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Increased insertion number leads to increased sperm transfer and fertilization success in a nursery web spider

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Abstract

Across animals, a male's fitness is largely dictated by his ability to fertilize eggs; and there exists a plethora of male adaptations associated with increasing fertilization success. In the nursery web spider, *Pisaurina mira*, males restrain females prior to and during copulation by wrapping them with silk. Previous research demonstrates that copulatory silk wrapping reduces a male's chance of being sexually cannibalized and increases the number of sperm transfer opportunities (termed insertions) that a male can achieve within a mating. While avoiding cannibalism provides an obvious survival benefit to males, the impact of insertion number on male fitness remains unknown. This study tested the hypothesis that increased insertion number realized through copulatory silk wrapping increases (1) the quantity of sperm transferred and (2) fertilization success. To accomplish this, we directly quantified the amount of sperm in male pedipalps (i.e. the male sperm storage organ) before mating and after obtaining one or two insertions. We also, indirectly quantified fertilization success by measuring the number of hatched offspring when males were capable of achieving one versus two insertions within a mating. In support of our hypotheses, we found that males transfer roughly twice the amount of sperm when achieving two insertions compared to one. We additionally found that the amount of sperm transferred is negatively related to female size. In terms of offspring number, females obtaining two insertions had more offspring compared to females obtaining only one insertion. These results show that males achieve a fertilization benefit from increased insertion number, which is obtained through the male behavior of copulatory silk wrapping.

Keywords: copulation duration, fertilization success, fitness, sexual conflict, sexual selection, sperm transfer

Differences observed in male and female reproductive strategies have been proposed to explain the evolution of sex-specific traits. For example, female fitness often depends on maximizing resource investment to developing offspring (Andersson, 1994; Bateman, 1948; Trivers, 1972) while male fitness is hypothesized to be dependent on maximizing the number of eggs fertilized (Andersson, 1994; Bateman, 1948; Parker, 1984). Positive selection is thus predicted, and often observed, on traits that allow males to secure matings and/or increase fertilization success.

In addition to facilitating copulations, many male-specific mating strategies or morphological traits may function to increase copulation duration, which can have important implications for sperm transfer and thus, sperm competition. Male traits such as "grasping traits" (e.g. Sakaluk, Bangert, Eggert, Gack, & Swanson, 1995), larger nuptial gifts (Svensson, Petersson, & Frisk, 1990) and barbed or spiny male genitalia (Edvardsson & Canal, 2006; Hotzy & Arnqvist, 2009) have all been documented to extend copulation duration. Male sagebrush crickets, *Cyphoderris strepitans*, for example, have

a grasping device called a gin trap, which secures females to males during copulation, prolongs the duration of copulation and increases the chance of complete transfer of the spermatophore to the female (Sakaluk, et al., 1995). As previously suggested, benefits of longer copulations include increased male fertilization success due to increased sperm transfer (e.g. Campbell & Fairbairn, 2001; Engqvist & Sauer, 2003; Pilastro, Mandelli, Gasparini, Dadda, & Bisazza, 2007; Schneider, Gilberg, Fromhage, & Uhl, 2006).

In some cases, the traits (including behaviors) that benefit male reproductive success can appear potentially harmful to female mating partners. For example, males of many species use "harassment" or "coercive" mating strategies (reviewed in Arnqvist & Rowe, 2013; Clutton-Brock & Parker, 1995), or possess morphological traits (e.g. larger body size, structures for grasping/holding) that appear to function in restraining females. Many of these male strategies/traits are proposed to increase the females' mating rates or copulation duration past their phenotypic optima (Arnqvist & Rowe, 2013; Parker, 1979, 2006). For example, prolonged copulation in females

can reduce foraging rates, increase risk of predation, injury, parasites, etc., and decrease control over fertilization and mate choice (reviewed in Arnqvist & Rowe, 2013). Indeed, cryptic female choice, the ability of females to exert choice on male sperm following copulation, is suggested to have evolved in response to primarily male-controlled copulations (Eberhard,1996; Thornhill & Alcock, 1983). Additionally, across a diversity of animal groups, females are observed to engage in behaviors associated with seeming attempts to terminate copulations, such as kicking their male mating partner (Edvardsson & Canal, 2006), sexual cannibalism (Elgar, Schneider, & Herberstein, 2000; Herberstein et al., 2011) or simply attempting to remove the male (e.g. Mazzi et al., 2009). Ultimately, in many taxa, there appears to be a conflict between the sexes in terms of copulation duration (reviewed in Arnqvist & Rowe, 2013).

