

**Mating rates and their adaptive value in orb-web spiders  
of the genus *Argiope* (Araneae: Araneidae)**

**Dissertation**

Zur Erlangung der Würde des Doktors der Naturwissenschaften  
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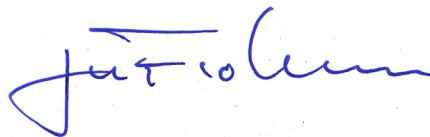
Klaas W. Welke

aus Gummersbach

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Professor Dr. J. Fromm  
Vorsitzender des Promotionsausschusses  
Biologie



SCHOOL OF BIOSCIENCES

Centre for Ecology & Conservation  
Cornwall Campus  
Penryn  
Cornwall  
TR10 9EZ

Telephone +44 (0)1326 371825  
Fax +44 (0)1326 253638  
Email [N.J.Royle@exeter.ac.uk](mailto:N.J.Royle@exeter.ac.uk)  
Web <http://www.uec.ac.uk/biology>

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**Use of English in thesis by Klaas W Welke**

To whom it may concern,

As a native English speaker I am writing in support of Klaas W Welke's thesis entitled "***Mating rates and their adaptive value in orb-web spiders of the genus Argiope***". Having read through Klaas's thesis I can confirm that the use of English is correct.

Yours sincerely

A handwritten signature in black ink, appearing to read "NJR", written in a cursive style.

Dr Nick J. Royle  
Senior Lecturer in Behavioural Ecology



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## Zusammenfassung

Im Tierreich finden sich zahlreiche Paarungssysteme, in denen die Geschlechterrollen von der traditionellen Ansicht abweichen, dass Männchen mit jeder Paarung ihren Fortpflanzungserfolg steigern und den Weibchen eine oder wenige Kopulationen für einen optimalen Fortpflanzungserfolg genügen sollten. Zahlreiche Spinnenarten, insbesondere Radnetzspinnen, weichen stark von diesen Geschlechterrollen ab, da Weibchen sich oft mehrfach verpaaren (Polyandrie) und Männchen auf maximal zwei Kopulationen beschränkt sind (Monogynie, Bigynie). Da die Paarungsraten innerhalb und zwischen den Arten stark variieren, bieten Spinnen ein geeignetes System, um den adaptiven Wert von Fortpflanzungsstrategien zu untersuchen.

In der Gattung der Wespenspinnen *Argiope* findet sich diese Kombination von Polyandrie und Monogynie und geht einher mit einer Reihe von extremen Anpassungen, insbesondere bei den Männchen. Häufig verstümmeln diese ihre paarigen Kopulationsorgane (Pedipalpen) und verstopfen mit den abgebrochenen Teilen die Kopulationsöffnungen der Weibchen. Hierdurch verhindern sie, mehr oder weniger effektiv, erfolgreiche Kopulationen möglicher Rivalen. Die beschädigten männlichen Pedipalpen werden hierdurch jedoch nutzlos, was sie auf ein Maximum von zwei Kopulationen in ihrem Leben beschränkt. Weiterhin werfen sich Männchen spätestens nach ihrer zweiten Verpaarung dem deutlich größeren Weibchen zum Fraß vor. Aufgrund dessen gibt es eine große Zahl an Männchen, die sich in ihrem Leben nur einmal verpaaren. Männchen, die ihre erste Kopulation überleben haben die Wahl zwischen zwei alternativen Paarungsstrategien. Entweder sie verpaaren sich mit demselben Weibchen erneut (Monogynie) oder sie machen sich auf die Suche nach einem Weiteren (Bigynie).

In meiner Arbeit habe ich mich hauptsächlich mit der Wespenspinne *Argiope bruennichi* beschäftigt, die sich in den vergangenen zehn Jahren zu einem Modellorganismus für die Untersuchung von Monogynie und Polyandrie entwickelt hat. Innerhalb einer *A. bruennichi* Population habe

ich die natürlichen Paarungsraten der Geschlechter bestimmt und in Laborexperimenten den adaptiven Wert von sexuellem Kannibalismus und Polyandrie untersucht. Des Weiteren habe ich den Wert von Polyandrie als eine Strategie zur Inzuchtvermeidung an *A. australis* getestet.

Mathematische Modelle postulieren, dass für die Entstehung von monogynen Paarungssystemen ein stark männchenlastiges Geschlechterverhältnis eine Grundvoraussetzung ist. Meine Feldbeobachtungen zeigten jedoch, dass das Verhältnis von Männchen zu Weibchen, die sich mindestens einmal paarten ('effective sex ratio') nahezu ausgewogen war. Das operationale Geschlechterverhältnis (OSR: paarungsbereite Männchen und Weibchen zu einem bestimmten Zeitpunkt) hingegen wies eine starke Veränderung im Verlauf der Saison auf. Da die Männchen deutlich kleiner als die Weibchen sind und schneller heranreifen, kommt es zu Beginn der Saison zu einem starken Männchenüberschuss. Für Männchen ist dieses frühe Heranreifen von Vorteil, da es ihnen ermöglicht, sub-adulte Weibchen zu belagern. Hierdurch erhöhen sie ihre Chancen mit einem virginen Weibchen zu kopulieren. Die Vermeidung von bereits verpaarten Weibchen ist höchst adaptiv in einem Paarungssystem in dem Männchen Weibchen durch Begattungspfpfropfen monopolisieren. Das OSR wird im Verlauf der Paarungssaison immer mehr zu Gunsten der Weibchen verschoben, da Männchen durch sexuellen Kannibalismus aus dem Paarungs-Pool ausscheiden. Entgegen den theoretischen Voraussagen hatte diese Dynamik im OSR keinen Einfluss auf die Paarungsraten der Geschlechter. Ein erwarteter Anstieg von Monogynie unter erhöhter Männchenkonkurrenz ließ sich nicht nachweisen. Männchen entschieden konditionell, ob sie sich zweimal mit demselben Weibchen verpaarten oder versuchten noch ein Weiteres zu finden. So neigten Männchen eher zu Monogynie, wenn ihr erstes Weibchen schwer und das lokale Angebot an unverpaarten oder sub-adulten Weibchen gering war. Die Häufigkeit der alternativen männlichen Paarungsstrategien erklärte auch den größten Teil der Polyandrie in der

Population, die mit einer durchschnittlichen Paarungsrate der Weibchen von 1.3 eher gering war.

In *A. bruennichi* sind Männchen in der Lage ihre Paarungsstrategien aktiv über ihre Kopulationsdauer zu beeinflussen. In einem Laborexperiment, in dem Geschwister oder Nicht-Geschwister miteinander verpaart wurden, konnte ich zeigen, dass Männchen eher eine bigyne Paarungsstrategie wählten, wenn sie zuerst mit einer Schwester verpaart wurden. Männchen verkürzten hierzu ihre Kopulationsdauer und somit auch ihre Überlebenswahrscheinlichkeit, da dies in direktem Zusammenhang miteinander steht. Dass Männchen die Paarung mit einer Schwester nicht von vornherein vermieden, hängt wahrscheinlich mit der starken Präferenz für virgine Weibchen zusammen.

Das *Argiope* Männchen sich den Weibchen nach der zweiten Kopulation zum Fraß vorwerfen stellt für sie möglicherweise geringe Kosten dar. Die Männchen sind nach zwei Kopulationen steril und verfügen über keine weiteren Paarungschancen in der Zukunft. Durch die Selbstaufopferung können die Männchen sogar noch ihren Vaterschaftserfolg steigern, denn wie ich zeigen konnte, wiesen Nachkommen kannibalistischer Weibchen eine erhöhte Überlebenswahrscheinlichkeit unter simulierten Winterbedingungen auf. Der Mechanismus dahinter bleibt noch ungeklärt, doch höchstwahrscheinlich liefern Männchen den Weibchen Nährstoffe, die im üblichen Beutespektrum nicht vorhanden sind. Die männliche Selbstaufopferung kann demnach als ein väterliches Investment in die Nachkommenschaft aufgefasst werden. Im Versuch ließ sich der Effekt auf die Nachkommen durch den Verzehr mehrerer Männchen nicht steigern, was darauf schließen lässt, dass Polyandrie in *Argiope* nicht allein aufgrund der Anhäufung materieller Vorteile entstanden ist.

Höchstwahrscheinlich dient Polyandrie als Strategie, um Inzucht und ihre Kosten zu vermeiden. Die Kombination von verwandten Genomen stellt eine hohe genetische Inkompatibilität zwischen Paarungspartnern dar und kann die Fitness beider Geschlechter drastisch herabsetzen. In *A. australis* zeigte sich schon nach einer Generation eine geringere Größe und verkürzte Lebenserwartung in ingezüchteten Tieren. Mechanismen zur Vermeidung dieser Kosten sind daher höchst adaptiv. Wespenspinnen-Weibchen scheinen durch ihre Genitalmorphologie ideal zu sein um Polyandrie als post-kopulatorischen Auswahlmechanismus zu nutzen. Sie besitzen zwei separate Spermienspeicher, die in unterschiedlichen Kopulationen befüllt werden. Dies legt nahe, dass Weibchen nach der Kopulation zwischen den verschiedenen Ejakulaten wählen können. Spermienzählungen bei *A. australis* zeigten starke Hinweise darauf, dass Weibchen gegen ihre Brüder diskriminieren. Dieser Effekt trat aber nur auf, wenn die Brüder nach Nicht-Geschwistern kopulierten. Ähnliche Ergebnisse wurden bereits in einer Studie an *A. lobata* gefunden und machen die Existenz von Inzuchtvermeidung durch post-kopulatorische Auswahlprozesse in *Argiope* sehr wahrscheinlich.

Diese Arbeit zeigt, wie wichtig genetische Vorteile für die Entstehung und den Erhalt von Polyandrie in der Gattung *Argiope* sind. Es fanden sich zwar materielle Vorteile von sexuellem Kannibalismus, die wiederum aber nicht im Zusammenhang mit Polyandrie standen. Weiterhin zeigt sich, dass Männchen einen hohen Einfluss auf die von ihnen gewählte Paarungsstrategie haben und sie ihre Entscheidung maßgeblich anhand der Qualität der Weibchen und der Verfügbarkeit zukünftiger Paarungspartner treffen.



## Summary

Within the animal kingdom we find plenty of mating systems that diverge from the concept of traditional sex roles which state that males increase their fitness with every additional mating while for females one or few copulations should be sufficient to achieve the optimal reproductive success. Spiders, especially orb weavers, diverge immensely from these traditional sex roles because females mate multiply and males are reduced to at most two copulations in their lifetime. Mating rates of spiders show a strong variation within and between species making them ideal model organisms to investigate the adaptive value of reproductive strategies. In the genus *Argiope* we find the combination of polyandry with low male mating rates (monogyny) and this goes along with many peculiar adaptations especially in males. Males often mutilate their paired genitalia (pedipalps) and plug up the genital openings of females. The effectiveness of mating plugs differs strongly between species. Mutilation of pedipalps renders them useless and therefore males are limited to at most two copulations in their lifetime. Moreover, males sacrifice themselves to the female at the latest after their second copulation. Males that survive their first copulation have the option to choose between two different mating strategies: males mate a second time with the same female (monogyny), or they continue mate search for another (bigyny).

