

**Sexual dimorphism and sexual selection in the highly dimorphic
orb-weaving spider *Argiope aurantia* (Lucas)**

Matthias W. Foellmer

A Thesis

In

The Department

Of

Biology

Presented in Partial Fulfilment of the Requirements

for the Degree of Doctor of Philosophy at

Concordia University

Montréal, Québec, Canada

April, 2004

© Matthias W. Foellmer



National Library
of Canada

Bibliothèque nationale
du Canada

Acquisitions and
Bibliographic Services

Acquisitions et
services bibliographiques

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

ISBN: 0-612-90384-2

Our file Notre référence

ISBN: 0-612-90384-2

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this dissertation.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de ce manuscrit.

While these forms may be included in the document page count, their removal does not represent any loss of content from the dissertation.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

**CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES**

This is to certify that the thesis prepared

By: **Matthias Foellmer**

Entitled: **Sexual Dimorphism and Sexual Selection in the Highly
Dimorphic Orb-Weaving Spider Argiope Aurantia (Lucas)**

and submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (Biology)

complies with the regulations of the University and meets the accepted standards with
respect to originality and quality.

Signed by the final examining committee:

Dr. S.T. Ali

Chair

Dr. C. Buddle

External Examiner

Dr. B. Woodside

External to Program

Dr. P. Albert

Examiner

Dr. D. Fairbairn

Thesis Co-Supervisor

Dr. J. Grant

Thesis Co-Supervisor

Approved by

Graduate Program Director

April 12, 2004

Dean, Faculty of Arts and Science

Abstract

Sexual dimorphism and sexual selection in the highly dimorphic orb-weaving spider

Argiope aurantia (Lucas)

Matthias W. Foellmer, Ph.D.

Concordia University, 2004

Extreme sexual size dimorphism (SSD) is relatively rare in animal species. Males are much smaller than females for example in anglerfish, some barnacles, and various spiders. Spiders (Araneae) are unique because this is the only free-living terrestrial taxon where extreme SSD is common. The factors involved in the evolution and maintenance of extreme SSD are still poorly understood. Spiders also exhibit a “shape” dimorphism, males having relatively longer legs than females. In this thesis, I estimate selection on adult males of the highly dimorphic orb-weaving spider *Argiope aurantia* to evaluate hypotheses about the adaptive significance of male size and shape in spiders. I use a multivariate approach to distinguish selection targeting different body components. My results suggest that selection tends to favour longer legs during mate search, and that this may result in net selection for overall larger males. During mating, the pattern of selection on males depends on the type of female with which they mate. Most males mate opportunistically with a moulting female when she is defenceless and are under strong selection for large body size due to interference competition over access to females. If males mate with mature, post-moult females, they face an 80% chance of a cannibalistic

attack by the female, but this does not result in selection on male body size. However, the longer males stay in copula, the more likely they are to be cannibalised. Relatively longer legs in males were not favoured during either form of mating and thus probably constitute an adaptation during mate search. Current hypotheses about the adaptive significance of small male size in spiders either predict a small male advantage during mate search or during mating with cannibalistic females, or assume that sexual selection for large size due to interference competition is absent or weak. Neither of these assumptions and predictions were upheld in my study.

Acknowledgements

I am most grateful to my supervisor Daphne Fairbairn for giving me great freedom in pursuing my project while at the same time providing me with exceptional support and advice. She kept me on track in moments when I seemed to lose focus over too many ideas, doubts, or both. My proficiency has tremendously improved through working with her

I am very thankful to my co-supervisor James Grant, who gave me a new “home” at Concordia after Daphne’s move to the Golden State. He has been of great help throughout my project and provided important suggestions.

My committee members Paul Albert and Grant Brown have always been accessible and have given good advice. Paul Albert supported the project logistically in the early stages by letting spiders flourish where flies should have been growing. I thank them both a lot.

Several kind people have contributed in one way or the other to make this part of my life a pleasant and enriching experience: Mattieu Bégin, Angéline Bertin, Ian Ferguson, Jeff Reeve, Laura Weir, Nathalie Brodeur, Jae-Woo Kim, Ivan Dolinsek, and last not least the ever enchanting Stefán Steingrímsson. I thank my various assistants whose dedicated work helped to make this thesis a success: Angela Wilby, Marie-Jose Breau, Genevieve Ring, Jamie Dubois, and Catherine Pollock. Thanks to Paul Widden for the digital photography and to the family Minder for letting me work on their property on Île Perrot.

My wife Christina Mackay has supported me all the way in the most loving fashion. Thank you so much!

Table of Contents

List of Figures	x
List of Tables	xii
Contribution of Authors.....	xiv
General Introduction	1
Chapter 1. Spontaneous male death during copulation in an orb-weaving spider.....	10
Introduction.....	11
Material and Methods	12
Mating behaviour of <i>Argiope aurantia</i>	12
Experimental observations.....	12
Experimental manipulation of individuals.....	13
Results.....	14
Discussion.....	16
Chapter 2. Males under attack: sexual cannibalism and its consequences for male morphology and behaviour in an orb-weaving spider	20
Introduction.....	21
Study system	25
Methods.....	27
Rearing.....	27
Experimental design	28
Analyses.....	30

Table of Contents (continued)

Results.....	31
Episode 1: Crossing the web.....	32
Episode 2: First courtship	32
Episode 3: First insertion	32
Episode 4: Re-approach	35
Episode 5: Second courtship.....	35
Female impact on male survival	36
Fertilization success and total selection on males.....	37
Discussion	38
Chapter 3. Selection on male size and shape during mate search in a sexually highly dimorphic orb-weaving spider	53
Introduction.....	54
Methods.....	57
Study species.....	57
Data collection	58
Data analysis	60
Results.....	62
Selection on male morphology	62
Effects on male condition	64
Discussion	66

Table of Contents (continued)

Chapter 4. Sexual selection favours large body size during opportunistic mating in dwarf males of an orb-weaving spider	84
Introduction.....	85
Methods.....	90
Data collection	90
Data analysis	93
Results.....	95
Direct observations	95
Selection on male morphology	96
Effect of male condition.....	98
Effect of the male's position on the web	98
Lost legs	99
Female size.....	100
Dispersion of males and the relative frequency of opportunistic mating	100
Discussion.....	101
General Conclusions	116
References.....	121
Appendix 1. Size and shape dimorphism in <i>Argiope aurantia</i>	135
Appendix 2. Multiple mating by females and the possible function of embolus caps ...	139
Introduction.....	139
Methods.....	140
Results.....	141

Table of Contents (continued)

Discussion	143
Appendix 3. The frequency of opportunistic mating in unrestricted and caged females	148

List of Figures

Figure 1. A: Adult male <i>Argiope aurantia</i> (preserved specimen). B: Adult female (left) and male <i>A. aurantia</i> on her web.	9
Figure 1.1. Appearance of males after insertion of the second palp.	19
Figure 2.1. Frequency distributions of prosoma width (A) for males ($n = 99$) and females ($n = 99$) used in the experiment and (B) for males ($n = 224$) and females ($n = 143$) from the source population.	50
Figure 2.2. Relative frequencies of attacked and killed males per episode and the number of males present per episode (on the secondary y-axis).	51
Figure 2.3. Timing of the female's attack during the first insertion.	52
Figure 3.1. Means within standard errors of the fixed morphological traits for males that were caught on moulting webs (MW) and on females' webs (FW).	80
Figure 3.2. General male size (PC1) as a function of observation date for males caught on their moulting web (MW) and for males caught on a female's web (FW).	81
Figure 3.3. Male condition (PC2) as a function of observation date for males caught on their moulting web (MW) and for males caught on a female's web (FW).	82
Figure 3.4. Log (opisthosoma area) as a function of log (prosoma width) for males on moulting webs (MW) and males caught on females' webs (FW).	83
Figure 4.1. Frequency distribution of the number of males per female for females caged in the experiment.	111
Figure 4.2. Univariate fitness surfaces (cubic spline estimates) for overall male body size (PC1) and male body size components with standard errors (based on bootstrapping).	112

List of Figures (continued)

Figure 4.3. Mean number of insertions per male (A) and the intensity of selection on overall male body size (B) as a function of the operational sex ratio (OSR)..	113
Figure 4.4. Relative frequency of insertion success (A) and mean overall size within standard errors (B) for males at different positions at the last scan before opportunistic mating..	114
Figure 4.5. Seasonal change in the frequencies of penultimate and adult females and adult males and their association patterns at the study site.....	115
Figure A1.1. The patella-tibia length / prosoma width ratio for all four leg pairs for males and females.	138
Figure A2.1. Frequency distribution of the number of pedipalp insertions received by females in experiment 1.	146
Figure A2.2. Frequency distribution of the number of mates per female in experiment 1.	147

List of Tables

Table 2.1. Regression coefficients with the associated standard errors and Wald statistics for logistic regression of female attack as a function of male morphology during each selection episode (episode 4 is omitted, because no male was attacked).....	45
Table 2.2. Multiple logistic regression analysis of the outcome of an attack during the first insertion as a function of insertion duration, general male size (PC1), and female prosoma width.....	46
Table 2.3. Means and standard errors for total number of eggs, number of fertilized eggs, and proportion of fertilized eggs laid by females that received one or two insertions.	47
Table 2.4. Analysis of covariance of the effect of total insertion duration controlled for number of insertions on the total number of fertilized eggs.	48
Table 2.5. Estimates of overall selection on male morphology during the mating interactions.....	49
Table 3.1. Factor loadings for the first and second extracted principal components after Varimax rotation for each sample.....	73
Table 3.2. Logistic regression analysis of the probability of finding a male on a web of a female as a function of general male size.....	74
Table 3.3. Multiple logistic regression analysis of the probability of finding a male on a web of a female as a function of fixed male morphological traits.....	75
Table 3.4. Univariate logistic regression analyses of the probability of finding a male on a web of a female as a function of fixed male morphological traits.....	76

List of Tables (continued)

Table 3.5. Results of a general linear model of general male size (PC1) as a function of web type and observation day for both samples.	77
Table 3.6. Results of a general linear model of condition (PC2) as a function of web type and observation day for both samples.....	78
Table 3.7. Results of a general linear model of log (opisthosoma area) as a function of web type and log (prosoma width) for both samples.	79
Table 4.1. Factor loadings for the first and second extracted principal components after Varimax rotation.	107
Table 4.2. Selection gradients with standard errors and <i>p</i> -values for the five fixed morphological traits and PC1 (general body size).	108
Table 4.3. Results of a multiple regression analyses of relative fitness as a function of number of males present per female, overall male body size (PC1), and male condition (PC2).	109
Table 4.4. Results of a multiple regression analyses of relative fitness as a function of number of males present per female, overall male body size (PC1), and the position of a male at the last scan before mating.	110
Table A1.1. Means and standard deviations for prosoma width and the average patella-tibia lengths for each of the four leg pairs (in mm).	136
Table A1.2. Results of the RMA (reduced major axis) regression of log(patella-tibia length) on log(prosoma width) for each leg pair for males and females.	137

Contribution of Authors

All chapters of this thesis were prepared for submission to peer-reviewed journals for publication. Dr. Daphne Fairbairn is the co-author of all articles. In her role as supervisor, she contributed to the planning of the studies, to the data analyses, and to the editing of the manuscripts.

Chapter 1 has been published as:

Foellmer, M. W. and D. J. Fairbairn. 2003. Spontaneous male death during copulation in an orb-weaving spider. *Proceedings of the Royal Society of London Series B - Biological Sciences* (Suppl.) 270: S183 -S185.

Chapter 2 has been published as:

Foellmer, M. W. and D. J. Fairbairn. 2004. Males under attack: sexual cannibalism and its consequences for male morphology and behaviour in an orb-weaving spider. *Evolutionary Ecology Research* 6: 163-181.

Chapter 3 has been submitted to *Oecologia* as:

Foellmer, M. W. and D. J. Fairbairn. Selection on male size and shape during mate search in a sexually highly dimorphic orb-weaving spider.

Chapter 4 will be submitted to *Behavioral Ecology* as:

Foellmer, M. W. and D. J. Fairbairn. Sexual selection favours large body size during opportunistic mating in dwarf males of an orb-weaving spider.

General Introduction

Sexual size dimorphism (SSD), a difference in the mean body size of males and females, is a common phenomenon in the animal kingdom. Females are the larger sex in the majority of species (most invertebrates and poikilothermic vertebrates), while males are typically larger than females in mammals and birds (Andersson, 1994; Abouheif and Fairbairn, 1997; Fairbairn, 1997). In a diverse set of phylogenetically unrelated taxa with female-biased SSD the most extreme cases of SSD occur. Males may reach less than one percent of the females' weight in some marine groups (e.g. anglerfish, barnacles), several parasitic species, rotifers, and various spiders (Ghiselin, 1974; Andersson, 1994; Vollrath, 1998). Males of such species are commonly referred to as dwarf males, although this should not automatically imply that extreme SSD is only the result of males shrinking over evolutionary time, as females might also have increased in size (Ghiselin, 1974; Coddington et al., 1997). Dwarf males were often originally ascribed to a different species than the females, and in the most extreme cases adult males represent little more than a testis attached to a female (Vollrath, 1998). Spiders, especially orb-weavers, are unique in that this is the only group of free-living terrestrial organisms in which extreme SSD is common (Ghiselin, 1974; Head, 1995; Vollrath, 1998). With the exception of rotifers, all highly dimorphic species have in common that adult females are relatively sedentary, while males have to engage in mate search, and orb-weavers and crab spiders have been seen as terrestrial analogues of barnacles and anglerfish (Ghiselin, 1974). The factors involved in the evolution and maintenance of extreme SSD are generally poorly understood (Andersson, 1994; Fairbairn, 1997; Blanckenhorn, 2000). Spiders offer a tangible system for study and the evolution of SSD in spiders has become a focus of

current research (e.g. Coddington et al., 1997; Prenter et al., 1999; Moya-Laraño et al., 2002; Higgins, 2002; Walker and Rypstra 2003).

Sexual size dimorphism is generally assumed to be adaptive (Fairbairn, 1990; Andersson, 1994; Fairbairn, 1997). Body size plays a central role in the life history of organisms and potentially affects fitness in many ways (Roff, 2002). SSD is predicted at equilibrium if the optimal size values differ for males and females - that is if net lifetime selection on body size differs for the sexes (Lande, 1980; Hedrick and Temeles, 1989; Reeve and Fairbairn, 2001). Sex-specific patterns of selection often arise as a consequence of the different reproductive roles of males and females (Andersson, 1994; Fairbairn, 1997). For example, selection for increased fecundity probably favours large size in females of many species of poikilotherms (Roff, 2002), while sexual selection operates mainly in males (Andersson, 1994). If body size is heritable, the genetic correlation between the sexes is less than 1 (i.e. is not absolute), and the sexes are under differential lifetime selection, SSD can evolve within given genetic, developmental, and physiological constraints (Lande, 1980; Reeve and Fairbairn, 2001; Badyaev, 2002). Genetic correlations measure the degree of co-variation in the genetic determination of male and female traits (Roff, 1997). While they are usually less than 1, they have the general effect that male and female traits do not respond independently to selection (Lande, 1980; Reeve and Fairbairn, 2001). This can lead transiently to suboptimal SSD, but it has been shown that SSD can evolve rapidly towards equilibrium values (Reeve and Fairbairn, 2001).

Various hypotheses have been put forward to explain the evolution and maintenance of extreme SSD in spiders (Darwin, 1871; Gerhardt, 1924; Ghiselin, 1974;

Vollrath and Parker, 1992; Head 1995; Coddington et al., 1997; Moya-Laraño et al., 2002). These emphasize either selection for large size in females or selection for small size in males, and there has been some controversy about the relative importance of these factors (Coddington et al., 1997; Vollrath and Parker, 1997). However, both types of selection can operate in concert, and the various hypotheses are not mutually exclusive. The best supported hypothesis to date is the “fecundity advantage hypothesis” (Gerhardt, 1924; Head, 1995). Female size correlates positively with fecundity in spiders both at the inter- and intraspecific level, and females show a greater variation in size than males (Marshall and Gittleman, 1994; Head, 1995; Prenter et al., 1999; Higgins, 2002). Within orb-weaving spiders, females appear to have increased in size over evolutionary time in several lineages independently, explaining most of the observed extreme size differences between males and females in this taxon (Coddington et al., 1997; Hormiga et al., 2000). It is less clear however, why males have stayed small or why they even have decreased in size in some lineages (Prenter et al., 1997, 1998; Walker and Rypstra, 2003; but see Vollrath, 1998; Schneider et al., 2000). In the absence of opposing selection, the genetic correlation between males and females would inevitably lead to a correlated increase in male size to some extent (Lande, 1980; Reeve and Fairbairn, 2001).

The “classic” hypothesis about the adaptive advantage of extremely small male size in spiders is the “sexual cannibalism hypothesis” (Darwin, 1871; Bristowe, 1929; Elgar, 1991). Sexual cannibalism occurs frequently in orb-weaving spiders (Elgar, 1992), and according to this hypothesis, small males have an advantage over larger ones, because they fall below a certain threshold above which the female detects approaching males or considers them as valuable prey either before or after copulation (Darwin, 1871;

Bristowe, 1929; Elgar et al., 1990; Elgar, 1991). This hypothesis has so far been evaluated directly only in a few orb-weaver species, with some (Elgar and Fahey, 1996) or no support (Uhl and Vollrath, 1998; Schneider et al., 2000).

The “gravity hypothesis” (Moya-Laraño et al., 2002) posits that large males should be at a disadvantage during mate search in many spider species, because body length is inversely proportional to climbing speed on vertical surfaces. Male spiders typically have to move through a three-dimensional habitat to reach females and thus likely have to climb vertical structures. Therefore, males are predicted to experience size-dependent mortality and/or mating success, with smaller males being favoured. The gravity hypothesis is an extension of Ghiselin’s (1974) small male hypothesis, according to which a sedentary life-style of adult females, combined with low population densities, results in low male-female encounter rates, and thus also in rare encounters between males. Hence, sexual selection for large male size due to male-male interference competition would be absent or weak. This would lead to early maturation of males at a small size and/or to traits being favoured that increase search efficiency in adult males. Arguments of why small males would be more efficient during mate search include increased agility of smaller males (e.g. Neems et al., 1998), and lower energy requirements of smaller males, allowing them to spend more time searching for females (e.g. Blanckenhorn et al., 1995). The gravity hypothesis adds a convincing argument as to why small males might be favoured in spiders. There is to date very little data on size-dependent male performance during mate search in highly dimorphic species, suggesting that intermediately sized males may be favoured (Vollrath, 1980; Christenson, 1990), or that male size is neutral during mate search (Andrade, 2003).

The “differential mortality hypothesis” (Vollrath and Parker, 1992) states that adult males suffer an increased mortality during mate search, compared to the sedentary females - that is they experience a stage-dependent, but not size-dependent mortality. As a result, the operational sex ratio (the ratio of adult males to females that are ready to mate [Emlen and Oring, 1977; Kvarnemo and Ahnesjö, 1996]) would become female-biased, relaxing male-male interference competition and in turn sexual selection for larger males. Absent sexual selection for large size in adult males would lead to selection for survival favouring reduced growth and therefore smaller size in males (Vollrath and Parker, 1992). The differential mortality hypothesis has been contested by comparative analyses that did not find the predicted relationship between life-history (sedentary vs. actively hunting females) and SSD (Prenter et al., 1997; Prenter et al., 1998). Male mortality during mate search has been found to be high in one species (Andrade, 2003), but it is not different from female mortality in another (Walker and Rypstra, 2003).

This thesis aims at improving our understanding of factors contributing to the evolution and maintenance of small male size in spiders by examining sexual selection on male body size in the highly dimorphic orb-weaving spider *Argiope aurantia*, a large, univoltine species common in early successional habitats (old fields) throughout eastern North America (Figure 1; Levi, 1968). In this species, the young spiderlings hibernate within the egg sac and emerge in April or May. Males and females reach maturity in July and August, with males starting to mature about one week earlier than females (Levi, 1968; Howell and Ellender, 1984). As is typical for all orb-weavers, juvenile males share the females’ sedentary life-style and spend almost all of their time on their webs where they rest and catch prey. After the maturation moult, adult males leave their web and

search for females (Foelix, 1996). With their last moult, males adopt a different body “shape”: adult males have relatively longer legs than juveniles or mature females (Foelix, 1996; Legrand and Morse, 2000; Appendix 1). Thus, to fully understand sexual dimorphism in spiders, it is important to also determine the adaptive significance of leg length independent of body size. Relatively longer legs in males have been hypothesized to confer an adaptive advantage during mating with cannibalistic females, during mate search, and during combat against other males (Elgar et al., 1990; Prenter et al., 1995; Bridge et al., 2000; Legrand and Morse, 2000). Throughout this thesis, I use a multivariate approach to examine selection on body size and leg length in males. Despite the widespread application of well established multivariate techniques to distinguish targets of selection (Lande and Arnold, 1983) in many taxa, to the best of my knowledge, no such study has been conducted so far for any spider.

Chapter 1 is an introduction to the mating behaviour of the species and provides the first experimental documentation for any species of sudden, programmed male death during copulation. Because of its focus on this peculiar aspect of the reproductive biology of *A. aurantia*, this chapter is complemented by Appendix 2, which describes the frequency of multiple mating by females.

Chapter 2 evaluates selection on male body size and leg length due to sexual cannibalism. The cannibalistic tendencies of females might not only affect male morphology, but also behaviour. It had previously been hypothesized that sexual cannibalism selects for short copulation duration, because of the increased risk that males would face of being attacked and caught (Elgar, 1995), and any effects of copulation duration might confound size effects. Therefore, in addition to examining selection on

male body size and leg length, I analyse the mating interaction with the aid of detailed video recordings and estimate selection on copulation duration.

In Chapter 3, I examine selection on male morphology during mate search. Due to the small size of males and the complexity of the habitat, individuals are notoriously difficult to follow in the field. Therefore, I conduct a cross-sectional study and compare male phenotypes before and after mate search. Size effects during mate search may also depend on energy reserves a male has available. Adult males do not feed while roving, and a size-dependent differential energy storage capacity may thus affect male performance during mate search. Therefore, I also investigate the scaling relationships between male size and energy reserves, and the differential loss of reserves.

Chapter 4 examines whether direct interference competition among males over access to females operates in *A. aurantia* and possibly generates sexual selection for large body size in males. As is typical for many orb-weavers, males cohabit with juvenile females and wait for them to undergo the maturation moult (e.g. Robinson and Robinson, 1980; Jackson, 1986). During the cohabitation period, many males may accumulate on a female's web, and may thus directly compete over access to the female (e.g. Robinson and Robinson, 1980, Elgar et al., 2003). It has been shown that larger males usually win staged contests over smaller ones even in dimorphic species (Elgar, 1998), but whether this actually translates into selection for large male size in a population is unknown. This will depend on how frequently males encounter other competitors and on whether females are defensible in the presence of other males (Legrand and Morse, 2000; Grant et al., 1995; Mills and Reynolds, 2003). Clarification of this issue is important, because both the differential mortality hypothesis and Ghiselin's (1974) small male hypothesis

assume that sexual selection favouring large size in males due to interference competition is absent or weak.

In summary, this thesis constitutes the first comprehensive multivariate analysis of sexual selection on male morphology in any spider. The examination of sexual selection in the highly dimorphic species *Argiope aurantia* allows an evaluation of hypotheses regarding the adaptive significance of male body size and thus the extreme sexual size dimorphism commonly found in spiders.

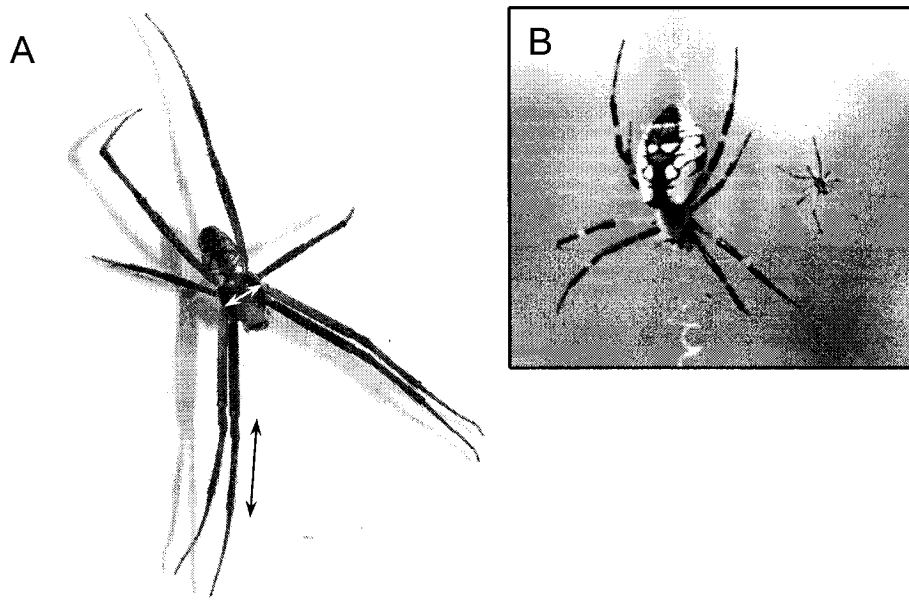


Figure 1. A: Adult male *Argiope aurantia* (preserved specimen). The markers depict traits on which selection was measured throughout this thesis. White arrow: prosoma width, black arrow: patella-tibia length, here highlighted only for the first right leg. B: Adult female (left) and male *A. aurantia* on her web.

Chapter 1. Spontaneous male death during copulation in an orb-weaving spider

An understanding of the mating behaviour of a species is a pre-requisite for examining sexual selection. This chapter is an introduction to the mating behaviour of *Argiope aurantia*, and offers the first experimental evidence of sudden, programmed death during copulation in any species. It is supplemented by Appendix 2, which investigates multiple mating by females.

This chapter was published in June 2003. Since then, Knoflach and Benjamin (2003) published a note describing the copulatory behaviour in the cob-web weaver *Tidarren sisypoides*. They suggest that males in this species also die during copulation. Therefore, this peculiar trait may have evolved at least two times independently in spiders.

Introduction

Mating can be deadly for males. Examples of sexual cannibalism where the female devours the male during or after copulation are well known, especially from many species of spiders and praying mantids (Elgar, 1992). In many cases, cannibalism is probably maladaptive for the males, and males try vigorously to escape the cannibalistic female (Elgar, 1992; Arnqvist and Henriksson, 1997; Maxwell, 1998). However, in some species of orb-weaving spiders and ceratopogonine midges, males apparently follow an adaptive strategy by sacrificing themselves and allowing the female to eat them (Blanke, 1975; Downes, 1978; Forster, 1992). In this context, sexual selection for male complicity in sexual cannibalism has been demonstrated for the redback spider (Andrade, 1996; 2000; Andrade and Banta, 2002).

Whether males assist or not, all of these cases have in common that males are killed by females. In contrast, anecdotal accounts suggest that males of some species in the cannibalistic orb-weaving spider genus *Argiope* may die spontaneously during copulation, without female collaboration (Gerhardt, 1933; Robinson and Robinson, 1980). Sasaki and Iwahashi (1995) showed that in *A. aemula*, males do not try to escape from the female after mating and die soon (often within one day) even when females are prevented from eating them.

Here we demonstrate that males of the orb-weaving spider *Argiope aurantia* experience programmed sudden death after onset of copulation. We show that all males exhibit the typical dead posture just after they insert their second pedipalp and before the female can bite them. Further we determine that this signals rapid cessation of heartbeat, and thus death, without female complicity.

Material and Methods

Mating behaviour of *Argiope aurantia*

In spiders, the pedipalps (the pair of extremities posterior to the fangs) are morphologically derived to function as copulatory organs (Foelix, 1996). Each palp inserts into one of the female's paired genital openings, which lead to separate sperm storage organs. Upon insertion, the distal bulb of the palp is inflated through increased hemolymph pressure, which leads to the coupling of the palp with the female's genital plate. In *A. aurantia*, males pursue two alternative mating tactics, depending on whether they encounter a juvenile or a mature female in the field (Robinson and Robinson, 1980). If they encounter a penultimate female (a juvenile just one moult from adulthood), they often cohabit with the female on her web and wait for her to undergo the maturation moult. They then try to copulate (i.e. achieve two pedipalp insertions) with the female during her moult, when she is defenceless and has no overt control over mating (opportunistic mating). If males encounter an already mature female, they court her and, if the female accepts, the male inserts the first palp. He then has to withdraw and re-court the female to achieve the second insertion. However, in this situation the female may attack the male at any time.