The observed mating strategy of male nursery web spiders, *Pisaurina mira*, suggests that a conflict might exist between females and males regarding copulation duration or the number of successful sperm transfer events achieved by males (i.e. male insertion number). In this cannibalistic species, males always constrain the female's movement by wrapping her legs with silk prior to and during copulation (Supplementary Fig. S1). Most virgin females are receptive to mating and appear to allow males to initially mount and silk-wrap them prior to transferring sperm (A. G. Anderson, personal observation). Sperm transfer relies upon independent paired external organs called pedipalps (Foelix, 2011), which function in sperm storage as well as transmission. In many spider species, including *P. mira*, males insert and transfer sperm using one pedipalp at a time (termed an insertion). In *P. mira*, females begin to appear aggressive towards their male mating partner immediately following the first insertion. Females begin to struggle in a seeming attempt to get out of the silk wrapping, suggesting an effort to shorten copulations. In response, males typically attempt to restrain and rewrap the females to achieve one more insertion (for a total of two), after which males quickly flee. Prior work has manipulated a male's ability to engage in copulatory silk wrapping and found that the silk wrapping reduces rates of postcopulatory sexual cannibalism and increases the likelihood of a male achieving two pedipalp insertions versus only one (Anderson & Hebets, 2016). Similarly, binding females in silk during mating has been linked to reduced rates of sexual cannibalism in two other spider species (*Caerostris darwini*: Gregorič, Šuen, Cheng, Kralj-Fišer, & Kuntner, 2016; *Nephila pilipes*: Zhang, Kuntner, & Li, 2011), as well as increased copulation duration in one species (Zhang et al., 2011). We hypothesize that silk wrapping in *P. mira* provides males increased fitness benefits by increasing copulation duration.

Although copulation duration is often correlated with increased fertilization success (i.e. increased offspring production given the number of eggs available) due to an increase in the quantity of sperm transferred (e.g. Arnqvist & Danielsson, 1999; Schneider et al., 2006; Svensson et al., 1990), this need not always be the case (Bukowski, Linn, & Christenson, 2001; Linn, Molina, Difatta, & Christenson, 2007; Schneider & Elgar, 2001; Snow & Andrade, 2004). For example, in the orb-weaving spider *Nephila clavipes*, increased copulation duration was not associated with the amount of sperm transferred, but was instead found to decrease female receptivity to future matings. Additionally, increased sperm transfer need not reflect the amount of sperm stored and available for fertilization as females of some species are known to manipulate sperm storage (e.g. Eberhard, 1996; Herberstein et al., 2011). Indeed, several studies have failed to find a relationship between copulation duration and fertilization success (Assis & Foellmer, 2016; Gilchrist & Partridge, 2000; Mazzi et al., 2009). Thus, in attempting to understanding male–female mating dynamics and potential costs and benefits

of copulation duration, it is important to directly quantify the relationships between copulation duration, sperm transfer, and fertilization success, as well as their relationship to female and male size.

This study uses the nursery web spider *P. mira* to test the hypothesis that increased insertion number, facilitated by copulatory silk wrapping, increases male fitness. Specifically, we predicted that two insertions (versus one) would increase (1) the quantity of sperm that males transferred to females and (2) the number of offspring that females produced.

