

COSTS AND BENEFITS OF COPULATORY SILK WRAPPING IN THE NURSERY
WEB SPIDER, *PISAURINA MIRA* WALCKENAER, 1837 (ARANEAE, PISAURIDAE)

by

Alissa G. Anderson

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Biological Sciences

(Ecology, Evolution and Behavior)

Under the Supervision of Professor Eileen A. Hebets

Lincoln, Nebraska

March, 2018

ProQuest Number: 10788541

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10788541

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

COSTS AND BENEFITS OF COPULATORY SILK WRAPPING IN THE NURSERY
WEB SPIDER, *PISAURINA MIRA* WALCKENAER, 1837 (ARANEAE, PISAURIDAE)

Alissa G. Anderson

University of Nebraska, 2018

Advisor: Eileen A. Hebets

Males and females share the same goal in sexual reproduction - increased offspring production and viability - yet specialized traits can evolve in one or both of the sexes that serve to increase individual reproductive fitness, sometimes at a cost to a mating partner. Exploring the costs and benefits of such traits in both sexes can provide insights into the role that male-female conflict play in the evolution of mating systems. Under this framework we have explored the evolution of the unique mating behavior observed in the cannibalistic nursery-web spider, *Pisaurina mira*, where males restrain females by wrapping them with silk prior to and during copulation. First, we tested the function of copulatory silk wrapping by pairing females with males that were experimentally manipulated to either be capable or not capable of silk wrapping. We found that males capable of wrapping females were (a) more likely to obtain two versus one opportunity to transfer sperm (termed insertions) and were (b) less likely to be cannibalized after or during copulation. Following from our initial study, we explored male benefits of increased insertion numbers, which revealed that increased insertion number corresponds to increased sperm transfer and higher fertilization success.

Specifically, males transfer roughly two times the amount of sperm when achieving two insertions compared to only one, and this increase in sperm transfer results in males having approximately 25% more offspring. Two additional studies exploring the costs and benefits of males avoiding sexual cannibalism revealed that copulatory silk wrapping results in (a) females losing out on fitness benefits of sexual cannibalism while (b) allowing males to mate multiply. Specifically, we found that consumption of a male mating partner leads to females producing heavier and longer-lived offspring, however, silk wrapping keeps females from obtaining these benefits. Further, regardless of this shared fitness benefit, we also found that cannibalism avoidance by males allows them to mate multiply and achieve higher offspring production. Overall, our results highlight how male and female reproductive strategies can differ, and can ultimately lead to adaptations that clearly benefit one sex while inflicting costs on the opposite sex.

PREVIEW

Copyright by Alissa G. Anderson

© 2018

Dedicated to my husband, Austin Davin Brooks and my baby girl, Olive Mae Brooks –

“I know there was something before you, I just can’t remember what it was.”

ACKNOWLEDGMENTS

I thank the Lincoln Parks and Recreation Department for granting permission to access and conduct research within Wilderness Park and Dr. Kiourtsis for supplying the dental silicone used within various aspects of this work. I also thank undergraduate students, Bridget Bickner, Jordan Harper and Kristen Kramer for their help with spider care, maintenance, etc. and the numerous graduate students (and non-students) who continually aided me in spider collections each year – Austin Brooks, Jay Stafstrom, Justin Buchanan, Malcolm Rosenthal, Rowan McGinley and Shivani Jadeja.

I thank committee members, Bill Wagner, Colin Meiklejohn, Dai Shizuka, Kristi Montooth and Susan Weller for their continued advice and support throughout the entirety of my program. I especially would like to thank my advisor, Eileen Hebets for her guidance and her willingness to allow me to pursue the research questions that I was most interested in. I would also like to thank members of the Basolo, Hebets, Shizuka and Wagner labs for providing feedback on research design and manuscripts. I thank the entire EEB community – staff, faculty and graduate students – for helping me through all aspects of my graduate career. Thank you to the faculty and students that listened to my yearly R&D and research seminars and thank you to the staff for making paper work, reimbursements and general graduate life much easier.

Finally, I would like to thank my loving family for their never-ending support. Thank you for being genuinely interested in all my “spider” research and for cheering me on through both the easier and tougher times. I especially would like to thank my husband for being a loving and supportive partner, and for being an amazing father to our beautiful baby girl.

GRANT INFORMATION

This work was funded by grants from the Animal Behavior Society (ABS), the American Arachnological Society (AAS) and the School of Biological Sciences at the University of Nebraska and by fellowships from the U.S. Department of Education – Graduate Assistance in Areas of National Need (GAANN).