Experimental observations

We conducted a series of observations to determine how frequently males die spontaneously during the second insertion, and whether males die regardless of the state of the female.

We observed 16 opportunistic matings during which males achieved two insertions. Two matings were observed naturally in the field. Ten cases involved caged

individuals in a field experiment, and in four instances field-caught individuals mated in the laboratory. Two of the latter matings were videotaped. These observations involved a total of 13 females. Two females had one male present on their web at the time of mating, the other 11 had two or more males on their web.

We also videotaped 99 staged matings between mature virgin females and males (laboratory-reared). Each individual was used only once. Mean adult age (days from maturation moult) \pm 1 s.d. was 5.5 ± 1.7 d for females, and 17.2 ± 2.2 d for males (the species is protandrous). Mean adult size (prosoma width at broadest point) \pm 1 s.d. was 4.0 ± 0.3 mm for females, and 2.1 ± 0.2 mm for males. Females were allowed to build a new orb-web in a mating cage (aluminium screen cages, 45 x 45 x 15 cm) before they were used in a trial. At the beginning of each trial, we placed males carefully into an upper corner of the cage without touching the female's web. Upon finding the female's web structures, males usually approached and courted the female. Mating trials lasted until males had achieved two insertions or until all mating interactions had ceased for at least $\frac{1}{2}$ h. We separated males from females after mating to prevent females from eating the males. We then inspected the males under a dissecting microscope.

Experimental manipulation of individuals

Nearly half of the palps inserted second were severely damaged (see Results). If palpal damage causes the death of males (pedipalps do not autotomize), experimentally detaching the palps might yield the same result and clarify the trigger of death. To test the hypothesis that detaching the palps causes the death of males and to describe the symptomatic pattern of male death, we compared males that had inserted both their palps with males that had their palps experimentally removed. We used field-collected

individuals, all virgin at the time of the experiment. Males were collected as adults and checked for virginity by inspecting their palps. Females were collected as penultimates and allowed to moult to maturity prior to the experiment. Individuals were assigned randomly to four treatments (two main effects and a double control). Males in the first treatment were allowed to insert both palps, but were separated from the female within 5 s after inserting the second palp, before she could bite them ($n = 7$). Males in the second treatment were held gently between two fibreglass screens glued to circular plastic frames. We then detached both palps using fine tweezers ($n = 8$). We held males of the third treatment similarly between fibreglass screens and quickly cut the first pair of legs through the femur or tibia to control for hemolymph leakage resulting from palp detachment ($n = 6$). Males of the fourth treatment (handling control) were held between the screens for a similar amount of time (2 min; $n = 7$). After their specific treatment, we placed each male in a Petri dish, poked him with tweezers to provoke a reaction and recorded his behaviour. We observed each male under the dissecting microscope to check for movement and heartbeat. These observations were recorded immediately after the respective treatment, and we checked again for heartbeat after 5, 15 and 30 min.

Results

During opportunistic mating, all males that achieved two insertions assumed the typical dead posture with legs folded under the body while still in copula (Figure 1.1a). Males competed over access to the female, and often attacked any male that had inserted a palp. They tried to pull out dead males that were anchored by means of their second inserted palp, but they succeeded in only three out of eleven cases. After 15 – 25 min females came to sit on the web and eventually pulled out the apparently dead male stuck

in their genital opening. They did not eat males right away, probably because their cuticle has to harden first. Thus none of the males survived opportunistic mating, even though females could not attack them.

During the staged matings with already mature females, all males vigorously tried to escape from the female by jumping off her body after a very short insertion of the first palp (median = 3.5 s, range = 1 - 27 s, n = 97). However, upon insertion of the second palp all males (n = 44) became completely motionless, and were pulled out by the female after a median duration of 8 s (range = 1 - 55 s). Males exhibited the dead posture (Figure 1.1b), and this was evident before the female could bite them (the female has to move the male towards her fangs to bite him). None of these males reacted when pinched with tweezers. Note that females attacked males with similar frequency during the first and second insertion (77%, n = 97, and 82%, n = 44, respectively; $\chi^2 = 0.4$, d.f. = 1, $p > 0.3$), and 38% (17/44) of males that inserted twice had visible bite marks. Thus in these cases the motionless males had been bitten before we separated males and females. Forty-five percent (20/44) had one palp ruptured, that is hemolymph was leaking out of a wound probably caused by torn membranes of the pedipalp. None had both palps ruptured. Ruptured palps were not found in any of the 22 males that were caught by the female after insertion of the first palp, and the difference between these groups is significant ($\chi^2 = 13.9$, d.f. = 1, $p < 0.001$). This suggests that only the palps inserted second were ruptured, either because females have to pull males out forcefully after the second insertion, or because second palps are damaged during intromission.

In our experiment with manipulated individuals, treatment type had an obvious effect on male response. All males in the “palps inserted” and “palps detached”

treatments exhibited the typical dead posture, whereas this occurred in only 33.3% and 0% of individuals in the “legs detached” and “handling control” treatments respectively (Fisher’s exact test, $p < 0.0001$). When poked with tweezers, no males that had inserted their palps reacted, whereas all males of the other treatments showed a reaction (Fisher’s exact test, $p < 0.0001$). All individuals except those in the “palps inserted” treatment maintained a heartbeat over the entire examination period. In contrast, the hearts of all males in the “palps inserted” treatment stopped beating within 15 min of insertion, and the heartbeat of these males was generally slower than that of males in the other treatments. Heartbeat could not be detected for one male (14%) at the first scan, for three males (43%) after 5min, and for the remaining three males after 15min. Thus, although detaching the palps results in some of the symptoms characteristic of males after the second insertion, insertion of the second palp itself with its accompanying processes (increase of hemolymph pressure to inflate the distal bulb of the palp) appears to be necessary to initiate the full cascade of events leading to the death of male *A. aurantia*.

One peculiar additional observation involved a male who inserted his first palp in a female, but re-courted a carcass of a *Tenebrio molitor* larva that was present in the web instead of re-courting the female. This male inserted his second palp in the carcass. Immediately upon inflation of the distal bulb of the palp, the male assumed the dead posture. The palp was not ruptured. Since the female was not in contact with the male, it is obvious that male death occurs even without contact with the female.

Discussion

Our results clearly indicate that male *A. aurantia* die as a consequence of inserting their second palp. Females do not appear to be complicit in their death. With insertion of

the second palp, a physiological process commences which leads to immediate (i.e. within seconds) lack of responsiveness and to certain death (as defined by the cessation of the heartbeat) within 15 min. The death of the male who mated mistakenly with the mealworm carcass suggests that this process initiates when the distal bulb of the palp is inflated, and requires neither actual contact with the female nor rupturing of the palp.

Determining the adaptive significance of sudden male death will be difficult, given that it appears to be a fixed trait (i.e. it occurred in all males sampled). Male self-sacrifice during copulation is seen as a form of terminal reproductive investment, and is expected to evolve when the benefits of this investment outweigh the cost of foregoing future mating opportunities (Buskirk et al., 1984; Johns and Maxwell, 1997). Males may benefit for two reasons. First, males may contribute their soma to increase the quantity and/or quality of the female's offspring. In *A. aurantia*, such an effect is possible, but it is probably not a major factor given the small size of males compared to females (see Andrade, 1996; Elgar et al., 2000). Second, males may increase their paternity, because females copulate longer with them and/or are less likely to re-mate. When mating with a mature female, males cannot gain extra copulation time, because females pull them out. However, males may gain a paternity advantage by dying during opportunistic mating. Males fight over access to the moulting female, and try to dislodge any male that has a palp inserted. Because the palps of dead males are fixed in the inflated state and are therefore harder to remove, dead males act as whole-body mating plugs, often preventing other males from copulating. On average 60% of females are mated opportunistically in the field (Chapter 4). Opportunistic mating is thus an important component in the

evolution of the mating system of this species, and we postulate that spontaneous male death has evolved in that context.

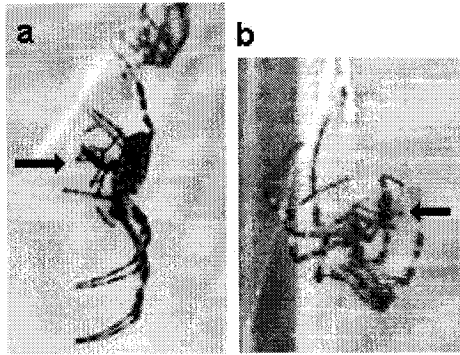


Figure 1.1. Appearance of males after insertion of the second palp. Arrows point to males in dead posture with legs folded under the body. (a) Mating with a moulting female: the female has come to sit on the web after the moult and the male's pedipalp is still anchored in the female's genital opening. At the top right corner is the female's exuvia visible. (b) Mating with an older mature female: the male has just been pulled out by the female and she is wrapping him with silk.

Chapter 2. Males under attack: sexual cannibalism and its consequences for male morphology and behaviour in an orb-weaving spider

This chapter examines sexual selection on males due to sexual cannibalism. Besides testing the “classic” sexual cannibalism hypothesis about the adaptive significance of small male size in spiders, selection on leg length and copulation duration is also examined. I use detailed video recordings to determine whether the copulation duration is under male or female control.

Introduction

Sexual selection is a powerful process shaping the morphology and behaviour of organisms (Andersson, 1994), leading for example to the evolution of impressive weaponry (e.g. Silverman and Dunbar, 1980), elaborate courtship displays (e.g. Petrie et al., 1991), complex genitalia (e.g. Simmons, 2001) or even self-sacrifice in males (Andrade, 1996). One potentially very potent factor resulting in selection on males in the context of reproduction is sexual cannibalism. Sexual cannibalism occurs when the female kills and devours the male before, during, or immediately after copulation (Elgar, 1992). It is known from a variety of invertebrate taxa, where females are usually larger than males, but occurs most frequently in praying mantids and spiders, and especially in highly dimorphic orb-weaving spiders (Elgar, 1992; Johns and Maxwell, 1997).

The fitness consequences of sexual cannibalism for males depend on the timing of cannibalism. If males are killed before copulation while they are approaching or courting the female, they achieve zero fitness. If males are cannibalized during or after copulation, after at least some sperm has been transferred, the net effect of sexual cannibalism on males will depend on the balance of the costs and benefits they incur (Buskirk et al., 1984; Johns and Maxwell, 1997; Andrade, 2003). Costs may consist of reduced sperm transfer before being killed and of losing any further reproductive opportunities. Benefits result if the contribution of the male's soma increases the quantity and/or quality of the female's offspring, or if the male increases his paternity, because the female may actually copulate longer with him or is less likely to re-mate (Andrade, 1996). Therefore, sexual cannibalism can be adaptive for males, and this has been demonstrated for the redback spider *Latrodectus hasselti* (Andrade, 1996, 2003; Andrade and Banta, 2002). Male *L.*

hasselti exploit the cannibalistic tendencies of females by enticing the females to eat them (Forster, 1992; Andrade, 1998). Similar self-sacrificial behaviour may also occur in other species (Grasshoff, 1964; Blanke, 1975; Downes, 1978; Sasaki and Iwahashi, 1995; Knoflach and Van Harten, 2001). However, in most species sexual cannibalism is probably maladaptive for the male and males typically approach females cautiously and try vigorously to escape after mating (Robinson and Robinson, 1980; Gould, 1984; Birkhead et al., 1988; Elgar, 1992; Lawrence, 1992; Prenter et al., 1994; Elgar and Fahey, 1996; Arnqvist and Henriksson, 1997; Maxwell, 1998; Uhl and Vollrath, 1998; Elgar et al., 2000; Johnson, 2001; Schneider and Elgar, 2001; Schneider et al., 2001).

Sexual cannibalism has been hypothesized to drive the evolution of various morphological and behavioural traits in male orb-weaving spiders (Araneoidea) (reviewed in Robinson, 1982; Elgar, 1992). For example, male body size has been considered to be under selection due to sexual cannibalism, because it might affect the risk of being attacked and/or the ability to escape. Small male body size has been suggested to be advantageous in highly sexually dimorphic species, because small males might fall below a certain threshold above which females would detect approaching males or consider them as valuable prey. Sexual cannibalism may thus be one factor contributing to the maintenance of extreme sexual size dimorphism in some species (Darwin, 1871; Elgar, 1991; Newman and Elgar, 1992; Elgar and Fahey, 1996; but see Uhl and Vollrath, 1998; Schneider et al., 2000). In contrast, large size may be favoured in the less dimorphic common garden spider *Araneus diadematus*, a species of the subfamily Araneinae, in which mating takes place on a mating thread outside the

female's web. Large males are better at escaping the female and achieve more pedipalp insertions (Elgar and Nash, 1988).

Leg length in males also appears to be important in the context of sexual cannibalism. Males typically use their legs to jump quickly off the female after copulation (Robinson and Robinson, 1980; Elgar et al., 1990; Prenter et al., 1995). In many Araneinae, males also use their legs to bring the female into the right position for intromission. Comparative data suggest that relatively longer legs may be favoured through a better ability to escape the female in species in which males are relatively small compared to females (Elgar et al., 1990), although direct evidence of such an effect is lacking. The significance of leg length during mating interactions involving cannibalistic females is unknown for other orb-weaver taxa. However, it is often reported that males lose legs while escaping the attacking female (e.g. Robinson and Robinson, 1980; Sasaki and Iwahashi, 1995). Thus, longer legs may actually be disadvantageous in some cases, because they offer a larger target for the female.

Sexual cannibalism may also influence the duration of copulation. Within spider families where sexual cannibalism is relatively common, copulation duration is shorter in cannibalistic genera than in non-cannibalistic ones (Elgar, 1995). This pattern could arise for two reasons. First, males staying longer in copula may face an increased risk of being cannibalized (Schneider and Elgar, 2001). Second, females may use cannibalistic attacks to control the copulation duration and therefore paternity of their mates (Elgar et al., 2000). Copulation duration is often positively correlated with fertilization success (e.g. Elgar et al., 2000; Schneider et al., 2000). Thus, in species in which males control the

duration of copulation males may often face a trade-off between current and future reproductive investment.

In this study we investigate whether copulation duration, male body size, and male leg length are under selection due to sexual cannibalism in the orb-weaving spider *Argiope aurantia* (Araneidae: Argiopinae). *Argiope aurantia* is a large orb-weaving spider species with pronounced sexual size dimorphism (SSD). Males are much smaller than females. The factors involved in the evolution and maintenance of extreme SSD, which occurs frequently in spiders, are a focus of current research (e.g. Vollrath and Parker, 1992; Coddington et al., 1997; Prenter et al., 1998, 1999; Legrand and Morse, 2000; Schneider et al., 2000; Moya-Laraño et al., 2002; Higgins, 2002; Walker and Rypstra, 2003). Yet our understanding of the adaptive significance of SSD in spiders is still poor and studies investigating the effect of sexual cannibalism on male size have so far focused almost exclusively on one genus, *Nephila* (Elgar and Fahey, 1996; Uhl and Vollrath, 1998; Schneider et al., 2000; Schneider and Elgar, 2001; but see Elgar et al., 2000). For example, almost nothing is known to date about the selective processes that determine male body size in *Argiope*, which belongs to a lineage in which extreme SSD has evolved independently from *Nephila* (Hormiga et al., 2000). In *Argiope keyserlingi*, females apparently allow smaller males to achieve longer copulations through sequential mate choice by timing the cannibalistic attack (Elgar et al., 2000). However, on average smaller males did not achieve longer copulations than larger males, and cannibalism is clearly not adaptive for males in this species (Elgar et al., 2000). The significance of this type of female choice for body size evolution remains therefore unclear.

Male spiders generally have relatively longer legs than females, probably as a result of sexual selection (Prenter et al., 1995; Foelix, 1996). Thus, to understand fully sexual dimorphism in spiders it is important to determine the adaptive significance of leg length independent of body size. Longer legs are thought to confer an advantage for example during mate search in a three-dimensional habitat and during agonistic interactions among males (Legrand and Morse, 2000; Bridge et al., 2000). Sexual cannibalism might select for relatively longer or shorter legs (see above). To our knowledge, no study has yet directly examined selection on leg length independent of body size in any spider.

In this paper we try to answer the following questions. 1) Is male body size under selection due to sexual cannibalism? 2) Is leg length in males under selection independent of body size? 3) Is the duration of copulation under male control, and if so, are shorter copulations favoured because of an increased likelihood of survival? To answer these questions we videotaped all interactions on females' webs and analyzed five selection episodes according to the approach, courtship and mating sequences occurring in this species.

Study system

In male spiders the pedipalps (the pair of extremities posterior to the fangs) are morphologically derived to function as copulatory organs. Each palp inserts into one of the female's paired genital openings, which lead to separate sperm storage organs. Male *A. aurantia* always try to insert both palps into the same female to complete copulation. If a male encounters the web of a mature female, he slowly crosses the web to reach the female, who rests at the hub of the web. Once in contact with the female, the male

commences his tactile courtship, which typically consists of very rapid tapping of the four anterior legs against the female's legs and body with intervening walkabouts over and around the female (for a detailed description of the courtship behaviour of this species see Robinson and Robinson, 1980). A receptive female responds to the male's efforts by assuming a characteristic acceptance posture. She lifts the anterior part of the body so that her body is held at an angle to the web plane and the first one or two pairs of legs may be lifted off the web. The male has to move under the female's body to reach the genital openings, which are situated anteriorly on the ventral surface of the opisthosoma, and he then inserts his first palp (Robinson and Robinson, 1980). After only a few seconds the male typically tries to escape the female by quickly jumping off. If he escapes, the male usually re-approaches by climbing back on his security thread. He has to court the female again in order to achieve a second insertion with the other palp. The female frequently attacks the male during pedipalp insertion (Foellmer and Fairbairn, 2003).

A unique feature of *A. aurantia* is that immediately after insertion of the second palp males become motionless and die within minutes (Foellmer and Fairbairn, 2003). Male self-termination probably evolved in a context other than sexual cannibalism. If male *A. aurantia* encounter a juvenile female just one moult from adulthood during their mate search, they often cohabit with the female and wait for her to undergo the maturation moult to pursue an alternative mating tactic. They try to copulate with the female during her moult, when she is defenceless (opportunistic mating) (Robinson and Robinson 1980). Males do not face cannibalistic attacks during opportunistic mating, but experience fierce competition over access to the female. Dead males probably act as

whole-body mating plugs, preventing other males from inserting their palps (Foellmer and Fairbairn, 2003). Therefore, because males die spontaneously after inserting their second palp, they can achieve a maximum of only two insertions.

Methods

Rearing

We reared individuals in the laboratory from egg sacs built by females that we collected in an old field on Île Perrot near Montreal, Quebec. Spiders were kept under controlled conditions (LD 16 : 8, Temp 26° : 20°C) in glass terrariums with chicken wire as web supports. We transferred penultimate females into individual aluminium screen cages (45 x 45 x 15 cm) where they moulted to maturity and remained until after the mating trial. We transferred adult males into individual plastic vials (50 x 23 mm) after they had left their moulting webs to search for females. All individuals were provided with water by gently spraying the webs. Young instars were fed freely flying *Drosophila melanogaster*, which they had to catch with their web. From approximately the fifth instar on, the diet was supplemented with small *Tenebrio molitor* larvae. We fed penultimate and adult females with two large *T. molitor* larvae three times per week. Adult males do not build catching webs but may scavenge prey present in the female's web. We provided them with bits of fresh liver once a week.

We examined whether the use of lab-reared individuals in our study would affect the power to detect any body size effect by testing whether the size range of our experimental males corresponded to the size range found in the source population (systematic sampling of adult individuals found within a 1030 m² area during the mating season in 2000). Mean prosoma width of experimental males and females was greater

than that of field caught individuals (Figure 2.1; males: $t = -16.1$, $P < 0.001$, d.f. = 139.4 [equal variances not assumed]; females: $t = -8.6$, $P < 0.001$, d.f. = 237.3 [equal variances not assumed]). This indicates that lab-reared individuals benefited from the constant supply of prey and ambient temperatures. Sexual size dimorphism (Female/male prosoma width ratio) was very similar (experimental spiders = 1.9, source population = 2.0). Prosoma width of experimental males was more variable than that of males measured in the natural population (Levene's test: $F = 17.7$, $P < 0.001$). Thus, the variation in the experimental population should be sufficient to detect selection on male morphology.

Experimental design

We measured the following traits for adults: male and female prosoma width (at the broadest point), male opisthosoma length, body length, and the average combined patella-tibia length for each pair of legs. Male prosoma length was estimated as body length minus opisthosoma length. All measurements were taken from the dorsal aspect under a dissecting microscope with individuals held gently between two lids of Petri dishes balanced with cotton. Repeatability of male prosoma length was 0.88, repeatability of all other measurements was > 0.97 ($n = 10$, $k = 3$ repeated measures). The video analysis revealed that the first insertion is always terminated by the male, not by the female (see Results). Therefore, in addition to the morphological traits we included the duration of the first insertion as a male behavioural trait in the analyses.

In the field males infrequently cohabit with mature females, and rarely is more than one male associated with a mature female. In a population in an old field in Quebec in 2000, 89.9% (187/208) of sampled webs with mature females had no male present during the mating season, 7.7% (16/208) had one male present, and 2.4% (5/208) had

more than one male present (see Chapter 4). A mature female is therefore unlikely to be courted by more than one male at any given time. Further, while several males may mate with a newly-moulted female (opportunistic mating), mature mated females do not frequently re-mate (Appendix 2). We therefore estimated selection on males using a simple experimental design in which we introduced one virgin adult male into a cage with one virgin adult female.

The pool of individuals available for mating on any given day consisted of females that had built at least one new catching web after the maturation moult (mean age in days after final moult = 5.5 d, s.d. = 1.7 d), and of males that were at least eight days after the maturation moult, because this species is protandrous (mean age = 18.1 d, s.d. = 2.4 d; male age had no effect on fitness [results not shown]). Males and females were chosen randomly from the available pool, and each male and female was used only once. Each mating trial began when we placed a male carefully into one of the upper corners of a screen cage containing a female without touching the female's web. Males that started walking around usually found web support threads quickly and approached the female on her web. Mating trials lasted until males had achieved two insertions or were killed, or until all mating interactions had ceased for at least $\frac{1}{2}$ h. We did not allow females to consume any males. All interactions ($n = 99$) were videotaped for later analysis. We weighed females immediately after the experiment to obtain an estimate of female condition (calculated as the residuals of the regression of female mass on prosoma width) on the day of the trial. We kept females until their natural death and stored any egg sacs in high humidity at 25°C for one month. The egg sacs were then preserved in alcohol and any spiderlings and sterile eggs were later counted under a dissecting microscope.

Analyses

For our analyses we distinguished five selection episodes during courtship and mating and scored male fitness for each episode as successful or unsuccessful (the criterion of success is given in parentheses): 1) crossing the web (male reaches hub), 2) first courtship (leads to insertion), 3) first insertion (male survives), 4) second approach (male reaches hub again), and 5) second courtship (leads to second insertion). For each stage we recorded whether the male was attacked (yes/no) and whether the male survived the attack (yes/no).

Principal components analysis of male morphological traits extracted only one component (PC1) explaining 93% of the total variance. We analyzed male fitness as a function of the general size of males using PC1 as the independent variable in univariate regressions. To examine selection on leg length independent of body size we performed multiple regression analyses with the average patella-tibia length of the first pair of legs and prosoma width as predictors. While representing only a single linear dimension, prosoma width is a reasonable indicator of body size in spiders (Foelix 1996). In multivariate regression models the partial regression coefficients estimate the effect of one trait holding the effect of other traits constant (Kleinbaum et al. 1998). We included only the first pair of legs to preserve power, since all leg measurements were highly correlated (Pearson $r > 0.97$). The first pair of legs is especially important during male-female interactions, because it is used along with the second pair in the male's tactile courtship to stimulate the female. It is also the longest leg pair, thus possibly offering the female a good target to catch the male. We used logistic regression to test models with dichotomous response variables (Kleinbaum et al. 1998). Predictors were either normally

distributed or successfully normalized through appropriate transformation. All variables were standardized to a mean of zero and a standard deviation of one to facilitate comparisons and to calculate selection gradients for overall selection on males during the mating interactions (using non-standardized data did not change the statistical conclusions).

Our goal in this paper was to determine whether male morphology and the duration of the first insertion are under selection due to sexual cannibalism. However, the likelihood of a male being attacked might also depend on other factors, for example characteristics of the female. To evaluate this effect, we conducted exploratory analyses of the following additional variables: female age, size, condition, and courtship duration.

Results

Females attacked males during all episodes of the mating interaction except during the re-approach of the males. The frequency of attacks was especially high during the first and second insertion where about 80% of males present during the respective episode were attacked (Figure 2.2). However, only during episode three (first insertion) were a substantial number of males (22/97: 23%) caught and killed. Only one male was killed during the first courtship and none during the other episodes. Almost all males (97/99: 98%) achieved the first insertion. Most males that survived the first insertion (69/75: 92%) courted the female again. Sixty-four percent (44/69) of these males achieved a second insertion (44% of all males). All males died spontaneously during the second insertion and were eventually pulled out by the female.

Episode 1: Crossing the web

All males approached the female slowly and with frequent hesitation, occasionally plucking at silk strands as they moved towards the female. Almost a third of all males (27/99: 27.3%) were attacked by the female, but this was independent of male morphology (Table 2.1). All males reached the hub.

Episode 2: First courtship

All females reacted to the courting male by assuming the acceptance posture. Ten percent (10/99) of males were attacked during courtship, and one was killed. Male morphology did not predict the probability of an attack during the first courtship (Table 2.1). Due to the low variance in male fitness (98% achieved the first insertion) we did not test for an effect of male morphological traits on fitness.

Episode 3: First insertion

The attack

Seventy-seven percent (75/97) of males were attacked during the first insertion (Figure 2.2). An attack commenced when the female collapsed over the male, flipping about 180°. The female then started to throw silk over the male while he was still inserted. Larger males were attacked more frequently (Table 2.1). Multiple regression analysis indicated that this effect was due to males that were attacked having relatively longer legs. However, neither effect was significant after correcting for multiple tests. The likelihood of being attacked was independent of the insertion duration ($n = 96$, $b \pm \text{s.e.} = -0.071 \pm 0.247$, Wald = 0.08, d.f. = 1, $P > 0.7$). Males always tried to escape from the female by jumping off her body (median insertion duration = 3.5 s, range = 1 - 27 s, $n = 97$). Some males whose first jump was blocked by the female's silk threads still

managed to escape by what appeared to be slipping through the layers of silk with extended legs. Males that got entangled in the female's threads were inevitably caught.

Twenty-two (29%) of the attacked males were caught and killed, while 53 (71%) males escaped and survived. The timing of the female's attack (seconds after palp insertion, Figure 2.3) did not differ between males that survived and those that did not ($U = 566, P > 0.8$). However, males that were killed had stayed inserted for a longer time after the onset of the female's attack than males that survived ($U = 358, P < 0.01$). Thus, the longer a male inserted his palp, the more likely he was to be caught. The likelihood of a male being killed once attacked was independent of general male size (PC1: $n = 75, b \pm \text{s.e.} = 0.243 \pm 0.255, \text{Wald} = 0.90, \text{d.f.} = 1, P > 0.3$). Likewise, neither male prosoma width nor patella-tibia length independently influenced the ability to escape (prosoma width: $n = 75, b \pm \text{s.e.} = 0.127 \pm 0.851, \text{Wald} = 0.02, \text{d.f.} = 1, P > 0.8$, patella-tibia length: $n = 75, b \pm \text{s.e.} = 0.133 \pm 0.854, \text{Wald} = 0.02, \text{d.f.} = 1, P > 0.8$, multiple logistic regression model: $\chi^2 = 1.03, P > 0.5$).