In my thesis I worked mainly on the wasp spider *Argiope bruennichi* which has turned out to be a model organism for research on monogyny and polyandry within the past 10 years. I determined natural mating rates of females and males in a population of *A. bruennichi* and in laboratory experiments I analysed the adaptive value of sexual cannibalism and polyandry. Further I determined the value of polyandry as inbreeding avoidance mechanism in *A. australis*.

Mathematical models have stated that monogyny in the absence of paternal care requires a male-biased sex ratio in order to evolve. However, my field study showed that the ratio of males to females that mated at least once during the season (effective sex ratio) was almost balanced. But there was a large variation

within the operational sex ratio (ratio of males to females that are ready to mate at a certain time). At the beginning of the season there is a strong male bias because the much smaller males mature earlier than the females. Males that mature early in the season are able to besiege sub-adult females which increases a male's chance to copulate with a virgin. Being the first male at a female is highly adaptive in a mating system with effective mating plugs. This male bias in the operational sex ratio changes with the ongoing season to a female bias because males are constantly sorted out of the mating pool by sexual cannibalism. This temporal variation within the operational sex ratio had no influence on the mating rates of males and females. Also spatial heterogeneity did not predict natural mating rates neither in males nor in females.

But I found that males made state dependent decisions on whether to mate twice with the same female or to copulate with two different females. Male mating strategies depended on the quality of the first female they mated with. Males were more willing to stay monogynous when their first female was heavy while bigynous males mated with relatively light females. Further males showed to be more prone to monogyny if future mating opportunities within close distance were absent. Over 50% of the males were bigynous in the observed population which explained the frequency of polyandry. Females had an average mating rate of 1.3 males.

Males in *A. bruennichi* are able to adaptively manipulate their copulation duration and I showed that males use this to discriminate against sisters. Males mated to a sister shortened their copulation duration and increased their chances to survive the female attack. Whereas males that were mated to a non-related female copulated for longer, had a higher risk of being cannibalised, and transferred more sperm. The high value of a virgin female and uncertainty about future mating opportunities may be reasons for males to mate with their sister at all.

In *Argiope* males perform self-sacrifice after their second copulation and this behaviour may have low costs for males because of their lacking future mating opportunities. Self-

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sacrifice may rather be beneficial because hereby males contribute to the fitness of their offspring. I was able to show that in *A. bruennichi* females that consumed a male produced offspring with increased survival abilities under climate and food stress. The mechanism behind this remains unclear but it is proposed that the consumption of a conspecific provides females with rare nutrients that cannot be found in usual prey items. This implies that male sacrifice in *Argiope* represents a paternal investment and that females gain material benefits through male consumption. But the effect on offspring quality could not be enhanced further by the consumption of several males which implies that this benefit alone does not explain the evolution of polyandry in *Argiope*.

Polyandry most likely evolved as a strategy to avoid or at least reduce the costs of inbreeding. Inbreeding may reduce the fitness of an individual substantially due to inbreeding depression. I was able to show that inbreeding depression is present in *A. australis* after a single generation of inbreeding. Inbred individuals suffered from reduced body size and shortened lifespans. Strategies to avoid inbreeding costs are therefore highly adaptive.

Entelegyne spiders are supposed to be capable of post-copulatory choice mechanisms such as cryptic female choice because of their peculiar genital morphology. Females possess two separate sperm storage organs that are filled during two different copulations. This suggests that females are able to discriminate between the ejaculates of different males. Indeed sperm counts in *A. australis* revealed that females stored less sperm of brothers if these had mated after a non-sibling male. Moreover, cryptic female choice has already been shown for *A. lobata* which suggests that polyandry in *Argiope* evolved as an inbreeding avoidance strategy.

In my thesis I was able to show that polyandry in *Argiope* most likely evolved for genetic benefits. Multiple mating of females allows them to lower the costs of inferior matings. Moreover, I found that males are able to control their copulation duration and may use this manipulation to either mate monogynously or bigynously. Which mating strategy a male performed depended mainly on the quality of the actual mate and on his future mating opportunities.

## General introduction

### The evolution of mating systems

Within the animal kingdom we find a large variety of male and female mating strategies reaching from life long associations between mates to a highly promiscuous mating behaviour in both sexes (Emlen and Oring 1977; Clutton-Brock 1989). Understanding the evolution of mating systems is one of the main goals of evolutionary biology because the specific mating rates have large implications on the degree of sexual conflict and cooperation between the sexes and on the intensity of competition within one sex (Arnqvist and Rowe 2005). Since Darwin, our view of mating systems and the female and male mating behaviour has been shaped by the idea that males compete for access to females and that females should be choosy (Darwin 1871). This concept derived from the idea that in sexually reproducing animals the interests of females and males diverge due to the different expenditure into reproduction. Males produce large numbers of cheap sperm and are potentially able to fertilise many females with a single sperm load. In contrast females produce only few very costly eggs and are not able to advance their reproductive success by mating multiply. This idea was supported by A.J. Bateman in 1948 who found in *Drosophila* that males increased their reproductive output with every further mating while females were not able to advance their fitness by mating multiply. However, upon the various animal mating systems we find plenty of deviations from Bateman's principle including bi-parental care and reversed sex-roles. The introduction of the parental investment hypothesis by R.L. Trivers (1972) helped understanding the evolution of different sex roles. The parental investment hypothesis describes that the sex with the lower costs of reproduction will compete for access to the other.

One of the most extraordinary mating systems can be found in spiders, especially in entelegyne spiders, where females mate multiply and males are reduced to at most two copulations in their lifetime. Due to their internal fertilisation and their notable genital morphology, orb-web spiders are considered ideal model organisms to investigate the consequences of polyandry and male paternity

protection strategies. In the following I will describe the evolution of polyandry and monogyny in more detail and I will discuss why research on spiders is potentially very fruitful in this context.

Females of many species mate more often than it would be necessary for fertilisation and the evolution of polyandry as an active female mating strategy is puzzling, particularly since mating has considerable costs for females (Rowe 1994). Referring to classical concepts females should be choosy due to the high costs of egg production and the costs associated with each mating event, for example reduced foraging activity, the risk of being seen by a predator and potential infections with sexually transmitted diseases (Jennions and Petrie 2000). All mating costs have to be outweighed by the benefits of polyandry otherwise female promiscuity may not evolve. In many species we find that females gain material benefits from multiple copulations because they get access to additional breeding territories, benefit from protection of several males and profit from nutrient donations at matings (Andersson 1994). These nutrient donations can consist of nuptial gifts such as small prey items or fecundity enhancing substances within the seminal fluid (Vahed 1998). Gathering material benefits of several males clearly is in the female's interest; however, often polyandry is not associated with these direct advantages. Here genetic benefits provide a reasonable explanation (Zeh and Zeh 1996, 1997; Jennions and Petrie 2000).

The possibility to trade up from males of poor quality may be one reason for additional matings of females (Kempnaers and Geert 1992). Another genetic advantage of mating multiply is the increased diversity in offspring which may be beneficial under uncertain environmental conditions (Loman et al. 1988) or reduce sibling competition (Ridley 1993).

More complex processes result from polyandry generated by the mixture of different ejaculates within the female body. First, sperm mixing can cause direct competition between different ejaculates for fertilisation success (Simmons 2001a; Elgar et al. 2003) which benefits females because they produce sons with more competitive sperm ('sexy sperm';

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Keller and Reeve 1995). Interactions between the female herself and the ejaculates may also occur and enable females to choose internally between several mating partners ('cryptic female choice'; Eberhard 1996; Birkhead 1998; Eberhard 1998). Evidence for cryptic female choice is scarce but the existing studies show that females mostly discriminate against males with incompatible genomes (Tregenza and Wedell 1998; Newcomer et al. 1999). Genetic incompatibilities between male and female genomes may arise from genomically imprinted genes, selfish genetic elements and from inbreeding (Zeh and Zeh 1997, 2001). Most of the empirical evidences for post-copulatory selection processes have been found in the context of inbreeding avoidance (Newcomer et al. 1999; Tregenza and Wedell 2002; Bretman et al. 2004). This suggests that the risk of inbreeding selected for female mechanisms to avoid being fertilised by a sibling male (Stockley et al. 1993; Cornell and Tregenza 2007).

In general, it is proposed that complex female reproductive tract morphologies enhance the opportunities for post-copulatory choice mechanisms (Hellriegel and Ward 1998). Spiders are due to their peculiar genital morphology particularly well suited for investigating sperm utilisation patterns (Eberhard 2004). Female spiders possess two separate genital openings that lead to two individual sperm storage organs (spermathecae). Spermathecae are filled independently during two copulation bouts which can be performed by different males (Foelix 1996). This spatial separation of ejaculates may facilitate post-copulatory selection processes in spiders (Eberhard et al. 1993; Elgar 1998; Hellriegel and Ward 1998).

In contrast to the common polyandrous female, spider males of many species have very low mating rates and often mate only once. Monogyny can be found across all taxa but evolved several times independently in spiders (Miller 2007). Many extraordinary male adaptations are associated with monogyny, for example life-long associations between mates, extreme sexual size dimorphism, and sexual cannibalism. In web-building spiders (Araneae) several convergent cases of male adaptations to monogyny can be found. Males are usually much smaller than females and may perform a suicidal mating behaviour by sacrificing

themselves to their mate. The most prominent example is certainly the Australian red-back spider *Latrodectus hasselti* in which males somersault onto the female fangs during copulation (Forster 1992; Snow and Andrade 2004). This self-sacrifice has two potential benefits for males: first they may prolong their copulation duration (mating effort) or second they provide a paternal investment to the female by sacrificing their body as nutrient donation (Buskirk et al. 1984; Simmons 1989).

In spider males the production of sperm is dislocated from the copulatory organs, the pedipalps (Foelix 1996). After males mature they place their sperm on a special sperm web and fill their pedipalps. In many species males are not able to refill their sperm and thus become sperm limited. Further, many spider males possess 'one-shot genitalia' which means that pedipalps can only be used once because males mutilate them during copulation and leave the broken-off parts inside the female genital opening (Levi 1975). These serve as mating plugs and are of different effectiveness in *Argiope* reaching from almost impassable for rivals as in *A. bruennichi* (Nessler et al. 2007) to easily vincible for successors as in *A. sector* (Nessler unpublished data). Functional sterility and sexual cannibalism limit male mating rates to at most two copulations and leave them with three possible reproductive strategies: first, males mate twice with the same female and completely monopolise her by plugging up both genital openings. Second, males partition their copulations on two different females. Third, males die after only a single copulation, either as a consequence of female aggression or as an active male mating strategy.

The evolution of monogyny without paternal care is not completely understood yet but theoretical work on this topic proposes that it may only be beneficial in combination with a highly effective paternity protection and under a strong male-biased sex ratio (Fromhage et al. 2005; Fromhage et al. 2008). In order to invade into a population of polygynous males, monogynists need to lift their reproductive success above the average in the population. In many orb-web spiders, especially in the wasp spiders *Argiope*, all of the above preconditions are met and there is a large variation among and within species in male mating strategies. This makes research on spider reproduction a promising field for the understanding of the

evolution of monogyny and its associated adaptations.