PREVIEW

TABLE OF CONTENTS

DEDICATION	v
ACKNOWLEDGEMENTS	vi
GRANT INFORMATION	vii
LIST OF TABLES AND FIGURES	xiv
OVERVIEW	1
References	7
CHAPTER 1: BENEFITS OF SIZE DIMORPHISM AND COPULATORY SILK WRAPPING IN THE SEXUALLY CANNIBALISTIC NUSERY WEB SPIDER, <i>PISAUURINA MIRA</i>	9
Abstract	10
<i>Key words</i>	10
Introduction	11
Methods	13
Results	14
Discussion	15
Acknowledgments	19

Tables and Figures	20
References	23
Supplementary Material	26
<i>Methods</i>	26
<i>Spider Collection and Maintenance</i>	26
<i>I. Size Dimorphism and Proxies of Fitness</i>	26
<i>Statistical Analyses</i>	27
<i>II. Silk Wrapping and Proxies of Fitness</i>	28
<i>Statistical Analyses</i>	30
<i>Results</i>	30
<i>Size Dimorphism and Proxies of Fitness</i>	30
<i>Tables and Figures</i>	32
CHAPTER 2: INCREASED INSERTION NUMBER LEADS TO INCREASED SPERM	
TRANSFER AND FERTILIZATION SUCCESS IN A NURSERY WEB SPIDER	
Abstract	36
<i>Key words</i>	37
Introduction	38
Methods	41

<i>Species Collection and Maintenance</i>	41
<i>Experiment I: Insertion number and sperm quantity</i>	42
<i>Statistical Analyses</i>	44
<i>Experiment II: Insertion number and offspring number</i>	44
<i>Statistical Analyses</i>	46
Results	47
<i>Experiment I: Insertion number and sperm quantity</i>	47
<i>Experiment II: Insertion number and offspring number</i>	48
Discussion	49
<i>Insertion number and sperm quantity</i>	49
<i>Insertion number and offspring number</i>	51
Acknowledgments	54
Tables and Figures	55
References	60
Supplementary Materials	66
<i>Figures</i>	66
 CHAPTER 3: FEMALE NURSERY WEB SPIDERS (PISAUINA MIRA) BENEFIT FROM CONSUMING THEIR MATE	 68

Abstract	69
<i>Key words</i>	69
Introduction	70
Methods	73
<i>Species Collection and Maintenance</i>	73
<i>Sexual Cannibalism and Fitness</i>	74
<i>Statistical Analyses</i>	76
Results	78
Discussion	79
Acknowledgments	84
Tables and Figures	85
References	88
CHAPTER 4: MALES MATE WITH MULTIPLE FEMALES TO INCREASE	
OFFSPRING NUMBERS IN A NURSERY WEB SPIDER	
Abstract	95
<i>Key words</i>	96
Introduction	97
Methods	99

<i>I. Male multiple mating and associated fitness</i>	99
<i>Species Collection and Maintenance</i>	99
<i>Multiple mating assays</i>	99
<i>Quantifying offspring production</i>	100
<i>Statistical Analyses</i>	100
<i>II. Population Demographics</i>	101
<i>Population Density and Sex Ratios</i>	101
<i>Male and Female Movement Patterns</i>	102
<i>Statistical Analyses</i>	102
<i>III. Mathematical model of mating opportunities</i>	103
<i>The general model</i>	103
<i>Parameterizing the model for <i>Pisaurina mira</i></i>	105
<i>Model Analysis</i>	106
Results	106
<i>I. Male multiple mating and associated fitness</i>	106
<i>II. Population Demographics</i>	107
<i>Population Density</i>	107
<i>Male and Female Movement Patterns</i>	107
<i>III. Mathematical model of mating opportunities</i>	108

Discussion	109
Acknowledgments	114
Table and Figures	115
References	119
Supplementary Material	122
<i>Appendix 4A: Parameter estimates and model assumptions</i>	122
<i>I. Parameter estimates used to model mating opportunities</i>	122
<i>II. Exploring effects of parameter estimates on model conclusions</i>	124
<i>III. Mate encounters in 3 dimensions</i>	127
<i>Tables and Figures</i>	129
<i>References</i>	134

LIST OF TABLES AND FIGURES

Figure 1.1 Male *Pisaurina mira* wrapping female with silk prior to copulation. _____ 20

