Electronic 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.

Variable	Female mean (mm) ± SE	Male mean (mm) ± SE	t-value	df	p-value
Carapace width	4.462 ± 0.086	4.000 ± 0.066	4.26	55.91	<0.001
Leg length	20.97 ± 0.382	29.53 ± 0.425	14.98	59.34	<0.001
Leg:body	0.135 ± 0.002	0.213 ± 0.001	39.66	41.63	<0.001

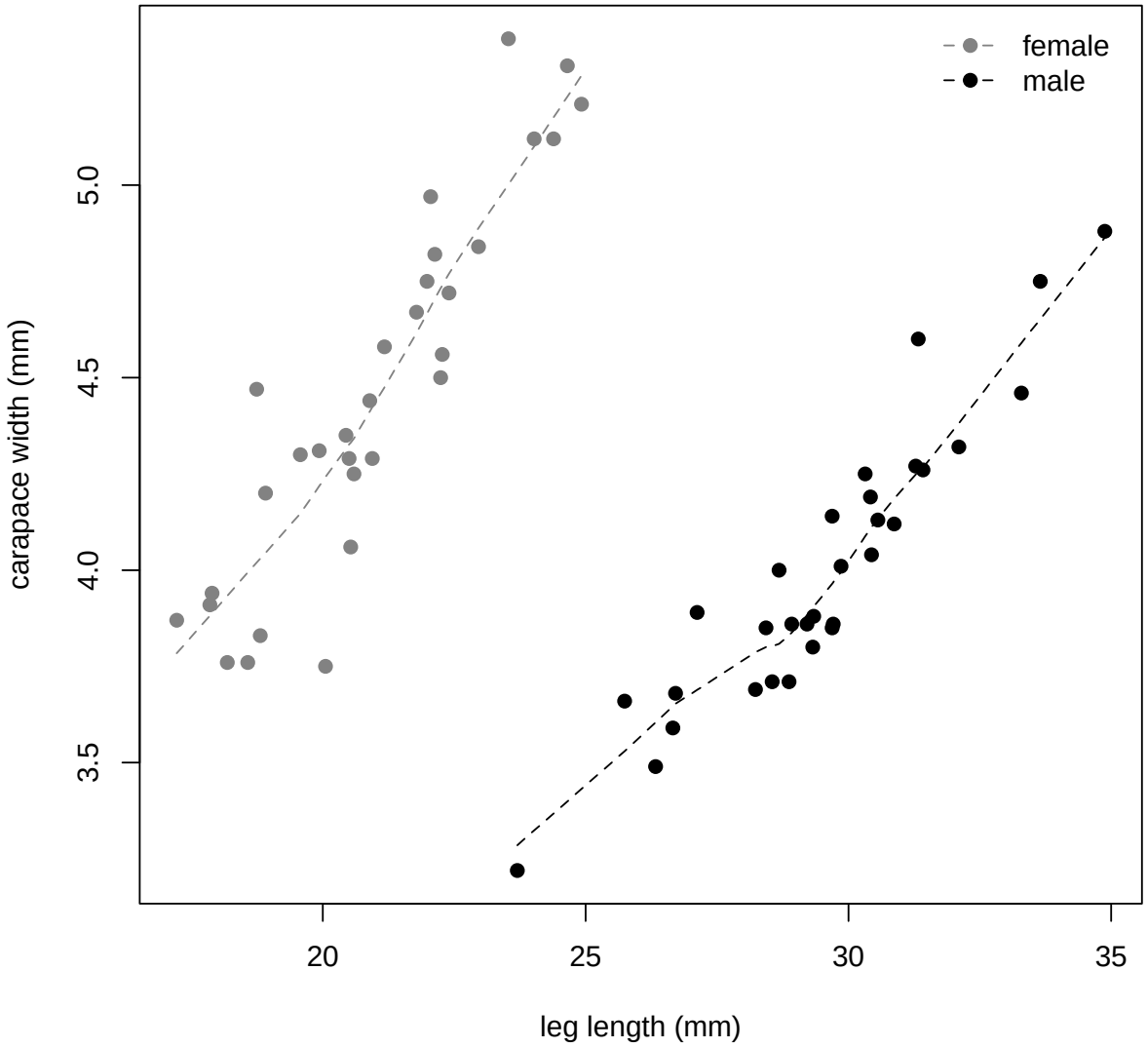

Figure S1. Relationship between carapace width (mm) and leg length (mm) for mature male and female *Pisaurina mira.* Dashed lines represent best-fit lines.

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).

Behavioral outcome	χ^2	***n***	**df**	**p-value**
Copulation	0.669	31	1, 29	0.416
Pre-copulatory cannibalism	2.640	31	1, 29	0.115
Post-copulatory cannibalism	2.294	21	1, 19	0.190
Insertions	0.782	19	1, 17	0.377