Methods

Species Collection and Maintenance

We collected immature female and male *Pisaurina mira* at night from Wilderness Park, Lancaster County, Lincoln, Nebraska, U.S.A. during 29 March–28 April 2015 and 4 April–26 April 2016. Collected individuals were transported to the University of Nebraska-Lincoln where they were individually housed in 87.3 × 87.3 × 112.7 mm clear plastic containers (763C, AMAC Plastics, Petaluma, CA, U.S.A.). We covered the outside of each container with opaque tape to maintain visual isolation between individuals. Spiders were maintained under a 12:12 h light:dark cycle, fed four crickets approximately 0.64 cm in length per week (Ghann's cricket farm, GA, U.S.A.) and provided water *ad libitum*. Spiders were checked each day for the presence of a molt and to determine the date of sexual maturity. Our research adhered to the ASAB/ABS guidelines for the treatment of animals in research, the legal requirements of the U.S.A., and all guidelines of the University of Nebraska–Lincoln. All animals were handled and maintained within the laboratory under proper conditions.

Experiment 1: Insertion Number and Sperm Quantity

To determine the relationship between insertion number and the amount of sperm transferred, we staged mating trials between 6 May and 21 May 2016 in which age-matched (15 days ± 2 days post maturation) females and males were randomly paired. Mating arenas and procedures were similar to those carried out in a previous study (Anderson & Hebets, 2016). We separated males from females at three time points: immediately after (1) the silk wrapping was laid (i.e. no sperm transferred/zero insertions), (2) the first insertion (one insertion), or (3) the second insertion (two insertions). From each of these three treatment groups, we took 15 males and quantified the amount of sperm remaining in each of their pedipalps. Sperm quantification took place immediately after the male was separated from the female.

To quantify sperm, we used methods adapted from Snow and Andrade (2005), which were originally adapted from Bukowski and Christenson (1997) and Bukowski et al. (2001). The modifications of methods were reported to improve a uniform distribution of sperm and reduce sperm clumping. Briefly, we removed each male's left and right pedipalps using soft forceps and dissecting scissors and then placed them into a 0.5 ml Eppendorf tube containing 75 µl of spider sperm counting solution. The counting solution consisted of 150 µl of a solution containing 10 ml of saline and 10 µl of Triton X detergent, which was then mixed with 10 ml of spider saline (Juusola & French, 1998). Within the Eppendorf tubes, each pedipalp was crushed using disposable pellet pestles (Fisher Scientific, Waltham, MA, U.S.A.). We vortexed each sample for 30 s and subsequently centrifuged each sample at 4000 revolutions/min for 10 min. We repeated the vortexing and centrifuging steps two additional times. Following sample preparation, we pipetted 10 µl of each sample into an improved Nebauer double-chamber hemocytometer (iNCYTO,

Korea). Using a Leica DM4000 microscope at 40× power, we performed blind counts (with respect to the pedipalp and the male's mating treatment) of the total number of sperm observed within the center counting grid. We repeated sperm quantification an additional time for each pedipalp sample to obtain an average number of sperm remaining within each pedipalp. The two counts were not statistically different within either the left (paired *t* test: t_{44}=_1.4274, P = 0.1605) or right (t_{44} = 1.5644, P = 0.1249) pedipalp, showing that sperm were equally distributed within our samples (Supplementary Fig. S2). Clumping of sperm was rarely observed and never exceeded more than six sperm per clump. To estimate the total number of sperm within each pedipalp, we multiplied our average sperm count of that pedipalp by 10 µl (our counting sample volume) and then by 75 µl (our total sample volume).

Size measurements of females and males were taken from preserved specimens following mating trials. All spiders were preserved in 70% EtOH, post death. Our body size measurement reflects the widest point of the carapace (often referred to as cephalothorax; anterior-most body part), which is fixed in size at maturity. We removed all appendages from each preserved spider and placed the carapace dorsal side up on a scale graduated in millimeters. We photographed each carapace using a Leica DM 4000 B microscope with a Di-agnostic Instruments Spot Flex digital camera. Measurements of carapace widths were taken from the digital photographs using the program PixelStick v.2.8 (https://roari ngapps.com/app/pixelstick).

Statistical analyses
All statistical analyses were carried out in R v.2.0 (R Foundation for Statistical Computing, Vienna, Austria). Across all analyses, we used parametric tests except when data were not normally distributed. We first compared the amount of sperm between unmated males' (zero insertion) left and right pedipalps using a paired *t* test. We then used a linear model to determine whether male size predicted the total amount of sperm stored in unmated males' pedipalps. Given that the amount of sperm stored in unmated males' pedipalps did not differ significantly (see Results), we took the absolute difference in the number of sperm remaining in each of the males' pedipalps. Within the one insertion treatment, we used this difference as an estimate of the amount of sperm transferred to the female. Using a linear model, we tested whether male size or female size influenced the amount of sperm transferred to the female.

