal Wallis test was used followed by Wilcoxon signed-rank tests for pair-wise comparisons (with a Benjamini-Hochberg procedure to correct for inflated type I errors).

Results

Copulation duration affected the time interval until first egg sac production (Kruskal-Wallis test: $X^2=40.4$, $df=5$, $p<0.001$). The time interval between mating and production of the first egg sac decreased sharply from approximately 140 days (median) when mating was interrupted after 1 min to 25 days when mating lasted 3 min. When copulation duration was extended from 5 min to uninterrupted mating, latency gradually decreased and leveled off to 14 days (Fig. 1a). The longest time lapse until first egg sac production was 276 days for a female that was *in copula* for only 1 min. The total number of egg sacs produced by a female was also affected by copulation duration (GLM: $X^2=31.8$, $df=5$, $p<0.001$). In particular when copulation was interrupted after 1 min, egg sac production was reduced. Most females that mated for 1 min produced no or only a few egg sacs (2.1 ± 0.8 egg sacs, mean \pm se, Fig. 1b). Females that were allowed to copulate for 10 min or longer produced similar numbers of eggs sacs (8.0 ± 0.5 egg sac per female, Fig. 1b). In addition, copulation duration, affected total mass of the egg sacs produced by a female (GLM: $X^2=24.8$, $df=5$, $p<0.001$) and spiderling hatching (GLM: $X^2=17.5$,



Copulation interruption treatments

Fig. 1 Effect of copulation interruption on latency to first egg sac production (a), total number of egg sacs produced by a female (b), their total mass (c) and the total number of spiderlings hatching from these egg sacs (d). Data are presented in box plots with the box representing the interquartile range (IQR), and the horizontal line the median. The whiskers extend to the largest data point less than the upper quartile

$df=5$, $p<0.003$). Similar as for time lapse until production of the first egg sac and production of total number of egg sacs, the effect of copulation interruption was strongest when females copulated for only 1 min and was absent when copulation lasted for 10 min or longer (Fig. 1c&d).

In the remating trials with females that had been reproductively inactive for 6 months, females from the 1-min copulation interruption treatment copulated on average 45 ± 4.4 min ($n=8$, second mating), while control females copulated on average 63 ± 6.0 min ($n=5$, second mating). 88% of the females (7/8) in the 1-min treatment produced egg sacs within 3 months following the second mating, while only 40% of control females (2/5) did. These five control females had already produced 9.4 ± 1.3 egg sacs and had copulated 55.4 ± 3.1 min during the first mating. Only two of the control females produced egg sacs (3 egg sacs each and only one viable egg sac per female) from the second mating, while the five females from the 1-min copulation treatment, produced 28 egg sacs in total of which 25 developed into spiderlings. Remating in young females occurred readily,

plus $1.5 \times IQR$ and the smallest data point greater than the lower quartile minus $1.5 \times IQR$, respectively. The individual data points beyond the whiskers can be considered outliers. The mean copulation duration of uninterrupted mating pairs (Control) was 54.1 ± 4.8 min (mean \pm SE, range 31–73 min). Number of replicates was 15 (1 min and 10 min) and 16 for the other treatments

irrespective of whether mating one day earlier was interrupted or not. All females accepted the male in the second mating trial. Thus, receptivity to another virgin male and the male's interest into females that had mated 24 h earlier was high regardless of duration of the previous mating event.

Discussion

Uninterrupted mating pairs of *S. grossa* copulate 40 to 60 min. When copulation was interrupted within the first 5 min of initiation, female reproductive performance was negatively affected, with females producing fewer egg sacs and fewer life offspring over their lifetimes than females that mated longer than 5 min. The duration of copulations that lasted longer than 10 min did not significantly affect reproductive success. This suggests that sperm transfer from the male to the female increases with copulation duration only during the first 10 min, and that copulation duration of 10 min is already sufficient for females to fertilize most

or all of her eggs. Furthermore, females that copulated for 10 min or longer typically produced their first egg sac around 2 weeks after mating. In contrast, early interruption of copulation significantly delayed production of the first egg sac, and in some instances, this was extreme, extending to over 200 days (or almost 7 months).

