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Benefits of size dimorphism and copulatory silk wrapping in the sexually cannibalistic nursery web spider, *Pisaurina mira*

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Abstract

In sexually cannibalistic animals, male fitness is influenced not only by successful mate acquisition and egg fertilization, but also by avoiding being eaten. In the cannibalistic nursery web spider, *Pisaurina mira*, the legs of mature males are longer in relation to their body size than those of females, and males use these legs to aid in wrapping a female's legs with silk prior to and during copulation. We hypothesized that elongated male legs and silk wrapping provide benefits to males, in part through a reduced likelihood of sexual cannibalism. To test this, we paired females of random size with males from one of two treatment groups—those capable of silk wrapping versus those incapable of silk wrapping. We found that males with relatively longer legs and larger body size were more likely to mate and were less likely to be cannibalized prior to copulation. Regardless of relative size, males capable of silk wrapping were less likely to be cannibalized during or following copulation and had more opportunities for sperm transfer (i.e. pedipalpal insertions). Our results suggest that male size and copulatory silk wrapping are sexually selected traits benefiting male reproductive success.

Keywords: behavior, evolution, antagonistic coevolution, cannibalism, mating system evolution, sexual conflict, sexual selection

1. Introduction

For most males, reproductive success is dependent on the ability to acquire mates and successfully fertilize a female's eggs [1,2]. Research across diverse animal taxa has documented elaborate courtship displays and seemingly coercive strategies that exist to secure matings (reviewed in [1–3]). In systems with the potential for high levels of sperm competition, males can increase fertilization success by preventing females from successfully remating through mate guarding or the production of sperm plugs and seminal toxins, among others (reviewed in [4]). For a small number of taxa in which females are sexually cannibalistic, however, males have an additional challenge—surviving a reproductive encounter. While cannibalism prior to sperm transfer results in zero male fitness, cannibalism after the transfer of sperm can be beneficial to a male [5,6]. Nonetheless, even post-copulatory sexual cannibalism carries a potentially high cost (e.g. reduced sperm transfer and loss of future reproductive opportunities) and can strongly influence the evolution of male mating strategies and associated morphologies [7,8].

Given the costs of sexual cannibalism, we expect positive selection for male traits/behaviors that reduce the likelihood of being eaten. Indeed, across cannibalistic spider taxa (Class Arachnida, Order Araneae), sexual cannibalism is commonly associated with extreme morphologies and striking mating systems (reviewed in [9,10]). For example, sexual size dimorphism (SSD) is often associated with sexual cannibalism [11,12]. It has been suggested that longer male legs have been selected to avoid pre-copulatory cannibalism in a number of orb-weaving spiders [13]. Additionally, larger male fishing spiders (*Dolomedes*...
fimbriatus) are more successful in avoiding cannibalistic attempts by females [14]. Many species with SSD, however, are not cannibalistic and the cause/effect relationship between SSD and sexual cannibalism is not well understood. Unusual behaviors such as death feigning [15], opportunistically mating with feeding females [16,17] and rendering females unconscious with sedative pheromones [18] are also presumably associated with avoiding sexual cannibalism. This study focuses on another particularly unusual mating behavior—the wrapping of females in silk by males in the sexually dimorphic nursery web spider Pisaurina mira (Walchenaer).

Pisaurina mira spiders exhibit a sexually dimorphic body shape with males presenting seemingly elongate front legs (forelegs), and an unusual mating strategy wherein a male will wrap a female’s legs with silk prior to and during sperm transfer [19] (figure 1). This unusual behavior has been postulated to reduce female mobility and the likelihood of sexual cannibalism [19], but this hypothesis has not been directly tested. A function in decreasing pre-copulatory cannibalism seems unlikely as receptive females appear to passively allow males to initially wrap them with silk. Following the first act of sperm transfer (i.e., the first insertion of the male’s pedipalp), however, females begin to engage in movements that look like an attempt to free themselves from the silk wrapping (supplementary material, video S1).

We hypothesized that size dimorphism influences the ability of P. mira males to initiate a female in copulation while silk wrapping enables them to acquire additional sperm transfer events (hereafter termed insertions) without being cannibalized. We test these hypotheses by randomly pairing phenotypically manipulated males (silk wrapping versus no silk wrapping) with females of variable size and assessing proxies of fitness.