To determine whether insertion number influences the amount of sperm transferred, we tested whether the difference in the number of sperm that remained in each male's pedipalps differed across our three insertion treatment groups (zero, one, two) using a Kruskal–Wallis test with Tukey like post hoc analyses. Furthermore, we performed an ANOVA with Tukey HSD post hoc analyses with the total amount of sperm remaining within males' pedipalps as the response variable and insertion treatment (zero, one, or two) as our predictor variable.

Experiment 2: Insertion Number and Offspring Number

To test the effect of increased insertion number (one versus two) on offspring number, we randomly paired a unique group of age-matched (15 days ± 1 day post maturation) virgin females and males for mating trials (N = 76) during 27 April–21 May 2015. In trials where copulation took place (N = 50), we controlled the number of insertions that males could acquire (one versus two) by separating the male and female with soft forceps immediately after the assigned numbers of insertions were obtained. During mating trials, we live-scored copulation success and the duration of each insertion as in experiment 1. If a male did not move within 30 min, or if a male

did not successfully copulate with the female 30 min, the trial ended. We excluded any trials where males failed to obtain the assigned number of insertions (one insertion: N = 2; two insertions: N = 8).

Post mating, females were maintained in the laboratory under the same conditions and diet described above. Females were checked each day for the production of an eggsac and subsequent offspring emergence. Offspring and eggsacs were removed from the mother's cage 3 days after offspring emergence. We counted the total number of offspring, as well as the number of eggs remaining within the eggsac to quantify the total number of eggs produced by the female. Following offspring quantification, we maintained females under the same controlled conditions and repeated this process if the female produced a second or third eggsac. All females were monitored for eggsac production until their death.

Quantification of male and female body sizes were carried out using the same methods described in experiment 1.

Statistical analyses
We first wanted to determine whether insertion number influenced a female's likelihood of producing at least one, two or three successful eggsacs. To do this we ran separate binomial generalized linear models (GLMs) with the likelihood of producing at least (1) one, (2) two, or (3) three successful eggsacs as our response variables and insertion number as our predictor variables. Because insertion number did not influence successful eggsac production (see Results), we proceeded with our analyses excluding females that produced zero successful eggsacs.

Next, in order for us to use offspring number as an indirect measure of fertilization success, we first confirmed that the total clutch size (i.e. number of offspring hatched þ eggs remaining in eggsac) laid by females did not vary across our insertion number treatment. We used a linear model to determine whether insertion number and female size influenced a female's total clutch size. Because we do not know whether females lay more than one eggsac in the field, we ran this model by looking at the total number of offspring and eggs that females produced across the season as well as within only females' first eggsacs.

Finally, we used a linear model to examine whether the total number of offspring produced was influenced by insertion number and female size. We again ran this model for the total number of offspring produced by a female and the number produced within each female's first eggsacs. Running a binomial logistic regression with the proportion of hatched offspring as the response variable provided the same results as our above model (analyses not included); therefore, we are confident that offspring number likely reflects fertilization success.

Results

Experiment 1: Insertion Number and Sperm Quantity

Despite the variability observed in the total amount of sperm stored in unmated males' pedipalps (N = 15; mean = 63,497.50 ± 4,911.08, range 11,700–109,200 sperm) male size (estimate = 12,737 ± 11,503, $F_{1,13}$ = 1.226, P = 0.288) did not influence this variability. Furthermore, prior to sperm transfer, the amount of sperm stored did not differ between the males' left and right pedipalps (t_{14}= –0.282, P = 0.782). Given that males have roughly the same amount of sperm stored between their two pedipalps prior to mating, the absolute difference in the amount of sperm remaining within the pedipalps after obtaining one insertion should reflect the approximate amount of sperm transferred to the female. Males transferred an average of 23,010 ± 3,802.798 sperm (N = 15; range 3,412–50,700)