Studies with other arthropods have reported positive, but not always linear, relationships between copulation duration and sperm transfer (Bukowski and Christenson 1997; Danielson-François 2006; Hinn and Niedzlek-Feaver 2001; Linn et al. 2007; Shandilya et al. 2021; Tong et al. 2021). For instance, the mean number of sperm transferred increases with copulation duration in the leafhopper *Balclutha incisa* (Bailey and Nuhardiyati 2005). Similar results were found for the black field cricket (*Teleogryllus commodus*), but in that species the relationship is asymptotic (Bussiégre et al. 2006). In spiders, a positive linear relationship was found in *Pisaura mirabilis* (Albo et al. 2013) and the golden orb-weaver *Trichonephila edulis* (Ceballos et al. 2015). However, in the Australian redback spider, *L. hasselti*, sufficient sperm for fertilization is transferred within the first 5 min of copulation (Snow and Andrade 2004). This is similar to the trend we report here for *S. grossa*, and suggests that sufficient sperm is transferred within the first 10 min of copulation in *S. grossa* regardless of the number of pedipalp insertions (only one palp was inserted in the copulation treatments that were interrupted after 10 min or earlier). Females that had copulated for 1 min produced few or no offspring at all and readily remated with a young virgin male after a 6-month period of reproductive inactivity. In response to remating, these females initiated the production of egg sacs from which spiderlings hatched. This result suggests that when copulation duration lasts less than 1 min insufficient sperm is transferred by the male to fully exploit a female's reproductive potential. In contrast, females from the unlimited copulation (control) cohort also readily remated after 6 months of reproductive inactivity, but produced no egg sacs or a few of poor quality following the second mating. The production of these 'dummy' egg sacs or lack of egg sac production suggests that resources for egg production have been exhausted in these females. However, as the sample size in these additional experiments were low, these results have to be interpreted with caution.

To what extent products transferred during mating (i.e., seminal fluids) other than sperm contribute to egg production has been little studied in spiders (Michalik and Ramírez 2014). For insects, it has been shown that substances from male ejaculate can influence female reproductive processes such as remating propensity, sperm storage, oögenesis, ovulation, and oviposition (Avila et al. 2011; Chapman 2001; Parker and Pizzari 2010; Shuker and Simmons 2014; Vahed 1998). These processes might also occur in spiders. A study

with wolf spiders showed that substances in male ejaculate reduced female receptivity to other males (Estramil and Costa 2007). When copulation was interrupted early in *S. grossa*, some females produced no egg sacs at all. These females may not have received sufficient amounts of sperm and or other essential substances to initiate egg laying. In other females that copulated for only 1 min, egg sac production was delayed. Sperm transferred during copulation are immobile, encapsulated and stored in the spermatheca of the female (Herberstein et al. 2011b; Tuni et al. 2020). In spiders, sperm is activated and eggs are fertilized when the female initiates egg-laying, which can take place for up to several months after copulation (Foelix 2011; Useta et al. 2007; Vöcking et al. 2013). Sperm activation requires a sequence of endocrinological and or neurological signals (Herberstein et al. 2011a). Thus, the initiation of egg fertilization appears to be actively controlled by the females. Courtship behaviors, such as nuptial gift-giving and web vibration can be indicators of male quality (Andrés and Cordero Rivera 2000; Eberhard et al. 2020; Pilastro et al. 2007). When copulation is interrupted early, females may consider these males as inferior and may wait for a second male to court and mate with. However, delay of reproduction can have severe fitness costs if the female dies prematurely. Once a critical temporal threshold is passed and no subsequent male mates with a female, then some of these females may wait no longer and produce their first egg sac to mitigate the risk of producing no offspring at all. Little is known how often *S. grossa* females encounter males in nature. *S. grossa* has a synanthropic lifestyle and its presence is often restricted to enclosed anthropogenic habitats, such as cupboards and sheds. In these confined areas, interactions between conspecifics can be frequent when densities are high, which in addition to human disturbances, can affect mating success. In a previous study (Dong et al. 2023), when two males compete for mating access to a female, copulation duration is significantly reduced or does not occur (in 18% of the trials). To what extent males compete for access to females in anthropogenic environments needs to be investigated in more detail. In natural environments, abiotic (e.g., rain and wind) and biotic disturbance (e.g., arrival of male rivals and prey in the female's web) may result in premature ending of courtship and copulation.