2. Material and methods
We collected immature male and female Pisaurina mira (Lancaster County, NE, USA) in Spring 2014. Spiders were maintained in the laboratory under controlled conditions. To assess the effect of size dimorphism and copulatory silk wrapping, we ran mating trials in which males were assigned to either a (i) wrap (n = 15) or (ii) no wrap (n = 16) treatment and paired with females of random size. We phenotypically manipulated males in each treatment by applying dental silicone (Take 1® Advanced™ Kerr, Orange, CA, USA) to either (i) the dorsal side of a male’s abdomen (wrap treatment) or (ii) a male’s spinnerets (no wrap treatment). During mating trials, we live-scored copulation success, pre- and post-copulatory cannibalism, and the number of insertions that a male obtained. We obtained body size measurements (i.e. carapace width (CW) and leg length) from preserved specimens following mating trials. For additional methodological details, see the supplementary material.

If size dimorphism in P. mira benefits males in terms of reproductive success, we predicted seeing males with higher male : female leg length or larger body size: (1a) achieve higher mating success and (1b) experience fewer pre-copulatory cannibalism events. We had no a priori predictions for the relationship between size dimorphism and post-copulatory cannibalism or insertion number, as we expected these to be influenced more by the silk wrapping. Specifically, we predicted that silk wrapping males would (2a) experience fewer cannibalism events during or after sperm transfer and (2b) experience two versus only one pedipalpal insertions. We predicted no relationship between silk wrapping and mating success or pre-copulatory cannibalism, as these both occur prior to silk wrapping. We used separate binomial linear regressions to test the effects of size and Fisher’s exact tests to examine the effects of silk wrapping on reproductive behavior. For details on statistical analyses, see the supplementary material.

3. Results

(a) Size dimorphism and proxies of fitness
Male P. mira have relatively longer legs and a larger leg length to body ratio than females, but females have a larger CW (i.e. larger body size) than males (supplementary material, table S1). As predicted, male : female leg length predicted copulation success ($X^2_{1,29} = 12.96, p < 0.0001$; figure 2a) and pre-copulatory cannibalism ($X^2_{1,29} = 7.76, p = 0.005$; figure 2b); results are identical for male : female CW (see the supplementary material). Males were more likely to copulate and less likely to be cannibalized when their legs were longer than those of their mating partner and male : female CW was larger. For all males (i.e. those in the wrap and those in the no wrap treatment), we found no effect of male : female leg length or body size (see the supplementary material) on a male’s likelihood of being cannibalized post-copulation (wrap: $X^2_{1,10} = 0.246, p = 0.620$; no wrap: $X^2_{1,8} = 0.412, p = 0.521$) or on the number of insertions obtained (wrap: $X^2_{1,10} = 0.300, p = 0.584$; no wrap: $X^2_{1,8} = 0.826, p = 0.363$). For simplicity, we performed both tests with the two male treatments combined and present these combined results in our figure (post-copulatory cannibalism: $X^2_{1,19} = 0.0002, p = 0.988$, figure 2c; insertion number: $X^2_{1,17} = 0.275, p = 0.600$, figure 2d).

(b) Silk wrapping and proxies of fitness
Copulation success and rates of pre-copulatory cannibalism did not differ between males that could and could not wrap females (Fisher’s exact test, $p = 0.458$; figure 2e and $p = 0.394$; figure 2f, respectively). In line with our predictions, copulatory silk wrapping affected rates of post-copulatory cannibalism and
the number of insertions a male obtained. Males that could not wrap females were more likely to be cannibalized after insemination compared with males that could wrap females (Fisher’s exact test, $p = 0.017$; figure 2g). Males in our wrap treatment were also more likely to achieve two insertions (versus one) compared with males in our no wrap treatment (Fisher’s exact test, $p = 0.049$; figure 2h).

4. Discussion

Size dimorphism and male silk wrapping in the nursery web spider *P. mira* are important for male mating success and survival. Mature males possess longer forelegs, relative to body size, than their female counterparts and this dimorphism is important for male reproductive success as males with relatively longer legs
were: (i) more likely to achieve a successful mating and (ii) less likely to be cannibalized prior to copulation. Using phenotypic manipulations to remove a male’s ability to wrap his mate, we also demonstrate that silk wrapping: (i) reduces the likelihood of cannibalism during or after sperm transfer and (ii) increases the number of insertions a male can achieve during mating.