during one pedipalp insertion. We found that insertion duration and male size did not predict the amount of sperm that single-inserting males transferred (insertion duration: estimate = 516.5 ± 450.1, $F_{1,11}$ = 1.116, P = 0.314; male size: estimate = −1,262.1 ± 12,975.3, $F_{1,11}$ = 0.010, P = 0.924); however, female size did (estimate =_32 274.8 ± 14 464.3, $F_{1,11}$ = 5.385, P = 0.041). Specifically, males transferred more sperm when mating with smaller females (Fig. 1). Although male's transferred more sperm to smaller females, we did not find any differences in female behavior (i.e. aggression during mating) across female sizes to help explain this result (Results not shown).

The absolute difference between sperm in males' pedipalps differed between our three treatment groups (Kruskal–Wallis test: χ^2_2 = 26.412, P < 0.0001; Fig. 2b). Specifically, males that obtained only one insertion (i.e. used only one pedipalp) had a larger difference in the number sperm remaining between their pedipalps compared to males that never inserted (P < 0.0001) and those that inserted twice (P < 0.0001). Furthermore, the total amount of sperm estimated in both pedipalps of males differed between our three treatment groups ($F_{2,42}$ = 9.041, P = 0.0005; Fig. 2a). Males had fewer sperm in their pedipalps after obtaining two insertions compared to zero insertions (P = 0.0003), but not compared to males obtaining one insertion (P = 0.153).

Experiment 2: Insertion Number and Offspring Number

The majority of females produced at least one successful eggsac (39 of 40), while 25 females produced two eggsacs and five females produced three eggsacs. A female's likelihood of producing at least one, two or three successful eggsacs was not influenced by the number of insertions obtained (one eggsac: odds ratio (OR) = 0.259, 95% confidence interval (CI) = 0.002–5.168, χ^2_1 = 0.768, P = 0.381; two eggsacs: OR = 2.167, 95% CI = 0.573–8.189, χ^2_1 = 51.587, P = 0.247; three eggsacs: OR = 2.00, 95% CI = 0.296–13.511, χ^2_1 = 29.624, P = 0.472).

There was no effect of insertion number or female size on the total number of eggs females produced (i.e. number of offspring + number of eggs remaining within the eggsac) within females' first eggsacs (insertion number: estimate = 8.330 ± 6.598, $F_{1,37}$ = 1.277, P = 0.266; female size: estimate = 4.860 ± 6.886, $F_{1,36}$ = 0.498, P = 0.485) or across all eggsacs laid by that female (insertion number: estimate = 23.14 ± 16.36, $F_{1,36}$ = 1.952, P = 0.171; female size:
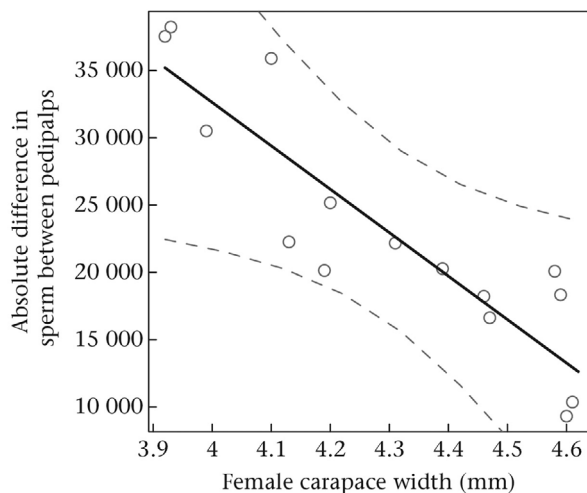
Figure 1. The absolute difference in sperm between male *Pisaurina mira's* pedipalps (N = 15) that achieved one pedipalp insertion during mating, which reflects the amount of sperm transferred to the female. Lines within the scatterplots represent model prediction and confidence intervals.
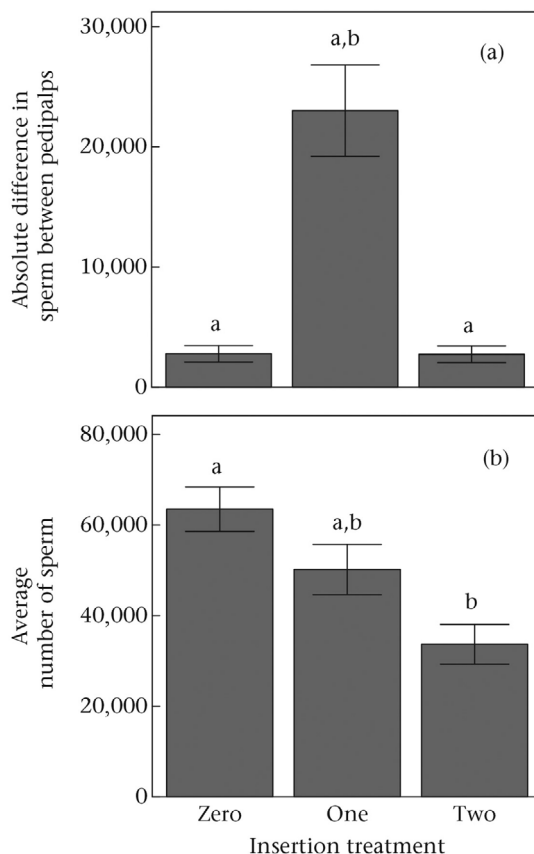
Figure 2. Average ±SE (a) total number of sperm and (b) absolute difference in the amount of sperm between male *Pisaurina mira's* left and right pedipalps after obtaining zero (N = 15), one (N = 15) or two (N = 15) insertions.