Figure 1.2 The probability of males (A) copulating, (B) being cannibalized pre-copulation, (C) being cannibalized post-copulation, and (D) obtaining two insertions as a function of male:female leg length (n = 31). The proportion of males that (E) copulated, (F) were cannibalized pre-copulation, (G) were cannibalized post-copulation and (H) achieved two insertions during copulation when allowed to wrap (n = 15) and not wrap (n = 16) females. The two males that obtained 1.5 insertions were excluded from the analyses presented in D and H. The asterisk signifies significance based on a p-value < 0.05. _____ 21

Table S1.1 Average (\pm SE) carapace width, leg length, and leg to body ratio for male and female *Pisaurina mira*. Results of independent t-tests represent differences between males and females and all three variables measured. _____ 32

Table S1.2 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). _____ 33

Figure S1.1 Relationship between carapace width (mm) and leg length (mm) for mature male and female *Pisaurina mira*. Dashed lines represent best-fit lines. _____ 34

Table 2.1 Model outputs testing the effect of insertion treatment, female size and male size on the number of offspring produced by females within their first egg sacs, as well as the total number of offspring produced by females' across all egg sacs. _____ 55

Figure 2.1 The absolute difference in sperm between male *Pisaurina mira*'s pedipalps (n = 15) that achieved one pedipalp insertion during mating reflects the amount of sperm transferred to the female. The absolute difference in sperm between pedipalps reflects the amount of sperm transferred to the female. Thus, males transferred more sperm to smaller females. Lines within the scatterplots represent model prediction and confidence intervals. _____ 56

Figure 2.2 The average (\pm SE) (A) total number of sperm and the (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. _____ 57

Figure 2.3 The 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. _____ 58

Figure 2.4 The number of days from mating that it took females to produce their first egg sacs across female size. Lines within the scatterplots represent model prediction and confidence intervals. _____ 59

Figure S2.1 A) Male *Pisaurina mira* wrapping a female's first two pairs of legs with silk prior to copulation, and (B) a female still wrapped in silk immediately after copulation ended. _____ 66

Figure S2.2 Female and male mass (g) and body size (mm) across our four kill/consumption treatments (K-Male: n = 29, K-Nothing: n = 27, K-Cricket: n = 24 and NK-Nothing: n = 28). _____ 67

Table 3.1 40x magnification of encapsulated male *Pisaurina mira*'s sperm within one of 25 large squares within the center counting grid of a haemocytometer. _____ 85

Figure 3.1 The effect of females killing and either consuming their mate (K-Male; n = 29), nothing (K-Nothing; n = 27), a size-matched cricket (K-Cricket; n = 24) or not killing their mate and consuming nothing (NK-Nothing; n = 28) on the number of offspring females produced (A) within their first egg sacs and (B) across the season. Boxes within the boxplots show the median and first and third quartile range, while the whiskers show the minimum and maximum values. Open circles represent outliers. ___ 86

Figure 3.2 The effect of females killing and either consuming their mate (K-Male; n = 25), nothing (K-Nothing; n = 18), a size-matched cricket (K-Cricket; n = 18) or not killing mate and consuming nothing (NK-Nothing; n = 14) on (A) offspring mass (mg), (B) log transformed time to egg sac production (days) and (C) the proportion of offspring alive across days monitored. Boxes within the boxplots show the median and first and third quartile range, while the whiskers show the minimum and maximum values. Open circles represent outliers. _____ 86

Table 4.1 The density (individuals/square meter) and proportion of male, female and juvenile *Pisaurina mira* within three plots measured across the season. Plot 1B, 2B and 3B represent the new plots created adjacent to their previous plots. The proportion of females reflects females that are available for copulation (i.e. that do not have egg sacs or spiderlings). _____ 114

Table 4.2 Metrics of male multiple mating potential taken from simulations of interactions between male and female *Pisaurina mira* occurring under early season and late season demographic conditions. Each cell contains the mean \pm standard deviation of the metric across 1000 replicate simulations. _____ 115

Figure 4.1 (A) The total number of males that achieved zero through five matings with different virgin females. (B) The total number of male *Pisaurina mira* that either mated or did not mate within the first through the fifth mating opportunity with a virgin female.