In my thesis I use a field study to assess the degree of polyandry in females and the reproductive strategies of males in *A. bruennichi*. I identify factors that influence males in their mating decisions and that account for polyandry. I compare my findings to the existing theoretical framework on monogynous mating systems. Further I present laboratory experiments in which I quantify the benefits of polyandry through material gains, arising from sexual cannibalism, as well as genetic benefits, in the context of inbreeding avoidance. I show the importance of inbreeding avoidance for *A. australis* by quantifying the degree of inbreeding depression and I test for the existence of cryptic female choice. Further I investigate the adaptive value of male self-sacrifice behaviour as a response to the threat of inbreeding.

### Study organisms

In orb weaving spiders (Araneidae), the combination of polyandrous females together with males that mate only once, monogynists, or twice in their lifetime, bigynists, is quite common. This extraordinary mating system bears a variety of peculiar adaptations especially in males and within the last 15 years spider mating behaviour and genital morphology has been intensively investigated including the genera *Nephila*, *Latrodectus* and *Argiope* (Schneider and Andrade 2011). Among these the wasp spiders (*Argiope*) have developed into a model system for research on the evolution of monogyny and polyandry.

In all *Argiope* species a pronounced sexual size dimorphism is prevalent and the much smaller males are attacked by the females during copulation (Gaskett et al. 2004; Herberstein et al. 2005; Schneider et al. 2006). This aggressive female behaviour may have evolved out of the predatory lifestyle of spiders but in *Argiope* it also represents a sexual conflict between males and females about copulation duration and the number of matings (Schneider et al. 2006). In order to keep the possibility of post-copulatory selection females are eager to prevent being monopolised by a single male while exactly this is in the male's interest. Interestingly, males in all *Argiope* species die

during their second copulation either through spontaneous death as in *A. aurantia* (Foellmer and Fairbairn 2003) or by sacrificing themselves to the female (Sasaki and Iwahashi 1995). Supposedly, this sacrifice has low costs for *Argiope* males because they are reduced to a maximum of two copulations anyway due to functional sterility (Andrade and Banta 2002). The lack of future mating opportunities may have selected for males to get cannibalised and increase their copulation duration (Schneider et al. 2006). The copulation duration in *Argiope* determines the number of sperm transferred by a male thus longer copulations translate into higher paternity shares under sperm competition (Elgar 1998; Simmons 2001b). Further males may perform better in mate plugging when cannibalised as was shown in *A. lobata* for example (Nessler et al. 2009a).

### Study aims

#### Polyandry in *Argiope*

##### a) Natural mating rates of females

In Chapter 1 I determine the natural mating rates of females in a confined population of *Argiope bruennichi* (Scopoli 1772). I observe all copulations during a whole mating season by monitoring each female of the population individually. Females of *A. bruennichi* are quite sedentary and therefore easy to observe while males leave their webs as soon as they mature and start roving around searching for females. By monitoring all females I collect data on female mating rates, male mating strategies (Chapter 2) and the sex ratios in the population. As predicted for monogynous mating systems I expect to find a male-biased effective sex ratio (ESR: ratio of males to females that mate at least once; Fromhage et al. 2005). This male bias in the ESR is supposed to arise from a highly dynamic operational sex ratio during the season (OSR: ratio of males to females that are ready to mate at a certain time; Emlen and Oring 1977). The OSR in *A. bruennichi* changes from a strong male bias in the beginning of the season to a complete female bias towards the end. I expect that females that mature early receive multiple copulations and those that mature towards the end of the season remain unmated. This dynamic in the OSR may lead to a male-biased ESR which is required for the evolution and maintenance of monogyny

## General introduction

(Fromhage et al. 2008). Further, female mating rates may be influenced by spatial patterns since spider males are attracted to airborne female pheromones (Gaskett 2007; Chinta et al. 2010). I predict that female clusters attract more males than patches with low female densities.

### *b) Sexual cannibalism*

Polyandry in spiders may have evolved for material benefits because sexually cannibalistic females earn an extra meal with every additional mating (Vahed 1998). However, in species with a high sexual size dimorphism the nutritional value of consuming a small male may be low (). In order to investigate possible genetic benefits of polyandry it is useful to quantify material benefits beforehand. In Chapter 3 I disentangle the effects of polyandry and sexual cannibalism by mating *A. bruennichi* females either multiply or only with a single male. Further I divide these groups into females that were experimentally prevented from male consumption and those that were allowed to consume their mates.

Previous studies failed to find fecundity enhancing effects of male consumption in *A. bruennichi* (Fromhage et al. 2003) therefore I focus on possible effects on offspring quality. If females gain material benefits through male consumption I expect to find a fitness increase in the cannibalism treatments only. If females benefit genetically and avoid incompatible mating partners or increase the diversity in their offspring I expect to find increased hatching rates and offspring of higher quality in the polyandry treatments.

### *c) Inbreeding avoidance*

In chapter 5 I use *A. australis* (Walckenaer 1805) to test for the existence of post-copulatory choice mechanisms, namely cryptic female choice, as a strategy to avoid inbreeding. I produce an inbred and an outbred line and I quantify the degree of inbreeding depression in *A. australis*. I compare lifespan, adult size, and adult weight of inbred and outbred individuals. If inbreeding depression is present detrimental incestuous matings should be avoided. Since pre-copulatory inbreeding avoidance mechanisms are lacking it is supposed that spider females use cryptic female choice to avoid or reduce the risk of inbreeding (Welke and Schneider 2009). In Chapter 5 I mate females to both a sibling and a non-sibling male

or vice versa. This enables females to choose between compatible and incompatible mating partners or to trade-up to superior males. By experimentally inducing males to copulate into two different genital openings I am able to count sperm numbers stored from each individual male. If polyandry serves as inbreeding avoidance mechanisms I predict that females store more sperm of non-sibling males.

## Monogyny

### *a) Natural mating rates of males*

Analogous to the determination of natural female mating rates I assess male mating strategies by observing all females and their copulations in a small *A. bruennichi* population (Chapter 2). I collect all males that fall victim to sexual cannibalism and determine their mating rates by combining my observational data with the frequency of genital mutilation in males. Males of *A. bruennichi* break their pedipalps in about 85% of their copulations therefore genital damage gives an accurate indication of a male's mating rate. I assess the frequency of different male mating strategies within the population (monogyny one copulation, monogyny two copulations, and bigyny) and determine which factors influence males in their mating decision. I expect high variation within male mating strategies during the season due to the high variability in male-male competition. As mentioned before, the operational sex ratio changes drastically during the season in *A. bruennichi*. In the beginning mostly males are adult while females mature slowly during the ongoing season. Thus we find a strong male bias in the beginning of the season which changes until the end to a complete female bias because males are constantly sorted out of the mating pool through sexual cannibalism. I expect that monogyny is most prevalent early in the season with strong male-male competition while bigyny becomes more beneficial as soon as the operational sex ratio becomes female-biased (Kvarnemo 1996).

### *b) Sexual cannibalism*

In chapter 3 I test the adaptive value of sexual cannibalism as material contribution of males to females. Sexual cannibalism from the male perspective can either serve as a mating effort by which males increase their copulation

duration and or males provide a paternal investment into their offspring by sacrificing their body to the female (Buskirk et al. 1984). If this is the case I expect to find a positive impact of male consumption on offspring quality. In a previous study on sexual cannibalism in *A. bruennichi* the existence of paternal investment has been rejected (Fromhage et al. 2003) but here only fecundity traits such as hatching rate and clutch size were compared between cannibalistic and non-cannibalistic females. In Chapter 3 I also test for positive effects of sexual cannibalism on offspring quality.

#### c) Inbreeding avoidance

While spider females most likely avoid the negative consequences of inbreeding with the help of cryptic female choice males are limited in their response to the threat of inbreeding. No pre-copulatory discrimination of males against sibling females has been found for any *Argiope* species yet. Therefore other mechanisms to reduce the costs of inbreeding should have evolved. In chapter 4 I investigate whether males of *A. bruennichi* adjust their mating behaviour to the risk of inbreeding and if they differ in the mating strategies performed when mating with a sister or a non-related female. It has been shown for *A. bruennichi* that males are able to adaptively manipulate their copulation duration to the presence or absence of female pheromones during their development (Nessler et al. 2009b). The copulation duration of *A. bruennichi* males directly influences the probability of being cannibalised or not. Males that copulate for longer than 10 seconds almost invariably fall victim to sexual cannibalism. Although females invariably attack the males at the onset of copulation it seems that *Argiope* males at least partly control the outcome of this attack by manipulating their copulation duration (Nessler et al. 2009b). Different from other spider species sexual cannibalism in *Argiope* takes place after the genital contact between mates has seized. By manipulating their copulation duration males may exercise different mating strategies: either sacrifice themselves to a female after one long copulation or opting for a second copulation with the same or a different female

#### Note

The individual chapters of this thesis should be comprehensible as they are, without need for

reference to other sections. A certain degree of overlap in the descriptions and explanations given is therefore inevitable.

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## Chapter 1: Determinants of natural mating success in the cannibalistic orb-web spider *Argiope bruennichi*

Stefanie M. Zimmer • Klaas W. Welke • Jutta M. Schneider

### Abstract

Monogynous mating systems (low male mating rates) occur in various taxa and have evolved several times independently in spiders. Monogyny is associated with remarkable male mating strategies and predicted to evolve under a male-biased sex ratio. While male reproductive strategies are well documented and male mating rates are easy to quantify, especially in sexually cannibalistic species, female reproductive strategies, the optimal female mating rate, and the factors that affect the evolution of female mating rates are still unclear. In this study, we examined natural female mating rates and tested the assumption of a male-biased sex ratio and female polyandry in a natural population of *Argiope bruennichi* in which we controlled female mating status prior to observations. We predicted variation in female mating frequencies as a result of spatial and temporal heterogeneity in the distribution of mature females and males. Females had a low average mating rate of 1.3 and the majority copulated only once. Polyandry did not entirely result from a male-biased sex-ratio but closely matched the rate of male bigamy. Male activity and the probability of polyandry correlated with factors affecting pheromone presence such as virgin females' density. We conclude that a strong sex ratio bias and high female mating rates are not necessary components of monogynous mating systems as long as males protect their paternity effectively and certain frequencies of bigyny stabilise the mating system.

### Introduction

Polyandry is a common phenomenon and requires an explanation as females in many species accept diverse costs of mating to gain more and/or more diverse sperm than required to fertilise their eggs (Hosken and Tregenza 2006). Numerous studies measured benefits and costs of multiple mating to females but our understanding of the evolution of polyandry is still incomplete (Halliday and Arnold 1987; Zeh and Zeh 1996; Arnqvist and Nilsson 2000; Jennions and Petrie 2000). It is assumed that benefits and costs of mating vary in magnitude, depending on how often a female mates (Arnqvist and Nilsson 2000). Optimal female mating rates are likely to balance various costs and benefits of mating but natural mating rates will also reflect trade-offs between the degrees of competition, conflict, and cooperation within and between the sexes (Arnqvist and Nilsson 2000). Optimal female mating rates should not be seen as fixed optima but as varying with different contexts e.g. defined by the degree of sexual conflict (Arnqvist and Nilsson 2000). Females of some insect species, for example, are capable of modulating their mating rate

depending on a set of environmental factors that affect the costs and benefits of mating (Gwynne 1993; Rowe et al. 1994; Rowe et al. 1996; Arnqvist 1997; Arnqvist and Nilsson 2000). Selection on male mating strategies may produce the side effect of removing the female mating rate from its optimum. In response, females will be under selection to develop adaptations that reduce the costs of mating and consequently shift the female mating rate back towards its optimum (Holland and Rice 1998).