estimate = 4.03 ± 17.07, $F_{1,36}$ = 0.056, P = 0.815). However, there was a significant effect of insertion number on the number of offspring produced within a female's first eggsacs and across all eggsacs produced by that female (Table 1). Results were the same when looking at the number of offspring produced within females' first eggsacs and when looking at females' total offspring production; therefore, we present figures only for females' total offspring numbers. Specifically, females that obtained two insertions had 25.25% more offspring (based on model predictions) compared to females that obtained only one insertion (Fig. 3). There was no effect of female and male size on offspring production (Table 1).

Given that males transfer more sperm to smaller females (see Results, experiment 1), and smaller females did not produce more

Table 1. Model outputs testing the effect of insertion treatment, female size and male size on the number of offspring produced by females within their first eggsacs, as well as the total number of offspring produced by females' across all eggsacs

Fixed effects	Estimate (±SE)	$F_{1,35}$	P
Number of offspring in first eggsac			
Insertion number	26.41±12.02	5.63	**0.023**
Female size	−5.52±13.37	0.62	0.437
Male size	−13.35±14.84	0.81	0.374
Number of offspring across all eggsacs			
Insertion number	31.40±16.97	4.59	**0.039**
Female size	−17.42±18.89	1.44	0.239
Male size	−11.54±20.96	0.30	0.585

Significant outcomes are shown in bold.
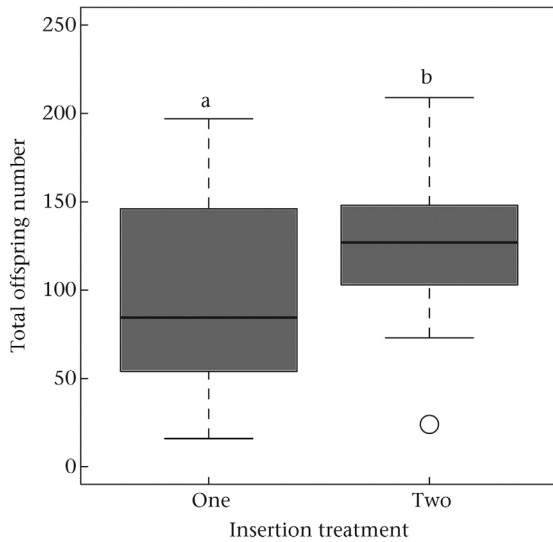
Figure 3. Total number of offspring produced by female *Pisaurina mira* after mating with a male that obtained either one or two pedipalp insertions. The solid black lines represent the median, the edges of the box show the first and third quartiles, the whiskers show the minimum and maximum and open circles show outliers.

offspring (see result above), we wanted to see whether female size was related to any other fitness variables measured (i.e. time to egg-sac production or offspring mass). We found that it took less time for smaller females to produce their eggsacs compared to larger females (estimate = 22.25 ± 15.72, $F_{1,37}$ = 2.004, P = 0.165; Fig. 4), but female size was not related to offspring mass (estimate= −0.00014 ± 0.00056, $F_{1,37}$ = 0.06, P = 0.808).