Decreases observed in the total number of males across mating virgin females is due to males being cannibalized either prior or after copulation. _____ 116

Figure 4.2 The total number of offspring that *Pisaurina mira* males fathered when achieving one through five matings with different virgin females. _____ 117

Table A4.1 Definitions and estimates of parameters and corresponding units used for our simulation of male-female encounters in *Pisaurina mira*. The source of each parameter estimate is noted. For those parameters that varied between the early and late phases of the growing season, both estimates are provided in respective order. All estimates are derived from data collected in this study, with the exception of the probability of predation h , which was derived from Wise and Chen (1999). _____ 128

Figure A4.1 The median number of mating opportunities per male as a function of mate detection radius, r . Boxplots represent the distribution of medians taken from 1000 replicate simulations for each value of r . The dashed line corresponds to a median mating opportunity number = 1. _____ 129

Figure A4.2 The median number of mating opportunities per male as a function of predation rate, f . Boxplots represent the distribution of medians taken from 1000 replicate simulations for each value of f . The dashed line corresponds to a median mating opportunity number = 1. _____ 130

Figure A4.3 A levelplot showing the median number of mating opportunities per male (colored regions) as a function of female and male movement probabilities. The values used to color the regions are averages of the median number of mating opportunities across the 1000 replicates of each combination of values. _____ 131

Figure A4.4 A levelplot showing the median number of mating opportunities per male (colored regions) as a function of mean female and male movement distances. The values used to color the regions are averages of the median number of mating opportunities across the 1000 replicates of each combination of values. _____ 132

PREVIEW

OVERVIEW

Males and females generally share the same goal in sexual reproduction – increased production and viability of offspring – yet factors such as differential investment in gamete production (eggs vs. sperm) (Bateman, 1948), realized operational sex ratio (Emlen & Oring, 1977) and differential investment in parental care (Trivers, 1972) (among others) can result in males and females attempting to achieve optimal fitness in different ways. For example, male fitness is predicted to be dependent on maximizing the number of eggs fertilized (Andersson, 1994; Bateman, 1948; Parker, 1984); thus, adaptations that allow males to secure matings and increase fertilization success should be under positive selection. Indeed, males often have exaggerated weapons, ornaments or courtship displays for gaining access to females. In contrast to males, females are expected to maximize their fitness by increasing quality (rather than quantity) of offspring and they can achieve this by investing resources into developing offspring and by being choosy in selecting mating partners (Andersson, 1994; Bateman, 1948; Trivers, 1972).

The reproductive strategies that evolve within each sex to maximize fitness may be in conflict with one another – specialized traits that evolve in one or both of the sexes to increase individual reproductive fitness may sometimes be costly to the other sex. Differences in optimal reproductive strategies have been proposed to explain the evolution of costly mating behaviors such as barbed or spiny male genitalia (e.g. Hotzy & Arnqvist, 2009), forceful copulations (Arnqvist & Rowe, 2013; Clutton-Brock & Parker, 1995), traumatic insemination (Stutt & Siva-Jothy, 2001), and sexual cannibalism (Elgar & Crespi, 1992a). Uncovering the function of a trait that appears potentially costly for

one of the sexes within the system provides unparalleled opportunities to understand how divergent reproductive strategies can influence male-female mating dynamics and the evolution of mating systems.

In an attempt to gain insight into the role that male-female mating dynamics plays in the evolution of mating systems, this dissertation focuses on uncovering the function of unusual behavior of copulatory silk wrapping in the nursery web spider, *Pisaurina mira*. In this species, all males wrap females' legs with silk prior to and during copulation (Bruce & Carico, 1988; Fig. 1); a behavior that appears potentially costly for females. Further, mating in *P. mira* follows a typical sequence of behaviors

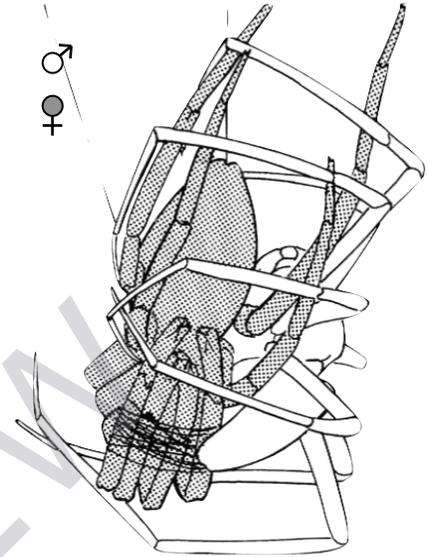


Figure 1 Male *Pisaurina mira* wrapping female's legs with silk prior to copulation. Adapted from Bruce & Carico (1988).