Reliable mating rates of wild animals are generally difficult to obtain and are often inferred from indirect evidence such as proximity of potential mating partners or courtship behaviour (Jones et al. 2002; Wanker 2002; Maklakov et al. 2005; Haddrill et al. 2008). However, such measures do not necessarily imply the successful fertilisation of eggs. Genetic methods used in the absence of field observations have the opposite problem, namely that it is unclear how many matings did not result in fertilisation. In invertebrates, quantitative field observations of copulation behaviour are particularly difficult to obtain,

requiring high logistic effort. Rodriguez-Munoz et al. (2010) determined natural mating rates through a combination of permanent video monitoring and subsequent DNA profiling. The results revealed a sex ratio very close to even in a wild population of field crickets, *Gryllus campestris*, and although male reproductive success varied more, both sexes had higher lifetime reproductive success when they had more mates.

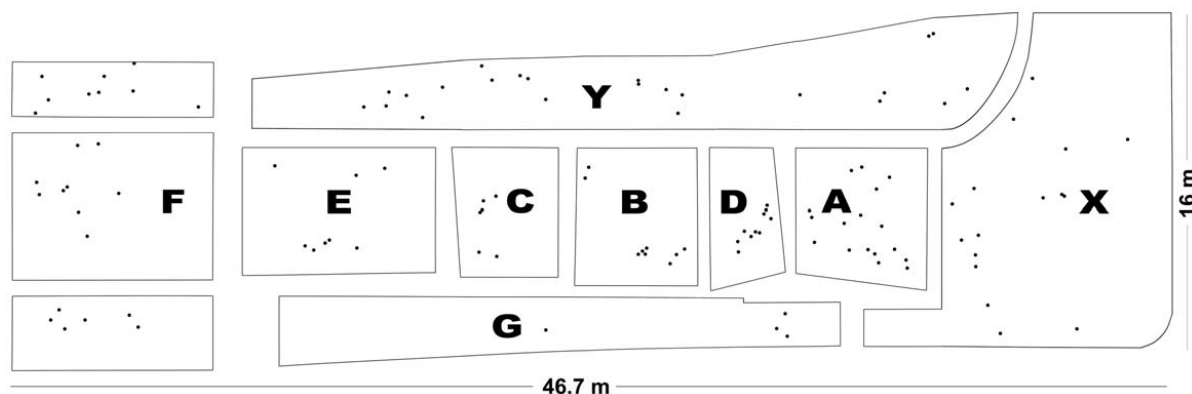
In cases where only one method is used to measure mating rates, observed values are upper or lower estimates of actual values. Moreover, data obtained in laboratory studies need not necessarily be representative of natural conditions (Arnqvist and Nilsson 2000; Reinhardt et al. 2007; Rodriguez-Munoz et al. 2010). These shortcomings and methodological difficulties may result in distorted views of actual mating rates and misjudgements of the operation of sexual selection and sexual conflict (Kokko and Jennions 2008). A well known example are the birds, in which genetic monogamy was generally assumed to be the prevailing mating system, until genetic evidence revealed that more than 70% of bird species perform extra-pair copulations (Griffith et al. 2002).

In contrast to the classical concept that males should generally be selected to mate with multiple females (Bateman 1948; Trivers 1972), it has recently been emphasised that males may sometimes be selected to invest strongly in paternity enhancement with one or two females only (Schneider and Fromhage 2010). These monogynous (males fertilise a single female) or bigynous (males fertilise two females) mating strategies occur in several taxa and have evolved several times independently in spiders (Schneider and Fromhage 2010). To increase their paternity with a single female, monogynous males evolved drastic mating strategies e.g. mate plugging by damaging genitalia in some *Argiope* species (Nessler et al. 2007), or facilitation of sexual cannibalism by somersaulting onto the fangs of females as described in some *Latrodectus* species (Andrade 1996; Segoli et al. 2008). The theory about the evolution of monogyny rests on the assumption of strong male competition for the fertilisation of females and the occurrence of a male-biased sex ratio combined with paternity benefits of increased mating investment (Fromhage et al. 2005). The sex ratio bias has to result in

different average mating rates of males and females to operate as a selection pressure on male monogamy (Fromhage et al. 2008), hence the traditional concept of the operational sex ratio (ratio of sexually active males to females; OSR) is not sufficient to describe this precondition as it does not relate to mating per se but to the potential for mating. An alternative concept is the effective sex ratio (ESR), defined as a ratio of males to females which includes only individuals that mate at least once (Fromhage et al. 2005). The degree of male bias in the ESR defines the average male mating rate. A bias of two, for example, means that the average male mating success is 0.5. Under these conditions, a monogynous mutant that can secure fertilisation of more than 50% of a female's offspring has a mating success above average. The ESR can be male biased as a result of a biased tertiary sex ratio, or because a proportion of females do not mate (e.g. because they mature too late). Theory relies on polyandry as a precondition for evolution and maintenance of monogyny and bigyny (Fromhage et al. 2005; Fromhage et al. 2008). A mixture between monogyny and bigyny was found in *Argiope bruennichi*, but contrary to theory, effective mate plugging and avoidance of mated females by males suggest that most females mate only once (Nessler et al. 2007; Schulte, Uhl and Schneider 2010).

In the present study, we investigated natural female mating rates and tested the assumptions of theory with respect to the degree of polyandry and a bias in the ESR. We used a population of the sexually cannibalistic wasp spider *Argiope bruennichi*, a species with pronounced protandry and a highly seasonal breeding biology. Copulations may occur shortly after the female is moulting and are very brief and easily missed. To control female mating status at the onset of the observation, we removed penultimate females to moult in the laboratory. We returned the females to their exact position in the morning after their moult and monitored them closely until they got mated. Subsequently we regularly scan-sampled females until the end of the mating season.

We tested the hypothesis that more males than females would mate due to temporal and spatial variation in female mating rates. We predicted high female mating rates early in the season when protandrous males have matured



**Figure 1:** Map of the study field with nine distinct patches (A–G, X, Y) of different sizes which were separated from each other by narrow paths. The whole field contains a size of 747.2 m<sup>2</sup>. Dots indicate the location of individual female spiders in their webs which were marked and observed during the mating season. Females rarely changed their web sites.

already and the OSR is male biased. Furthermore, we predicted higher mating rates in female clusters that may attract more males. Correspondingly, we expected that late maturing females would remain unmated as well as females located at the edges of the population. In addition, we measured female body mass which is a proxy of fecundity and may cause variation in female mating rates as well.

## Materials and Methods

The field study took place from July 2009 until August 2009. The study site was open grassland in Hamburg (permission was given verbally by the owner) bordered by unsuitable habitat on all sides (highway, road and gravel). The meadow had a shallow slope to one side and the density of the vegetation decreased down the slope. The whole field (747.2 m<sup>2</sup>) was divided into nine patches of different sizes, which were separated from each other by narrow paths (Figure 1). These paths and uncovered areas within each patch were used to step on by the examiners to minimise destruction of webs and web supporting vegetation.

The study started after the first female had matured. Each immature female web location was marked with a bamboo pole and received an individual number. Additionally, the distance to the nearest female neighbour was measured and the female neighbours within a radius of 1 m, 2 m, 3 m and 5 m were counted. Every day, we moved carefully through the field and noted the number of males staying

close to sub-adult females' webs or in their web.

During daily controls, the developmental status of each penultimate female was closely monitored. The swelling of the external genital structure of female spiders (epigyne) can be used to forecast the date of their final moult. When females are freshly moulted to sub-adulthood no swelling is present whereas close to final moult the swelling looks like that of an adult female but is still covered with a cuticular layer. Based on the epigyne swelling females were categorised in seven groups, the last of which were about to mature. In order to ensure that all copulations could be observed, penultimate females were removed from the study site as soon as they reached the last category. The removal occurred with great care not to damage the web or interfere with the surroundings. Thereby, we intended to minimise disturbance for males that were already present near the web. The categorisation ensured that we collected sub-adult females as shortly as possible before their final moult. In the laboratory, the collected sub-adult females were kept in individual Perspex frames (36 x 36 x 6.5 cm), where they built characteristic orb-webs. All spiders were fed with ca. 20 *Drosophila spec.* and one *Calliphora spec.* and sprayed with water once per day. In the morning after females moulted to maturity, which occurred within  $3.56 \pm 1.55$  days after capture, they were weighed on an electronic balance to the nearest 0.001 mg and they were individually marked with a unique combination of colour dots on their opisthosoma. Then the females were returned

with the frame to the original web site and the frames were attached to the ground with tent pegs. The first adult female was released on the 10<sup>th</sup> of July, the last one on the 7<sup>th</sup> of August 2009. The interval between female removal and maturation did not affect female mating rate (monogamous or polygamous) (Logistic regression:  $\chi^2=0.51$ ,  $P=0.47$ ,  $N=98$ ) nor the time required until copulation occurred (*delay factor*;  $r=-0.07$ ,  $P=0.48$ ).

Previous studies on this and other spider species found that most matings happened just after the female's final moult (Miyashita and Hayashi 1996; Schulte et al. 2010). The females were observed for eight hours per day, which corresponds to the largest part of the available daylight period. If a virgin female did not copulate on a given day, she was carried back in her frame to the laboratory and released again the following day(s), until she mated. After having mated at least once, females were left in the field so as to maintain a natural occurrence of virgin and mated females. Based on previous field observations (Schulte et al. 2010), we assumed that the attractiveness of females rapidly declines after copulation, because matings with mated females are mechanically difficult due to the presence of mating plugs and the expected paternity gain is close to zero (Nessler et al. 2007). When a male visited a female, we noted the following: his size and number of legs; the time of his first appearance; the times of his first contact with the frame, the web, and the female; the beginning and duration of courtship and copulation; the number of copulatory insertions; and the occurrence of sexual cannibalism or male escape from a female attack.

When males survived the copulation and left the web, they were captured and marked with a specific colour dot (with non-toxic PELIKAN Plaka paint) on their opisthosoma before they were released again. In this way they could be identified if they reappeared. After sexual cannibalism, the wrapped males were carefully taken out of the females' web and were frozen at  $-80^{\circ}\text{C}$  in the freezer in the laboratory for further measurements. Overall, 121 females were recorded and observed at the study site over the whole mating season. Of these, we excluded 11 females whose mating status was unclear because they were already adult when found. One sub-adult and one adult

female disappeared in the laboratory and eight adult females escaped in the field before their mating rate could be measured. The mating of one virgin female in the field was missed and the last virgin female during the mating season did not get mated. Hence, mating rates of 98 females could be determined.

Data analyses were carried out with JMP 7.0.2. Most data were analysed using logistic regressions. Not normally distributed data were analysed with the non-parametric Wilcoxon Signed-Rank test and these tests are indicated with the results. We used an ANOVA to test the dependent spatial and temporal variables of male visitors' accumulation on females' web, as well as a multiple regression for the dependent variables on male activity. Descriptive statistics are given as mean  $\pm$  standard error of the mean (SE) unless stated otherwise and sample sizes may differ between analyses due to missing data.