Leg loss

Fifty-seven percent (30/53) of surviving males lost at least one leg, whereas only 14% (3/22) of males that were not attacked lost one leg during the first insertion ($\chi^2 = 11.65, \text{d.f.} = 1, P < 0.001$). This suggests that males often autotomize legs to increase their chance of escaping the attacking female. Note that a comparison of males that survived the attack versus those that were caught was not possible, because we could not determine with confidence which legs males had autotomized before they were definitely caught by the female. Surviving males were most likely to autotomize a leg of the first pair of walking legs. Of the 30 males that lost at least one leg, 23 lost at least one leg of

the first pair, but none of the three hind leg pairs. Two males lost one leg of the first pair and another leg, and five males lost one leg of the hind three pairs. We tested whether males with longer legs were more likely to autotomize legs in an attack, thereby possibly compensating for a greater risk of being caught. Absolute patella-tibia length did not predict leg autotomy (univariate logistic regression: $n = 53$, $b \pm \text{s.e.} = -0.072 \pm 0.291$, Wald = 0.06, d.f. = 1, $P > 0.8$). Multiple logistic regression of leg loss on prosoma width and patella-tibia length indicated a non-significant trend for small males with long legs to be more likely to lose at least one leg of the first pair in the attack (patella-tibia length: $n = 53$, $b \pm \text{s.e.} = 2.032 \pm 1.091$, Wald = 3.47, d.f. = 1, $P < 0.1$; prosoma width: $n = 53$, $b \pm \text{s.e.} = -2.301 \pm 1.165$, Wald = 3.90, d.f. = 1, $P < 0.05$; model $\chi^2 = 4.56$, $P > 0.1$).

Selection during the first insertion

To determine whether the cannibalistic behaviour of females resulted in selection on insertion duration during this episode, we included all males present during the episode in the analysis, i.e. males that were not attacked as well as those attacked. Insertion duration was a significant predictor of survival during the first insertion ($n = 96$, $b \pm \text{s.e.} = -0.600 \pm 0.262$, Wald = 5.24, d.f. = 1, $P < 0.025$). Overall larger males with relatively longer legs were more likely to be attacked and several males were killed. This could lead to differential survival during the first insertion, even though the likelihood of being killed was independent of male morphology. However, there was no selection on general male size (PC1: $n = 97$, $b \pm \text{s.e.} = 0.081 \pm 0.244$, Wald = 0.11, d.f. = 1, $P > 0.7$) and no direct selection on male prosoma width and patella-tibia length during this episode (prosoma width: $n = 97$, $b \pm \text{s.e.} = 0.418 \pm 0.748$, Wald = 0.31, d.f. = 1, $P > 0.5$; patella-tibia length: $n = 97$, $b \pm \text{s.e.} = -0.321 \pm 0.750$, Wald = 0.18, d.f. = 1, $P > 0.6$;

multiple logistic regression model: $\chi^2 = 0.416$, $P > 0.8$). This suggests that the effect that male morphology had on the likelihood of being attacked was too weak and/or too few males were killed to result in statistically detectable selection on male size or leg length during this episode.

Episode 4: Re-approach

Of the 75 males that survived the first insertion only six (8%) did not re-approach the female. These six males had not lost more legs than the males that re-approached (Fisher's exact test: $P > 0.6$). Re-approaching males typically quickly climbed up the security thread which they had fastened on the female's web prior to copulation. None was attacked by the female.

Episode 5: Second courtship

All females reacted to the courting male by assuming the acceptance posture. Twenty-five percent (17/69) of males were nevertheless attacked during courtship, but none was killed. Male morphology did not predict the probability of an attack during the second courtship (Table 2.1). Only 44 (64%) of the courting males achieved the second insertion, although all females assumed the acceptance posture and no male was killed. Males that did not manage to insert their second palp tried several times to insert, but jumped off without actually inserting. The probability of achieving a second insertion was not influenced by general male size ($n = 69$, $b \pm \text{s.e.} = 0.085 \pm 0.261$, Wald = 0.11, d.f. = 1, $P > 0.7$), prosoma width or patella-tibia length (prosoma width: $n = 69$, $b \pm \text{s.e.} = 0.737 \pm 0.796$, Wald = 0.86, d.f. = 1, $P > 0.3$; patella-tibia length $n = 69$, $b \pm \text{s.e.} = -0.614 \pm 0.796$, Wald = 0.60, d.f. = 1, $P > 0.4$; multiple logistic regression model: $\chi^2 = 0.987$, $P > 0.6$). Further, males that had lost at least one leg during the first insertion did not

achieve the second insertion less frequently than males that had not lost a leg (60% vs. 76%, $\chi^2 = 1.28$, d.f. = 1, $P > 0.2$). Males that did not achieve the second insertion courted the female longer ($n = 69$, $b \pm \text{s.e.} = -1.154 \pm 0.356$, Wald = 10.52, d.f. = 1, $P < 0.005$). However, it is impossible to distinguish whether a long courtship was cause or consequence of males being unable to insert.

Female impact on male survival

During the first episode, older females were slightly more likely to attack web-crossing males than younger ones ($n = 99$, $b \pm \text{s.e.} = 0.28 \pm 0.14$, Wald = 3.91, d.f. = 1, $P < 0.05$), but this was without consequence for the males, because none was killed. During the third episode, we found a non-significant trend for a decreasing likelihood of male survival with increasing female size. To test for an effect of female size, we performed a multiple logistic regression analysis, where the full model included insertion duration, PC1 (general male size), female prosoma width, and all resulting interaction terms (Table 2.2). None of the interaction terms were significant. When we dropped these from the model, the insertion duration remained the sole significant predictor of male survival during the first insertion. Finally, during the fifth episode the likelihood of inserting the second palp decreased with increasing female age ($n = 69$, $b \pm \text{s.e.} = -0.40 \pm 0.18$, Wald = 5.08, d.f. = 1, $P < 0.05$). In summary, female characteristics had no effect on male survival, but female age was negatively correlated with the probability of inserting the second palp. Note however, that none of these trends were significant after Bonferroni correction for multiple tests within episodes.

Fertilization success and total selection on males

Ninety-two females constructed egg sacs (mean number egg sacs = 4.25, s.d. = 1.65, Range 1 - 7). Of these, 13 only built sacs that were completely empty, and another 48 females built at least one sac that was completely empty. The number of empty sacs was independent of the number of palpal insertions received ($\chi^2 = 12.8$, d.f. = 10, $P > 0.2$). Therefore, empty egg sacs were excluded from the following analyses, since they do not convey any information about fertilization success.

We used the total number of fertilized eggs for all egg sacs combined as a measure of male fertilization success. However, since females probably construct fewer sacs in the wild than in the lab (Tolbert, 1976) due to a higher mortality rate, we also analyzed fertilization success for only the first egg sac. We report these results where different from the analysis of all sacs.

Males that achieved two insertions fertilized more eggs than males that achieved one insertion, and this was highly significant for the first egg sac (Table 2.3). In contrast, the total number of eggs laid by females did not differ between females that received one or two insertions.

Since the probability of male survival during the first insertion decreased with increasing insertion duration, males may face a trade-off during the first insertion, if the insertion duration is positively related to fertilization success. We tested this hypothesis by regressing the number of fertilized eggs on the duration of the first insertion for males that achieved only one insertion. The duration of the first insertion was not a significant predictor of fertilization success ($\beta \pm \text{s.e.} = -0.064 \pm 0.075$, d.f. = 40, $t = -0.86$, $P > 0.3$). Thus we could not detect any trade-off between fertilization success and survival during

the first insertion. Similarly, the total insertion duration (insertion 1 and 2 combined) was unrelated to the total number of eggs fertilized, when the number of insertions was controlled for (Table 2.4). Note that the duration of the second insertion (median = 8 s, range = 1 - 55 s) includes time that males were already dead (Foellmer and Fairbairn, 2003).

To estimate overall selection on male morphology we included all males in the analyses, i.e. also those that did not achieve any insertion. We performed univariate and multivariate regression analyses in the same way as earlier, but we employed two different estimates of male fitness. First, we used the number of fertilized eggs converted to relative fitness (absolute fitness divided by the mean absolute fitness) to calculate selection gradients (Lande and Arnold, 1983). Second, we used the probability of achieving two insertions, because the sample size in the analyses of fertilization success was reduced due to females failing to lay any eggs. We did not detect overall selection on general male size or direct selection on male prosoma width and patella-tibia length during the mating interactions with either method (Table 2.5).

Discussion

This study examined in detail the consequences of the cannibalistic behaviour of females on male size, male leg length and copulation duration in *Argiope aurantia*. Females attacked males during all episodes of the mating interactions except episode 4 when males climb back up to the female on their security thread, but most frequently during the first and second insertion.

Our results suggest that pre-copulatory cannibalism may be rare in this species. Instead, cannibalism occurred almost exclusively during the first insertion (23% of males

were killed), when males were apparently most vulnerable. This pattern is very similar to that observed in other highly dimorphic orb-weaving spider species in which the male has to cross the web to reach the female (Elgar and Fahey, 1996; Uhl and Vollrath, 1998; Elgar et al., 2000; Schneider et al., 2000; Schneider and Elgar, 2001). The crossing of the female's capturing device therefore seems generally unlikely to put males at an increased risk. Nevertheless, Elgar and Fahey (1996) noted that larger males in *Nephila plumipes* elicited more aggressive movements by females during the web crossing and inferred selection against large male size during this phase. In *A. aurantia* there was a trend for males of larger general size to be attacked more frequently during the first insertion, which appeared to be due to attacked males having longer legs for their size measured as prosoma width. In all other situations larger males were not more likely to be attacked than smaller ones. An absence of an effect of male size on attack frequencies was also found in *N. edulis* (Schneider et al., 2000), but most studies do not distinguish between attacking and catching a male.

In *A. aurantia* male size or leg length had no effect on the probability of surviving an attack. Further, although larger males tended to be attacked more frequently, this did not translate into larger males being overall less likely to survive the first insertion. The effect of male morphology on the likelihood of being attacked was probably too weak and/or too few males were killed to result in statistically detectable selection on male morphology during this episode. Consequently, male size was neutral with respect to male survival during the mating interactions. In addition, the size of the male was unrelated to fertilization success, suggesting that larger males do not successfully transfer more sperm to the female. In the congener *A. keyserlingi* there was a non-significant

trend for small males to be cannibalized more frequently (Elgar et al., 2000). In *Nephila* spp. male size had on average no effect on the likelihood of being cannibalized (Uhl and Vollrath, 1998; Schneider et al., 2000; Schneider and Elgar, 2001). In *N. edulis* small males achieved greater fertilization success than large males, but this was unrelated to cannibalism (Schneider et al., 2000). Instead, small males employed a more efficient mating tactic (Schneider et al., 2000). Hence, there is no evidence to date that male size is under selection due to sexual cannibalism in highly dimorphic orb-weaving spiders.

Male size appears to be more relevant in less dimorphic species. In the orb-weaver *Araneus diadematus* larger males achieved more pedipalp insertions with a given female, because they were better at escaping the attacking female (Elgar and Nash, 1988). In fishing spiders of the genus *Dolomedes* large males were also better at escaping the female, although large males may not gain more palp insertions with a given female (Arnqvist and Henriksson, 1997; Johnson, 2001). Nevertheless, an escaped male will have the opportunity to search for another mate. This suggests that in less dimorphic species, sexual cannibalism may often select for larger male size, at least in spiders.

Males that survived the attack autotomized legs of the first and longest leg pair most frequently, but the absolute length of the first leg pair did not predict leg autotomy. Only small males with long legs for their size tended to lose legs more often. This could mean that males of this particular proportion might be at a disadvantage, but our results do not allow a firm conclusion at this point. In any case, a male's ability to achieve a second insertion was not affected by leg loss they incurred. Lost legs may thus only become important if the male does not achieve a second insertion with the same female and has to move on to search for another female. Since no other study has yet examined

selection on leg length independent of body size in the context of sexual cannibalism, comparisons are not possible.

The present study along with others (see above) suggest that extremely small male size relative to female size does not increase male survival during mating in cannibalistic species. Rather, male size appears to be neutral during cannibalistic interactions in species with pronounced SSD, whereas larger males may be favoured in less dimorphic species. Phylogenetic reconstruction of the evolution of extreme SSD in orb-weavers suggest that SSD has evolved several times in the Araneoidea, and that females generally increased over evolutionary time while males stayed small (Hormiga et al, 2000). Thus, as the sexes diverged, there was probably first selection for larger male size in cannibalistic species, since this seems to be the case in less dimorphic ones. In addition, male-male competition likely selects in several species for larger size, even in highly dimorphic species (Christenson and Goist 1979; Elgar and Fahey, 1996; Chapter 4). This means that during the course of their evolution there must have been strong selection against an increase in male size, for example during mate search or the juvenile stage (e.g. Ghiselin, 1974; Vollrath and Parker 1992), to counteract selection for large male size and the increase in size due to the genetic correlation with females (Lande, 1980, Reeve and Fairbairn, 2001). Schneider et al. (2000) have shown a small male size advantage during mating in an orb-weaver *Nephila edulis*, but this is to date is only direct evidence of this kind. Further demonstrations of selective factors that might have given rise to and/or are maintaining extreme SSD in spiders will be necessary to resolve the controversy concerning its evolution and adaptive significance (see Coddington et al., 1997; Vollrath, 1998).

The copulation duration during the first insertion is under male control in *A. aurantia*, and selection appears to favour short copulation duration during the first insertion. Males that had their first pedipalp inserted longer after the onset of the female's attack were more likely to be caught. The first insertion lasts a median duration of 3.5 seconds and we could not detect any correlation between insertion duration and fertilization success. This suggests that sperm is transferred rapidly and in an all or nothing fashion as has been found for example in *Gasteracantha cancriformis* (Bukowski et al., 2001). Males that achieve only one pedipalp insertion fertilize fewer eggs than those that insert both palps, a pattern found also in other spiders (e.g. Arnqvist and Henriksson, 1997). In addition to this immediate cost of sexual cannibalism males leave one of the female's paired sperm receptacles uninseminated. Another male transferring sperm into the empty receptacle would almost inevitably greatly reduce the first male's fertilization success, since sperm from both spermathecae are likely to be used by the female to fertilize the eggs (see e.g. Schneider et al., 2000; Schneider and Elgar, 2001). Sexual cannibalism is perhaps not the only selective process favouring short insertion duration in this species. During opportunistic mating males that have a palp inserted are frequently attacked by other males (Foellmer and Fairbairn, 2003). Thus rapid sperm transfer is likely to be advantageous in this situation too.

Although our study was not designed to test hypotheses about female attack motivation, the fact that larger males were more frequently attacked could point to a mechanism of overt female choice. However, females did not receive more pedipalp insertions from smaller males. Thus mate choice is unlikely to account for cannibalistic attacks in *A. aurantia*. Moreover, females risk being sperm limited by killing their mate.

Cannibalism has been hypothesized to be maladaptive in the fishing spider *Dolomedes fimbriatus* where females may similarly risk staying only partially inseminated (Arnqvist and Henriksson, 1997). According to this hypothesis, females are selected to be non-discriminate, aggressive foragers as juveniles, and are genetically constrained to behave aggressively towards mates. This model thus assumes that populations are not at genetic equilibrium with regards to female behaviour, and it would be of great interest to test this hypothesis in *A. aurantia*.

Other female characteristics besides the cannibalistic behaviour had no significant effect on male survival in our study. However, our exploratory analyses suggested that males may tend to be less successful in inserting the second palp into older females. Perhaps this is related to the gradual hardening of the female's cuticle. For example, in *Nephila clavipes* males achieve greater fertilization success when mating within a few hours after the female's final moult while her cuticle is still soft (Eberhard, 1996 and refs therein). Perhaps it is more difficult for male *A. aurantia* to insert a palp into a "hard" female. However, this does not explain why all males that tried to insert their first palp were successful, regardless of female age.

We estimated selection on male traits imposed by sexual cannibalism using only virgin females. Does this sufficiently reflect the male's risk of being cannibalized in the field where they may encounter both virgin and mated females? Mated females do not behave more aggressively towards an approaching or courting male than virgin females (Appendix 2), and we have not yet observed any male being killed by a female during the approach. Females that are unreceptive to courting males often signal this unresponsiveness by web-shaking or brushing-off the male, rather than with overt

aggression (Appendix 2). Thus encountering a non-virgin female does not put the male at increased risk of being killed by the female.

In conclusion, male body size does not appear to be under selection due to sexual cannibalism in *A. aurantia*. Hence sexual cannibalism is unlikely to be an important factor contributing to the maintenance of the extreme sexual size dimorphism in this species, and this is in line with findings in other highly-dimorphic orb-weavers. Our study is the first one to examine selection on male leg length in a spider. We did not find significant net or direct selection on leg length in the context of sexual cannibalism. Sexual cannibalism does select for very short copulation duration during the first insertion. This supports the hypothesis that sexual cannibalism is a major factor driving the evolution of male copulatory behaviour in spiders.

Table 2.1. Regression coefficients with the associated standard errors and Wald statistics for logistic regression of female attack as a function of male morphology during each selection episode (episode 4 is omitted, because no male was attacked). The effect of general male size (PC1) was evaluated in univariate regressions. For prosoma width and patella-tibia length the partial regression coefficients are given from the bivariate model containing both traits.

Episode	Variable	<i>b</i>	s.e.	Wald	d.f.	<i>P</i>
1.	General male size (PC1)	0.420	0.235	3.19	1	< 0.1
Crossing						
the web	Prosoma width	-0.206	0.694	0.09	1	> 0.7
(<i>n</i> = 99)	Patella-tibia length	0.620	0.694	0.80	1	> 0.3
2. 1 st	General male size (PC1)	-0.006	0.335	0.00	1	> 0.9
courtship						
(<i>n</i> = 99)	Prosoma width	1.094	0.967	1.28	1	> 0.2
	Patella-tibia length	-1.096	0.977	1.26	1	> 0.2
3. 1 st	General male size (PC1)	0.523	0.263	3.94	1	< 0.05
insertion						
(<i>n</i> = 97)	Prosoma width	-1.095	0.758	2.09	1	> 0.1
	Patella-tibia length	1.641	0.793	4.28	1	< 0.05
5. 2 nd	General male size (PC1)	0.361	0.297	1.48	1	> 0.2
courtship						
(<i>n</i> = 69)	Prosoma width	0.980	0.877	1.25	1	> 0.2
	Patella-tibia length	-0.585	0.865	0.46	1	> 0.4

Table 2.2. Multiple logistic regression analysis of the outcome of an attack during the first insertion as a function of insertion duration, general male size (PC1), and female prosoma width. The effect of the three main predictors was tested in a model only containing the main predictors. The contribution of the interaction terms was tested in the full model. The full model was significant ($\chi^2 = 16.21$, d.f. = 7, $P < 0.025$).

Variable	<i>b</i>	s.e.	Wald	d.f.	<i>P</i>
Insertion duration	-1.086	0.382	8.08	1	< 0.005
General male size (PC1)	0.449	0.290	2.40	1	> 0.1
Female prosoma width	-0.549	0.293	3.52	1	< 0.1
Insertion duration x female prosoma width	-0.407	0.448	0.83	1	> 0.3
Insertion duration x male prosoma width	0.116	0.446	0.07	1	> 0.7
Male prosoma width x female prosoma width	0.056	0.389	0.02	1	> 0.8
Insertion duration x male prosoma width x female prosoma width	-0.621	0.644	0.93	1	> 0.3

Table 2.3. Means and standard errors for total number of eggs, number of fertilized eggs, and proportion of fertilized eggs laid by females that received one or two insertions.

Values are given for first egg sacs and all egg sacs that contained eggs combined.

Unequal sample sizes are due to empty egg sacs (excluded from analysis, see text).

		One insertion		Two insertions		<i>U</i>	<i>P</i>
		Mean	s.e.	Mean	s.e.		
First egg sac n = 74	Total number of eggs	338.3	13.5	366.4	22.0	560.0	> 0.2
	Number of fertilized eggs	247.6	17.9	347.0	26.8	342.5	< 0.001
All egg sacs n = 78	Total number of eggs	1108.5	62.5	983.3	99.0	586.0	> 0.1
	Number of fertilized eggs	596.0	45.8	783.5	79.4	573.0	< 0.1

Table 2.4. Analysis of covariance of the effect of total insertion duration controlled for number of insertions on the total number of fertilized eggs. The residuals of the model were normally distributed. The model is not significant ($F = 2.15$, d.f. = 2, $P > 0.1$, adjusted $R^2 = 0.03$).

Source	d.f.	Mean Square	F	P
Total insertion duration	1	81422.1	0.53	> 0.4
Number of insertions	1	574106.3	3.71	< 0.1
Error	73	154632.6		

Table 2.5. Estimates of overall selection on male morphology during the mating interactions. a) Selection gradients with standard error and t-statistic obtained from a univariate LS regression (PC1) and a multivariate LS regression (prosoma width and patella-tibia length) with total number of fertilized eggs as the response ($n = 78$); b) Logistic regression coefficients with standard error and Wald-statistic obtained from a univariate logistic regression (PC1) and a multivariate logistic regression (prosoma width and patella-tibia length) with the probability of achieving the second insertion as the response ($n = 99$).

a)

Variable	β	s.e.	t	P
General male size (PC1)	0.033	0.068	0.48	> 0.6
Prosoma width	-0.169	0.207	-0.82	> 0.4
Patella-tibia length	0.191	0.207	0.92	> 0.3
Model: $F = 0.44$, $P > 0.6$				

b)

Variable	b	s.e.	Wald	d.f.	P
General male size (PC1)	0.131	0.204	0.41	1	> 0.5
Prosoma width	0.555	0.615	0.81	1	> 0.3
Patella-tibia length	-0.407	0.612	0.44	1	> 0.5
Model: $\chi^2 = 1.14$, $P > 0.5$					

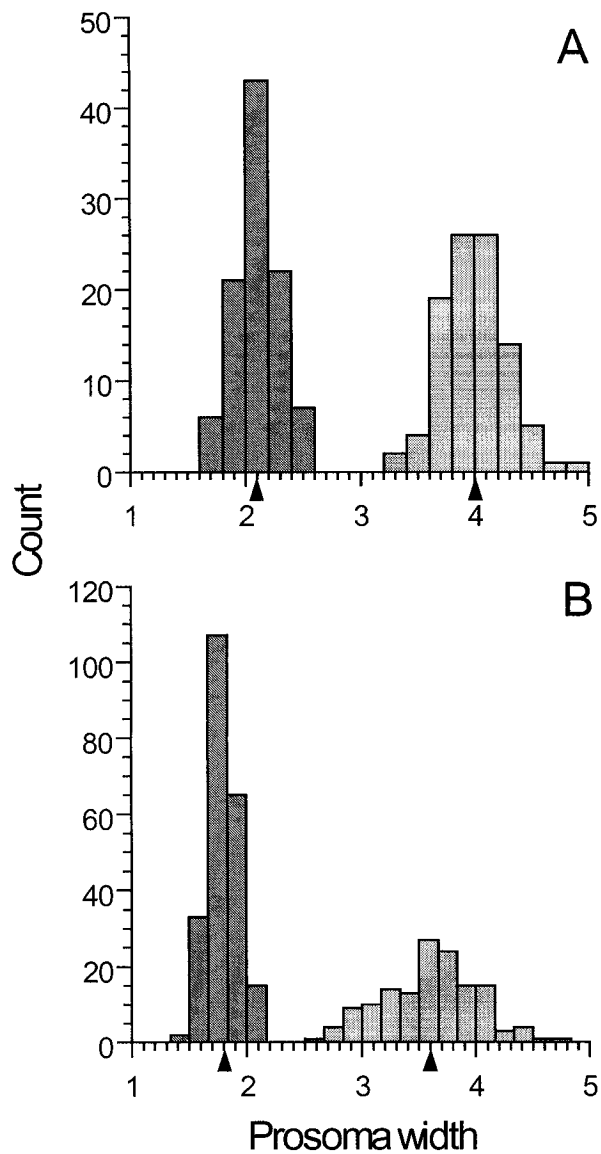


Figure 2.1. Frequency distributions of prosoma width (A) for males ($n = 99$) and females ($n = 99$) used in the experiment and (B) for males ($n = 224$) and females ($n = 143$) from the source population. Males are shown in dark grey, females in light grey. The arrow heads point to the respective means of the distributions.

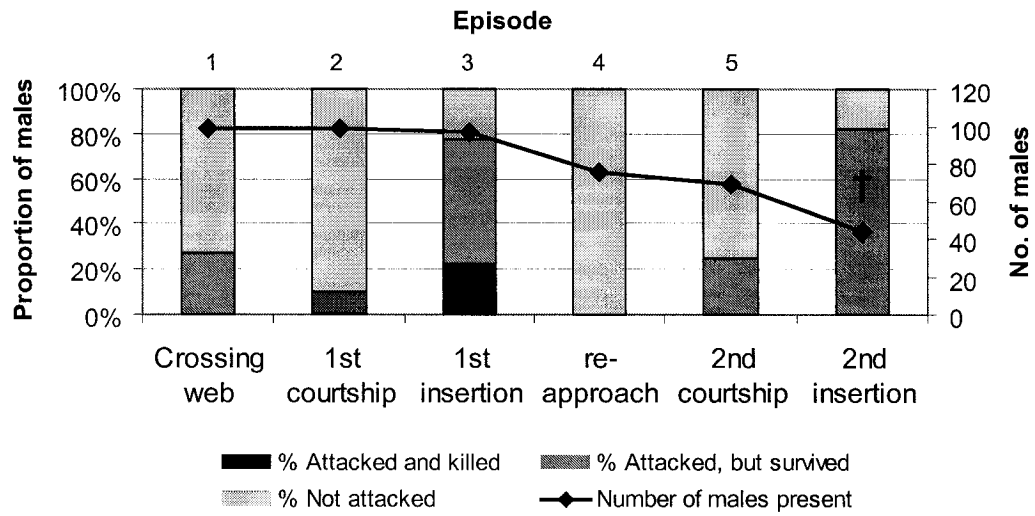


Figure 2.2. Relative frequencies of attacked and killed males per episode and the number of males present per episode (on the secondary y-axis). †All males die spontaneously during the second insertion (see text).

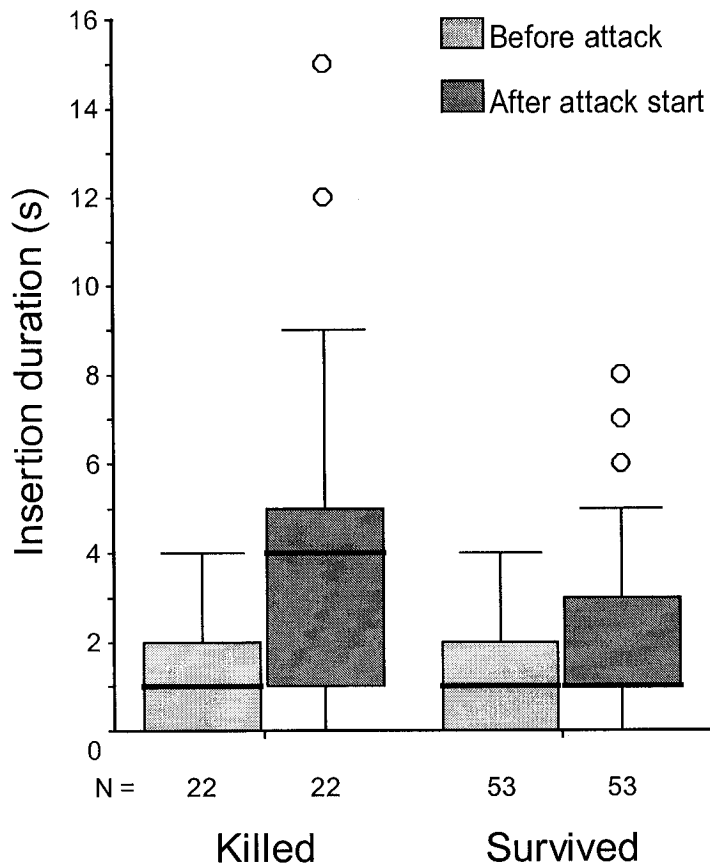


Figure 2.3. Timing of the female's attack during the first insertion. Before attack: time (seconds) males had inserted their palp when the female attacked. After attack: time males stayed inserted after onset of the female's attack. Box plots show medians (centre horizontal line), 25th and 75th percentile (upper and lower box border), highest and lowest values (whiskers), and outliers (outside 1.5 box lengths; open circles).