Though males with relatively longer legs obtained mating advantages, male leg length and CW are strongly correlated (see the supplementary material and figure S1), making it difficult to confirm a direct benefit of longer legs per se. Our results do suggest, however, a reproductive benefit of males being larger (or closer in size) relative to their mating partner, a pattern found across a number of distinct taxa, including red-sided garter snakes [20], Drosophila [21,22] and the tobacco moth [23]. In addition to increasing mating success, longer male legs (and larger males) also reduced the risk of precopulatory sexual cannibalism, a pattern consistent with other cannibalistic species ([11,12,24] but see [25]). With precopulatory cannibalism resulting in a complete loss of fitness for males, it is not surprising to observe larger body size and/or grasping traits that reduce the risk of cannibalism. In P. mira, males’ relatively longer legs appear to aid in their ability to manipulate, wrap and restrain females. As has been previously suggested across multiple cannibalistic orb-weaving spiders [13], we propose that longer leg length is a sexually selected trait in male P. mira.

Sexual cannibalism can lead to obvious male fitness costs (e.g. lost potential to secure future matings or reduction of sperm transferred) [7,8], yet sexual cannibalism can also be an adaptive male strategy [5,6]. In the dark fishing spider (Dolomedes tenebrosus), male consumption by females significantly increases offspring number, weight and survival [6]. It is currently unknown if P. mira males receive any fitness benefits from being cannibalized. Given that silk wrapping reduces the likelihood of post-copulatory sexual cannibalism, however, we hypothesize that male silk wrapping evolved to enable males to obtain increased benefits from multiply mating.

In addition to reducing rates of sexual cannibalism, we found that silk wrapping increases the number of insertions that a male can obtain. We suspect that a male’s ability to achieve more insertions corresponds to increased sperm transfer, which in turn corresponds (to some degree) to fertilization success. In the cannibalistic orb-weaving spider, Argiope aurantia, males experienced 25% higher fertilization success when achieving two versus one insertion [26]. Additionally, the transfer of more sperm might out-dilute or displace rival sperm, thereby increasing the proportion of the female’s eggs fertilized by a given male [2,4]. In preliminary trials, we find evidence that female P. mira mate multiply (primarily through forced copulations), suggesting a potential benefit of increased sperm transfer.

Though females may also benefit from mating with larger males, the striking behavior of copulatory silk wrapping may reflect an evolutionary history of conflicting reproductive strategies between males and females. Within mating trials, females attempt to be freed from the silk wrapping, which suggests they may incur a cost by being wrapped (e.g. missed post-mating cannibalism). Yet, virgin females often passively allow the wrapping to take place. Costs versus benefits of wrapping for virgin versus mated females and males may differ substantially as sperm competition could play an important role in this system. This study provides strong evidence that size dimorphism and copulatory silk wrapping have been sexually selected through fitness benefits to males, but the putative fitness cost(s) of these traits for females remain unknown.

References


Supplementary materials

**Benefits of sexual size dimorphism and copulatory silk wrapping in the sexually cannibalistic nursery web spider, *Pisaurina mira***

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**Methods**

*Spider collection and maintenance*

We collected immature male and female *Pisaurina mira* at night from Wilderness Park, Lancaster County, Lincoln, NE from 2 May – 12 May 2014. Spiders were individually housed at the University of Nebraska-Lincoln in 87.3 mm X 87.3 mm X 112.7 mm clear plastic containers (763C, AMAC Plastics, Petaluma, CA). We covered the outside of each container with opaque tape to visually isolate individuals. Spiders were maintained under a 12 h light/12 h dark cycle, fed three 7 mm juvenile crickets per week (*Gryllus sigilatus*, Ghann’s cricket farm, GA, USA) and provided water *ad libitum*.

*I. Size Dimorphism and Proxies of Fitness*

If male-biased size dimorphism in *P. mira* benefits males in terms of reproductive success, we predicted to see males with higher male:female leg length: (1a) achieve higher mating success and (1b) experience fewer pre-copulatory cannibalism events. We predicted no relationship between size dimorphism and post-copulatory cannibalism or insertion number.