## Results

### *Mating rates*

On average, the 98 observed females attracted  $2.61 \pm 0.23$  visitors (range 0–14) that at least reached the frame in which the female had her web; only one female had no visitor and remained unmated. However, 66 males (on average 0.8 males per female) left the female web without proceeding to courtship and copulation. Because *A. bruennichi* males may use either one or both of their pedipalps with the same female, we distinguish a female's mating rate (defined as the number of males that performed at least one copulatory insertion with this female) from her copulation rate (defined as the sum of copulatory insertions performed with this female by any of her mates).

The average mating rate of females was  $1.30 \pm 0.05$  (0–4 range) and based on 98 females, of which 72 females (73.5%) mated with only a single male, 24 females (24.5%) mated with two males, one female (1%) mated with three males, and one female (1%) mated with four males.

We could not determine the final copulation rate for five females. We just found dead males in the webs of these five females and we did not know if they had copulated once or twice with the females before they were killed. Hence, copulation rate is known for 93 of the 98 females. The average copulation rate of females

was  $1.38 \pm 0.07$  (0–4 range); 63 females (67.74%) copulated once, 24 females (25.81%) copulated twice, four females (4.3%) copulated three times, and only two females (2.15%) copulated four times. Of the 24 females that copulated twice, 15 females copulated once with each of two different males, whereas nine females copulated twice with the same male. No differences were detected (Binomial:  $\chi^2=1.52$ ,  $P=0.22$ ,  $N=24$ ). Four females achieved their copulation rate of three through a single copulation with one male and a double copulation with another male. Only two females had four copulations; one through single copulations with four different males and the other one through double copulations with two different males.

The effective sex ratio (ESR), defined as the ratio of males to females that mate at least once (Fromhage et al. 2005), was 1.29 resulting from 98 females and 126 males that met the definition. The male bias may in fact be lower and the true ESR closer to even for the following reasons: The number of females may be higher because bigynous males were counted once although the 1<sup>st</sup> mates of 22 (17.5%) bigynous males were unknown. Furthermore, we did not account for the number of females that only received a copulation from a bigynous male which could have inflated the number of males that mated and the male bias in the ESR would indeed be reduced. According to theory (Fromhage et al. 2005), this relatively weakly biased ESR implies that monogyny will be favoured by selection if and only if monogynists' expected paternity share is  $>77.5\%$ .

For further analyses we group females in two categories concerning their mating success (the number of mates a female had during the entire mating season): those that received one mating and those that received more than one mating. Female mating success was not a function of female adult weight (Logistic regression:  $\chi^2=0.31$ ,  $P=0.58$ ,  $N=98$ ). Below we analyse whether female mating success was influenced by spatial and temporal factors.

#### *Spatial patterns*

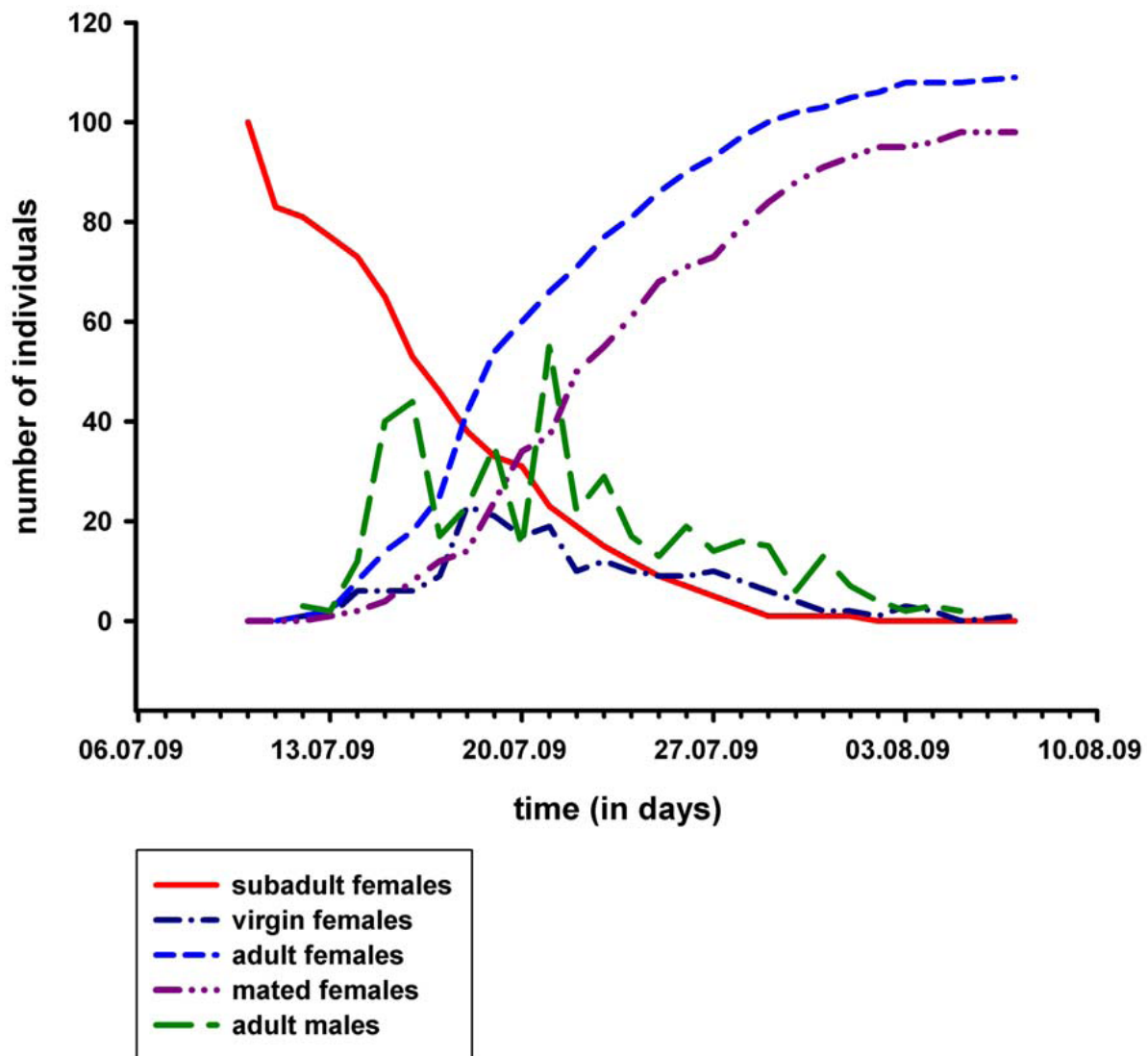
Females were scattered over the whole study site although there were several small clusters (Figure 1) so that nearest neighbour distances varied (see Material S1). The local density, measured as the number of other females in a

radius of 1–5 m or as the distance to the nearest neighbour regardless of their mating status, did not correlate with the number of male visitors nor with the copulations that a female received (Table 1). However, females were less likely to mate with more than one male if the number of virgin females in the population was relatively high (Logistic regression:  $\chi^2=4.48$ ,  $P=0.03$ ,  $N=98$ ).

**Table 1:** Correlations between female copulation frequencies or the number of male visitors per female with different measures of female density.

Variable	With variable	<i>r</i>	<i>N</i>	<i>P</i>
Copulation frequency per female	♀♀ in 1 m radius	-0.11	98	0.3
	♀♀ in 2m radius	-0.1	98	0.34
	♀♀ in 3 m radius	-0.11	98	0.3
	♀♀ in 5 m radius	-0.14	98	0.16
	distance of nearest neighbours	-0.03	98	0.77
Male visitors per female	♀♀ in 1 m radius	0.12	109	0.23
	♀♀ in 2 m radius	-0.04	109	0.65
	♀♀ in 3 m radius	0.002	109	0.99
	♀♀ in 5 m radius	-0.01	109	0.89
	distance nearest neighbours	-0.07	109	0.48

Notably, many males waited in close proximity to sub-adult females, either in their own webs or in the outer region of a female's web. Usually in such cases there were one or two males (in one case even three males) associated with a given sub-adult female. The number of males waiting at a female's web depended on the female's developmental state. Females close to their final moult attracted more males ( $0.77 \pm 0.14$ ;  $N=26$ ) than females that were more than a week away from adulthood ( $0.2 \pm 0.11$  males;  $N=21$ ; Wilcoxon Signed-Rank test:  $\chi^2=9.7$ ,  $P=0.002$ ). In order to avoid pseudo-replication through repeated sightings of the same males we analysed this pattern further using a single day of the mating season. We picked day six because on that day the ratio of sub-adult females to adult males was balanced. On this day, the number of males at a sub-adult female's web was significantly predicted by an ANOVA ( $F_{14,35}=4.62$ ,  $r^2=0.65$ ,  $P=0.0001$ ) with Patch ID ( $F_{8,41}=3.61$ ,  $P=0.004$ ) and female developmental state ( $F_{6,43}=7.48$ ,  $P<.0001$ ) as factors.



**Figure 2:** Daily numbers of sub-adult (red line) and adult females (blue dashed line) and adult males (green dashed line) in the population. Adult females are also separated according to their mating status (virgin females (dark blue dashed line) and mated females (purple dashed line)). The mating season started at the 12<sup>th</sup> of July with the 1<sup>st</sup> adult virgin female.

#### Temporal patterns

The mating season lasted 30 days (see Figure 2). Accordingly, the daily availability of virgin females was highest during the first eight days, reached a peak on the 18<sup>th</sup> of July with 23 freshly moulted females and then declined: 50% of the females moulted to maturity during the first week. Roving males were seen until the 6<sup>th</sup> of August and the daily counts fluctuated during the first two weeks of the season and steadily declined during the last two weeks. The number of roving males was always higher than the number of virgin females and showed two major peaks, one early and one later in the

season (Figure 3). These peaks in male activity were not reflected in daily female mating rates which varied between one and two throughout the course of the season (Figure 3). Accordingly, the variation in female mating rates (female mating rates are categorised as 'one' or 'larger than one') was not explained by the number of active males (Logistic regression:  $\chi^2=0.97$ ,  $P=0.32$ ,  $N=98$ ) nor by the day in the season on which the females matured (Logistic regression:  $\chi^2=0.04$ ,  $P=0.85$ ,  $N=98$ ).

Fluctuations in male activity, measured as the number of male visitors per female per day, were significantly explained by how many



virgin females were present in the population ( $F_{1,24}=4.58$ ,  $P=0.0001$ ) but not by the day of the season ( $F_{1,24}=0.65$ ,  $P=0.52$ ; multiple regression:  $F_{2,22}=11.1$ ,  $r^2=0.5$ ,  $P=0.0005$ ). Hence, high numbers of virgin females increase male activity but decrease female mating rates (see above).