Chapter 3. Selection on male size and shape during mate search in a sexually highly dimorphic orb-weaving spider

Mate search, or the “roving phase”, is a critical period in a male’s life. Males compete against each other to find females that are available as mates and are thus under sexual selection. However, they also face various predators while roving through the vegetation and are therefore also under viability selection, which some authors also call “natural selection” (as opposed to sexual selection). This chapter examines the effects of selection on male morphology during the roving phase.

Introduction

In most animal species, males and females differ conspicuously in morphological attributes, such as size or colour of body components. Ultimately, all manifestations of sexual dimorphism are probably rooted in the different reproductive roles of males and females and the resulting patterns of natural and sexual selection acting on the sexes (reviewed in Andersson, 1994). The most prevalent form of sexual dimorphism in the animal kingdom is sexual size dimorphism (SSD) (Andersson, 1994; Fairbairn, 1997). Males are typically larger than females in mammals and birds, but in the vast majority of species (invertebrates and poikilothermic vertebrates), females are generally the larger sex (Abouheif and Fairbairn, 1997; Fairbairn, 1997). Accordingly, SSD in spiders is almost always female-biased, but spiders are unique in that it is the only terrestrial taxon where extreme SSD is common (Ghiselin, 1974; Andersson, 1994; Vollrath, 1998). In certain genera of orb-weaving spiders (Orbiculariae) females may weigh over a hundred times more than males (Head, 1995; Vollrath, 1998). A comparable magnitude in SSD is otherwise found only in some marine and endoparasitic taxa and rotifers (Ghiselin, 1974; Andersson, 1994; Vollrath, 1998). Spiders thus offer a tangible system to study the evolution and maintenance of extreme SSD, yet SSD in spiders remains poorly understood (e.g. Coddington et al., 1997; Vollrath, 1998; Walker and Rypstra, 2003).

Generally, SSD can evolve if body size is heritable and if the genetic correlation between the sexes is less than 1 (Lande, 1980). SSD will then evolve as a result of different net selection pressures acting on the body sizes of males and females within given genetic and physiological constraints (e.g. Lande, 1980; Reeve and Fairbairn, 2001). Several hypotheses have been put forward about how natural and sexual selection

might have contributed to the evolution and maintenance of extreme SSD in spiders (Darwin, 1871; Gerhardt, 1924; Ghiselin, 1974; Vollrath and Parker, 1992; Schneider et al., 2000; Moya-Laraño et al., 2002). Recent evidence suggests that within orb-weaving spiders females have increased in size over evolutionary time in several lineages independently (Coddington et al., 1997; Hormiga et al., 2000) and that selection for increased female fecundity is the major force driving this trend (Marshall and Gittleman, 1994; Head, 1995; Prenter et al., 1999; Higgins, 2002). It is less clear, however, why males have stayed small or even have decreased in size in some cases (Prenter et al., 1997; 1998; Walker and Rypstra, 2003; but see Vollrath, 1998; Schneider et al., 2000). In orb-weaving spiders, males switch from a sedentary to a roving life-style after their maturation moult to search for females and no longer build catching webs (Foelix, 1996). Therefore, one can distinguish three distinct episodes in a male orb-weaving spider's life during which male body size might be under selection: the juvenile phase, mate search (i.e. the "roving phase"), and mating. A new hypothesis focuses on the roving phase as an episode generating selection for small male size (Moya-Laraño et al., 2002). According to this "gravity hypothesis", smaller males might have an advantage over larger ones in species such as orb-weaving spiders, where males move through a three-dimensional habitat and have to climb to reach females. Smaller males are expected to be able to climb faster than larger males, because the average speed achievable is inversely proportional to body length (Moya-Laraño et al., 2002). Thus, smaller males might be better at finding females, which would give them an advantage in scramble competition, and might also be better at escaping predators. As predicted by the gravity hypothesis,

SSD in spiders seems to increase with increasing height of female habitat (Moya-Laraño et al., 2002).

A small male advantage in scramble competition has so far been demonstrated mostly in aerial species (some insects and birds), where small males are favoured either because they are more agile, or because they have an energetic advantage (reviewed in Blanckenhorn, 2000). Generally, in species where mature males have to feed to maintain stamina, small males should have an energetic advantage because their lower metabolic requirements enable them to spend more of their time and energy searching for females and mating (Reiss, 1989; Blanckenhorn et al., 1995; Blanckenhorn, 2000). However, in species where adult males do not or only rarely feed, such as in orb-weaving spiders, large males might actually be at an energetic advantage, because of their lower mass-specific metabolic rate (Calder, 1984; West et al., 2000). Large males may also be able to store disproportionately more reserves than small males if this capacity scales allometrically with size (Calder, 1984), and these effects might trade-off with the gravity-advantage of small males. Indeed, it has been suggested that intermediate sized males might have a higher probability of successfully travelling to a female's web in the orb-weaver *Nephila clavipes* (Vollrath, 1980; Christenson, 1990).

In this study we investigate selection on male body size during mate search in the sexually highly dimorphic orb-weaving spider *Argiope aurantia*. We use a multivariate approach to isolate selection targeted at different components of size (Lande and Arnold, 1983; Brodie et al., 1995). This is particularly important because adult (but not juvenile) male spiders generally have relatively longer legs than females (Foelix, 1996). Thus, to understand fully sexual dimorphism in spiders it is important to determine the adaptive

significance of leg length independent of body size. Longer legs in male spiders have been hypothesized to confer an advantage during mate search and also during agonistic interactions among males and during mating with cannibalistic females (Elgar et al., 1990; Prenter et al., 1995; Legrand and Morse, 2000; Bridge et al., 2000), but no study has yet examined the independent effects of selection on leg length and body size other than in the context of sexual cannibalism (Foellmer and Fairbairn, 2004). Therefore, we examine direct and net selection on both body size and leg length during mate search in *A. aurantia*.

In this paper we present the results of a cross-sectional study comparing males that were found and measured before and after a phase of mate search. We examine whether male body size and/or leg length is under selection during the roving phase in *A. aurantia*. Previous hypotheses predict that smaller males and/or males with relatively longer legs should be favoured during mate search, i.e. they should be more likely to be found with females. Further, we investigate the relationship between male size, storage capacity of energy reserves, and loss of condition (energy reserves) during mate search. This will help to clarify whether large males might be at an energetic advantage in orb-weavers.

Methods

Study species

Argiope aurantia is a large orb-weaving spider common in successional habitats (old fields) throughout eastern North America (Levi, 1968). Sexual size dimorphism is pronounced; females are about 3-4 times longer than males (Levi, 1968). Males mature on average about a week earlier than females and leave the web on which they moulted to

adulthood to search for females. Males pursue two alternative mating tactics, depending on the type of female they encounter (Robinson and Robinson, 1980; Foellmer and Fairbairn, 2003). If a male encounters a penultimate female (a female just one moult from adulthood), he typically cohabits with her on peripheral web structures and waits for her to moult. During cohabitation, males may occasionally scavenge prey items present in the female's web. Males try to mate with the female while she is moulting and thus completely defenceless (opportunistic mating). On the other hand, if a male encounters a mature female, he courts her on her web and, if the female is receptive, may mate with her. However, males show a strong preference for penultimate females (see Foellmer and Fairbairn, 2003), and throughout the mating season are mostly associated with penultimate females that are close to moult (Chapter 4). Males that do not achieve copulation in either situation search for another female.

Data collection

We sampled two populations in Quebec, Canada. One population was located on Île Perrot (45°22'N, 73°55'W), 31km southwest of Montreal (sampled in 2000, called sample 1 hereafter), the other was near Phillipsburg (45°04'N, 73°03'W), 65km southeast of Montreal (sampled in 2002, referred to as sample 2). Both sites were old fields with invading shrubs and abundant goldenrod (*Solidago* sp.). To examine whether male morphology is under selection during the roving phase, we compared adult males collected on their own moulting webs (i.e. before they engaged in mate search) with males collected from webs of females that were potential mates. Since male *A. aurantia* frequently cohabit with penultimate females, but only rarely with mature females (see above), most males were collected from penultimate females close to moult. We did not

collect males that were cohabiting with penultimate females that were not close to moult, because these females might still relocate, before males have an opportunity to mate with them. Thus, mate search cannot be considered at least potentially completed in these cases. Females close to moult are recognized by the presence of a typical moulting web (with extensive barrier webs, but without visceral spirals). Mature females can be identified by the presence of the pronounced scape on the epigyne (the sclerotized genital plate on the ventral side of their abdomen). Penultimate females are similar to mature females in general appearance, but do not yet have a developed epigyne. Adult males are easily distinguishable from juveniles by their developed pedipalps, a dark brown colour and elongated legs.

We thoroughly searched the vegetation on each side of a given path using water sprayers to increase the visibility of web structures (Tolbert, 1976). Sampling regimes differed slightly between samples. For sample 1, the collection of adult males was done as part of a larger field study using a 1030 m² area through which parallel transects were placed 1.5 m apart. We searched for males on their moulting webs along 10 randomly chosen 20 m long transects on about every second day. The whole study area was searched almost daily for penultimate females and associated males. Because we searched more frequently and a larger area for males on females' webs than for males on moulting webs, we collected more males on females' webs (n = 111) than males on moulting webs (n = 52). All individuals were measured on the same day of collection. For sample 2, we haphazardly established paths within an approximately 1000 m² area. We then searched for males along these paths on three days per week. We collected 51

males on moulting webs and 58 males on females' webs, and measured all males the day following collection.

We measured the following traits of males: prosoma width (at the broadest point), opisthosoma length and width (at the broadest point), and the combined patella-tibia length of each of the eight legs. We used the average patella-tibia length of each leg pair for later analysis. If one leg of a pair was missing, the length of the remaining leg was used. All measurements were taken from the dorsal aspect under a dissecting microscope with individuals held gently between two lids of Petri dishes balanced with cotton. Repeatability of all measurements was > 0.89 ($n = 10$, $k = 3$ repeated measures). Prosoma width and patella-tibia length are fixed traits, i.e. they do not change after the final moult. Opisthosoma dimensions do change with the nutritional status of the individual. Adult males rarely feed (see above). Therefore, the opisthosoma of males shrinks during the adult life as the stored reserves in the opisthosoma are used up (Foelix, 1996), and opisthosoma dimensions can be used to estimate male condition.

Data analysis

Our general approach was to estimate selection on males using logistic regression (Kleinbaum *et al.*, 1998) with web type as the response variable. We set the response to 0, if the male was found on his own moulting web and to 1, if the male was found on a female's web. Because we did not follow individuals from web to web, we cannot estimate selection gradients *sensu* Lande and Arnold (1983). However, our data allows us to estimate selection on male morphology qualitatively by comparing males found before and after they had engaged in mate search. We employed regression models in an analogous fashion to selection gradient analysis. Univariate models are used to estimate

net selection on a trait, while multivariate models estimate direct selection on a given trait, independent of the other traits in the model (Lande and Arnold, 1983; Fairbairn and Reeve, 2001). Linear coefficients estimate directional selection. Nonlinear coefficients estimate the curvature of the fitness function and thus indicate stabilizing or disruptive selection.

Variables were log-transformed prior to analysis to normalize distributions. We performed a principal components analysis (PCA) with Varimax rotation on all variables measured for each sample separately to obtain a variable representing overall male size. Varimax rotation optimizes factor solutions by making large factor loadings larger and small factor loadings smaller, so that variables are ideally correlated with only one of the extracted factors (SPSS Inc., 1997). For each sample, PCA extracted two components. The first component (PC1) of the rotated solution explained most of the variance (63% for sample 1, 66% for sample 2) and was highly correlated with the fixed morphological traits (Table 3.1). The second component (PC2) explained 27% (sample 1) and 28% (sample 2) of the variance and was highly correlated with opisthosoma length and width. Thus, PC1 can be interpreted as an estimator of overall fixed male size, and PC2 as an estimator of male condition.

We analyzed male fitness as a function of the general size of males using PC1 as the independent variable in univariate regressions. We also estimated direct selection on fixed body size components in multivariate regression models and net selection using univariate regression. We did not attempt to estimate nonlinear components for the multivariate model to preserve power. For regression analyses, prosoma width and patella-tibia lengths were standardized to a mean of zero and a standard deviation of one

to facilitate comparisons. Note that because we cannot estimate selection gradients, we report the untransformed logistic regression coefficients.

Results

Selection on male morphology

In sample 2, all fixed morphological traits of males that were caught on females' webs were on average larger than those of males that were caught on their own moulting web (Figure 3.1). In sample 1 no such trend is observed. Accordingly, logistic regression suggests significant selection for overall larger males during the roving phase in sample 2, but not in sample 1 (Table 3.2). The quadratic terms are not significant for either sample, indicating that overall male size was not under stabilizing or disruptive selection. Examination of direct selection on male body components using multiple logistic regression revealed the following pattern (see Table 3.3). In sample 1, we detected significant direct selection for longer patella-tibia of the third leg pair. There appeared to be also a tendency for selection to favour males with a narrower prosoma, but this was not significant after correcting for multiple comparisons. Note that because we have performed 19 significance tests within each sample, our α -level for a Type I error rate of 5% is 0.0027. However, correcting for multiple tests has not become standard in selection analyses (e.g. Blanckenhorn et al., 2003). Therefore, we also indicate results that are significant at the 0.05 level. In sample 2, none of the traits in the model were significant independently of each other. Interestingly, the sign of the coefficient for prosoma width is also negative, but just not significant. Tolerances in both analyses were all > 0.02 , suggesting that multicollinearity was not a problem in our multiple regression models, because tolerances > 0.001 are generally acceptable (see Fairbairn and Preziosi, 1996).

None of the fixed body components were under net selection in sample 1 (Table 3.4). For sample 2 our analyses suggest that the patella-tibia for all legs were under directional net selection, and that longer legs were favoured. Nevertheless, if we apply the strict criterion $\alpha = 0.0027$, then none of the coefficients are significant. In this case, however, the coefficients for all legs were consistently significant and the differences between means obvious (see Figure 3.1). Thus we do consider this result relevant. Taken together, these results suggest that in sample 2 there was selection for generally larger size in males, but that this was due to selection for longer legs, with direct selection on prosoma width tending to be negative.

We tested whether the size of males caught on moulting webs and of males caught on females' webs changed over the season to determine whether a seasonal effect could be a possible confounding factor in our analyses (Figure 3.2). In sample 1, general male size (PC1) is not a function of maturation date ($y = -0.07 - 0.01x$; $n = 50$, $F = 0.29$, $p > 0.5$), but the size of males on females' webs increased over the season ($y = -0.67 + 0.04x$; $n = 111$, $F = 7.59$, $p < 0.01$). In sample 2, maturation date was negatively correlated with PC1 ($y = 0.13 - 0.09x$; $n = 51$, $F = 7.15$, $p < 0.025$), whereas the size of males on females' webs did not change significantly as the season progressed ($y = 0.50 - 0.01x$; $n = 54$, $F = 0.13$, $p > 0.7$). This could mean that the result suggesting selection for larger males during mate search is confounded by maturation date. Instead of larger males, males that mature earlier might be selected for, and the size effect would then just be due to the correlation between size and maturation date. To control for seasonal effects we modelled general male size (PC1) as a function of web type (fixed factor) and observation day (covariate) (Table 3.5). The interaction term web type x observation day

was not significant in either sample. When these were dropped from the models, observation day was a significant predictor of male size in sample 1 (Table 3.5). In sample 2, the effect of web type was highly significant, while observation day was marginally significant (see Figure 3.2). Thus, inclusion of sampling date does not change our conclusions concerning selection on male size. Further, almost all males matured during the first half of the season (see Figure 3.2), and potential mates were abundant during the second half and males were accordingly associated with them. Given this, it is difficult to see why late maturing males would not be able to reach a female other than for the fact that they were smaller.

Effects on male condition

Adult males are expected to lose condition over time, because they rarely feed. We tested whether males lose condition during mate search while controlling for observation date by modelling condition (PC2) as a function of web type (fixed factor) and observation day (covariate) (Table 3.6, Figure 3.3). In sample 1 the interaction term web type x observation day was not significant. When the interaction term was dropped from the model, both web type and observation day were strong predictors of male condition. In sample 2 the interaction term was significant, and also the web type effect. Thus, males did lose condition during mate search in both samples. Males may also mature with less stored reserves for their size, i.e. in poorer condition, towards the end of the season, because the amount of reserves becomes less important. Thus, males might trade-off the amount of stored reserves with the achieved size at the maturation moult as the season progresses. To examine the temporal effects on male condition, we regressed condition (PC2) on observation day for males caught on their moulting web and males

caught on a female's web separately, because loss of condition in these two groups reflects different phenomena. The condition (PC2) of males on their moulting webs is negatively correlated with maturation date in sample 2 ($y = 1.14 - 0.10x$; $n = 51$, $F = 10.24$, $p < 0.01$), but this is not significant in sample 1 ($y = 0.95 - 0.04x$; $n = 50$, $F = 2.52$, $p > 0.1$). However, regression diagnostics identified an outlier in terms of the influence on the regression line (see Figure 3.3; Cook's $d \times [n-k-1] = 0.46 \times 48 = 22.08 >$ the critical value of 16.39 [Kleinbaum et al., 1998, p. 731]). If this data point is excluded from the analysis, then the regression equation becomes $y = 1.09 - 0.06x$ ($n = 49$, $F = 6.00$, $p < 0.02$), and we would see the same trend as in sample 2. Thus, there is a general trend for males moulting later in the season to be in poorer condition. In sample 1, males that were caught on females' webs were also in poorer condition as the season progressed ($y = 0.27 - 0.03x$; $n = 111$, $F = 5.06$, $p < 0.05$). In sample 2, no such trend was observed ($y = -1.04 + 0.04x$; $n = 54$, $F = 2.40$, $p > 0.1$). The discrepancy between samples could be due to the extended sampling period in sample 1. Because we searched for males on females webs for longer in 2002, we might have been more likely to find males in poor condition at the end of the season. Indeed, restricting the comparison to the same time period in the two samples (Julian dates 30 Jul. – 15 Aug.) renders the relationship non-significant ($y = 0.08 - 0.02x$; $n = 91$, $F = 0.62$, $p > 0.4$).

To test whether males store or lose energy reserves disproportionately with size, we need to examine the scaling relationship between the amount of stored reserves and body size. For this purpose, size-corrected condition indices, such as a principal component or residuals, are not useful. Instead, we used as an estimate of the amount stored reserves the opisthosoma area calculated as the ellipse defined by opisthosoma

width and length (opisthosoma area = $[\text{width}/2] \times [\text{length}/2] \times \pi$), which allowed us to express stored reserves as a function of a suitable size measurement (prosoma width). We first regressed opisthosoma area on prosoma width for males caught on their moulting web. The 95% confidence interval of the slope of the regression of log-transformed variables included the slope of 2, which is the expectation of isometry when an area is regressed on a linear trait (sample 1: $b = 1.89$, 95% CI = 1.03 – 2.76; sample 2: $b = 1.73$, 95% CI = 0.80 – 2.67). Therefore, larger males are not able to store reserves disproportionately compared to smaller males. We then tested whether males lose stored reserves disproportionately with size by including all males in the analysis. If loss of energy reserves is a function of male size, the slope of opisthosoma area regressed on prosoma width should differ between males caught on moulting webs and males caught on females' webs. In a general linear model with log opisthosoma area as the response, web type as a fixed factor, and log prosoma width as the covariate, the effect of both web type and prosoma width were highly significant in both samples, whereas the interaction term was not (Table 3.7, Figure 3.4). This confirms that males lose condition as they search for females and shows that loss of condition does not depend on male size.

Discussion

Contrary to the prediction of the gravity hypothesis, we did not detect selection for small male size during the roving phase in *Argiope aurantia*. Overall larger males were actually more likely to reach a female's web in one of our two sampled populations, and this appeared to be the result of selection for longer legs. We did not find any evidence for stabilizing or disruptive selection on male body size or leg length.

Female *A. aurantia* build their webs about 80 cm above the ground between tall herbs or bushes (Enders, 1974). While this in itself is likely to represent a substantial climb for a 5 mm long male, a male will often have to travel a long distance (several meters) through the vegetation to find a mate (Foellmer, personal observation, see also Legrand and Morse, 2000). Therefore, gravity is potentially an important factor in this species. There are several explanations why we might not have found a small male advantage in *A. aurantia*. According to the gravity hypothesis small males would be favoured because they are able to climb faster and are thus better at finding females and/or at escaping predators. To what extent scramble competition might operate in *A. aurantia* is unknown. Males typically cohabit with penultimate females for days and wait for them to moult. By the time a female moults, in most cases more than one male has arrived on the web (McCook, 1890; Chapter 4), so perhaps larger (i.e. slower) males often have enough time to reach a female. Most females are mated opportunistically in the field (Chapter 4), thus finding a female's web first might not be an important factor in determining male life time fitness in *A. aurantia*. Nevertheless, males are expected to be vulnerable to predation during mate search (Vollrath and Parker, 1992; Foelix, 1996; Andrade, 2003). *A. aurantia* is diurnal and males travel during the day (Foellmer, personal observation); they are therefore at risk of being caught by visually hunting predators such as jumping spiders (Wise, 1993). Larger males have longer legs and are thus likely to be better runners at least on horizontal structures, because they are capable of longer strides (Foelix, 1996). In wolf spiders, larger individuals achieve a higher speed in one species, but not in another (Amaya et al., 2001). However, wolf spiders are adapted to move in a more two-dimensional habitat; their legs are all of similar length

and equipped with adhesive hairs (Foelix, 1996). This makes comparisons between wandering spiders and orb-weaver males difficult. Nevertheless, it might be that it is the ratio of vertical to horizontal structures a male has to travel on that determines which body features have a selective advantage during mate search and that gravity might become important only in species where females live high up in trees.

In our first sample, we detected a tendency for selection directly targeting prosoma width, favouring males with a narrower prosoma. We also found significant direct selection for longer legs of the third pair. However, this did not translate into significant net selection for either trait, probably due to the phenotypic correlation between these and the other traits that were not targeted by selection (Lande and Arnold, 1983; Price, 1984). The third leg pair is the shortest of all leg pairs and is probably constraining the locomotive performance of a male on substrate other than silk threads (Foelix, 1996). Shorter third legs likely confer an advantage in the context of web construction and prey handling on the web, and this probably balances selection for longer third legs during mate search. Selection favouring a narrower prosoma seems surprising as it is believed that the same conditions that favour longer legs in males also favour a broader prosoma, because it accommodates much of the leg musculature (Prenter et al., 1995; Foelix, 1996). However, we detected a similar tendency also in sample 2, and it is conceivable that narrower males might have an advantage when moving through the vegetation.

Our study is the first to investigate selection on leg length during mate search independently of body size in any spider. Previous work on highly dimorphic spider species suggested stabilizing selection on male body size during mate search in *Nephila*

clavipes, based on the finding that males grouped in intermediate size classes were more likely to be caught again after interweb travel than males in extreme size classes (Vollrath, 1980; Christenson, 1990). Andrade (2003) has shown that males suffer from a high mortality during mate search in the redback spider *Latrodectus hasselti*, but that mortality was statistically independent of male size. However, there was a non-significant trend for larger males to be more likely to survive, and, as pointed out by Andrade (2003), the power of the statistical test was relatively low. Redback spiders build their webs near the ground, so it is perhaps not surprising that a small male advantage due to the gravitational force was not detected in this species. In the less dimorphic wolf spider *Hygrolycosa rubrofasciata*, mortality during mate search appears to be independent of body size, but increases with increasing mate-searching activity (Kotiaho et al., 1998a; 1999). Clearly, more studies on highly dimorphic species are needed to evaluate to what extent males suffer from size-selective mortality during mate search.

We found that in *A. aurantia*, stored energy reserves estimated as opisthosoma area scaled isometrically with prosoma width at maturation. Therefore, larger males were not able to store disproportionately more reserves than smaller ones. Males lost condition (i.e. energy reserves) during mate search, and the rate of condition loss seemed to be independent of male size. This is somewhat surprising, as larger males can be expected to lose condition at a lower rate per unit mass, because the mass-specific metabolic rate scales generally as body mass^{-1/4} (West et al., 2000). Perhaps our sample sizes were not large enough to detect such an effect or the occasional food intake by males is sufficient to overcome any effects due to differences in mass-specific metabolic rate. However, although the universal scaling relationships between metabolic rate and body mass have

been confirmed in a variety of spiders (e.g. Anderson, 1970; Greenstone and Bennet, 1980; Watson and Lighton, 1994), it has been suggested that the relationship between body mass and mass-specific metabolic rate can even be positive in males of a wolf spider (Kotiaho et al., 1998b). An investigation of sex-specific metabolic rates in *A. aurantia* might be interesting, since to our knowledge this has so far been done for only two spider species (Watson and Lighton, 1994; Kotiaho, 1998). In any case, based on our data male *A. aurantia* of a particular size are not expected to have an advantage during mate search or mating due to their energy storage capacity.

We detected a trend for earlier maturing males to be in better condition. Our condition index reflects the amount of stored energy reserves a male has available for his adult life span and thus probably reflects an allocation decision with respect to overall achieved size versus stored reserves for future use (Roff, 2002). Males that begin their adult life with fewer reserves after moulting need not necessarily be of poorer quality in terms of their immediate performance, e.g. during combat – as long as they have sufficient energy. However, they are expected to have lower endurance, i.e. they cannot search for suitable females or wait for females to moult as long as males in better condition. Therefore, the negative relationship between male condition and maturation date suggests that males may trade off achieved adult size with the amount of stored reserves. Males start to mature about a week earlier than females and as the season progresses energy reserves might become less important, because females start to mature and potential mates are thus more readily available. This is reflected by the fact that during the first 2 1/2 weeks that males were associated with females, the condition of these males did not appear to change and only later in the season were males in poorer

condition. Thus early maturing males in high condition probably lost more condition, because they had to wait on average longer for penultimate females to moult. We do not know to what extent males die of starvation before they get to mate, but it is known that adult male orb-weavers generally senesce rapidly and die within weeks (e.g. Andrade, 2003). Nevertheless, male *A. aurantia* die during insertion of their second pedipalp and therefore will most often mate with only one female (Foellmer and Fairbairn, 2003), so only energy needed to achieve a single mating is likely to be important.

In conclusion, we detected selection for large body size during mate search in male *A. aurantia* in one out of two sampled populations, while male body size seemed to be neutral in the other. Thus we did not find support for the gravity hypothesis and it is still unclear during which stage selection might favour small male size in *A. aurantia*. Males lose condition during mate search, but males of a particular size are unlikely to be at an energetic advantage. Given that during mating with cannibalistic females male size appears to be neutral (Foellmer and Fairbairn, 2004), and that during opportunistic mating large males are favoured (Chapter 4), selection for smaller males might operate in the juvenile stages. Selection for overall larger males during mate search led to significant net selection for longer legs, but not for a broader prosoma. This suggests that relatively longer legs in males are the result of selection during the roving phase, supporting an existing hypothesis about the adaptive advantage of leg length in male spiders (Prenter et al., 1995; Foelix, 1996; Legrand and Morse, 2000). Finally, it should be pointed out that selection often varies in space and time (e.g. Blanckenhorn et al., 1999; Ferguson and Fairbairn, 2000; Jann et al., 2000). Experiments that directly investigate how body size and leg length determine a male's ability to move through

different types of vegetation would further improve our understanding of the significance of the roving phase for body size evolution in male orb-weaving spiders.

Table 3.1. Factor loadings for the first and second extracted principal components after Varimax rotation for each sample. Fixed traits are: prosoma width (PROWI) and the average patella-tibia lengths of the four leg pairs (PATI1 to PATI4). Variable traits are: opisthosoma width (OPIWI) and length (OPILE). Sample 1: n = 161. Sample 2: n = 105.

	Sample 1 (2000)		Sample 2 (2002)	
	PC1	PC2	PC1	PC2
PROWI	0.882	0.277	0.916	0.244
PATI1	0.934	0.259	0.954	0.233
PATI2	0.938	0.240	0.969	0.196
PATI3	0.915	0.217	0.944	0.211
PATI4	0.934	0.263	0.959	0.210
OPIWI	0.343	0.860	0.149	0.951
OPILE	0.168	0.928	0.292	0.909

Table 3.2. Logistic regression analysis of the probability of finding a male on a web of a female as a function of general male size. Given are the coefficients with the associated standard errors for the linear and quadratic terms of the univariate models. Coefficients for linear terms are estimated without quadratic terms in the model. Coefficients that are significant after correction for multiple comparisons are in bold.