Discussion

In *P. mira*, increased insertion number, enabled by male copulatory silk wrapping (Anderson & Hebets, 2016), results in increased sperm transfer and offspring number. Males transferred roughly twice the amount of sperm when obtaining two insertions compared to one.
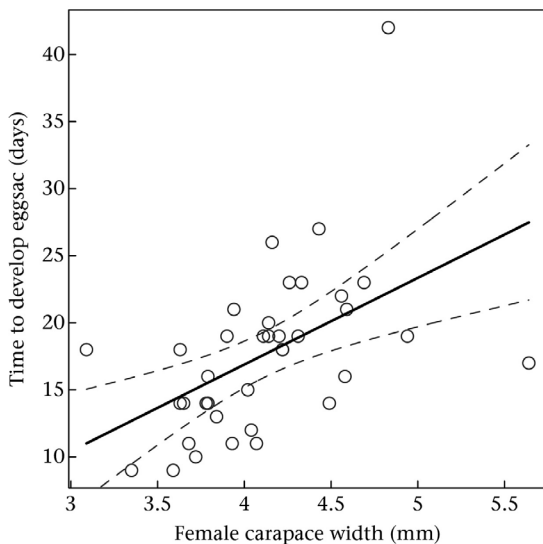
Figure 4. Number of days from mating that it took females to produce their first eggsacs across female sizes. Lines within the scatterplots represent model prediction and confidence intervals.

Additionally, males obtaining two insertions had approximately 25% more offspring compared to males obtaining only one insertion. Below, we discuss these findings in more detail, including their evolutionary implications within the context of sexual selection and sexual conflict.

Insertion Number and Sperm Quantity

Results of our sperm counts support the prediction that increased insertion number increases the amount of sperm transferred to the female. Comparing the amount of sperm between the paired sperm storage organs (i.e. pedipalps) of males that obtained zero, one or two insertions revealed a large difference for males that obtained one insertion as compared to males that obtained zero or two insertions. These results suggest that males transfer roughly the same amount of sperm during each pedipalp insertion, thus doubling the amount of sperm transferred to the female when achieving two insertions compared to one. Furthermore, there were fewer sperm remaining in the pedipalps of males that obtained two insertions compared to males that obtained zero insertions. There were also fewer sperm (although not statistically significant) compared to males that obtained one insertion. Given that males store roughly the same amount of sperm within each of their pedipalps, we presume that the absolute difference in the amount of sperm remaining in each pedipalp for males that inserted only once reflects the amount of sperm that was transferred to the female.

We observed a significant amount of variation in the amount of sperm stored in unmated males pedipalps and this variation was not explained by any of the tested variables. In some species, male size is positively related to increased sperm quantity and increased sperm transfer (e.g. Assis & Foellmer, 2016; Ceballos, Jones, & Elgar, 2015; Wiernasz, Sater, Abell, & Cole, 2001), but we found no evidence that male size influences the initial amount of sperm stored in male's pedipalps or the amount of sperm that males transfer to female *P. mira*. Similar levels of variation in the number of sperm stored in virgin males' pedipalps has been observed in other spider species (e.g. Bukowski et al., 2001: range 15,667–75,222 total sperm; Schneider et al., 2006: range 600–11,7400; Snow & Andrade, 2004: mean = 105,359 ± 10,660), and in some of these cases, male size similarly did not explain the observed variation (Schneider et al., 2006; Snow & Andrade, 2004). We did find that males transferred less sperm to larger females, which is opposite of results found within many other animal species (e.g. crickets, *Acheta domesticus* and *Gryllodes supplicans*: Gage & Barnard, 1996; moths, *Plodia interpunctella*: Gage, 1998; crayfish, *Austropotamobius italicus*: Rubolini et al., 2006). Given that we found no evidence that larger *P. mira* females were more fecund, it may not be surprising that males did not favor larger females. However, it is currently unclear why males transferred more sperm to smaller females. One possibility is that males may have a greater potential to monopolize smaller females within the context of sperm competition, assuming that smaller females have smaller spermathecae (Ramos, Coddington, Christenson, & Irschick, 2005). For example, males might be able to fill an entire spermathecae with sperm if it is small enough, thus preventing any additional males from contributing their sperm. This idea could be tested by first comparing spermathecal size across female sizes and then comparing the proportion of the spermathecae filled with sperm following copulation for small versus large females.