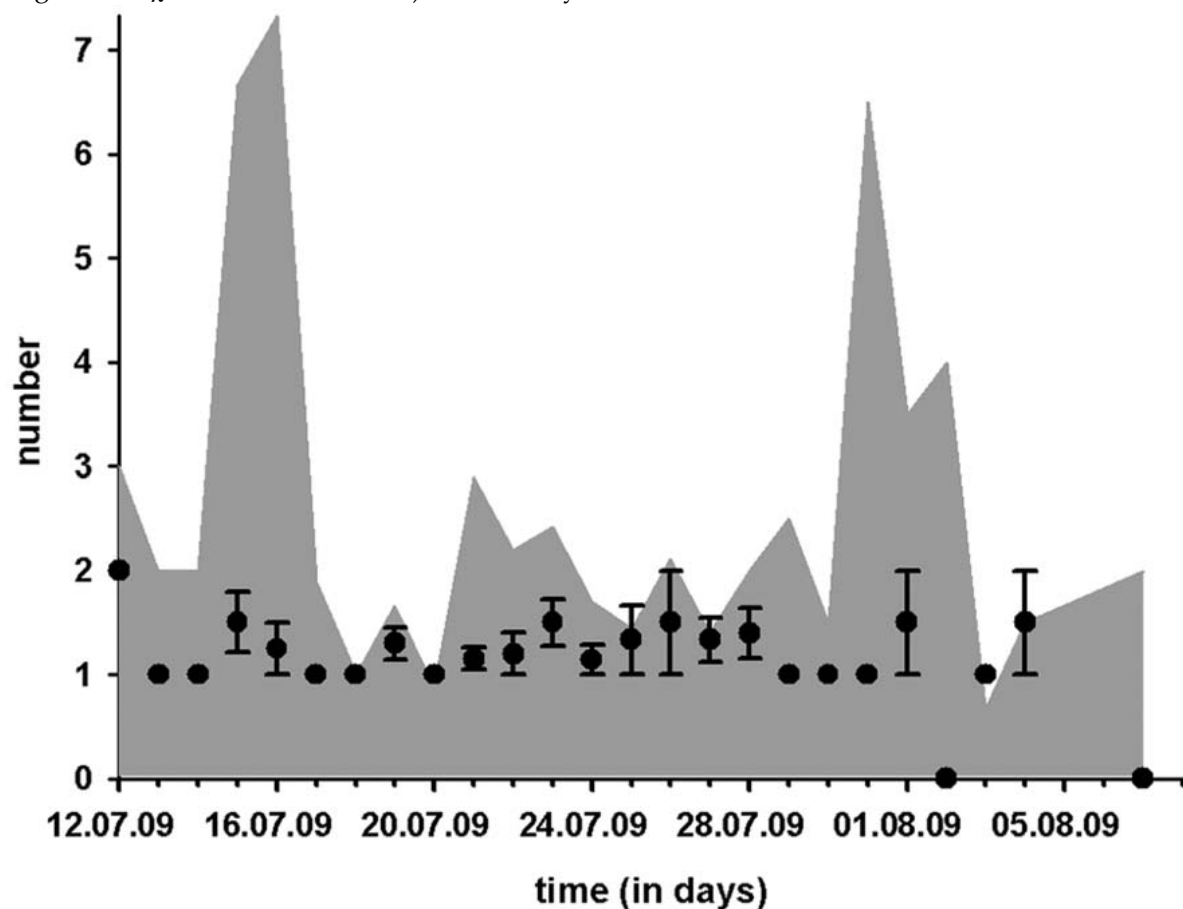
#### Female attractiveness

All females except one received at least one copulation but not every female was mated soon after she appeared on the meadow. In fact, only 17.4% of the females received a copulation within the first two hours of their release. Many females ( $N=55$ ) had to be repeatedly released on up to five consecutive days. We categorised females' mating latency as  $\leq 2$  h or  $>2$  h. Females in these two categories did not differ significantly in any of the following variables: day of first (Logistic regression:  $\chi^2=0.33$ ,  $P=0.57$ ,  $N=98$ ) and last release (Logistic regression:  $\chi^2=2.0$ ,  $P=0.16$ ,  $N=98$ ); female size (Logistic regression:  $\chi^2=2.87$ ,  $P=0.09$ ,  $N=98$ ); and nearby

female density in the field (Logistic regression: 1 m radius:  $\chi^2=0.003$ ,  $P=0.95$ ,  $N=98$ ; 2 m radius:  $\chi^2=0.002$ ,  $P=0.97$ ,  $N=98$ ; 3 m radius:  $\chi^2=0.007$ ,  $P=0.93$ ,  $N=98$ ; 5 m radius:  $\chi^2=0.05$ ,  $P=0.83$ ,  $N=98$ ; nearest neighbour distance:  $\chi^2=0.65$ ,  $P=0.42$ ,  $N=98$ ). 40.7% of these females (33 of 81 females) were actively rejected by visiting males (20 by one male, 13 by several males), while 59.3% simply had no visitors prior to their first copulation. Rejected females did not differ from unvisited females in the above parameters (all  $P>0.05$ ).

#### Discussion

Contrary to our predictions about the mating system of *Argiope bruennichi*, we found surprisingly low variance in female mating rates over the course of the season. Polyandry was rare and female mating rates were not related to temporal and spatial homogeneity in the distribution of mature females and males. The small variance in female mating rates is



**Figure 3:** The grey area shows the ratio of adult males per virgin female calculated for each day of the season. The ratio is generally male biased with two distinct peaks early and late in the season. The dots depict daily female mating rates. If more than one female mated on a particular day, mean and SE of mating rates are given.

likely explained by life-history and mating decisions of males and it remains unclear whether observed mating rates are merely male imposed or in which degree they reflect female interests.

Many orb-weaving spiders, including all species of the genus *Argiope*, are characterised by a pronounced sexual size dimorphism and small male size is generally explained by selection on protandry (Higgins 2002; Miller 2007). Protandry means that males mature before females and if females mature progressively, a dramatic shift in the operational sex ratio is expected from an early male bias to a late female bias. The latter is expected if males are removed from the mating market for example through sexual cannibalism (Kasumovic and Andrade 2009; Schneider and Andrade 2011) which is the case in our study system. As a consequence of this pattern, we expected strong competition among protandrous males over the earliest maturing females and an elevated mating rate in these females. High rates of polyandry of early females and low mating rates of males may have the effect that late maturing females remain unmated because no males are available any more. However, we did not find the expected pattern and only a single female did not mate. Males were found active throughout the season and there was even a second peak of male abundance during the second half of the season, strongly opposing the idea of protandry. This finding suggests that males keep maturing at least during the first half of the season and/or that males survive long enough to wait for opportunities to copulate with virgins. Below we discuss male life-history decisions and male choice as possible explanations for the observed pattern of low female mating rates and low variance in female mating rates.

Males of the sexually cannibalistic spider *L. hasselti* were shown to change their development time in response to pheromones that provide cues of female and male density. Without female odour cues, males delayed maturation in favour of reaching a better condition to survive mate searching and competition for sparse females. With female odour cues, males speeded up development and matured smaller and in poorer condition (Kasumovic and Andrade 2006). As suggested for several further species with similar mating

systems (Kasumovic and Andrade 2006), males of *A. bruennichi* may also be able to flexibly truncate or prolong the penultimate instar and adjust the timing of maturation to the presence of pheromone cues emitted by females in the vicinity. As the local presence of volatile cues varies over time, such a mechanism could lead to the continuous maturation of males as long as virgin females mature. Although pheromone dependent maturation has not yet been studied in *A. bruennichi*, the potential is present as males of this species are known to adjust their mating strategy to the past perception of female sex pheromones (Nessler et al. 2009).

The perception of female availability and female mating status through volatile pheromones provided the base for our prediction of spatial variation in mating rates. As female webs in high density patches should attract more males we expected higher mating rates. However, although more female cues were likely present in high densities, this did not result in elevated rates of mating or male visitation in this study. In contrast, a study on *Nephila plumipes* showed that females formed clusters between which the opportunity of males to find suitable mates differed (Kasumovic et al. 2008). In some areas, males encountered many females and accumulated on female webs, probably leading to increased female mating rates. In contrast, other locations were difficult to find for males and consequently females in these patches were never reached by males and remained unmated. In comparison to *Nephila*, the distribution of *Argiope* species is generally less patchy. Females may occur in small clusters but distances between clusters are short (Kasumovic et al. 2009).

Low incidences of polyandry and the absence of a correlation between the number of male visitors and female mating rates may be a result of male discrimination against mated females (Schulte et al. 2010). Avoiding mated females appears highly adaptive in view of the paternity protection mechanisms in *A. bruennichi*: mated females usually have at least one of their two genital openings plugged with a genital sclerite of the first male to mate (Nessler et al. 2007). Such mating plugs are highly effective, and although most males inseminate and plug only one side, a following male apparently cannot detect which side is virgin (Nessler et al. 2007). Hence, a male that

copulates with a half-mated female has a 50% risk of using a plugged side. If sperm transfer fails in this case and the male falls victim to sexual cannibalism, he will not have any reproductive success. The finding that mated females appear to be relatively unattractive raises the question of whether extreme male mating strategies such as self-sacrifice and genital damage are really necessary to protect a male's paternity.

In accordance with an earlier field study (Schulte et al. 2010), some males even abandoned virgin females after a brief inspection of the web. The majority of freshly moulted virgin females had to be taken to the field several times before they received a copulation. While one half of the females were not visited by males, 40.7% were actively rejected by a visiting male at least once before they received a copulation. However, female mating latency was not related to her size, the time in the season nor to female density. Female mating success was lower if more other virgin females were present, suggesting that males were more likely to leave a virgin if alternative mating opportunities were available and that females compete for mates. Females are known to emit male-attracting pheromones (Gaskett 2007) and variation in the quantity of such pheromones may explain why male activity in our study correlated with the number of virgin females. Experimental tests with varying pheromone dosages are required to substantiate this speculation.

Pheromones are mostly found on the webs of virgin females (Chinta et al. 2010) suggesting that males perceive a female only after or during the moult to maturity. However, our observations are in accordance with reports from *A. aurantia* (Foellmer and Fairbairn 2005) in which males position themselves in the vicinity of females that are about to mature. Thereby they likely increase the probability to mate first with a virgin but the competition with the other males surrounding the same female may be severe.

By removing sub-adult females (along with any associated sensory cues) from the males for a few days, we may have interfered with male mating strategies. The time between the removal of the sub-adult female and the returning of the freshly moulted adult varied but the length of this time period did not influence her mating rate or mating latency.

Even though we made sure to leave the web and the surrounding vegetation intact, we cannot exclude that female removal had an effect. At worst, this manipulation may have increased male mortality and influenced the sex ratio so that we underestimated female mating rates. Even though we would not expect major distortions, future studies should account for male guarding of penultimate females and simulate this situation to assess whether it provides a fundamentally different scenario. In addition, the nature of the cues used by males to identify sub-adult females close to maturity needs to be investigated. Perhaps pheromones are already present at this stage, at dosages too low to be detected by gas chromatography-mass spectrometry (Chinta et al. 2010), but high enough to be detectable by male spiders. Alternatively, there might be an additional volatile substance that is used as a cue for female's reproductive status, receptivity, and attractiveness.