Response: web type		b	s.e.	p	b	s.e.	p
		(linear)			(quadratic)		
Sample 1	PC1 (size)	0.28	0.18	0.1244	0.23	0.17	0.1692
Sample 2	PC1 (size)	0.71	0.23	0.0018	0.03	0.19	0.8655

Table 3.3. Multiple logistic regression analysis of the probability of finding a male on a web of a female as a function of fixed male morphological traits. Given are the coefficients with the associated standard errors for the linear terms of the multivariate model for each sample. Coefficients that are significant after correction for multiple comparisons are in bold. For explanation of abbreviations see Table 3.1.

Response: web type		b (linear)	s.e.	p
Sample 1	PROWI	-0.84	0.40	0.0354
	PATI1	-0.75	0.72	0.2930
	PATI2	0.65	0.69	0.3449
	PATI3	1.40	0.45	0.0020
	PATI4	-0.48	0.63	0.4438
Sample 2	PROWI	-0.94	0.51	0.0677
	PATI1	-0.64	1.43	0.6516
	PATI2	0.76	1.56	0.6279
	PATI3	0.48	0.67	0.4724
	PATI4	0.81	0.97	0.4066

Table 3.4. Univariate logistic regression analyses of the probability of finding a male on a web of a female as a function of fixed male morphological traits. Given are the coefficients with the associated standard errors for the linear and quadratic terms of the univariate models. Coefficients for linear terms are estimated without quadratic terms in the model. For explanation of abbreviations see Table 3.1.

Response: web type		b (linear)	s.e.	p	b (quadratic)	s.e.	p
Sample 1	PROWI	-0.11	0.17	0.5109	0.29	0.16	0.0665
	PATI1	-0.05	0.17	0.7640	0.09	0.13	0.4917
	PATI2	0.02	0.17	0.8991	0.19	0.14	0.1864
	PATI3	0.21	0.18	0.2207	0.30	0.17	0.0835
	PATI4	-0.02	0.17	0.9232	0.20	0.14	0.1767
Sample 2	PROWI	0.27	0.19	0.1585	0.00	0.15	0.9815
	PATI1	0.43	0.20	0.0361	0.18	0.17	0.2986
	PATI2	0.48	0.21	0.0216	0.18	0.18	0.3255
	PATI3	0.46	0.21	0.0252	0.15	0.19	0.4293
	PATI4	0.46	0.21	0.0280	0.13	0.17	0.4372

Table 3.5. Results of a general linear model of general male size (PC1) as a function of web type and observation day for both samples. A: influence of the main effects after the interaction term was dropped from the model. B: statistics for the interaction and the error term from the full model. All models were significant ($p < 0.01$).

		Source	SS	df	F	p
Sample 1	A	web type	0.0052	1	0.01	0.942
		observation day	5.5612	1	5.78	0.017
		Error	152.0517	158		
	B	web type x observation day	2.8120	1	2.96	0.087
		Error	149.2397	157		
Sample 2	A	web type	13.2350	1	14.98	0.000
		observation day	3.2613	1	3.69	0.057
		Error	90.0960	102		
	B	web type x observation day	1.8262	1	2.09	0.151
		Error	88.2699	101		

Table 3.6. Results of a general linear model of condition (PC2) as a function of web type and observation day for both samples. Sample 1: (A) influence of the main effects after the interaction term was dropped from the model; (B) statistics for the interaction and the error term from the full model. Sample 2: the interaction term was significant and the full model is shown. All models were significant ($p < 0.001$).

	Source	SS	df	F	p
Sample 1	A web type	8.7406	1	10.96	0.001
	observation day	5.9926	1	7.51	0.007
	Error	126.0171	158		
	B web type x observation day	0.0413	1	0.05	0.821
	Error	125.9758	157		
Sample 2	web type	17.6700	1	29.46	0.000
	observation day	1.3441	1	2.24	0.138
	web type x observation day	7.2411	1	12.07	0.001
	Error	60.5850	101		

Table 3.7. Results of a general linear model of log (opisthosoma area) as a function of web type and log (prosoma width) for both samples. A: influence of the main effects after the interaction term was dropped from the model. B: statistics for the interaction and the error term from the full model. All models were highly significant ($p < 0.001$).

	Source	SS	df	F	p
Sample 1	A web type	0.216	1	32.88	0.000
	log (PROWI)	0.338	1	51.47	0.000
	Error	1.050	160		
	B web type x log (PROWI)	0.005	1	0.77	0.382
	Error	1.045	159		
Sample 2	A web type	0.445	1	56.95	0.000
	log (PROWI)	0.397	1	50.78	0.000
	Error	0.814	104		
	B web type x log (PROWI)	0.003	1	0.43	0.512
	Error	0.810	103		

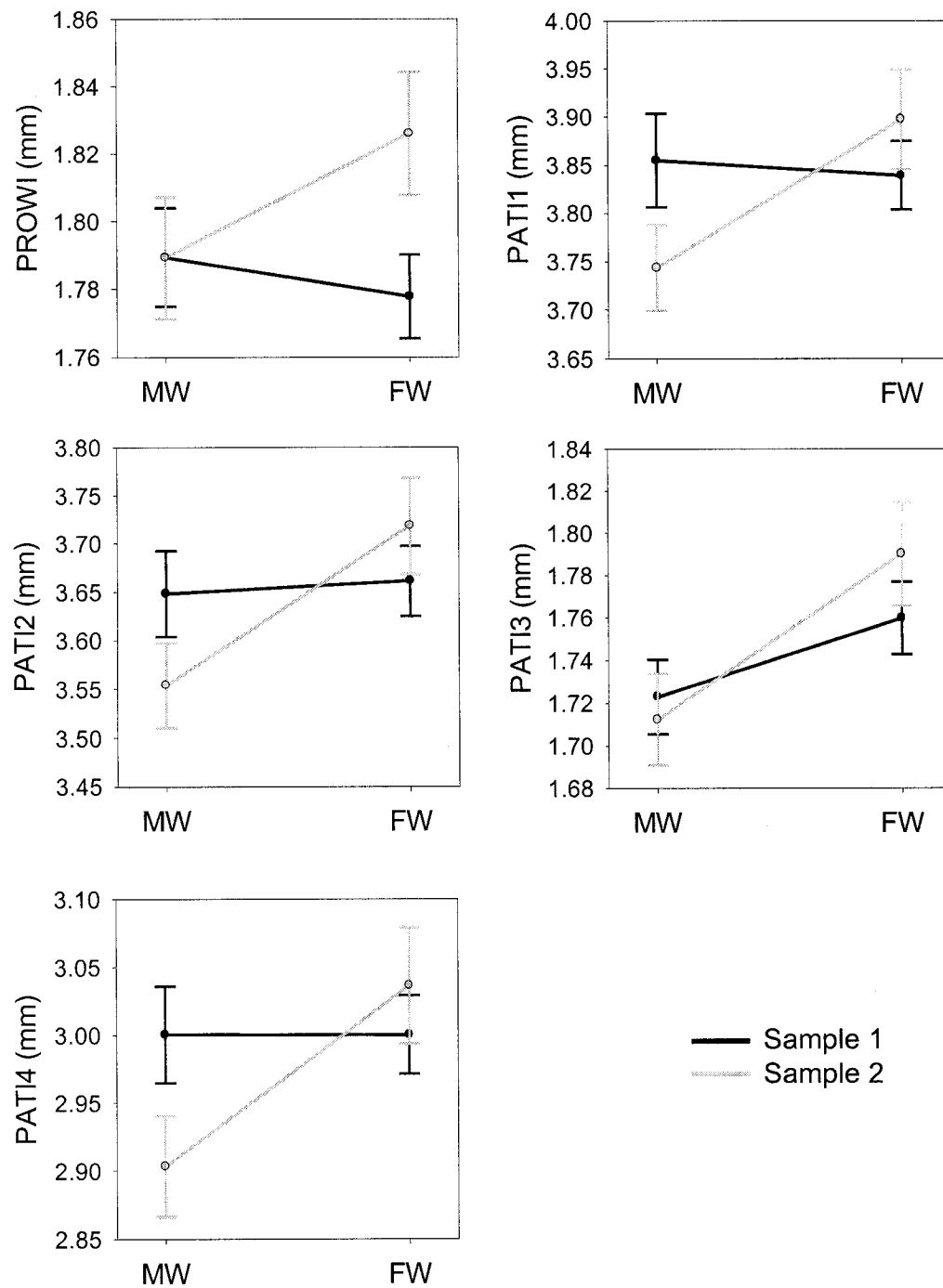


Figure 3.1. Means within standard errors of the fixed morphological traits for males that were caught on moulting webs (MW) and on females' webs (FW). Fixed traits are: prosoma width (PROWI) and the average patella-tibia lengths of the four leg pairs (PATI1 to PATI4). Sample 1: n = 52 MW, 111 FW. Sample 2: n = 51 MW, 58 FW.

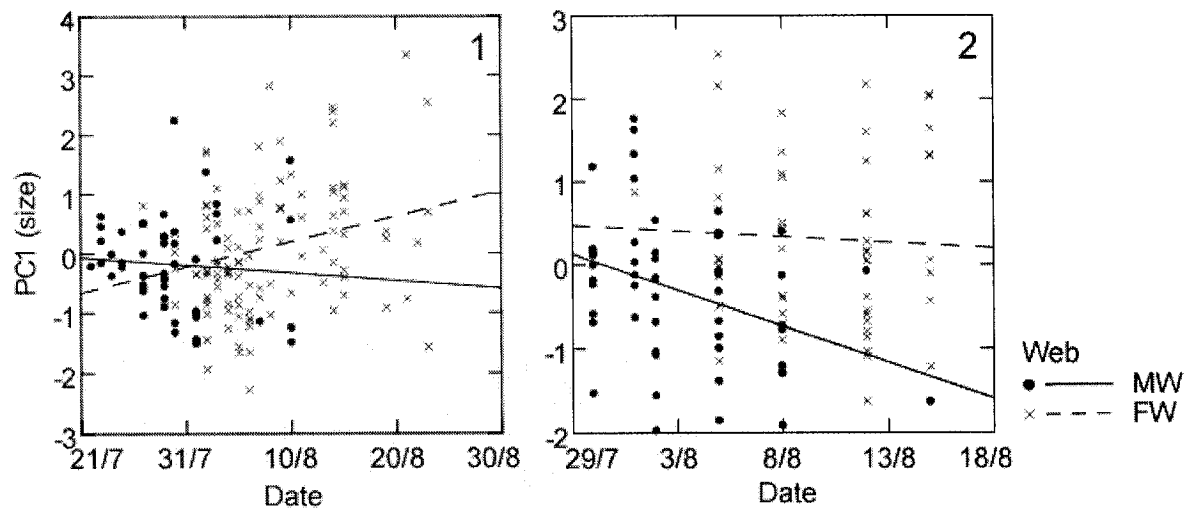


Figure 3.2. General male size (PC1) as a function of observation date for males caught on their moulting web (MW) and for males caught on a female's web (FW). 1: sample 1; 2: sample 2.

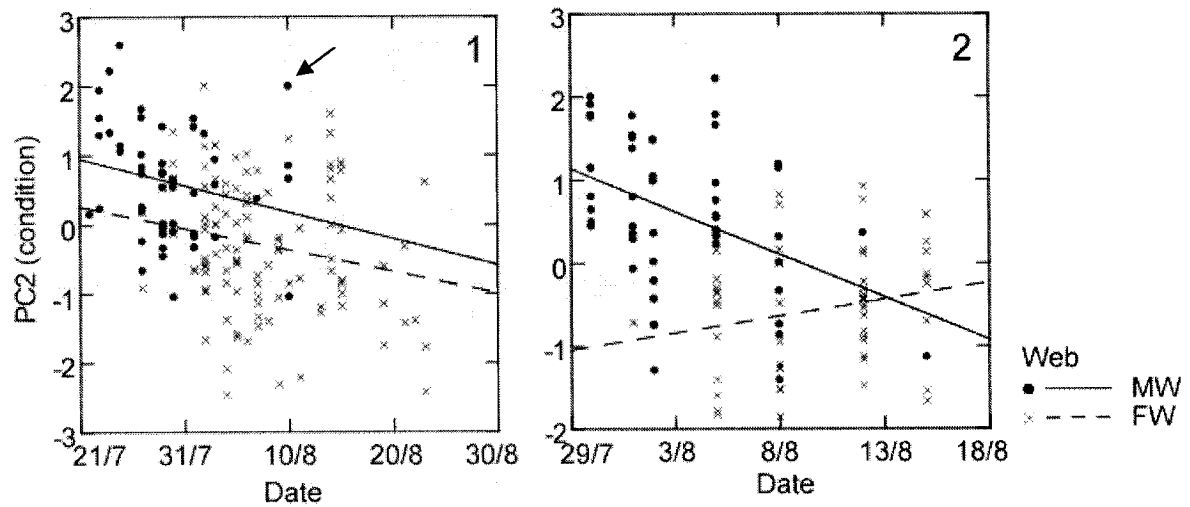


Figure 3.3. Male condition (PC2) as a function of observation date for males caught on their moulting web (MW) and for males caught on a female's web (FW). 1: sample 1; 2: sample 2. The arrow in part 1 points to an outlier in terms of its influence on the regression equation as identified by regression diagnostics (see text). The regression line shown here is based on the model containing the outlier.

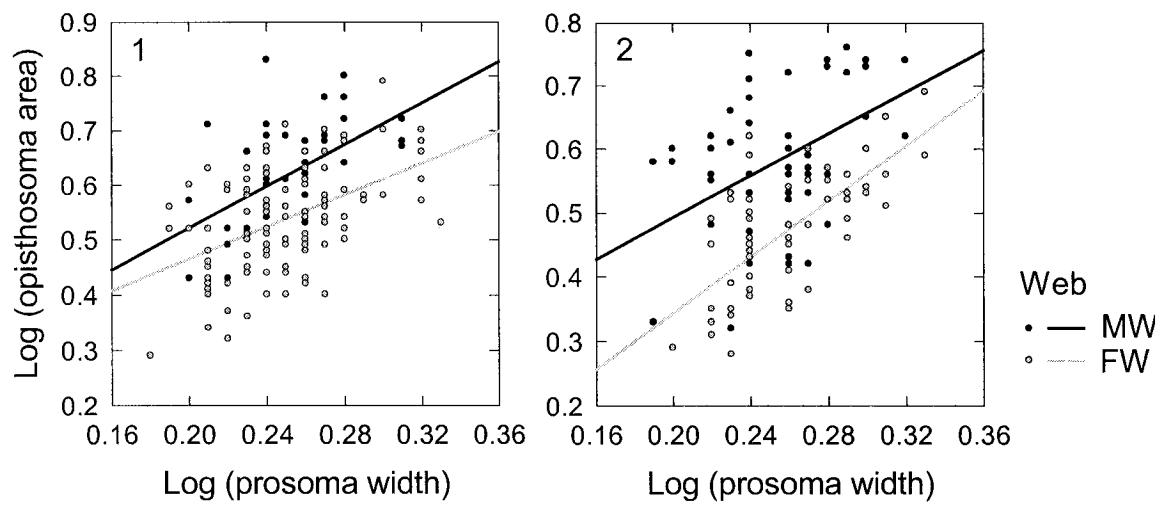


Figure 3.4. Log (opisthosoma area) as a function of log (prosoma width) for males on moulting webs (MW) and males caught on females' webs (FW). 1: sample 1; 2: sample 2.

Chapter 4. Sexual selection favours large body size during opportunistic mating in dwarf males of an orb-weaving spider

Opportunistic mating occurs when a male takes advantage of a situation where the female is either defenceless or at least distracted. In some species, males try to mate with the female while she is feeding (e.g. Elgar and Fahey, 1996). In *Argiope aurantia*, males try to mate while the female undergoes her final moult. In this situation, the female has no overt control over mating, and any males present on her web at the time of her moult have to compete for access to the female. This chapter estimates sexual selection due to male-male competition during opportunistic mating.

Introduction

Sexual size dimorphism (SSD), a difference in the mean body size of males and females, is a common phenomenon in the animal kingdom. Males are usually larger than females in homoiotherms, whereas females are typically the larger sex in poikilotherms (Andersson, 1994; Abouheif and Fairbairn, 1997; Fairbairn, 1997). The spiders (Araneae) are particularly interesting with regards to their pattern of SSD. In most species SSD is female biased, but in certain genera of orb-weaving spiders (Orbiculariae) and crab spiders (Thomisidae) extreme SSD occurs: females are much larger and may weigh over a hundred times more than males (Head, 1995; Vollrath, 1998). A size difference between the sexes of comparable magnitude is otherwise found only in some marine and parasitic taxa and rotifers (Ghiselin, 1974; Andersson, 1994; Vollrath, 1998). Spiders are thus the only terrestrial taxon where extreme SSD is common and the evolution and maintenance of extreme SSD in spiders has become a focus of current research. As yet, we are only beginning to disentangle the factors that are involved in this process (e.g. Vollrath and Parker, 1992; Coddington et al., 1997; Prenter et al., 1998; 1999; Schneider et al., 2000; Moya-Laraño et al., 2002).

Sexual size dimorphism is generally assumed to be adaptive (Fairbairn, 1990; 1997). Body size plays a central role in the life history of organisms and potentially affects fitness in many ways (Roff, 2002). Provided that body size is heritable, selection should lead to an optimal body size that maximizes fitness within given genetic and physiological constraints (Lande, 1980; Reeve and Fairbairn, 2001; Roff, 2002). The optimal body size often differs for males and females as a consequence of their different reproductive roles (Andersson, 1994; Fairbairn, 1997), and SSD can evolve in response

to different net selection acting on males and females if the genetic correlation between the sexes is less than 1 (Lande, 1980; Reeve and Fairbairn, 2001). A number of sex-specific selection regimens have been proposed to explain the evolution and maintenance of extreme SSD in spiders (Darwin, 1871; Gerhardt, 1924; Ghiselin, 1974; Vollrath and Parker, 1992; Schneider et al., 2000; Moya-Laraño et al., 2002). There is good support for the hypothesis that fecundity selection favouring large size in females is an important factor (Marshall and Gittleman, 1994; Head, 1995; Prenter et al., 1999; Higgins, 2002; see also Reeve and Fairbairn, 1999), and females appear to have increased in size over evolutionary time in several orb-weaving spider lineages independently (Coddington et al., 1997; Hormiga et al., 2000). However, what keeps males small or why they have even decreased in size in some lineages is still not well understood (Prenter et al., 1997; 1998; Walker and Rypstra, 2003; but see Vollrath, 1998; Schneider et al., 2000). In the absence of opposing selection, the genetic correlation between males and females would inevitably lead to a correlated increase in male size to some extent (Lande, 1980; Reeve and Fairbairn, 2001).

In orb-weavers, as in all web-building spiders, males leave their own web after their final moult and search for mates (Foelix, 1996). Male-male competition over access to females likely generates sexual selection for large body size in males of many spider species (reviewed in Elgar, 1998). Several studies have shown that large males usually win fights against smaller males - even in highly dimorphic species (Christenson and Goist, 1979; Vollrath, 1980; Miyashita, 1993; Elgar and Fahey, 1996; Elgar et al., 2003; but see Elgar and Bathgate, 1996). However, while such studies provide important information on the functional relationship between body size and the likelihood of

winning a contest, they do not tell us how relevant male interference competition is in nature – that is whether male body size is actually under selection in a population. For example, males may only rarely encounter each other in nature because of low population densities (Ghiselin, 1974; Legrand and Morse, 2000). Sexual selection for large size might thus be weak or absent, and this has been considered to be an important factor for the evolution of extreme SSD (Ghiselin, 1974; Legrand and Morse, 2000). The differential mortality hypothesis (Vollrath and Parker, 1992) states that sexual selection for large male size due to male-male competition would generally be weak in sit-and-wait predators, such as orb-weavers, due to an even or female-biased operational sex ratio (OSR, the ratio of adult males to females that are ready to mate [Emlen and Oring, 1977; Kvarnemo and Ahnesjö, 1996]), which in turn would be the result of high male mortality during mate search. On the other hand, if the temporal distribution of receptive females is dispersed, if for example females reach sexual maturity asynchronously, the OSR might become highly male-biased (e.g. Grant et al., 1995; Lindström and Seppä, 1996). Such an effect seems probable in many orb-weaving spiders. First, many species have been described as protandrous, that is males enter the pool of individuals ready to mate earlier than females (Foelix, 1996). Second, males tend to cohabit with penultimate females (females just one moult away from sexual maturity) for days or even weeks and many males may accumulate on a given female's web during that period (McCook, 1890; Robinson and Robinson, 1980; Robinson, 1982; Howell and Ellender, 1984; Jackson, 1986; Hill and Christenson, 1988; Alayon Garcia and de Armas, 1990; Elgar and Bathgate, 1996). An increasing OSR by definition increases competition among males, but the form of competition depends on the ability of males to defend females. At highly

male-biased OSRs, resource defence might become uneconomical for males and the system might switch from interference to scramble competition, potentially changing patterns of selection on males (Grant et al., 1995; Grant et al., 2000; Grant and Foam, 2002; Mills and Reynolds, 2003).

This study examines sexual selection on male body size and body size components due to male-male competition over access to females in the highly dimorphic orb-weaving spider *Argiope aurantia*. In *A. aurantia*, males mature on average about one week earlier than females (Howell and Ellender, 1984). Males cohabit with penultimate females and wait for the female to undergo her final moult to reach sexual maturity. Anecdotal accounts suggest that at the time of a female's moult more than one male is often present on a given female's web (McCook, 1890; Robinson and Robinson, 1980; Howell and Ellender, 1984). Males try to mate with the female while she is moulting and fight vigorously over access to the female (Robinson and Robinson, 1980; Foellmer and Fairbairn, 2003). The female is completely defenceless during her moult and cannot resist a male's mating attempt and thus cannot exert any overt mate choice (opportunistic mating: Robinson and Robinson, 1980; Foellmer and Fairbairn, 2003). Therefore, any pattern of pre-copulatory sexual selection on males in the context of opportunistic mating can unambiguously be ascribed to male-male competition. Males can also pursue an alternative mating tactic, if they encounter an already mature female during mate search. They usually approach and court a mature female directly and, if she is receptive, may mate with her (Robinson and Robinson, 1980; Foellmer and Fairbairn, 2003). In this situation, males do not face competing males but rather a cannibalistic female. Despite

the fact that female attacks occur frequently, male body size is not under selection due to sexual cannibalism in this species (Foellmer and Fairbairn, 2004).

In *A. aurantia*, the sexes are not only dimorphic in size, but also in “shape”. Adult males have relatively longer legs than females and, unlike in females, leg length scales positively allometrically with prosoma width (Appendix 1). Relatively longer legs in males are typical for all spiders (Foelix, 1996). Therefore, to understand fully sexual dimorphism in spiders, it is important not only to examine selection on body size, but also to determine the adaptive significance of leg length independent of body size. Longer legs in male *A. aurantia* are favoured during mate search (Chapter 3), but not during mating with cannibalistic females (Foellmer and Fairbairn, 2004). Males use their legs extensively during fights and an advantage of relatively long legs during agonistic encounters seems probable (see Bridge et al., 2000), but no study has yet examined direct selection on leg length during combat in any spider. In this study, we determine mating success of male *A. aurantia* during opportunistic mating and estimate sexual selection on male body size and leg length using a multivariate approach to isolate selection targeted at the different components of size (Lande and Arnold, 1983; Brodie et al., 1995; Fairbairn and Reeve, 2001). Males lose a significant amount of energy reserves during mate search (Chapter 3). We therefore also test whether male condition affects mating success. Finally, we put our results into the ecological context of the species by determining male dispersion with respect to available females and estimate the frequency of opportunistic mating in the field.

Methods

Data collection

We established a study site in an old field located on Île Perrot (45°22'N, 73°55'W), 31km southwest of Montreal in Quebec, Canada in 2000. Our general approach was to collect penultimate female *Argiope aurantia* that were close to their final moult with any cohabiting males and to temporarily cage these until the female moulted. This was necessary because it is otherwise unfeasible to get a good estimate of male mating success during opportunistic mating in the field given that opportunistic mating occurs rarely at any given time and that males that do not achieve two insertions usually rove off soon after the female's moult. A preliminary study showed that opportunistic mating occurs at similar frequencies, whether individuals are caged or unrestricted (Appendix 3).

In a 1030 m² area we placed parallel transects 1.5 m apart and searched for penultimate and adult females along these transects two to three times per week during the mating season. We recorded female status (penultimate with catching web, penultimate close to moult, or adult) and number of cohabiting males. Females close to moult are recognized by the presence of a typical moulting web (with extensive barrier webs, but without visceral spirals). Mature females can be identified by the presence of the pronounced scape on the epigyne (the sclerotized genital plate on the ventral side of their abdomen). Penultimate females are similar to mature females in general appearance, but do not yet have a developed epigyne. We tagged web sites of penultimate females and inspected tagged sites for female status almost every day. Any penultimate female which we judged to be close to moult and that had one or more males cohabiting was collected

with the associated male(s). The females were immediately released into individual wood/fibreglass screen cages (30 x 30 x 24 cm) where they could build new moulting webs. We measured and marked all collected males and checked them for signs of pedipalp insertion on the same day of collection. In *Argiope aurantia*, the relatively large cap of the sperm transferring tube, the embolus (Levi, 1968; Foelix, 1996), breaks in 96% of insertions (Appendix 2). This is easily detectable with a dissecting microscope and thus constitutes a good estimator of male insertion success. We use embolus breakage as an indicator of pedipalp insertion throughout this paper. Note that this means that we might have missed about 4% of palp insertions. Male *A. aurantia* use each palp only once and invariably die during the second insertion (Foellmer and Fairbairn, 2003); they therefore can achieve a maximum of two insertions.

The morning following the day of capture, after the female had constructed a new web, we released all males into the cage with the female with whom they had been originally found. This was done in random order and into a corner where support structures of the female's web were not attached. We performed scan samples three times per day (10:00, 13:00, 16:00) and recorded female status (moulted or not) and the position of males. We distinguished the following three positions a male could assume: at the hub (very close to the female, touching her with one or more legs), on the web (on the actual web plane between the frame and the hub), and elsewhere (in the barrier web, on other peripheral web structures such as support threads, or somewhere else in the cage). Note that females moult at the hub, hanging from a short moulting thread. The first scan was done at 16:00h on the day of release, giving males at least 6 hours to assume new positions on the web (males typically find and enter a female's web rapidly after release

into a cage). If individuals were interacting at the time of a scan, we observed and recorded any interactions (fights, mating) until these had ceased. After a female had moulted, any dead males were preserved and any other males were inspected for palp insertions, and then released where originally found. Therefore, these males had the opportunity to search again for other mates. Adult females were measured and also released where found.

We measured the following traits of males: prosoma width (at the broadest point), opisthosoma length and width (at the broadest point), and the combined patella-tibia length of each of the eight legs. We used the average patella-tibia length of each leg pair for later analysis. If one leg of a pair was missing, the length of the remaining leg was used. For females we measured prosoma width. All measurements were taken from the dorsal aspect under a dissecting microscope with individuals held gently between two lids of Petri dishes balanced with cotton. Repeatability of all measurements was > 0.89 ($n = 10$, $k = 3$ repeated measures). Prosoma width and patella-tibia length are fixed traits, i.e. they do not change after the final moult. Opisthosoma dimensions do change with the nutritional status of the individual. Adult males rarely feed; the opisthosoma of males therefore shrinks during the adult life as the stored reserves in the opisthosoma are used up (Foelix, 1996). Opisthosoma dimensions can therefore be used to estimate male condition.

We caged a total of 54 females with 129 males over the period from 28 July 2000 to 29 August 2000. Five caged females (9.3%) moulted to the penultimate stage, which means that males were associated with antepenultimate females when collected. In four of these cases we had suspected females to be antepenultimate based on their small size

and general appearance. Four females (7.4%) moulted to maturity before we could introduce males. Only one male was caged twice and did not achieve any insertion in either case. This male was included in the analyses only once. Therefore, our analyses are based on 45 females and 111 males. We caged a mean number of 2.5 males per female, ranging from 1 – 7 (Figure 4.1). Males were caged for a mean duration of 1.9 days (range 1 - 4).