(Fig. 2) that reveal the potential for differing mating strategies for each sex. Most virgin females are receptive to mating and allow males to mount and silk wrap them prior to

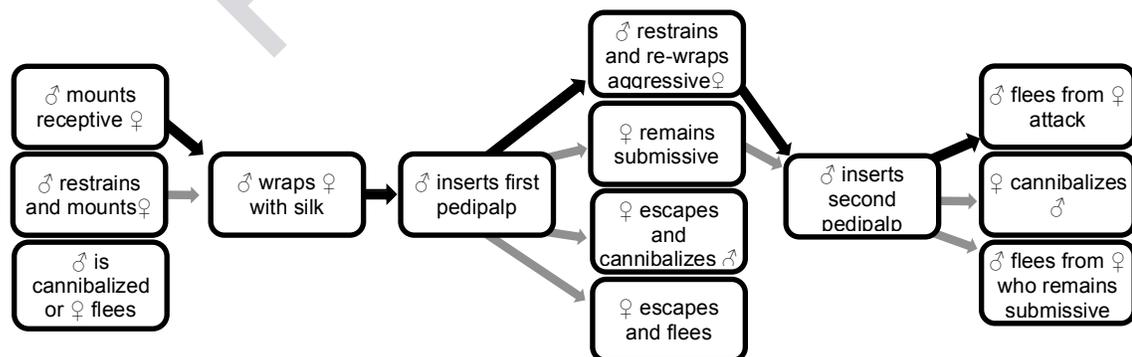


Figure 2. Overview of typical actions and reactions of male and female *P. mira* observed in un-manipulated mating trials carried out in the laboratory. The mating sequence begins when a male locates and approaches a mature female. The **black** arrows indicate the most common mating sequence observed.

transferring sperm. In some cases, males forcibly mount seemingly unreceptive females and once restrained, males then silk wrap the female. After males insert one of their two pedipalps (the male sperm storage organ) to transfer sperm (hereafter called an insertion), females often become aggressive. Males will typically restrain and re-wrap females and achieve one more insertion for a total of two insertions (and longer copulation durations), at which point they quickly flee from aggressive females.

In my first chapter, published in *Biology Letters*, I tested the current function of copulatory silk wrapping by pairing virgin females of random size with virgin males that were experimentally manipulated to either be capable or not capable of silk wrapping. Specifically, I placed dental silicone on either the male's spinnerets (no wrap) or on the dorsal side of the male's abdomen (wrap). While the silk wrapping had no effect on the likelihood of copulation or on sexual cannibalism prior to copulation, I found that males capable of wrapping females were less likely to be cannibalized after or during copulation and were more likely to obtain two versus one opportunity to transfer sperm (termed insertion) (Anderson & Hebets, 2016). When the silk wrapping was not present, females usually attacked and cannibalized males immediately after the first insertion (see Fig. 2). Because females typically show aggression after the first insertion, regardless of whether the wrapping is present or not, we presume that the silk acts as a physical restraint allowing males the opportunity to safely obtain a second insertion. Overall, results from this study suggest that silk wrapping is a sexually selected trait that benefits male reproductive success. Results from this first study led me to explore the potential costs and benefits of increased insertion number and sexual cannibalism avoidance for both males and females within the remaining chapters of my dissertation.

In addition to facilitating copulation, many male-specific mating strategies or morphological traits may function to increase copulation duration. Male “grasping traits” (Sakaluk et al., 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 copulations. Given that copulatory silk wrapping increases the time males spend copulating through increased insertion number, I tested the hypothesis that increased insertion number corresponds to increased sperm transferred and higher fertilization success. Results from this experiment are reported in my second chapter, published in *Animal Behaviour*. In brief, by manipulating male insertion number, I found that males transfer roughly twice the amount of sperm when achieving two insertions compared to only one, and this increase in sperm transfer results in males having approximately 25% more offspring (Anderson & Hebets, 2017).

Copulatory silk wrapping reduces rates of post-copulatory sexual cannibalism. Sexual cannibalism may be a beneficial female strategy resulting in an immediate meal, or, if occurring before mating, may act as a form of mate rejection (Eberhard, 1996; Prenter et al., 2006). Further, sexual cannibalism during sperm transfer may act as a mechanism for females to control the duration of copulation and thus male fertilization success, especially if cannibalism terminates sperm transfer (Elgar et al., 2000). While cannibalism may benefit females in several ways, cannibalism can lead to male fitness costs such as the lost potential to secure future matings or reduced sperm transfer (Elgar & Crespi, 1992; Elgar & Schneider, 2004). Sexual cannibalism can also be an adaptive male strategy (Andrade, 1996; Schwartz, 2013). For example, in the fishing spider, *Dolomedes tenebrosus*, consumption of the male by females significantly increases