Theory suggests that a monogynous male mating strategy can evolve when two conditions are met. First, monogynous males should possess a mechanism of paternity protection not available to polygynous males (Fromhage et al. 2005; Fromhage et al. 2008). This is true for *A. bruennichi*, in which monogynous males have the potential of monopolising a female by leaving mating plugs in both of her genital openings (Nessler et al. 2007; Schneider and Lesmono 2009). Second, there should be a male-biased effective sex ratio (ESR; based on males and females that mate at least once). The male bias in the ESR is required if the high investment in monopolisation is to elevate male mating success above the average.

However, our data reveal only a weak bias of 1.29 in the study population which then requires a very potent paternity protection with a success of over 77%. Otherwise a monogynous mating strategy would not be better than polygyny. Indeed, plugging success and effectiveness are very high (above 80% in the laboratory) in *A. bruennichi* (Nessler et al. 2007).

Female mating rates were unexpectedly low but still above one. The proportion of doubly mated females was partly explained by the proportion of bigynous males [Welke, Zimmer, Schneider, unpublished]. Bigynous males escape sexual cannibalism after their first mating and search for a new female instead of

re-mating with the same one. The presence of bigynous males elevates female mating rates and increases the need for paternity protection even under an even sex ratio and male discrimination against mated females. At the same time, bigynous males will lower the ESR. A potential source of error may have caused an underestimation of female mating rates. Mated females remained in the field and may have received additional copulations that took place undetected, e.g. because they occurred at night. However, field observations of focal females at night *A. bruennichi* suggest that copulations rarely if at all occur at night (SMZ, personal observation). Nevertheless, 22 unmarked mated males were found which means that their first copulation was missed. This strongly implies that a proportion of copulations occurred either outside the observation times or outside the observed population. These unseen 1<sup>st</sup> copulations could have occurred with virgin or mated females. The latter case would not affect the ESR while unnoticed once-mated females would reduce the male bias in the ESR. Hence, our main conclusions may be robust with respect to unnoticed copulations.

Overall, our results suggest a weak ESR bias in *A. bruennichi* and support the theoretical prediction that even a weak bias is sufficient to favour monogyny given that monogynists possess efficient mechanisms of paternity protection (Fromhage et al. 2005). On the other hand, as the frequency of monogyny increases and monogynists compete increasingly with each other, the fitness of this strategy decreases, potentially leading to a mixture of mono- and bigynous mating strategies that is maintained by negative frequency-dependent selection (Fromhage et al. 2008). Since any increase in the frequency of bigynous males would necessarily elevate the degree of polyandry and hence the need for paternity protection, it seems likely that the coexistence of monogyny and bigyny stabilised the mating system.

To our knowledge, this study provides the first study design to examine natural female mating rates in a population of orb-web spiders. Further studies on different populations, and on other species, are required to fully understand the selection factors that have shaped the remarkable mating system of this and other species.

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**Supplementary material S1: Nearest neighbour distances in metre between females**

Female ID	Patch	Nearest Neighbour Distance in metre
A2	A	1,198
D1	D	0,22
C5	C	0,156
Y14	Y	1,26
X4	X	2,19
F4	F	1,31
A1	A	0,87
C1	C	0,625
D2	D	0,37
X5	X	3,675
F2	F	0,625
F5	F	0,49
D6	D	0,22
X2	X	1,25
Y4	Y	0,81
Y6	Y	0,44
X1	X	0,19
A3	A	0,37
B3	B	0,2
E2	E	0,5
B1	B	0,19
D4	D	0,218
A4	A	0,531
B4	B	0,55
Y1	Y	1,25
X6	X	1,25
F1	F	0,25
F3	F	0,625
A5	A	0,375
C2	C	0,625
A6	A	0,37
F7	F	1,1
Y8	Y	0,87
A7	A	0,813
X7	X	2,2
D3	D	0,375
Y2	Y	1,1
Y3	Y	0,68
E1	E	0,5
X3	X	0,755
F6	F	0,25
F9	F	0,9
Y7	Y	0,68
E4	E	1,34
D7	D	0,28
B2	B	0,49
E6	E	3,75
F12	F	0,84
Y11	Y	0,25
B5	B	0,55
E5	E	0,25
F11	F	3,19
X9	X	0,875
X8	X	0,55
X10	X	0,19
C3	C	0,72
Y10	Y	0,375
Y9	Y	1,28
X11	X	0,55

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A9	A	0,531
C4	C	0,156
F8	F	0,9
Y12	Y	0,187
Y13	Y	3,76
A8	A	0,813
F16	F	1,22
F17	F	0,78
D5	D	0,219
E3	E	1,38
F15	F	0,66
F13	F	0,84
B6	B	0,19
Y15	Y	0,78
A10	A	0,75
X13	X	1,4
X12	X	2,85
D8	D	0,5
A11	A	0,49
E7	E	1,38
X15	X	2,2
F14	F	1,31
B7	B	0,25
F20	F	0,69
A13	A	1,16
X14	X	0,755
F19	F	0,69
G1	G	0,63
Y17	Y	0,78
Y16	Y	0,875
F18	F	0,66
E8	E	0,25
Y18	Y	0,44
F10	F	0,49
F22	F	0,78
A12	A	0,313
Y19	Y	3,76
F23	F	1,22
C7	C	0,72
A14	A	0,375
X16	X	1,4
Y21	Y	0,375
Y20	Y	0,187
G2	G	0,84
Y22	Y	0,87
F21	F	3,19
G3	G	0,63
A15	A	0,81
F24	F	2,125
Y5	Y	0,875
D9	D	0,218
Y23	Y	1,25
B8	B	0,49
B9	B	0,55



## Chapter 2: Conditional monogyny: female quality predicts male faithfulness

Klaas W. Welke • Stefanie M. Zimmer • Jutta M. Schneider

### Abstract

Male monogyny in the absence of paternal investment is arguably one of the most puzzling mating systems. Recent evidence suggests that males of monogynous species adjust their life-history and their mating decision to shifting spatial and temporal selection regimes. In the cannibalistic wasp spider *Argiope bruennichi* males can be either monogynous or mate with a maximum of two females. We studied factors underlying male mating decisions in a natural population over a whole mating season. We documented all matings and categorized the males into single-mated and double-mated monogynous as well as bigynous males. We found that all categories were continuously present with relatively stable frequencies despite changes in the operational sex ratio. Males were more likely monogynous when copulating with relatively heavy and old females and otherwise bigynous. Our results imply that males make conditional mating decisions based on the quality of the first female they encounter but do not adjust their mating tactic to the local selection regime.

*Keywords:* monogyny, polyandry, mate choice, alternative reproductive tactics, sexual cannibalism, *Argiope bruennichi*.

### Introduction

In males the variety of mating tactics is high and often different tactics are associated with an intra-sexual morphological polymorphism (Gross 1985; Zatz et al. 2011). In many species small males use a 'sneaker' or 'parasitic' tactic to get access to females that are guarded by larger, 'bourgeois' males (Emlen 1997; Taborsky 1998). Furthermore, male mating tactics may differ in their optimal mating rate. In species with traditional sex roles, males are known to maximize their fitness by increasing their mating rate whereas multiply mating in females does not necessarily elevate fitness (Bateman 1948; Trivers 1972). However, in many cases males mate at a lower than maximum rate, for example when they provide parental care (Emlen and Wrege 2004). These deviations from traditional sex roles are of particular interest for the understanding of mating system evolution and the expression of alternative tactics. While sex role reversal and bi-parental care is well explained by the general theory (Parker 1985; Kvarnemo 1996), low male mating rates with low or even no paternal care (here called monogyny) are less well understood. By theory, monogynous mating systems are suggested to evolve if they are associated with a highly efficient paternity protection such that the fertilization success of monogynous males is higher than the average of a polygynous

strategy (Fromhage et al. 2005). The evolution of paternity protection, however, only makes sense under a male biased effective sex ratio (ratio between males and females that mate at least once) and a high degree of sperm competition within a species.

Monogyny can be found within several taxa such as insects (Ross and Keller 1995; Boomsma et al. 2005) and fishes (Vollrath 1998) but is particularly common in spiders, especially web spiders, where it evolved several times independently (Miller 2007). Monogyny is associated with curious adaptations like life-long associations between males and females (Vollrath 1998), extreme sexual size dimorphism (Higgins 2002; Miller 2007), genital damage (Monnin and Peeters 1998; Nessler et al. 2007; Kuntner et al. 2008), and sexual cannibalism (Elgar 1992). A well known spider example for monogyny is the black widow spider *Latrodectus hasselti*. Males of *L. hasselti* sacrifice themselves to the female by somersaulting into her fangs during copulation (Andrade 1996). By doing this males are able to increase their copulation duration and thus their paternity share (Snow and Andrade 2004). Monogynous spider species are well known for their peculiar genital morphology and species from the genera *Argiope* and *Nephila* are well studied in this aspect (Schneider et al. 2006; Nessler et al. 2007;

Foellmer 2008; Kuntner et al. 2009; Ghione and Costa 2011). Spider males possess two secondary copulatory organs, the pedipalps, which are often damaged during copulation and each pedipalp can only be used once in a lifetime (one shot genitalia) (Foelix 1996; Miller 2007; Schneider et al. 2008; Schneider and Fromhage 2010; Schneider and Andrade 2011). In many species these broken-off genital parts serve as mating plugs within the paired insemination ducts of the female and increase male fertilization success under sperm competition as is the case in *Argiope bruennichi* (Nessler et al. 2007). Hence, due to their genital morphology these spider males are limited to a maximum of two copulations in their lifetime while females can mate multiply. Thus, considering the mating rate, a male has the choice between two main reproductive tactics: (i) they may invest everything into a single female and mate monogynously or (ii) they may copulate with two different females and mate bigynously. While monogynous males are able to completely monopolize a female by plugging both of her genital openings, bigynous males only partly protect their paternity in a single mating because they will always leave one of the spermathecae unused and available for rival males.

In a mathematical model Fromhage et al. (2008) have shown that monogyny and bigyny can coexist within populations as alternative tactics of a mixed strategy. Fromhage et al. (2008) proposed the two mating tactics of orb-web spiders (monogyny and bigyny) to be conditional reproductive tactics and indeed there is accumulating evidence that males of monogynous spider species are plastic in their behavioral or physiological response to local selection regimes. A recent study on *L. hasselti* demonstrated a remarkable adaptive plasticity in monogynous males as the male maturation could be induced by the presence of female pheromones at a cost of body size and condition (Kasumovic and Andrade 2006). *A. bruennichi* males were shown to alter their mating behavior depending on the presence or absence of female pheromones (Nessler et al. 2009). Indeed, male spiders detect female mating status through pheromones that virgin females emit (Stoltz et al. 2007; Chinta et al. 2010; Thomas 2011) and the presence of such cues may provide a male with information about local competition and current and future mate availability. Thus

pheromones may have an impact on the choice of the best reproductive tactic.

A common measurement for local competition is the operational sex ratio (OSR: ratio of sexually active males to fertilizable females at a given time; Emlen and Oring 1977; Kvarnemo 1996). The OSR is said to have an influence on the expression of alternative reproductive phenotypes and often underlies variation during and within mating seasons. Especially in monogynous orb-web spiders the OSR changes drastically throughout the mating season. Most of these species are seasonally breeding with one generation per mating season and a first male advantage. As a consequence, protandry (early male maturation) is favored by selection because it increases a male's chance of encountering virgin females. Here the OSR will initially be male biased and this bias will decrease during the season as mated males disappear from the mating pool due to genital damage and sexual cannibalism, while females continue to mature. Only few studies have yet investigated empirically which factors determine whether a male is monogynous or bigynous (Nessler et al. 2009; Welke and Schneider 2010) and no study has done this under field conditions.