Data analysis

We performed a principal components analysis (PCA) with Varimax rotation on all variables to obtain a variable representing overall male size. Varimax rotation optimizes factor solutions by making large factor loadings larger and small factor loadings smaller, so that variables are ideally correlated with only one of the extracted factors (SPSS Inc., 1997). PCA extracted two components. The first component (PC1) of the rotated solution explained most of the variance (64%) and was highly correlated with the fixed morphological traits (Table 4.1). The second component (PC2) explained 26% of the variance and was highly correlated with opisthosoma length and width. Therefore, PC1 can be interpreted as an estimator of overall fixed male size, and PC2 as an estimator of male condition.

The individuals in our experiment were temporarily confined in cages. However, because we continuously caged males according to their natural association pattern and because we released any males that did not achieve both insertions at the original site and allowed them to achieve further mating, we consider each male as an independent data point in the analyses. We estimated selection on male body size and body size components using standard selection gradient analysis (Lande and Arnold, 1983;

Fairbairn and Reeve, 2001). Prior to analysis we standardized variables to z-scores with a mean of zero and a standard deviation of one: $z_i = (x_i - \bar{x})/s_x$. Our fitness measure, insertion success, was converted to relative fitness by dividing individual absolute fitness by the mean absolute fitness: $w'_i = w_i/\bar{w}$. We estimated net selection (direct selection and indirect selection through selection on phenotypically correlated characters combined) on a given trait using univariate regression models. The linear model ($w'_i = c + \beta_{uni} z_i$) estimates directional selection, whereas the quadratic model ($w'_i = c + \beta' z_i + 0.5 \gamma_{uni} z_i^2$) estimates the univariate nonlinear selection gradient and thus the curvature of the fitness function (indicating either stabilizing or disruptive selection). Note that the univariate linear selection gradient is equivalent to the selection intensity (standardized selection differential). We estimated direct linear selection on body size components using a multivariate regression model ($w'_i = c + \sum \beta_{multi,j} z_{ij}$) containing all fixed male morphological traits, but not PC1. The multivariate model thus estimates selection directly targeted at a given trait holding the other traits in the model constant. We did not attempt to estimate multivariate nonlinear gradients to preserve power. Because the residuals from the regression analyses were not normally distributed, we used the randomization software RT (Manly, 1992) for significance testing of regression coefficients (i.e. selection gradients) by randomizing the dependent variable 9999 times. To visualize the pattern of selection we calculated cubic spline estimates of the univariate fitness surfaces for all fixed morphological traits (Schluter, 1988).

Results

Only two males had one palp already used when collected. Here we present the results of analyses involving only palp insertions achieved during our experiment; however, inclusion of the two palp insertions achieved prior to collecting did not change any of the results. In our experiment, forty-two males (37.8%) did not achieve any insertion, 22 (19.8%) achieved one insertion, and 47 males (42.4%) achieved two insertions. Females received on average 2.6 (standard deviation = 1.2) insertions, while mating with a mean of 1.6 (standard deviation = 0.8) males. Two females (4.4%) did not receive any insertion, three (6.7%) received one insertion, 20 (44.4%) received two and the remaining 20 more than two (3-6) insertions.

Direct observations

While males were waiting for females to moult, we observed three fights between males, that is in 0.9% out of a total of 329 scan samples performed, excluding mating events. Given that males were on average caged for 1.9 days, this means that per cage, males spent an estimated total time of 25 min fighting during cohabitation. We observed opportunistic mating involving seven females, and in all cases males were interacting agonistically. In four cases males were fighting frantically to insert a pedipalp, and we saw two males being attacked during their first insertion. In the three other cases we started our observation after one male had already achieved two insertions and was dead, stuck in the female. In all seven cases, other males tried to pull out dead males and succeeded in three instances (see Foellmer and Fairbairn, 2003).

In four cases the female was found at one point at the periphery of her web, and in one instance we directly observed the female relocating as a consequence of what looked

like males “probing” the female (involving the drumming against the female with the front legs, similar to courtship behaviour). All of these females eventually returned to the hub, where they were mated.

Selection on male morphology

The univariate linear selection gradients for general male size (PC1), prosoma width (PROWI), and the average patella-tibia length of each of the four leg pairs (PATI1 – 4) were all positive and significant, whereas none of the univariate nonlinear and multivariate linear gradients was significant (Table 4.2). Thus there was no evidence of stabilizing or disruptive selection. Further, none of the traits was directly targeted by selection independently of the other traits included in the multivariate model (Table 4.2). Taken together, this suggests that males were under directional selection for overall large body size during opportunistic mating, leading to significant net selection for a broader prosoma and longer legs (Figure 4.2).

Since the number of males per female varied considerably, the selective environment (i.e. the local OSR) was not equal for all males. Therefore, competition over access to the female can be expected to vary accordingly and to affect the intensity of selection on male body size and male mating success in general. We tested this by including the number of males per female in the analysis together with general male size (PC1). Both the number of males per female and male body size were significant predictors of male mating success (main effects after dropping the non-significant interaction term: males per female, $\beta = -0.135$, s.e. = 0.050, $n = 111$, $p = 0.0076$; PC1, $\beta = 0.259$, s.e. = 0.077, $n = 111$, $p = 0.0012$), but selection on male size did not appear to vary in intensity, as indicated by the non-significant interaction term ($\beta = 0.009$, s.e. = 0.054, n

= 111, $p > 0.8$). Hence, our data suggest that mean male mating success decreases as the number of males present on a female's web, and thus the local OSR, increases (Figure 4.3A). Males are thus under strong selection for large body size when they have to compete with other males, irrespective of the number of males present on the web. Nevertheless, visual inspection of selection intensities estimated for overall male size (PC1) for different levels of the local OSR separately (i.e. for cases with one, two, three, and more than three males present per female) reveals a tendency for the intensity of selection to increase with increasing number of males per female, and this effect might level off at higher values for the OSR (Figure 4.3B). Note, however, that none of the selection intensities for the different OSR categories was statistically significant (OSR = 1: $p > 0.4$; for all other groups: $0.1 > p > 0.05$). In the case of single males per female, selection for large male size would not be expected, but in the other cases nonsignificance was probably due to low power because of reduced sample sizes. When we increased power by grouping all cases with more than two males per female together ($n = 69$), selection on size was significant ($\beta = 0.331$, s.e. = 0.116, $p = 0.006$). Therefore, larger sample sizes would be required to confirm the subtle effects the local OSR might have on the selection intensity as suggested here.

The number of males per female was uncorrelated with collection date ($r_s = -0.002$, $n = 111$, $p > 0.9$). However, overall male size (PC1) did increase as the season progressed ($r_p = 0.277$, $n = 111$, $p < 0.01$). Therefore, the intensity of selection on male body size might change over the season. To test this, we performed a multiple regression of relative fitness on collection date and PC1. A change of the selection intensity with time would be indicated by a significant interaction term. However, the interaction term

was not significant ($\beta = 0.004$, s.e. = 0.011, $n = 111$, $p > 0.7$; main effects after dropping the interaction term from the model: collection date, $\beta = -0.001$, s.e. = 0.011, $n = 111$, $p > 0.9$; PC1, $\beta = 0.305$, s.e. = 0.081, $n = 111$, $p = 0.0002$). Hence we could not detect any seasonal variation in the intensity of selection on male body size during opportunistic mating.

Effect of male condition

To test whether male condition had an effect on male mating success, we performed a multiple regression analysis with condition (PC2), male body size (PC1), and number of males per female as predictors of relative fitness with all resulting interaction terms (Table 4.3). None of the interaction terms was significant. When these were dropped from the model, male condition was not a significant predictor of male mating success. This suggests that energy reserves did not limit male competitive success.

Effect of the male's position on the web

With respect to achieving insertions, males that assume a position close to the female shortly before or during her moult can be expected to have an advantage over males that are farther away, for example in the barrier web. We tested this using only cases with more than one male per female ($n = 102$). Males that were closer to the female at the last scan prior to mating achieved more insertions than males that were farther away (Figure 4.4A; $\chi^2 = 15.0$, d.f. = 4, $p < 0.005$). However, overall male size (PC1) did not differ for males that were at the hub, on the web, or elsewhere at the last scan (Figure 4.4B; $F = 0.75$, d.f. = 2, $p > 0.4$). Consequently, both male position prior to mating and male body size were significant predictors of male mating success independently of each

other (Table 4.4). The larger the male and the closer the male was to the female prior to her moult, the higher the male's mating success.

If a male's position prior the female's moult is so important, it might be advantageous for a male to keep the most favourable position over time. However, the number of pedipalp insertions was not associated with the relative frequency with which males were recorded at the hub during the caging period (two insertions: $\bar{x} = 13.9\%$, one insertion: $\bar{x} = 14.6\%$, no insertion: $\bar{x} = 10.5\%$, Kruskal Wallis $\chi^2 = 2.43$, $p > 0.25$), and many males were actually never seen at the hub (median for all three insertion groups = 0%). This suggests that males are not able to defend a hub position, which is in line with the finding that males found at the hub prior to mating were not larger than other males. Note also that more than one male may be present at the hub with the female; it is thus not a position that one male can occupy exclusively.

Lost legs

Thirty-one males (27.9%) lost at least one leg during opportunistic mating (McNemar test $p < 0.001$). Males that lost at least one leg were smaller than those that did not (PC1: $\bar{x} = -0.38$, s.e. = 0.12 vs. $\bar{x} = 0.15$, s.e. = 0.12; $t = 3.17$; d.f. = 89.8; $p < 0.005$; equal variances not assumed). Twenty-five males (22.5%) were initially missing at least one leg. However, this did not affect male mating success or the intensity of selection on male size (main effects in a general linear model with relative fitness as the response after dropping the non-significant interaction term: missing leg, $F = 0.180$, d.f. = 1, $p > 0.6$; PC1, $F = 13.726$, d.f. = 1, $p < 0.001$; interaction term, $F = 1.335$, d.f. = 1, $p > 0.2$). Therefore, male competitive ability was not adversely affected by initially missing legs, but smaller males were more likely to lose a leg during opportunistic mating.

Female size

If the size of available females changes over the season, then this could affect male fitness, because of the presumed higher fecundity of larger females. However, the size of females in our experiment was not correlated with maturation date ($r_p = -0.139$, $n = 45$, $p > 0.3$).

Dispersion of males and the relative frequency of opportunistic mating

To evaluate whether selection for large male body size during opportunistic mating is relevant, we have to determine the relative occurrence of opportunistic mating in the field. Our transect searches revealed the following pattern. Penultimate females that are close to their final moult were never frequent in the study population relative to younger penultimate females (e.g. still with catching webs) and mature females. From the males' perspective, therefore, penultimate females close to moult were both spatially and temporally rare and dispersed (Figure 4.5A). Yet, about half of all males that were cohabiting with females were associated with penultimates close to moult, and most penultimate females close to moult were found with cohabiting males during most of the mating season (Figure 4.5B-C). Further, the male to female ratio was much higher for penultimate females close to moult than for other penultimate or adult females and equalled at least two most of the time, even though females with no males were included in this calculation (Figure 4.5D). Consequently, the dispersion of males cohabiting with females (all three types of females considered) was significantly clumped over almost the entire mating season (Figure 4.5E). If we weight the percentage of penultimate females close to moult that are associated with males (Figure 4.5B) by the actual number of these females in the field at a given time (Figure 4.5A) and multiply this by the proportion of

opportunistically mated females in the experiment (0.95), we obtain that on average 62% of females were mated opportunistically at our site. Taken together, this suggests that males show a strong preference for penultimate females close to moult and that opportunistic mating occurs frequently in *A. aurantia*.

Discussion

Our results clearly demonstrate pre-copulatory sexual selection on male *Argiope aurantia* due to male-male competition over access to newly-moulted adult females. When males compete for these “opportunistic” matings, larger males are more successful. All measured fixed morphological traits, prosoma width and average patella-tibia length of each of the four leg pairs, were under significant net selection, favouring an increase in trait dimensions. We did not find any evidence for stabilizing or disruptive selection. Further, none of the traits was directly targeted by selection independent of the others, indicating that selection favours overall large body size. The absence of evidence for selection favouring longer legs when body size is held constant (i.e. in the multivariate model) does not support the hypothesis that relatively longer legs in males are favoured during male contests (Prenter et al., 1995; Bridge et al., 2000).

During competition for opportunistic matings, males may gain advantage through both scramble and interference competition. Males interact aggressively while cohabiting with a female, and once the female has commenced moulting they fight fiercely over access to the female and often attack any male that has inserted a pedipalp (see also Robinson and Robinson, 1980; Foellmer and Fairbairn, 2003). Almost 30% of males lost at least one leg and the probability of leg loss was higher for smaller males. Legs that were lost were probably autotomized in self-defence during escalated contests (Foelix,

1996; Punzo, 1997). This indicates that interference competition is important during opportunistic mating and that large males have an advantage during combat. However, we also found that the proximity of a male to the female on the web prior to the female's moult was a significant predictor of male mating success. Males that were at the hub at the last scan prior to mating did not differ in size from males that were further away from the female, and position predicted male mating success independently of body size. Males do not defend the hub position, unlike in other orb-weavers (e.g. Christenson and Goist, 1979; Elgar and Fahey, 1996), and therefore, success in maintaining position near the hub, and hence in obtaining matings, appears to be due to scramble competition rather than direct contest. Thus, both interference and scramble competition probably operate during opportunistic mating in *A. aurantia*, and interference competition results in selection for large body size in males.

Males congregated on webs of penultimate females close to moult and the number of males per female was very variable, ranging from one to seven. Therefore, males may find themselves with no or varying degrees of competition. However, most males (92%) faced at least one competitor, and the number of males per female, i.e. the local OSR, had no significant overall effect on the intensity of sexual selection on male body size. Nevertheless, mean male mating success did decrease with increasing local OSR, and the comparison of selection intensities at different levels of the OSR suggests that selection intensity increases as the OSR increases from one to three, but may then level off. When three or more males are present with the female, "sneaking" an insertion, for example while other males are fighting, can be expected to become a viable option (Grant et al., 1995; Kvarnemo et al., 1995), and this is perhaps the reason why the intensity of

selection on body size might level off at increasingly male-biased OSRs (see Grant et al., 1995; Mills and Reynolds, 2003). Selection on body size in males was significant even when only cases with three or more males per female were considered, indicating that interference competition was still important at higher levels of the OSR. However, the possibility that the intensity of selection levels off at high OSRs due to sneaking by smaller males remains. Unfortunately, our data do not allow for a firmer conclusion at this point.

Male condition did not influence male mating success. During mate search, adult male *A. aurantia* do not actively forage and hence lose condition (i.e. energy reserves) (Chapter 3). Our results suggest that males arriving on a female's web nevertheless have enough energy reserves to successfully compete over access to the female. Note that in our experiment males were unlikely to be able to feed, because no prey item was in any of the webs that females constructed in the cages. In *A. aurantia*, male condition is negatively correlated with maturation date, perhaps because males trade off energy reserves with achieved adult size (Chapter 3). Males start to mature about a week earlier than females and all males have become adult by the middle of the mating season (Chapter 3); later maturing males thus might not need as many reserves as early maturing males and may therefore invest relatively more in achieved body size (Roff, 2002). Our finding that male body size but not condition affected mating success supports this argument.

We did not detect any seasonal effects influencing male mating success or the intensity of selection on male body size. In our experiment, the number of males per female was not correlated with collection date. The transect counts showed that

penultimate females close to moult were spatially and temporally rare and dispersed, and the ratio of males per penultimate female close to moult was strongly male-biased throughout most of the mating season. In contrast, the sex ratio was female-biased for penultimate females not close to moult and, importantly, for mature females during the entire season. This suggests that male-male competition is irrelevant when males approach older mature females (see Foellmer and Fairbairn, 2004), and also that many adult females are probably unavailable as mates at any given time (Appendix 2). Finally, the size of females in our experiment did not correlate with maturation date. Therefore, earlier maturing males did not have the advantage of mating with larger and presumably more fecund females, as had been suggested for other species (Miyashita, 1993).

Most females are mated opportunistically in *A. aurantia*, and sexual selection for larger body size due to male interference competition is therefore an important component determining lifetime fitness in this species. During mating with mature cannibalistic females neither body size nor leg length appears to be under selection (Foellmer and Fairbairn, 2004). This raises the question to what extent finding a female first, i.e. scramble competition “on the ground” (vs. on the web), might be important (e.g. Schwagmeyer and Wootner, 1986; Legrand and Morse, 2000). Males have days to reach a given penultimate female and accumulate on webs of these females accordingly. Given that no male can assume a favourable position on the web and defend it, reaching a penultimate female’s web first is unlikely to be important. Nevertheless, some males mate with mature, “post-moult” females, and most females mate with only one or two males (Appendix 2). Thus, even when other aspects such as sperm competition are ignored, being first likely becomes relevant in this situation. During mate search, i.e. the

“roving phase”, viability selection varies, but longer legs tend to be favoured, leading to selection for overall larger male size (Chapter 3). Therefore, in *A. aurantia* there is probably overall significant selection for larger body size in males during the adult stage. This means that we should expect viability selection favouring smaller size during the juvenile stages to counteract selection for large size during the adult stage, given that male size has changed little over evolutionary time in this lineage (Lande, 1980; Hormiga et al., 2000; Reeve and Fairbairn, 2001).

Our study demonstrates the importance of recognizing the OSR at a local level (Lawrence, 1986; Grant et al., 1995). Even as the overall sex ratio becomes female-biased due to male mortality during mating and mate search, the local OSR remains male-biased until the end of the mating season, leading to significant sexual selection for larger males due to male-male competition. Therefore, the mechanism proposed by Vollrath and Parker (1992), that sexual selection due to male-male competition would be relaxed because of a high male mortality during mate search, is unlikely to be relevant. The fact that males are able to congregate on webs of penultimate females close to moult even at the end of the season argues further against a high travel mortality. Indeed, the proposed stage-dependent mortality difference between males and females has not been found in a burrowing wolf spider (Walker and Rypstra, 2003; but see Andrade, 2003), and the prediction of the differential mortality hypothesis that SSD should be related to life-history differences (sit-and-wait vs. actively hunting foragers) in spiders was not supported (Prenter et al., 1997; 1998). Given that male cohabitation with penultimate females is a common phenomenon and that large males have been shown to win in contests in many orb-weavers (e.g. Robinson and Robinson, 1980; Alayon Garcia and de

Armas, 1990; Elgar et al., 2003), sexual selection for larger size in males due to male interference competition probably operates in other highly dimorphic orb-weavers.

Recently, small males have been shown to employ a more efficient mating tactic in the absence of direct competitors in *Nephila edulis* (Schneider et al., 2000). Male size is very variable in this species; small males can approach the female directly, while larger males insert their palps through a whole they cut into the web. As a result, smaller males achieve longer copulations and hence higher fertilization success than larger ones (Schneider et al., 2000). However, the overall significance of this is uncertain, given that larger males often prevent smaller ones from mating in *N. edulis* (Elgar et al., 2003). Nevertheless, this example provides the first evidence of a small male mating advantage in a spider and shows possible limits to achieving an ever larger size in males.

In conclusion, we have shown that in *Argiope aurantia* male-male competition over access to females selects for overall larger size in males, but not for relatively longer legs, as had previously been proposed (e.g. Bridge et al., 2000). The intensity of selection on male size was overall independent of the local OSR (i.e. the number of males per female on a web), but this issue warrants further investigation, as the selection intensity might actually initially increase with increasing local OSR and then level off at higher OSR levels. Our results suggest that sexual selection for large male size may be stronger and more common than previously thought in highly dimorphic species, which is at odds with current hypotheses about the evolution of extreme SSD in spiders (Ghiselin, 1974; Vollrath and Parker, 1992). Future studies should explore how viability selection during juvenile stages might counteract sexual selection for large male size in highly dimorphic spider species.

Table 4.1. Factor loadings for the first and second extracted principal components after Varimax rotation. Fixed traits are: prosoma width (PROWI) and the average patella-tibia lengths of the four leg pairs (PATI1 to PATI4). Variable traits are: opisthosoma width (OPIWI) and length (OPILE). n = 111.

	PC1	PC2
PROWI	0.913	0.228
PATI1	0.942	0.252
PATI2	0.941	0.223
PATI3	0.918	0.263
PATI4	0.938	0.256
OPIWI	0.398	0.811
OPILE	0.127	0.933

Table 4.2. Selection gradients with standard errors and p -values for the five fixed morphological traits and PC1 (general body size). Relative fitness is based on insertion success. β_{uni} = univariate linear gradients; γ_{uni} = univariate nonlinear gradients; β_{multi} = multivariate linear gradients. Coefficients that are significant after correction for multiple significance tests are in bold. For explanation of abbreviations see Table 4.1. All analyses: $n = 111$.

Trait	β_{uni}	s.e.	p	γ_{uni}	s.e.	p	β_{multi}	s.e.	p
PC1	0.303	0.077	0.0004	0.005	0.054	0.9677			
PROW	0.283	0.078	0.0003	0.071	0.058	0.5535	0.214	0.189	0.2652
PAT11	0.256	0.079	0.0016	0.100	0.060	0.4162	-0.344	0.327	0.2963
PAT12	0.278	0.078	0.0008	0.116	0.058	0.3371	0.272	0.274	0.3349
PAT13	0.269	0.078	0.0005	0.088	0.055	0.4054	0.004	0.223	0.9860
PAT14	0.272	0.078	0.0011	0.088	0.057	0.4658	0.153	0.293	0.5978

Table 4.3. Results of a multiple regression analyses of relative fitness as a function of number of males present per female, overall male body size (PC1), and male condition (PC2). The coefficients of the main effects were obtained from the model after the interaction terms had been dropped. Coefficients that are significant after correction for multiple significance tests are in bold. N = 111.

Variable	β	s.e.	<i>p</i>
Number of males per female (MPF)	-0.134	0.051	0.0097
Body size (PC1)	0.259	0.077	0.0010
Condition (PC2)	-0.017	0.076	0.8180
Interaction MPF x PC1	-0.044	0.060	0.4633
Interaction MPF x PC2	-0.005	0.059	0.9283
Interaction PC1 x PC2	-0.077	0.204	0.7190
Interaction MPF x PC1 x PC2	0.069	0.057	0.2336

Table 4.4. Results of a multiple regression analyses of relative fitness as a function of number of males present per female, overall male body size (PC1), and the position of a male at the last scan before mating. Only cases with two more males were included (n = 102). The coefficients of the main effects were obtained from the model after the interaction terms had been dropped. Coefficients that are significant after correction for multiple significance tests are in bold.

Variable	β	s.e.	p
Number of males per female (MPF)	-0.061	0.055	0.2705
Body size (PC1)	0.258	0.077	0.0012
Position before mating (PBM)	-0.457	0.122	0.0003
Interaction MPF x PC1	0.393	0.247	0.1143
Interaction MPF x PBM	-0.179	0.106	0.0963
Interaction PC1 x PBM	0.540	0.329	0.1077
Interaction MPF x PC1 x PBM	-0.164	0.108	0.1332

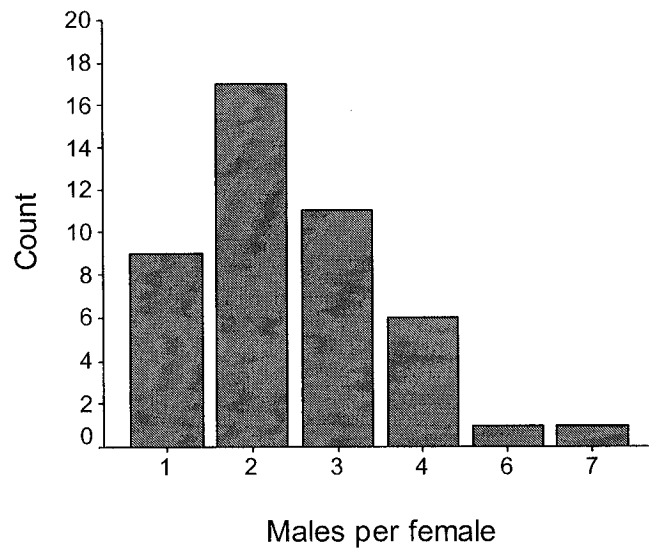


Figure 4.1. Frequency distribution of the number of males per female for females caged in the experiment. Females: $n = 45$; males: $n = 111$.

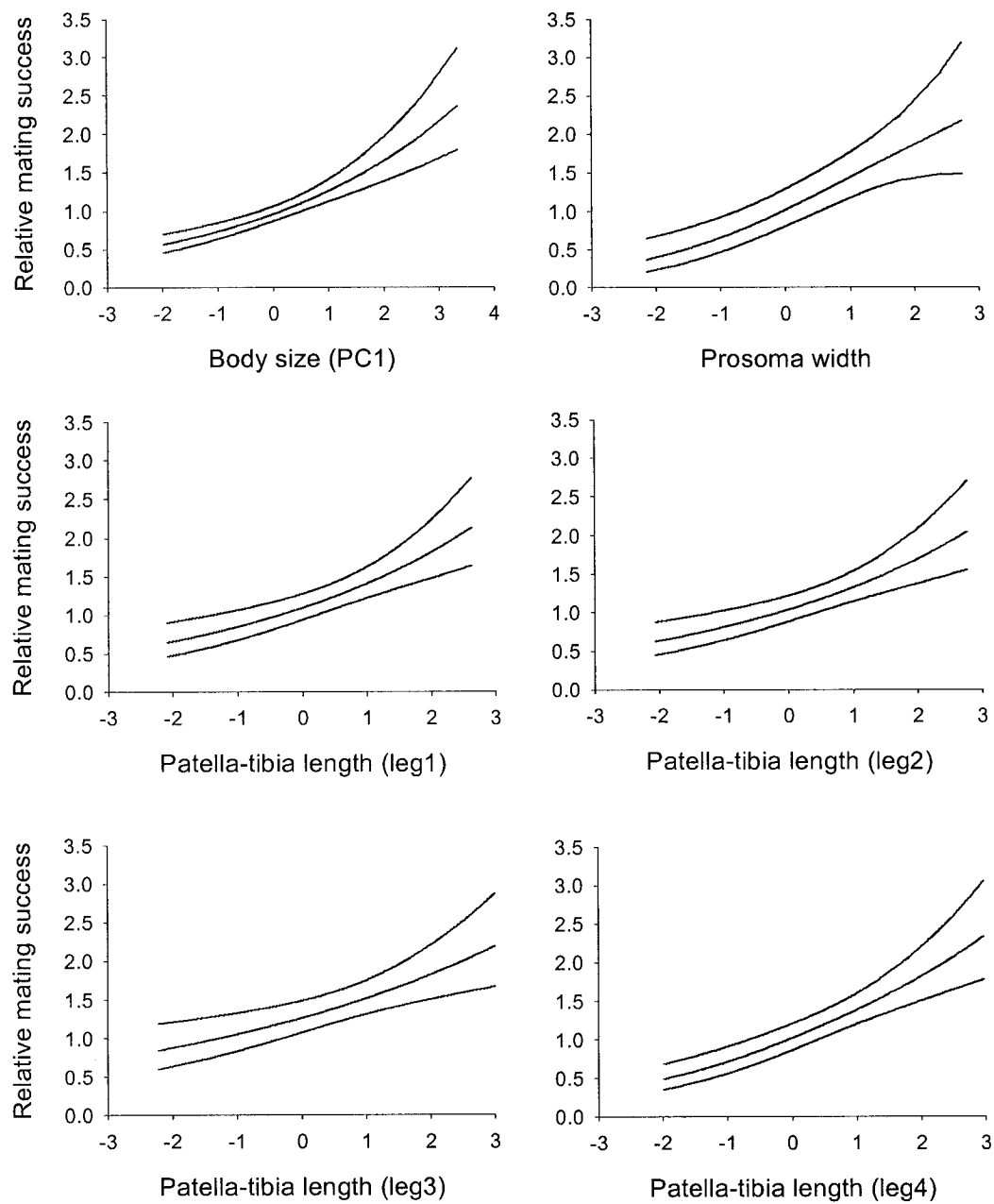


Figure 4.2. Univariate fitness surfaces (cubic spline estimates) for overall male body size (PC1) and male body size components with standard errors (based on bootstrapping).