Since *S. grossa* can achieve maximum reproductive success when mating duration is as low as 10 min, an important question is why copulation extends well beyond this time. Long copulation decreases time spent in other activities such as foraging or feeding, and also increases the risk of precocious mortality from natural enemies via predation or parasitism or from other factors such as extreme weather events (Daly 1978). In many organisms, copulation behavior plays a role in mate guarding and paternity (insects, Alcock 1994;

birds, Møller and Birkhead 1991). In spiders, copulation duration has been studied in relation to body size, female mating frequency, risk of predation, and sexual cannibalism (Elgar 1995). For instance, in the sexually cannibalistic spiders, *L. hasselti* and *Argiope bruennichi*, sexual cannibalism prolongs copulation duration and sperm transfer, which in turn increases paternity under sperm competition (Andrade 1996; Schneider et al. 2006). In *S. grossa*, sexual cannibalism is not prominent and size of the male does not affect copulation duration (Dong et al. 2023). Scott et al. (2018) found that *S. grossa* females that mated previously were not receptive to a second mate. However, we found that females readily remated with a second male, 24 h after mating with a first male, irrespective of whether copulation with the first male was interrupted or not. This additional experiment was replicated with only a limited number of females, because the propensity of these females to remate was 100%. The time lapse between first and second mating events may affect the willingness of females to mate with another male. Extended copulation, may affect the outcome of sperm competition when females mate with more than one male and sperm transfer increases with copulation duration thereby numerically diluting sperm inseminated by subsequent males (Arnqvist and Nilsson 2000; Bukowski and Christenson 1997; Snow and Andrade 2004). Female cryptic choice and production of a mating plug are other factors that affect male reproductive success and their impact may depend on the copulation duration (Eberhard 2004; Uhl et al. 2010).

This study has reported how copulation duration influences reproductive outcome in a false widow spider, *S. grossa*, under laboratory conditions. These experiments reflect constraints on copulation duration that may occur through natural factors such as male-male competition, the presence of a predator, physical disturbance by another organism or by weather-related factors such as wind and rain. For example, a previous study showed that copulation duration is significantly shorter when two males compete for access to an individual *S. grossa* female than when a male was alone (Dong et al. 2023). The ‘winner’ was frequently attacked by the ‘loser’, leading to early termination in copulation. Here, a minor stimulus—tapping—was also sufficient for male and female spiders to terminate copulation early. Physical interruptions happening during the early stages of copulation leads to decreased reproductive outcome and thus generates fitness costs for females and males. Given the prevalence of potentially interruptive factors, we anticipate that selection favors rapid and efficient transfer of sperm during copulation, maximizing reproductive output even under very short copulation duration. However, this was only partially true in *S. grossa* since in this species copulation is up to several times longer than necessary in terms of maximizing reproductive output. Preliminary

results suggest that extended copulation duration does not decrease the female receptivity to other males. Prolonged copulation duration may serve other functions, for example, play a role in cryptic female choice, thus influencing the reproductive success of males when a female mates with multiple males. Future studies should investigate the effect of prolonged copulation on the outcome of sperm competition and explore the mechanistic parameters that determine paternity (sperm activation, fertilization of eggs), but also the costs and benefits of extended copulation in terms of male and female fitness.

Data availability Data is deposited in Dryad. <https://doi.org/10.5061/dryad.k98sf7mcn>

Declarations

The authors declare that they have no financial or non-financial conflicts of interest that are related to this paper submitted for publication. The spiders reared in the laboratory in accordance with the ASAB/ABS Guidelines for the treatment of invertebrates.

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