Size measurements were taken from preserved specimens following mating trials (see *II. Silk Wrapping and Proxies of Fitness*). All spiders were preserved in 70% EtOH post-death. To
measure leg length, we removed the first right leg from preserved individuals, and allowed them to air dry. Legs were placed lateral side up on a glass slide that sat atop a scale graduated in mm and were photographed using a Leica DM 4000 B microscope with a Di-agnostic Instruments, Inc. Spot Flex digital camera. Our body size measure reflected the widest point of the carapace (often referred to as cephalothorax; anterior-most body part), which is fixed in size at maturity. To measure carapace width, we removed all appendages from each preserved spider and photographed the carapace using the same set up described previously. Measurements of leg length and carapace width were taken from the digital photographs using the program PixelStick ver. 2.8.

Additional photographs of males were taken prior to mating trials as a precaution in case males were cannibalized during the trial. Live males were placed on size scale graduated slides (mm) and then covered by a petri dish. Covering the male with the petri dish caused them to completely stretch out their legs. We used a Nikon COOLPIX S6100 digital camera held perpendicular to the table surface to take photographs. For a subset of ten males, we used paired t-tests to confirm that leg length and body size measurements did not differ between our two measurement protocols ($t_9 = 0.545$, $p = 0.599$ and $t_9 = -0.987$, $p = 0.349$, respectively). In the event that males were cannibalized during mating trials, pre-mating photographs were used for morphological measurements.

*Statistical Analyses*

To assess differences between male and female carapace width, leg length, and leg to body ratios we used t-tests. From our body measurements we found that carapace width and leg length are strongly correlated for both males and females (males: $R^2 = 0.869$, df = 29, $p < .0001$; females: $R^2 = 0.829$, df = 29, $p < 0.0001$; Figure S1). To account for male and female size differences we
used the ratio of male to female leg length and the ratio of male to female carapace width in the following analyses. We used separate binomial logistic regressions to test our predictions that male:female leg length and male:female carapace width (CW) affects copulation success and pre-copulatory cannibalism. We also used separate binomial logistic regressions to determine if male:female leg length and male:female CW affects post-copulatory cannibalism and the number of insertions a male obtained. Because there is an effect of our silk ablation treatment on post-copulatory cannibalism and insertion number (see results) we also performed separate binomial logistic regressions for both treatments. Within our analysis, looking at the effect of male:female leg length and CW on number of insertions, we called a male successful if he obtained two insertions (versus one). For this analysis we excluded the two trials where the females cannibalized the male in the middle of the male’s second insertion.

II. Silk Wrapping and Proxies of Fitness

If silk wrapping increases male reproductive success, especially through decreased risk of sexual cannibalism, we predicted that silk wrapping males would (2a) experience fewer cannibalism events during or after sperm transfer and (2b) experience more pedipalpal insertions. We predicted no relationship between silk wrapping and copulation success or pre-copulatory cannibalism. To test these predictions, we randomly assigned males to a (i) wrap ($n = 15$) or (ii) no wrap treatment ($n = 16$). We ablated a male’s silk wrapping ability by covering the male’s spinnerets with dental silicone (Take 1® Advanced™, Kerr, Orange, CA). To control for any potential effects of our treatment, we placed dental silicone on the dorsal side of a male’s abdomen in the wrap treatment. To apply the dental silicone treatment, we first placed males into a freezer for approximately three minutes to slow their movement. We carefully restrained males onto a Styrofoam block by pinning down their legs and then applied dental silicone to the pre-
assigned body region (i.e. wrap – dorsal abdomen; no wrap – spinnerets). Males were never restrained for more than one minute. Once released, we gave males 30 minutes to recover prior to the start of the mating trial. Prior to trials we also confirmed by visual inspection that the ‘no wrap’ treatment males were unable to lay down any silk.

Mating trials took place from 21 May – 2 June 2014 between 1200 and 1800h and consisted of a randomly paired virgin male and female 15 days ± 1-day post-maturation. Trials were run within 200 mm dia. X 190 mm ht. covered, plastic arenas (250C, Pioneer Plastics, North Dixon, KY) with filter paper (185 mm dia.) placed on the arena floor. To allow spiders to climb, we placed a plastic cylinder (42 mm dia. X 156 mm ht.) covered with fiberglass screen wire at the center of the arena. Two strips (40 mm wid.) of screen wire were placed on opposing walls that extended the height of the arena. The central cylinder and screen wire strips were connected near the top of the arena using two wooden craft sticks (9 mm wid.). Between mating trials we wiped down the arenas with 70% EtOH and replaced the filter paper. We placed females in mating arenas approximately 24 hours prior to the male’s introduction.