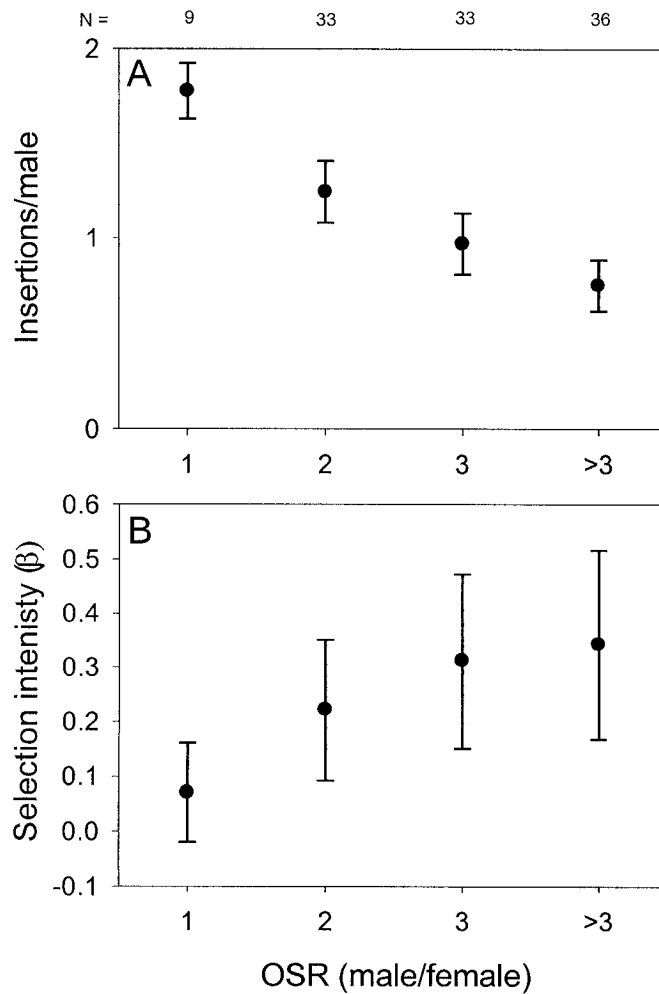


Figure 4.3. Mean number of insertions per male (A) and the intensity of selection on overall male body size (B) as a function of the operational sex ratio (OSR). Error bars represent standard errors. Selection intensities are the regression coefficients from the univariate regression of relative fitness on male body size (PC1) performed for the different OSR categories separately. The numbers on top of the upper panel indicate the sample sizes for each category. See text for further details.

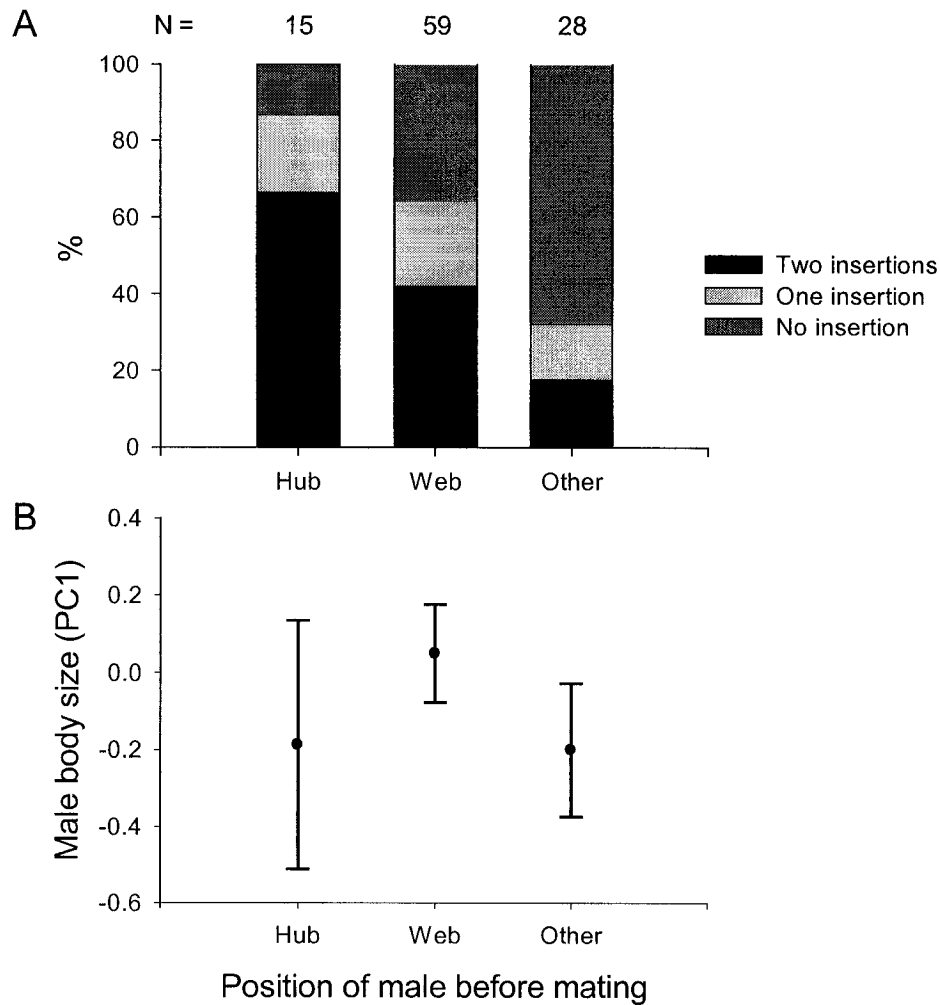


Figure 4.4. Relative frequency of insertion success (A) and mean overall size with standard errors (B) for males at different positions at the last scan before opportunistic mating. The category “Other” includes males that were in the barrier web, on other peripheral web structures, or somewhere else in the cage. Only cases with two or more males are included ($n = 102$). The numbers on top of the bars indicate the sample sizes for each group of males.

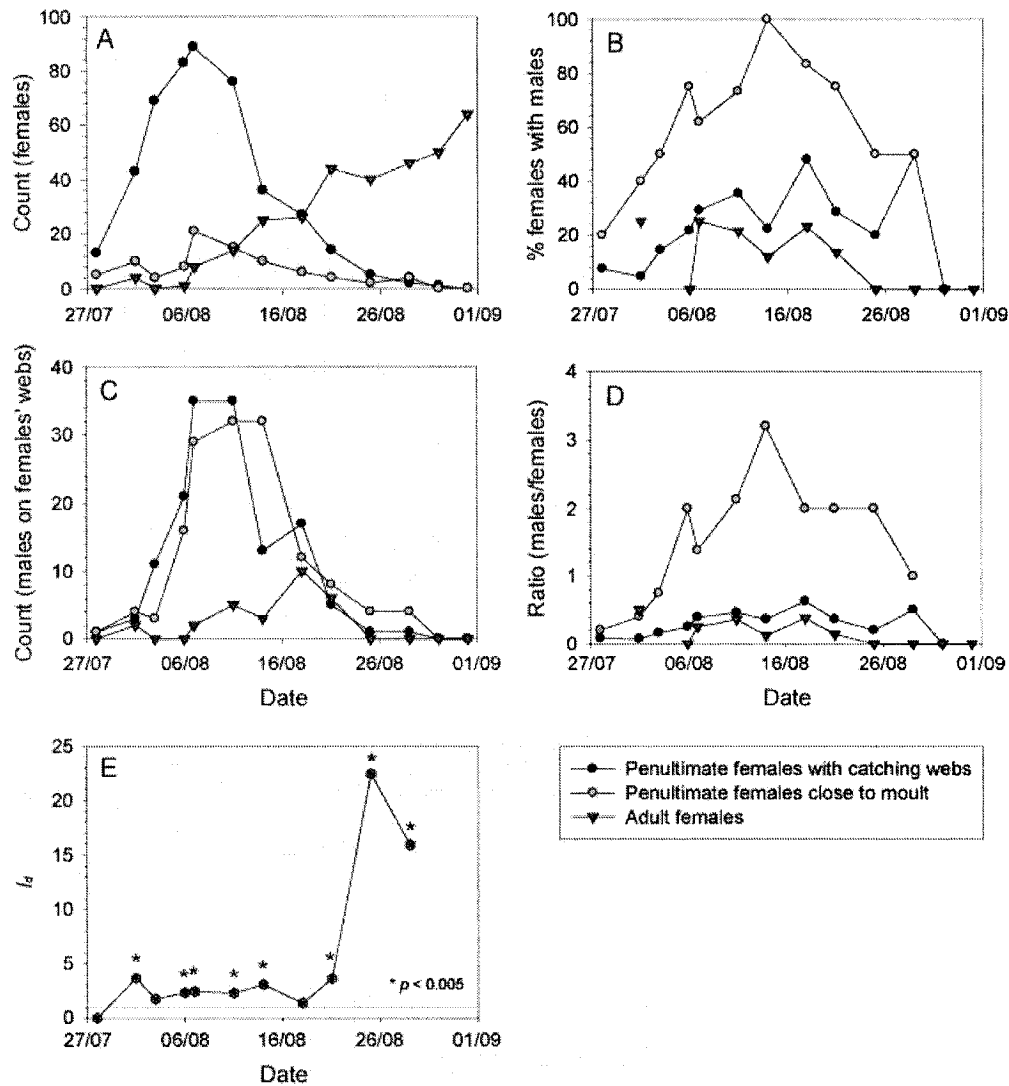


Figure 4.5. Seasonal change in the frequencies of penultimate and adult females and adult males and their association patterns at the study site. A: absolute frequency of penultimate females with catching webs, penultimate females close to moult, and adult females. B: relative frequency of the three types of females that were found with cohabiting males. C: absolute frequency of adult males cohabiting with each of the three types of females. D: cohabiting male to female ratio for the three types of females. E: Morisita's index of dispersion for males per female for all three types of females combined; the grey line at $I_d = 1$ denotes a random distribution.

General Conclusions

This thesis examines sexual selection on males of the highly dimorphic orb-weaving spider *Argiope aurantia*. The main endeavour of this thesis was to investigate selection on male morphology and to relate the obtained results to the evolution and maintenance of extreme sexual size dimorphism (SSD), but other important aspects of the reproductive biology of *A. aurantia* have also emerged, such as the sudden, programmed death of males during copulation.

During mate search, selection on male morphology may vary between populations, but selection tends to favour longer legs, and this may result in net selection for overall larger males. Thus, contrary to the prediction of the gravity hypothesis (Moya-Laraño et al., 2002), small males do not have an advantage during mate search. Further, the distinct shape of adult males with their relatively longer legs appears to be an adaptation during mate search and probably increases their running speed (see also Legrand and Morse, 2000). The adoption of a search-adapted morphology is reminiscent of male anglerfish that metamorphose into dwarf males with a streamlined body shape and large tail fin (Andersson, 1994; and refs. therein).

During mating, the pattern of selection on males depends on the type of female they mate with. Most male *A. aurantia* mate opportunistically – that is with a moulting female. Due to the fact that females close to their final moult are scarce in space and time, males aggregate on penultimate females' webs and have to compete over access to the female. Males are under strong sexual selection for large body size due to male-male interference competition, even though the number of competitors on females' webs varies considerably and males seem unable to defend a female. On the other hand, if males mate

with mature females that have recovered from the maturation moult, they face an 80% chance of a cannibalistic attack by the female, but this appears to be without consequence for male body size. In contrast, very short insertion duration with rapid sperm transfer is favoured during cannibalistic matings as males that stay too long in copula increase their risk of being caught by the female. In neither situation did I detect selection directly targeting leg length in males, and therefore relatively long legs do not appear to improve a male's ability to escape an attacking female or to win a fight against another male, as had previously been suggested (Prenter et al., 1995).

In summary, I did not find any indication that small size is favoured in adult male *A. aurantia*. Instead, large size often confers an advantage. These results have the following implications with respect to SSD evolution. First, the recently proposed gravity hypothesis (Moya-Laraño et al. 2002), which states that larger males should be at a disadvantage during climbing due to gravity, may, despite its great appeal, not apply for the many spider species which dwell in the upper herb layer. Overall, males probably cover greater distances on horizontal or mildly inclined structures than on vertical ones, and gravity may only be of marginal importance for the evolution and maintenance of extreme SSD in spiders. Size-selective mortality favouring larger males during mate search is also inconsistent with the differential mortality hypothesis, which assumes random, size-independent mortality (Vollrath and Parker, 1992). Second, the long held view that sexual cannibalism is an important factor selecting for very small size in males does not hold for *A. aurantia*, and in the light of the accumulating evidence against the sexual cannibalism hypothesis it seems no longer justified to present it as one of the most probable explanations of extreme SSD in spiders. Third, sexual selection for large body

size in males due to interference competition, which is ubiquitous in less dimorphic species (Andersson, 1994), may indeed also occur commonly in species with dwarf males. Previous hypotheses have assumed that interference competition would be absent or weak in extremely dimorphic species and have emphasized a low male-female encounter rate and female-biased operational sex ratios (Ghiselin, 1974; Vollrath and Parker, 1992). Given that male cohabitation and aggregation on webs of females have been reported from several dimorphic spider species (e.g. Robinson and Robinson, 1980; Jackson, 1986), and that large males typically win in contest (e.g. Elgar, 1998), interference competition may generate selection for large size in those cases as well, weakening the explanatory power of hypotheses emphasizing its absence or weakness.

Obviously, body size is determined through selection operating throughout the life-history of animals, and selection on adult individuals is only one aspect of this. In *A. aurantia*, selection for large size during adulthood is probably counterbalanced by viability selection favouring reduced growth during the juvenile stages – compared to females, at least. Unfortunately, it will be almost impossible to measure selection during juvenile stages as small individuals that moult regularly are extremely difficult to follow. Therefore, we might never get an estimate of net lifetime selection in spiders.

Considering phylogenetic information will provide further insight into the probable processes involved in the evolution and maintenance of extreme SSD. Within the large orb-weaver clade that includes the genus *Argiope*, females have probably increased in size over evolutionary time, while males have stayed small (Hormiga et al., 2000). This means that the optimal size for females changed, but not that for males (this being of course an oversimplification for clarity). Thus, for some still unknown reason,

females in this lineage (as opposed to other ecologically similar lineages where females have stayed small) have been able to achieve very large size, and hence high fecundity, through faster and/or longer growth. This should have led to a decrease in the number of females reaching adulthood, due to mortality costs of growing longer or faster (Roff, 2002). In turn, a male-bias in the operational sex ratio may have resulted and consequently an *increase* in male-male competition as the sexes diverged in size. Thus, it is possible that male size was not only pulled up through the between-sex genetic correlations, but was also selected upwards through an intensified sexual selection due to male-male competition (and perhaps also through sexual cannibalism – see Chapter 2). However, a lower female density would probably also lead to a decrease in the number of males that reach females, assuming that an increased search time for males results in an increased mortality during mate search. Therefore, the OSR, and hence the intensity of male-male competition, might not have changed at all. Under this scenario, the optimum size for males would not have changed. Thus, after a probable initial increase due to the between-sex genetic correlations, males would have returned to their previous and unchanged equilibrium size (Lande, 1980; Reeve and Fairbairn, 2001). Of course, the factors important for the initial evolution of extreme SSD may have differed from those responsible for its maintenance. Species in which males have apparently dwarfed over the course of evolution seem to fit a Ghiselin (1974) scenario for the evolution of extreme SSD (Legrand and Morse, 2000).

In conclusion, I have shown that none of the current hypotheses about the adaptive significance of small male body size in spiders (relative to female size) is likely to apply to *Argiope aurantia*. All of these hypotheses focus on at least one aspect of

selection during the adult stage, such as selection during mate search or mating. My results suggest that males commonly experience selection for large size as adults, and therefore viability selection against achieving a large size is predicted to occur in the juvenile stages.

References

- Abouheif, E. and D. J. Fairbairn. 1997. A comparative analysis of allometry for sexual size dimorphism: assessing Rensch's rule. *The American Naturalist* 149:540-562.
- Alayon Garcia, G. and L. F. de Armas. 1990. Observaciones sobre la conducta reproductiva de *Argiope trifasciata* (Araneae: Araneidae) en el Sur de La Habana . *Poeyana* 398:1-17.
- Amaya, C. C., P. D. Klawinski, and D. R. Formanowicz. 2001. The effects of leg autotomy on running speed and foraging ability in two species of wolf spider, (Lycosidae). *American Midland Naturalist* 145:201-205.
- Anderson, J. F. 1970. Metabolic rates of spiders. *Comparative Biochemistry and Physiology* 33:51-72.
- Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, New Jersey.
- Andrade, M. C. B. 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science* 271:70-72.
- Andrade, M. C. B. 1998. Female hunger can explain variation in cannibalistic behavior despite male sacrifice in redback spiders. *Behavioral Ecology* 9:33-42.
- Andrade, M. C. B. 2000. Sexual selection and male mating behavior in a cannibalistic spider. Ph.D. thesis. Cornell University.
- Andrade, M. C. B. 2003. Risky mate search and male self-sacrifice in redback spiders. *Behavioral Ecology* 14:531-538.
- Andrade, M. C. B. and E. M. Banta. 2002. Value of male remating and functional sterility in redback spiders. *Animal Behaviour* 63:857-870.

- Arnqvist, G. and S. Henricksson. 1997. Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. *Evolutionary Ecology* 11:255-273.
- Birkhead, T. R. 1988. Sexual cannibalism in the praying mantis *Hierodula membranacea*. *Behavior* 106:112-118.
- Blanckenhorn, W. U., U. Kraushaar, and C. Reim. 2003. Sexual selection on morphological and physiological traits and fluctuating asymmetry in the yellow dung fly. *Journal of Evolutionary Biology* 16:903-913.
- Blanckenhorn, W. U. 2000. The evolution of body size: what keeps organisms small? *Quarterly Review of Biology* 75:385-407.
- Blanckenhorn, W. U., C. Morf, C. Mühlhäuser, and T. Reusch. 1999. Spatiotemporal variation in selection on body size in the dung fly *Sepsis cynipsea*. *Journal of Evolutionary Biology* 12:563-576.
- Blanckenhorn, W. U., R. F. Preziosi, and D. J. Fairbairn. 1995. Time and energy constraints and the evolution of sexual size dimorphism - to eat or to mate? *Evolutionary Ecology* 9:369-381.
- Blanke, R. 1975. Untersuchungen zum Sexualverhalten von *Cyrtophora cicatrosa* (Stoliczka) (Araneae: Araneidae). *Zeitschrift für Tierpsychologie* 37:62-74.
- Bridge, A. P., R. W. Elwood, and J. T. A. Dick. 2000. Imperfect assessment and limited information preclude optimal strategies in male-male fights in the orb-weaving spider *Metellina mengei*. *Proceedings of the Royal Society of London, B* 267:273-279.

- Brodie, E. D. I., A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. *Trends in Ecology and Evolution* 10:313-318.
- Bukowski, T. C., C. D. Linn, and T. E. Christenson. 2001. Copulation and sperm release in *Gasteracantha cancriformis* (Araneae: Araneidae): differential male behaviour based on female mating history. *Animal Behaviour* 62:887-895.
- Buskirk, R. E., C. Frohlich, and K. G. Ross. 1984. The natural selection of sexual cannibalism. *American Naturalist* 123:612-625.
- Calder, W. A. 1984. *Size, Function, and Life History*. Harvard University Press, Cambridge, MA.
- Christenson, T. E. 1990. Natural selection and reproduction: A study of the golden orb-weaving spider, In D. A. Dewsbury [ed.], *Contemporary Issues in Comparative Psychology*. Sinaur, Sunderland, Massachusetts.
- Christenson, T. E. and K. C. Goist. 1979. Costs and benefits of male-male competition in the orb weaving spider, *Nephila clavipes*. *Behavioral Ecology and Sociobiology* 5:87-92.
- Coddington, J. A., G. Hormiga, and N. Scharff. 1997. Giant female or dwarf male spiders? *Nature* 385:687-688.
- Darwin, C. 1871. *Sexual Selection and the Descent of Man*. Murray, London.
- Downes, J. A. 1978. Feeding and mating in the insectivorous Ceratopogoninae (Diptera). *Memoirs of the Entomological Society of Canada* 104:1-61.
- Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton, New Jersey.

- Elgar, M. A. 1991. Sexual cannibalism, size dimorphism, and courtship behavior in orb-weaving spiders (Araneidae). *Evolution* 45:444-448.
- Elgar, M. A. 1992. Sexual cannibalism in spiders and other invertebrates, pp. 128-155 In M. A. Elgar and B. J. Crespi [eds.], *Cannibalism. Ecology and Evolution among Diverse Taxa*. Oxford University Press, Oxford, New York, Tokyo.
- Elgar, M. A. 1995. The duration of copulation in spiders: comparative patterns. *Records of the Western Australian Museum Supplement* 52:1-11.
- Elgar, M. A. 1998. Sperm competition and sexual selection in spiders and other arachnids, pp. 307-339 In T. R. Birkhead and A. P. Møller [eds.], *Sperm Competition and Sexual Selection*. Academic Press, San Diego.
- Elgar, M. A. and R. Bathgate. 1996. Female receptivity and male mate-guarding in the jewel spider *Gasteracantha minax* Thorell (Araneidae). *Journal of Insect Behavior* 9:729-737.
- Elgar, M. A. and B. F. Fahey. 1996. Sexual cannibalism, competition, and size dimorphism in the orb-weaving spider *Nephila plumipes* Latreille (Araneae: Araneoidea). *Behavioral Ecology* 7:195-198.
- Elgar, M. A. and D. R. Nash. 1988. Sexual cannibalism in the garden spider *Araneus diadematus*. *Animal Behaviour* 36:1511-1517.
- Elgar, M. A., N. Ghaffar, and A. F. Read. 1990. Sexual dimorphism in leg length among orb-weaving spiders: a possible role for sexual cannibalism. *Journal of Zoology* 222:455-470.

- Elgar, M. A., J. M. Schneider, and M. E. Herberstein. 2000. Female control of paternity in the sexually cannibalistic spider *Argiope keyserlingi*. *Proceedings of the Royal Society of London, B* 267:2439-2443.
- Elgar, M. A., F. E. C. De Crespigny, and S. Ramamurthy. 2003. Male copulation behaviour and the risk of sperm competition. *Animal Behaviour* 66:211-216.
- Emlen, S. T. and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215-223.
- Fairbairn, D. J. 1990. Factors influencing sexual size dimorphism in temperate waterstriders. *American Naturalist* 136:61-86.
- Fairbairn, D. J. 1997. Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics* 28:659-687.
- Fairbairn, D. J. and R. F. Preziosi. 1996. Sexual selection and the evolution of sexual dimorphism in the water strider, *Aquarius remigis*. *Evolution* 50:1549-1559.
- Fairbairn, D. J. and J. P. Reeve. 2001. Natural selection, pp. 35-54 In C. W. Fox, D. A. Roff, and D. J. Fairbairn [eds.], *Evolutionary Ecology. Concepts and Case Studies*. Oxford University Press, Oxford.
- Ferguson, I. M. and D. J. Fairbairn. 2000. Sex-specific selection and sexual size dimorphism in the water strider *Aquarius remigis*. *Journal of Evolutionary Biology* 13:160-170.
- Foelix, R. F. 1996. *Biology of Spiders*. Oxford University Press, New York.

- Foellmer, M. W. and D. J. Fairbairn. 2003. Spontaneous male death during copulation in an orb-weaving spider. *Proceedings of the Royal Society of London, B (Suppl.)* 270:S183 -S185.
- Foellmer, M. W. and D. J. Fairbairn. 2004. Males under attack: sexual cannibalism and its consequences for male morphology and behaviour in an orb-weaving spider. *Evolutionary Ecology Research* 6:163-181.
- Forster, L. M. 1992. The stereotyped behaviour of sexual cannibalism in *Latrodectus hasselti* Thorell (Araneae: Theridiidae), the Australian redback spider. *Australian Journal of Zoology* 40:1-11.
- Gerhardt, U. 1924. Neue Studien zur Sexualbiologie und zur Bedeutung des sexuellen Größendimorphismus der Spinnen. *Zeitschrift für Morphologie und Ökologie der Tiere* 1:507-538.
- Gerhardt, U. 1933. Neue Untersuchungen zur Sexualbiologie der Spinnen, insbesondere an Arten der Mittelmeerländer und der Tropen. *Zeitschrift für Morphologie und Ökologie der Tiere* 27:1-75.
- Ghiselin, M. T. 1974. *The Economy of nature and the Evolution of Sex*. University of California Press, Berkeley.
- Gould, S. J. 1984. Only his wings remained. *Natural History* 9:10-18.
- Grant, J. W. A. and P. E. Foam. 2002. Effect of operational sex ratio on female-female versus male-male competitive aggression. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 80:2242-2246.

- Grant, J. W. A., M. J. Bryant, and C. E. Soos. 1995. Operational sex ratio, mediated by synchrony of female arrival, alters the variance of male mating success in Japanese medaka. *Animal Behaviour* 49:367-375.
- Grant, J. W. A., C. L. Gaboury, and H. L. Levitt. 2000. Competitor-to-resource ratio, a general formulation of operational sex ratio, as a predictor of competitive aggression in Japanese medaka (Pisces: Oryziidae). *Behavioral Ecology* 11:670-675.
- Grasshoff, M. 1964. Die Kreuzspinne *Araneus pallidus* - ihr Netzbau und ihre Paarungsbiologie. *Natur und Museum* 94:305-314.
- Greenstone, M. H. and A. F. Bennet. 1980. Foraging strategy and metabolic rate in spiders. *Ecology* 61:1255-1259.
- Head, G. 1995. Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (class Araneae). *Evolution* 49:776-781.
- Higgins, L. 2002. Female gigantism in a New Guinea population of the spider *Nephila maculata*. *Oikos* 99:377-385.
- Hill, E. M. and T. E. Christenson. 1988. Male residency on juvenile female *Nephila clavipes* (Araneae: Araneidae) webs. *Journal of Arachnology* 16:257-259.
- Hormiga, G., N. Scharff, and J. A. Coddington. 2000. The phylogenetic basis of sexual size dimorphism in orb-weaving spiders (Araneae, Orbiculariae). *Systematic Biology* 49:435-462.
- Howell, F. G. and R. D. Ellender. 1984. Observations on growth and diet of *Argiope aurantia* Lucas (Araneidae) in a successional habitat. *Journal of Arachnology* 12:29-36.

- Jann, P., W. U. Blanckenhorn, and P. I. Ward. 2000. Temporal and microspatial variation in the intensities of natural and sexual selection in the yellow dung fly *Scathophaga stercoraria*. *Journal of Evolutionary Biology* 13:927-938.
- Johns, P. M. and M. R. Maxwell. 1997. Sexual cannibalism: who benefits? *Trends in Ecology and Evolution* 12:127-128.
- Johnson, J. C. 2001. Sexual cannibalism in fishing spiders (*Dolomedes triton*): an evaluation of two explanations for female aggression towards potential mates. *Animal Behaviour* 61:905-914.
- Kleinbaum, D. G., L. L. Kupper, K. E. Muller, and A. Nizam. 1998. *Applied Regression Analysis and Other Multivariable Methods*, 3 ed. Duxbury Press, Pacific Grove.
- Knoflach, B. and A. Van Harten. 2001. *Tidarren argo* sp. nov. (Araneae: Theridiidae) and its exceptional copulatory behaviour: emasculation, male palpal organ as a mating plug and sexual cannibalism. *Journal of Zoology* 254:449-459.
- Knoflach, B. and S. P. Benjamin. 2003. Mating without sexual cannibalism in *Tidarren sisypoides* (Araneae, Theridiidae). *Journal of Arachnology* 31:445-448.
- Kotiaho, J. S. 1998. Sexual differences in metabolic rates of spiders. *Journal of Arachnology* 26:401-404.
- Kotiaho, J., R. V. Alatalo, J. Mappes, S. Parri, and A. Rivero. 1998a. Male mating success and risk of predation in a wolf spider: a balance between sexual and natural selection? *Journal of Animal Ecology* 67:287-291.
- Kotiaho, J., R. V. Alatalo, J. Mappes, M. G. Nielsen, S. Parri, and A. Rivero. 1998b. Energetic costs of size and sexual signalling in a wolf spider. *Proceedings of the Royal Society of London, B* 265:2203-2209.