Insertion Number and Fertilization Success

We found that males that obtained two insertions had approximately 25% more offspring than singly inserted males. In a study by Foellmer and Fairbairn (2004), they similarly experimentally

manipulated male *Argiope aurantia* to obtain either one or two pedipalps insertions, and found that females had approximately 25% more offspring when males achieved two insertions compared to just one. In *P. mira*, given that we found no effect of insertion number on the total number of eggs that females produced, we presume that offspring number reflects fertilization success. Furthermore, we also carried out statistical analyses using the proportion of hatched offspring as our response variable, and found complementary results. Taken together, males transfer approximately twice the amount of sperm to females but achieve only a 25% increase in offspring number and no significant increase in total eggs produced. Recent work in the wolf spider *Schizocosa malitiosa* demonstrated cryptic female choice in sperm use (Albo & Costa, 2017) and it is possible that something similar is happening in *P. mira*. Regardless, we currently are unclear how double the amount of sperm transferred translates into a 25% increase in offspring number.

Although males were found to transfer more sperm to smaller females, female size had no effect on the number offspring females produced. It is possible that on the controlled laboratory diet, larger females, which likely have higher metabolic demands, required more resources for general maintenance and therefore had to invest less into egg and offspring development. In line with this prediction, we found that smaller females produced their eggsacs faster. Increased sperm transfer itself could trigger faster egg development and laying, which might explain why smaller females developed their eggs faster. Alternatively, if small females are able to invest more in offspring development (given identical diets with larger females that may have more metabolic demands), males may be able to assess this and adjust their amount of sperm transfer accordingly. Future work is required to tease apart these hypotheses.

Beyond increased offspring number, another potential benefit to increased insertion number falls within the realm of sperm competition. Fromhage, Uhl, and Schneider (2003) found that male *Argiope bruennichi* that were allowed obtain two insertions within a mating, compared to one, reduced the paternity of the second male that mated with the same female. Additionally, in the same way that males have paired pedipalps, females also have paired genital organs and copulatory ducts. In many spider species, these ducts lead to separate sperm storage organs (Austad, 1984; Foelix, 2011). If males obtain only one insertion, they leave one spermathecae free of sperm, which may strongly influence male paternity success if the female mates with an additional male (Eberhard, 2004). This may be especially true if females are capable of controlling which spermathecae they use to fertilize their eggs (Eberhard, 1996). Preliminary studies confirm that female *P. mira* can remate in the laboratory (A. G. Anderson, personal observation), however, factors influencing male paternity success have yet to be explored. Given that sperm competition can be an important driver of sexually selected traits (Parker, 1970, 1984; Simmons, 2001) future work will explore the effects of copulatory silk wrapping and increased copulation duration when females mate with additional males. We might expect that females have evolved a means by which they can control sperm use given that males have evolved a means to restrain females while they obtain increased insertion numbers.

In conclusion, copulatory silk wrapping exhibited by male *P. mira* allows males to increase the number of pedipalp insertions within a mating (Anderson & Hebets, 2016), which corresponds to increased quantity of sperm transferred and male fertilization success. This finding suggests that this sex-specific trait evolved in order for males to increase their own reproductive fitness. Although *P. mira* females often attempt to terminate copulation and cannibalize their male mating partner, it is currently unknown whether male silk wrapping imposes any cost on females. Regardless, the behaviors exhibited by both male and female *P. mira* during mating suggest differing mating goals and a potential conflict over the duration of copulation.

Supplementary material — Supplementary material related to this article follows the References.

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