A trial began once we placed the male in the arena at the farthest point from the female’s current location. During mating trials, we live-scored copulation success, pre and post-copulatory cannibalism, and the number of insertions that a male obtained. We categorized cannibalism events as pre-copulatory cannibalism if it occurred prior to a male’s insertion or as post-copulatory cannibalism if it occurred after or during a male’s insertion. Quantification of the above variables was not preformed blindly because the dental silicone made male treatment obvious to the observer. In mating trials, the dental silicone worked as expected - all of the males in the no wrap treatment were unable to wrap females, while all males in the wrap treatment successfully wrapped the females with silk.
Statistical Analyses

To determine if copulation success, pre-copulatory cannibalism and post-copulatory cannibalism differed between males that could and could not wrap females, we used Fisher’s Exact Tests. To determine if the number of insertions a male obtained differed between our two treatments we also used a Fisher’s Exact test. Because males obtained one or two insertions, we compared the proportion of males that achieved two versus one insertion. For this analysis we excluded two trials where the females cannibalized the male in the middle of the male’s second insertion. All statistical analyses were conducted using R software (ver. 3.0.3).

Results

Size Dimorphism and Proxies of Fitness

Female *P. mira* have a larger carapace width (*i.e.* larger body size) than males, but males have relatively longer legs and a larger leg length to body ratio (Table S1). As presented in the main text, males were more likely to copulate and less likely to be cannibalized when their legs were longer than those of their mating partner. Similarly, males were more likely to copulate ($\chi^2_{1,29} = 15.87, p < 0.0001$) and less likely to be cannibalized prior to copulation ($\chi^2_{1,29} = 7.84, p = 0.005$) when male:female CW was larger.

Again, similar to our results for male:female leg length, for all males (*i.e.* those in the wrap and those in the no wrap treatment) we found no effect of male:female CW on a male’s likelihood of being cannibalized post-copulation (wrap: $\chi^2_{1,10} = 1.136, p = 0.286$; no wrap: $\chi^2_{1,8} = 0.746, p = 0.388$) or on the number of insertions obtained (wrap: $\chi^2_{1,10} = 0.826, p = 0.363$; no wrap: $\chi^2_{1,8} = 0.966, p = 0.326$). We also present results with the two male treatments combined
(post-copulatory cannibalism: \( \chi^2_{1,19} = 1.811, p = 0.178 \); insertion number: \( \chi^2_{1,17} = 0.253, p = 0.615 \)).

In order to further explore the role of size on male reproductive behavior, we used the residuals of a correlation between male leg length and male carapace width as the predictor variable for our behavioral outcomes (i.e. likelihood of copulation, pre-copulatory cannibalism, post-copulatory cannibalism and obtaining two versus one insertion). Here we found that the relationship between male body size and leg length has no effect on any of our behavioral outcomes (Tables S2).

**Tables and Figures**

**Table S1.** Average (± SE) carapace width, leg length, and leg to body ratio for male and female *Pisuarina mira*. Results of independent t-tests represent differences between males and females and all three variables measured.

<table>
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<tr>
<th>Variable</th>
<th>Female mean (mm) ± SE</th>
<th>Male mean (mm) ± SE</th>
<th>t-value</th>
<th>df</th>
<th>p-value</th>
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<td>4.462 ± 0.086</td>
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<td>Leg length</td>
<td>20.97 ± 0.382</td>
<td>29.53 ± 0.425</td>
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<td>Leg:body</td>
<td>0.135 ± 0.002</td>
<td>0.213 ± 0.001</td>
<td>39.66</td>
<td>41.63</td>
<td>&lt;0.001</td>
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Figure S1. Relationship between carapace width (mm) and leg length (mm) for mature male and female *Pisaurina mira*. Dashed lines represent best-fit lines.
Table S2. Results from binomial linear regressions using the residuals of a correlation between male leg length and male carapace width as the predictor variable on the likelihood of copulation, pre-copulatory cannibalism, post-copulatory cannibalism and obtaining two versus one insertion (i.e. behavioral outcome).

<table>
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<th>Behavioral outcome</th>
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<th>$n$</th>
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<td>21</td>
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<td>0.782</td>
<td>19</td>
<td>1, 17</td>
<td>0.377</td>
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