- Kotiaho, J., R. V. Alatalo, J. Mappes, and S. Parri. 1999. Sexual signalling and viability in a wolf spider (*Hygrolycosa rubrofasciata*): measurements under laboratory and field conditions. *Behavioral Ecology and Sociobiology* 46:123-128.
- Kvarnemo, C., E. Forsgren, and C. Magnhagen. 1995. Effects of sex ratio on intra- and inter-sexual behaviour in sand gobies. *Animal Behaviour* 50:1455-1461.
- Kvarnemo, C. and I. Ahnesjö. 1996. The dynamics of operational sex ratios and competition for mates. *Trends in ecology and evolution* 11:404-408.
- Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in phylogenetic characters. *Evolution* 34:292-305.
- Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210-1226.
- Lawrence, S. E. 1992. Sexual cannibalism in the praying mantid, *Mantis religiosa*: a field study. *Animal Behaviour* 43:569-583.
- Lawrence, W. S. 1986. Male choice and competition in *Tetraopes tetrapphthalmus*: effects of local sex ratio variation. *Behavioral Ecology and Sociobiology* 18:289-296.
- Legrand, R. S. and D. H. Morse. 2000. Factors driving extreme sexual size dimorphism of a sit-and-wait predator under low density. *Biological Journal of the Linnean Society* 71:643-664.
- Levi, H. W. 1968. The spider genera *Gea* and *Argiope* in America (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology* 136:319-352.
- Lindström, K. and T. Seppä. 1996. The environmental potential for polygyny and sexual selection in the sand goby, *Pomatoschistus minutus*. *Proceedings of the Royal Society of London, B* 263:1319-1323.

- Manly, B. F. J. 1992. RT A Program for Randomization Testing. CASM1-16.
- Marshall, S. D. and J. L. Gittleman. 1994. Clutch size in spiders: is more better?
Functional Ecology 8:118-124.
- Maxwell, M. R. 1998. Lifetime mating opportunities and male mating behaviour in sexually cannibalistic praying mantids. *Animal Behaviour* 55:1011-1028.
- McCook, H. C. 1890. American Spiders and their Spinning Work, Vol II. The Wistar Institute of Anatomy and Biology, Philadelphia.
- Mills, S. C. and J. D. Reynolds. 2003. Operational sex ratio and alternative reproductive behaviours in the European bitterling, *Rhodeus sericeus*. *Behavioral Ecology and Sociobiology* 54:98-104.
- Miyashita, T. 1993. Male-male competition and mating success in the orb-web spider, *Nephila clavata*, with reference to temporal factors. *Ecological Research* 8:93-102.
- Moya-Laraño, J., J. Halaj, and D. H. Wise. 2002. Climbing to reach females: Romeo should be small. *Evolution* 56:420-425.
- Newman, J. A. and M. A. Elgar. 1992. Sexual cannibalism in orb-weaving spiders: an economic model. *American Naturalist* 138:1372-1395.
- Petrie, M., T. Halliday, and C. Sanders. 1991. Peahens prefer peacocks with elaborate trains. *Animal Behaviour* 41:323-332.
- Prenter, J., R. W. Elwood, and S. Colgan. 1994. The influence of prey size and female reproductive state on the courtship of the autumn spider, *Metellina segmentata*: a field experiment. *Animal Behaviour* 47:449-456.

- Prenter, J., W. I. Montgomery, and R. W. Elwood. 1995. Multivariate morphometrics and sexual dimorphism in the orb-web spider *Metellina segmentata* (Clerk, 1757) (Araneae, Metidae). *Biological Journal of the Linnean Society* 55:345-354.
- Prenter, J., W. I. Montgomery, and R. W. Elwood. 1997. Sexual dimorphism in northern temperate spiders: implications for the differential mortality model. *Journal of Zoology* 243:341-349.
- Prenter, J., R. W. Elwood, and W. I. Montgomery. 1998. No association between sexual size dimorphism and life histories in spiders. *Proceedings of the Royal Society of London, B* 265:57-62.
- Prenter, J., R. W. Elwood, and W. I. Montgomery. 1999. Sexual size dimorphism and reproductive investment by female spiders: a comparative analysis. *Evolution* 53:1987-1994.
- Punzo, F. 1997. Leg autotomy and avoidance behavior in response to a predator in the wolf spider, *Schizocosa avida* (Araneae, Lycosidae). *Journal of Arachnology* 25:202-205.
- Reeve, J. P. and D. J. Fairbairn. 1999. Change in sexual size dimorphism as a correlated response to selection on fecundity. *Heredity* 83:697-706.
- Reeve, J. P. and D. J. Fairbairn. 2001. Predicting the evolution of sexual size dimorphism. *Journal of Evolutionary Biology* 14:244-254.
- Reiss, M. J. 1989. *The Allometry of Growth and Reproduction*. Cambridge University Press, Cambridge.
- Robinson, M. H. 1982. Courtship and mating behavior in spiders. *Annual Review of Entomology* 27:1-20.

- Robinson, M. H. and B. Robinson. 1980. Comparative studies of the courtship and mating behavior of tropical araneid spiders. *Pacific Insects Monograph* 36:1-218.
- Roff Derek. *Life History Evolution*. 2002. Sunderland, MA, Sinauer Associates Inc.
- Sasaki, T. and O. Iwahashi. 1995. Sexual cannibalism in an orb-weaving spider *Argiope aemula*. *Animal Behaviour* 49:1119-1121.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* 42:849-861.
- Schneider, J. M. and M. A. Elgar. 2001. Sexual cannibalism and sperm competition in the golden orb-web spider *Nephila plumipes* (Araneioidea): female and male perspectives. *Behavioral Ecology* 12:547-552.
- Schneider, J. M., M. E. Herberstein, F. E. de Crespigny, S. Ramamurthy, and M. A. Elgar. 2000. Sperm competition and small size advantage for males of the golden orb-web spider *Nephila edulis*. *Journal of Evolutionary Biology* 13:939-946.
- Schneider, J. M., M. L. Thomas, and M. A. Elgar. 2001. Ectomised conductors in the golden orb-web spider, *Nephila plumipes* (Araneioidea): a male adaptation to sexual conflict? *Behavioral Ecology and Sociobiology* 49:410-415.
- Schwagmeyer, P. L. and S. J. Wootner. 1986. Scramble competition polygyny in thirteen-lined ground squirrels: the relative contribution of overt and competitive mate searching. *Behavioral Ecology and Sociobiology* 19:359-364.
- Silverman, H. B. and M. J. Dunbar. 1980. Aggressive tusk use by the narwhale (*Monodon monoceros* L.). *Nature* 284:57-58.
- Simmons, L. W. 2001. *Sperm Competition and its Evolutionary Consequences in the Insects*. Princeton University Press, Princeton.

- SPSS Inc. 1997. SPSS for Windows 8.0. 444 North Michigan Ave., Chicago, IL, USA
- Suter, R. B. 1990. Courtship and the assessment of virginity by male bowl and doily spiders. *Animal Behaviour* 39:307-313.
- Tolbert, W. W. 1976. Population dynamics of the orb-weaving spiders *Argiope trifasciata* and *A. aurantia* (Araneae: Araneidae): density changes associated with mortality, natality, and migration. University of Tennessee.
- Uhl, G. and F. Vollrath. 1998. Little evidence for size-selective sexual cannibalism in two species of *Nephila* (Araneae). *Zoology* 101:101-106.
- Vollrath, F. 1980. Male body size and fitness in the web-building spider *Nephila clavipes*. *Zeitschrift für Tierpsychologie* 53:61-78.
- Vollrath, F. 1998. Dwarf males. *Trends in Ecology and Evolution* 13:159-163.
- Vollrath, F. and G. A. Parker. 1992. Sexual dimorphism and distorted sex ratios in spiders. *Nature* 360:156-159.
- Vollrath, F. and G. A. Parker. 1997. Reply to: Giant female or dwarf male spiders? *Nature* 385:688.
- Walker, S. E. and A. L. Rypstra. 2003. Sexual dimorphism and the differential mortality model: is behaviour related to survival? *Biological Journal of the Linnean Society* 78:97-103.
- Watson, P. J. and J. R. B. Lighton. 1994. Sexual selection and the energetics of copulatory courtship in the Sierra dome spider, *Linyphia litigiosa*. *Animal Behaviour* 48:615-626.

- West, G. B., J. H. Brown, and B. J. Enquist. 2000. The origin of universal scaling laws in biology, pp. 87-112 In J. H. Brown and G. B. West [eds.], *Scaling in Biology*. Oxford University Press, New York, NY.
- Wise, D. H. 1993. *Spiders in ecological webs*. Cambridge University Press, New York, NY.

Appendix 1. Size and shape dimorphism in *Argiope aurantia*

In *Argiope aurantia*, adult males are much smaller than females and have relatively longer legs. This species thus exhibits a dimorphism in size and “shape”. Relatively longer legs in males than in females are typical for all spiders (Foelix 1996). To quantify the size and shape differences between males and females in *A. aurantia*, I provide several statistics which are based on measurements of individuals from the Île Perrot population (see Chapters 3 and 4). Table A1.1 lists summary statistics for the fixed morphological traits prosoma width and average patella-tibia lengths for each of the four leg pairs for males and females, and gives the degree of dimorphism expressed as the ratio (female/male) for each trait. Figure A1.1 depicts the shape dimorphism between males and females. Males have relatively longer legs than females: the patella-tibia length/prosoma width ratios for all four leg pairs are significantly larger for males than for females. The description of the shape dimorphism is supplemented by Table A1.2, which gives the allometric relationships between the patella-tibia lengths and prosoma width for all four leg pairs for both sexes. In males, all four leg pairs scale positively allometrically with prosoma width, which is indicated by the fact that the 95% confidence intervals for the regression slopes do not contain a slope of one. In females, all legs scale isometrically with prosoma width, except for the third leg pair, which shows a slope slightly higher than one. The various hypotheses regarding the adaptive significance of relatively longer legs in males are discussed in Chapters 2, 3, and 4.

Table A1.1. Means and standard deviations for prosoma width and the average patella-tibia lengths for each of the four leg pairs (in mm). Given is also the degree of dimorphism as the ratio (female / male) for each trait. Measurements taken from adults of the Île Perrot population in 2000 (see Chapters 3 and 4).

Trait	Males (n = 223)		Females (n = 165)		Dimorphism (Female / male)
	Mean	SD	Mean	SD	
Prosoma width	1.784	0.121	3.513	0.455	2.0
Patella-tibia 1					
length	3.844	0.359	5.989	0.782	1.6
Patella-tibia 2					
length	3.656	0.349	5.799	0.760	1.6
Patella-tibia 3					
length	1.745	0.163	3.283	0.462	1.9
Patella-tibia 4					
length	3.003	0.284	5.395	0.730	1.8

Table A1.2. Results of the RMA (reduced major axis) regression of log(patella-tibia length) on log(prosoma width) for each leg pair for males and females. C.L. = confidence limit

		Intercept	Slope	Low 95% C.L.	High 95% C.L.
Males	Leg 1	0.211	1.487	1.365	1.625
	Leg 2	0.183	1.512	1.385	1.657
	Leg 3	-0.132	1.488	1.344	1.654
	Leg 4	0.098	1.509	1.374	1.664
Females	Leg 1	0.217	1.026	0.970	1.085
	Leg 2	0.194	1.043	0.991	1.097
	Leg 3	-0.093	1.115	1.039	1.197
	Leg 4	0.149	1.068	1.000	1.142

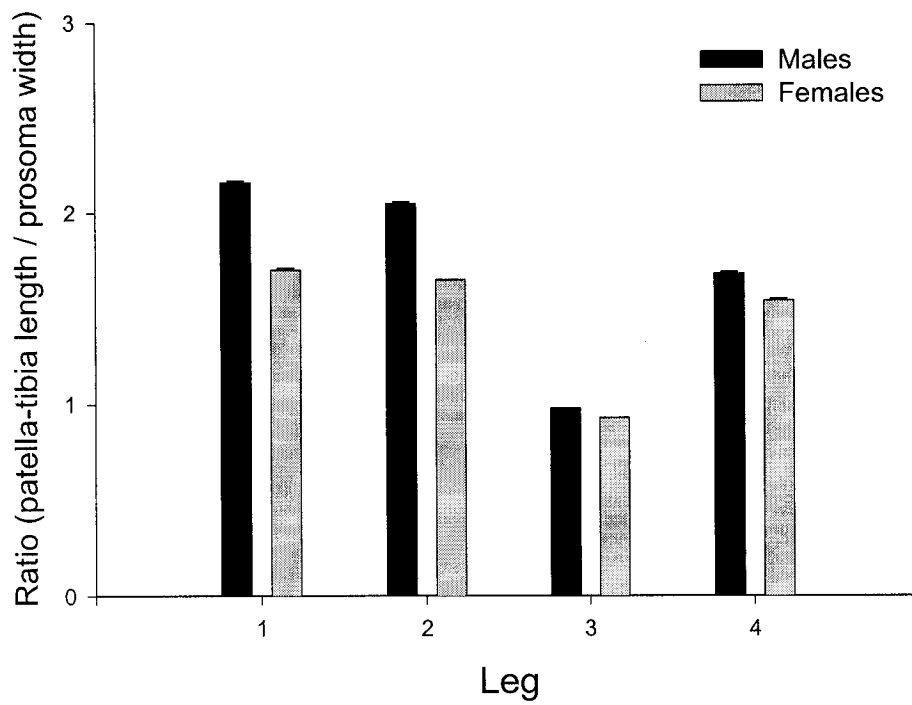


Figure A1.1. The patella-tibia length / prosoma width ratio for all four leg pairs for males and females. Note that standard errors are indicated. The ratios are significantly larger for males than for females for all four leg pairs (all comparisons: $p < 0.0001$).

Appendix 2. Multiple mating by females and the possible function of embolus caps

Introduction

Multiple mating by females appears to be common in spiders (reviewed in Elgar 1998). In some species, females are receptive for only a limited time after their final moult or after their first mating, and males may engage in mate guarding during this period (e.g. Elgar & Bathgate 1996). In *Argiope aurantia*, several males may mate with a given female during opportunistic mating (Chapter 1 and 4). In this situation the female has no overt control over mating and the dead bodies of mated males appear to function as whole-body mating plugs (Chapter 1). This raises the question to what extent “older” females, i.e. those that have recovered from their final moult, may mate multiply, because dead males certainly cannot “guard” them. In the context of this thesis, the following question is of interest: Do adult females mate with more than one male, and do mated females behave differently towards males than virgin females, especially with respect to cannibalistic attacks? Answering this question will clarify whether selection on males due to sexual cannibalism was adequately assayed in Chapter 2. It will also elucidate the strength of competition among males over access to females. For example, if mature females only mate once, at any given time most adult females will probably not be available for mating and the operational sex ratio will be male-biased throughout much of the mating season (see Chapter 4). Here I determine female mating frequency by sequentially providing adult males as prospective mates.

Levi (1968) noted male embolus caps found in the copulatory ducts of female *A. aurantia*. These caps break off frequently during copulation (in about 95% of cases, personal observation), but the frequency with which these are retained in the female is unknown. The caps are relatively large and might influence a male's ability to insert a palp or transfer sperm (but see Schneider et al. 2001). Therefore, I also examine to what extent male embolus caps are retained in the female and thus might affect remating frequency.

Methods

I collected 23 penultimate females, 20 adult females and 51 adult males haphazardly along a road side in Swanton, Vermont on 31 July and 3 August 2001. All females were placed into aluminium screen cages (45x45x15cm) where they built new webs and kept in a greenhouse room (natural light cycle; mean high temperature = 32.1°C, s.d. = 2.7°C; mean low = 20.3°C, s.d. = 3.2°C). The general protocol for feeding females and conducting mating trials followed the one described in Chapter 2, except that trials were not videotaped but instead continuously observed. Mating trials lasted until males had achieved two insertions or were killed, or until all mating interactions had ceased for at least 1/2 h. Females were not allowed to consume any males.

I conducted two experiments; the assignment of individuals was always random. Males that did not insert any palp in a trial and that were not harmed by the female were reused. The first experiment was to clarify whether and to what extent adult females re-mate. Penultimate females were divided into two groups. In the first group ($n = 11$), one male was introduced to each female to induce opportunistic mating. In both groups (second group: $n = 12$), one male was introduced to each female on the day following the

female's final moult (females that were discovered moulted in the morning were considered to have moulted the preceding day), and every day after. Each female was observed until she was unreceptive to a courting male, a male that was accepted by the female could not insert any palp, or males repeatedly approached the female but did not court her. If a male did not approach the female at a given trial the male was exchanged with another male. In the second experiment, I introduced one male to each of 14 females that were already mature when collected.

At the end of each experiment all individuals were preserved in alcohol. I dissected the epigyna of all females to determine the frequency with which broken-off emboli caps remain in the female.

Results

In the first experiment, on average 4.4 males were introduced per female (standard deviation = 2.2, range = 2 – 11, $n = 23$). Females received a mean number of 2.0 insertions (standard deviation = 1.0, range = 1 – 5, $n = 23$, Figure A2.1) while mating with a mean number of 1.6 males (standard deviation = 1.0, range = 1 – 5, $n = 23$, Figure A2.2). Both the number of received insertions and the number of mates did not differ for females that received at least part of their insertions during opportunistic mating (insertions: $\bar{x} = 2.00$; mates: $\bar{x} = 1.45$) and those that did not (insertions: $\bar{x} = 2.10$; mates: $\bar{x} = 1.75$; insertions: Fisher's Exact test, $p > 0.7$, $n = 23$; mates: Fisher's Exact test, $p > 0.6$, $n = 23$). Note that in this comparison insertions received during opportunistic mating are included. Nine females (39.1%) became unreceptive to courting males, seven females (30.4%) were still receptive at the last trial, but in each case the male tried only briefly to insert, and then left the female swiftly without inserting any palp. In the remaining seven

cases males repeatedly approached the female, but did not court her. Whether this means that females were not receptive is not clear; however, I have never seen a male approaching a virgin female and not courting her. Thus, females likely mate with only one or two males once they have reached reproductive maturity.

In the second experiment, of 14 females collected as adults, one (7.1%) was receptive and received one insertion, three females (21.4%) were receptive but the male did not insert, nine females (64.3%) were not receptive, and one female (7.1%) was not courted. Hence almost all mature females collected in the field did not appear to be available for males as suitable mates.

In order to evaluate whether behaviour toward approaching males differs between mated and virgin females, I performed a paired test involving females from the first experiment, comparing attack frequencies for the first and the second male. First and second males were attacked at similar frequencies (22% vs. 44%, $n = 9$, McNemar test, $p = 0.625$). However, because the sample size was small for the paired test, I also compared the attack frequencies of all mated females from these experiments with those of the virgin females from the experiment described in Chapter 2. Again, mated females attacked approaching or courting males at a similar frequency as virgin females (32%, $n = 31$ vs. 35%, $n = 99$, $\chi^2 = 0.1$, d.f. = 1, $p > 0.7$). Therefore, males that approach mated females do not appear to be at an increased risk of being attacked. Note that not a single male was killed during the approach or courtship. Further, even when non-receptive females are considered separately, they did not always attack males; 46.7% attacked and 53.3% reacted non-aggressively by shaking the web and/or brushing-off the male. These results suggest that a male is not at a greater risk of being attacked when approaching a

mated female, and that the effects of cannibalism on males were adequately assayed in Chapter 2.

Males in the first experiment performed a total of 47 pedipalp insertions. The embolus caps of 45 palps (95.7%) broke off and 43 caps (91.5%) were not found with the palps. Instead, 40 caps (85.1%) were found in female copulatory ducts. Therefore, broken embolus caps constitute a good estimator of male insertion success and can also be used to estimate the number of insertions received by a female. A comparison of females in the two experiments showed that females mated in experiment 1 contained a mean number of 1.7 embolus caps (standard deviation = 1.1, range 0 – 5, $n = 23$), whereas females collected in the field contained, minus the one insertion received in the experiment, on average 2.3 caps (standard deviation = 0.9, range 0 – 4, $n = 20$), although this difference was not significant (Fisher's Exact test: $p > 0.1$). Note that *A. aurantia* exhibits, like most orb-weavers, ipsilateral insertion – that is for example the right palp inserts into the right copulatory duct of the epigynum.

Discussion

When female *Argiope aurantia* were given the opportunity to mate with one male each day from the day of the final moult on, most females mated with only one male, and this was independent of whether the first mating occurred opportunistically during the female's moult. However, this was not entirely due to females becoming sexually unreceptive. In about 30% of cases the female was clearly receptive to the male's courtship and assumed the typical acceptance posture. The male on the other hand appeared to try to insert but withdrew soon without inserting any palp. I also observed this behaviour on three occasions in the field. Males abandoned the female's web,

although the female had accepted them. I could catch the male each time and confirm that none had inserted a palp. Given that most broken-off embolus caps are actually retained in the copulatory ducts of females, it is possible that these prevent further insertions and thus function as mating plugs. Nevertheless, embolus caps do not prevent other males from inserting during opportunistic mating, when every male who reaches the female appears to be able to successfully insert a palp, unless prevented by the body of a dead male stuck in the female (Foellmer & Fairbairn, 2003). It is thus possible that the caps are only effective in females whose cuticle has hardened after the moult.

In one exceptional case, the female received five insertions from five different males. Perhaps more spacious copulatory ducts allowed for more insertions, but this example indicates that even older females might potentially mate multiply and may often be prevented from doing so by the presence of the embolus caps in their ducts. On the other hand, most females collected as adults in the field were clearly not sexually receptive anymore. These females were collected in the early mating season when males were abundant and many of the females were probably opportunistically mated (see Chapter 4). On average they contained slightly more embolus caps than the females from the first experiment, but this difference was not statistically significant. During the opportunistic mating experiment in Chapter 4, 48.9% of females mated with one male, 35% of females mated with two males, and 11.1% with more than two males. Taken together, these results suggest that most female *A. aurantia* generally mate with only one or two males. Given that most males are found with penultimate females in the field and probably mate opportunistically (Chapter 4), the likelihood of multiple mating is probably reduced for females beyond the day of the moult.

With respect to previous chapters of this thesis these results have the following implications. First, at any given time, many mature females are probably unavailable for mating for males, further intensifying competition among males (Chapter 4). Second, mated females do not attack approaching or courting males more frequently than virgin females. Males are therefore not at an increased risk when approaching a mated female and sexual cannibalism was thus adequately assayed in Chapter 2.

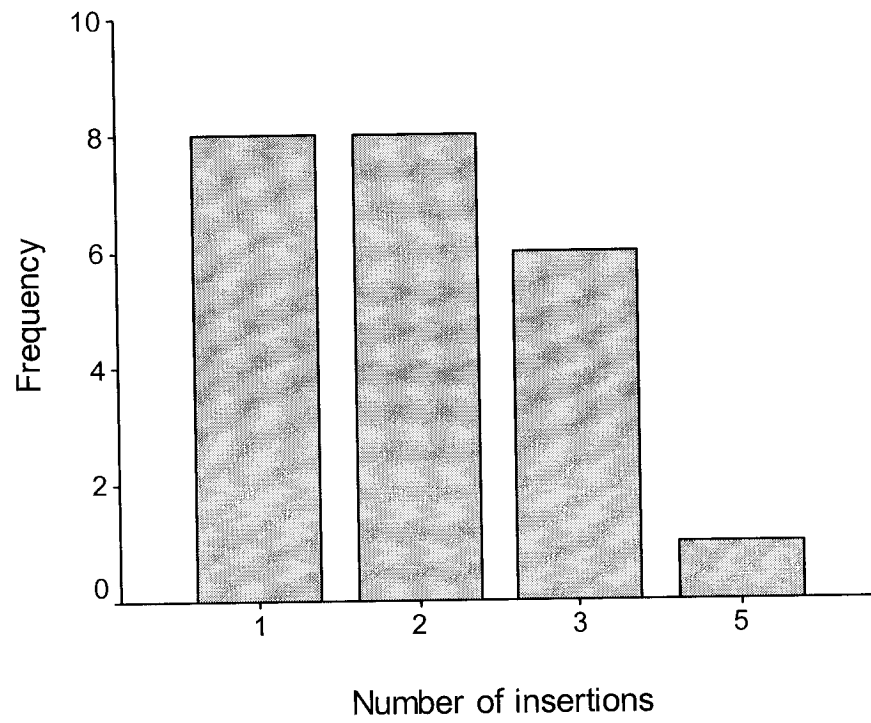


Figure A2.1. Frequency distribution of the number of pedipalp insertions received by females in experiment 1.

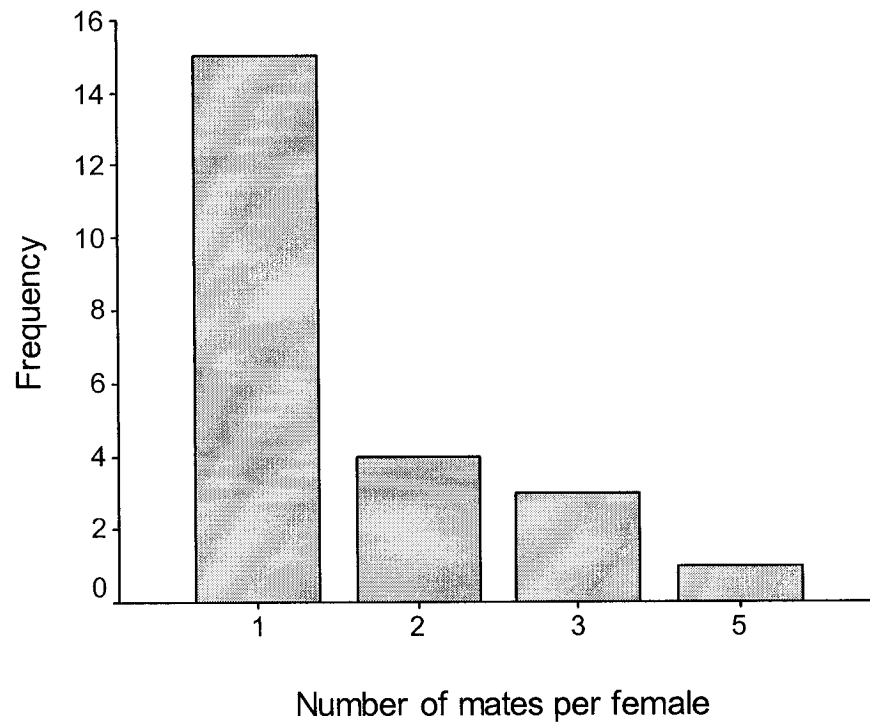


Figure A2.2. Frequency distribution of the number of mates per female in experiment 1.

Appendix 3. The frequency of opportunistic mating in unrestricted and caged females

To evaluate whether experimental caging of individuals affects the frequency of opportunistic mating, I conducted a preliminary study in 1999. I compared the frequency with which dead males that had inserted both palps opportunistically were found in the webs of unrestricted females in the field with the frequency with which those males were found with females that were caged. In an old field near Griffin, Quebec, 5km northwest of Stanstead at the US border, I found nine females freshly moulted that I had visited the day before and that I had observed to be attended by a median number of three males per female. Seven out of nine females (79%) had one dead male in the web, and all of these males had inserted both palps. For comparison, I caged 16 penultimate females, each with three males in aluminium screen cages (45 x 45 x 15 cm), allowing the females to build a new web first (see Chapter 4 for details). Twelve out of 16 females (75%) were found with one dead male in her web, one female (6%) with two dead males. All dead males had inserted both palps. The frequencies with which dead males were found with females were not significantly different (Fisher's Exact test, $p > 0.9$). Further, during the opportunistic mating experiment in Chapter 4, females received a mean number of 2.6 insertions (standard deviation = 1.2), based on broken tips. Appendix 2 shows that 40 out of 45 broken embolus caps (89%) were retained in female copulatory ducts. Eighty-nine percent of 2.6 insertions equals 2.3 caps, which also equals the number of caps found in wild-caught females (Appendix 2). Thus, mating occurred at natural frequencies in the experiment in Chapter 4.