Here we investigated under natural conditions which factors influence males of the orb-web spider *A. bruennichi* in their mating decision, namely whether to invest maximally in a single female or whether to opt for inseminating two females. We used a small confined population where it was possible to closely observe all females as well as the roving males during a whole mating season. We inspected all females in this population before the mating season started and removed them from the study site shortly before their final molt. After females matured in the laboratory we brought the freshly molted virgins (maturity reached at most 24 hours before) back to the field and observed all visiting and copulating males and recorded courtship and mating behavior. We determined the frequency of alternative reproductive tactics throughout the mating season and the cues by which males adjust their mating behavior. We predicted varying frequencies of bigynous males as the season and the OSR changes from male biased to female biased as well as between patches with different densities of virgin females. Furthermore, we expected physical condition of

males and the value of mating partners to influence males in their mating decision.

## Materials and methods

### *Study species*

The European wasp spider *Argiope bruennichi* (Scopoli 1772), an entelegyne orb weaver, is present in Europe from the Mediterranean up to Scandinavia. It inhabits dry meadows as well as marsh areas with high densities of crickets as main prey items. *Argiope bruennichi* is characterized by a strong sexual size dimorphism with much smaller males than females. In the laboratory, females are highly cannibalistic and 80% of males are killed during their first copulation (Schneider et al. 2006). Genital damage is very common in *A. bruennichi* and occurs in 85% of copulations into unused genital openings (Nessler et al. 2007). The broken-off pieces remain in the female insemination duct in 97% of the cases and are highly effective in their function as mating plugs (Nessler et al. 2007). By plugging female insemination ducts males secure their paternity share and the fertilization success of future rivals is drastically reduced (Nessler et al. 2007; Schneider and Lesmono 2009). However, the two separate sperm storage organs of females can only be inseminated in two separate copulations as males can use only one of their pedipalps at a time (Schneider et al. 2006; Nessler et al. 2007; Uhl et al. 2007).

### *Study site*

The study was conducted in July and August 2009 on a meadow in Hamburg-Moorfleet, Germany (53°30'38"N, 10°6'4"E). The meadow was bordered by a street on two sides, the motorway, and unsuitable habitat on another. We fenced in the observational area of app. 750 m<sup>2</sup> with barrier tape to prevent passers-by from crossing it. The study site was divided into nine patches of different sizes. By cutting down the vegetation between the patches we created paths for the observers and prevented spiders from building their webs in this area (see Zimmer et al. 2012). Male mate search seemed unaffected by our paths because we saw males and threads of roving males crossing it.

### *Field observations*

Before the mating season started we went to the field site weekly to check the female

development. As soon as the first adult females appeared we located all female *A. bruennichi* on the meadow by carefully searching for webs. To improve visibility of webs, we used aerosol cans filled with water. Each female web location was marked with a bamboo stick and was visited daily. During these daily checks, we categorized the developmental state of all sub-adult females on the basis of their epigyne swelling. While young sub-adults have no such swelling, the differentiated genital area protrudes below a cutaneous layer in females close to maturation. Thereby we were able to predict the time of their final molt. Females characterized as very close to their final molt (we ensured to leave the sub-adults in the field as long as possible) were removed from the field and were brought to our laboratory at the University of Hamburg. There we housed them in Perspex frames (30 × 30 × 6 cm), sprayed them with water immediately, and fed them with 1 *Calliphora* sp. and approx. 20 *Drosophila* sp. (daily dosage throughout their laboratory stay). As soon as females had molted to adulthood, between 3.56±1.55 days after capture, we measured their adult weight and marked them individually for recognition in the field on their opisthosoma with moisture-proof, non-toxic color dots (Pelikan PLAKA).

Within 24 hours after maturation, females were taken to their collection site inside the Perspex frames where they were fixed with tent pegs on the meadow. We observed females individually for a minimum of four hours after their release. After this focal observation period, all adult females, mated and unmated, were monitored in intervals of 10–15 minutes for the presence of courting, copulating, or cannibalized males. Copulation durations were recorded using stop watches. Depending on the number of females we brought to the field we were between two and six observers. Each one observed one or several females. Observations ended at sunset when visibility was reduced to a minimum and daily observation periods were about 8 hours. If females were not mated within this period they were returned to the laboratory and were brought back to the field on the following days until they were mated. All mated females remained in the field and were scan sampled as mentioned above for the whole mating season. Most of the females stayed within close vicinity to the place where we had released them. All females were easily

identifiable due to their individual color marking.

If males were cannibalized after copulation, we rescued them from the female fangs and stored them in plastic vials until they were frozen in the laboratory at  $-80^{\circ}\text{C}$  for further measures. Males that were not cannibalized after copulation were marked with waterproof colors (Pelikan PLAKA) on their opisthosoma using a fine brush or a blade of grass as soon as they left the female's web. Sometimes we painted a male's leg or parts of his prosoma but they continued their mate search unaffected. Additionally, we noted for each male its number of legs before and after copulation for individual recognition.

#### Mating season

On July 10<sup>th</sup> we found two freshly molted adult females in the field, one of them copulating, and considered this as the start of the mating season. We took the individuals into the laboratory to remove them from the study site and to avoid further unobserved copulations. From this day on, we started daily trips to the field site. The last sub-adult male in the field was sighted on the 24<sup>th</sup> of July 2009 and the last copulation was seen on the 7<sup>th</sup> August. Hence, the mating season ended after 29 days. The last adult female (adult: 07.08.2009) did not mate with any male. Overall we observed 98 females, although 121 females were on our study site, and collected 128 males (one male was lost).

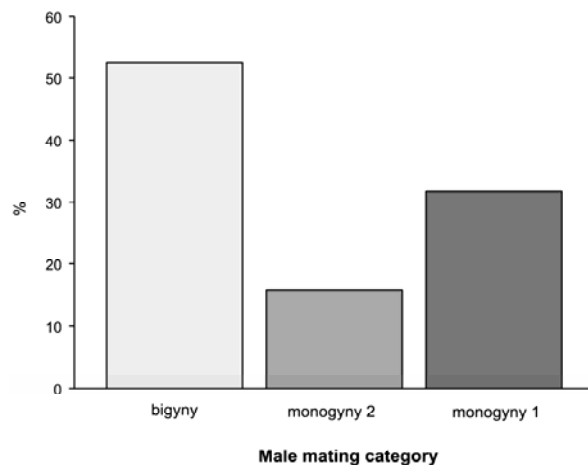
#### Laboratory work

In the laboratory, we measured tibia-patella-length of the first pair of legs and prosoma length of all collected males under a dissecting microscope using the measuring tool of Leica IM500. Additionally, we examined each male's pedipalps for possible breakage damage. Because we knew from our observations which pedipalp a male had used for copulation we were able to combine the data of color marking, number of legs, and used pedipalp to identify individual males when they approached a new female. We used these data to assign males to either the monogynous or the bigynous mating tactic. Males could be monogynous because (i) they were killed and cannibalized after their first copulation (forced monogyny **M1**) or because (ii) they mated twice with the same female (voluntarily monogynous **M2**). Bigynous males mated two times with different females

(**B**). Males were classified as bigynous whenever they had clearly survived their first copulation and continued mate search, even if we did not witness one of his two copulations (see results for details). For example, among observed copulations with the result of sexual cannibalism, several males had both of their pedipalps damaged clearly indicating an unobserved previous copulation with a different female. If data for pedipalp use were missing we excluded this individual from the analyses of mating tactic.

For calculating the OSR we divided the number of adult females (mated and virgin) in the field on each day by the number of adult males on or close to female webs plus those males that had courted or mated with females during that day. Besides the OSR we also measured the distance of each female to her closest female neighbor and we determined the total number of females within a radius of 1, 2, 3, and 5 meters.

The reproductive success of each male was estimated by calculating the paternity share of each male with his female(s). The paternity share of males that had mated with polyandrous females can be determined with high accuracy from the copulation duration of each male. The relationship between copulation duration, the use of plugged or unplugged copulatory ducts and sperm transfer as well as relative paternity are well known for *A. bruennichi* (Schneider et al. 2006; Nessler et al. 2007; Schneider and Lesmono 2009). A male's reproductive success depends on his duration of copulation and that of his rival(s). Therefore we translated the



**Figure 1:** Frequencies of male mating tactics (monogyny 1 copulation, monogyny 2 copulations, bigyny) in the focal population.

copulation durations of rival males into percentages and used this to calculate paternity shares. Furthermore paternity is influenced by the presence of mating plugs. Past studies have shown that the presence of a mating plug reduces the paternity share of a successor to approx. 10% (Nessler et al. 2007; Schneider and Lesmono 2009). Therefore we allocated males a paternity share of 10% if they had copulated into a plugged genital opening. By taking all this into account we were able to estimate individual paternity shares.

#### Statistical analyses

Statistical analyses were conducted using the JMP IN 7.0.2 for Windows (SAS Institute Inc., Cary, NC, USA). Data were tested for the distribution of the residuals and parametric statistics were applied if the requirements were fulfilled. Otherwise we used a t-test for unequal variances, a Welch-Anova, a Kruskal-Wallis Anova ( $\chi^2$ ), Mann-Whitney-U test (Z), or Chi-square test ( $\chi^2_2$ ) as implemented in JMP. All tests are two-tailed. All data are presented as mean  $\pm$  standard deviation.

## Results

#### Male mating rates

The ratio of monogynous to bigynous males was balanced in our study population with 52.5% that mated with two females (bigynous B) and 47.5% with one (monogynous M) ( $N=101$ , Figure 1).

We observed bigynous males either during their first ( $N=27$ , 4 males fled before marking) or their second copulation ( $N=22$ ) but rarely during both: 18.5% (5 out of 27) of color marked bigynous males were recaptured after their first copulation and were unambiguously assigned to both of their mating partners. On average these males had covered a distance of  $6.5 \pm 5.4$  m (range 4-16 m) between the two females and the journey took them  $2.4 \pm 2.7$  days (range 0-7 days).

The monogynous males either copulated once (M1, males cannibalized after a single copulation; 32 of 48) or twice (M2: 16 of 48) with the same female. In the M1 group it cannot be decided whether males would have followed a bigynous or a monogynous tactic if only they had survived their first copulation. Hence, for analyses we divide monogynous males into

**Table 1:** Male phenotypes, mating behavior, and female features of the three male reproductive tactics

	Monogyny (1 copulation)	Monogyny (2 copulations)	Bigyny	Test statistic	P
Duration of 1 <sup>st</sup> cop (s)	8.9 $\pm$ 4.21 (25)	6.61 $\pm$ 2.62 (14)	7.14 $\pm$ 2.82 (32)	Welch-Anova: $F_{2,68}=2.3$	0.12
Duration of 2 <sup>nd</sup> cop (s)	-	68.78 $\pm$ 34.14 (15)	62.47 $\pm$ 78.62 (22)	$X^2=2.69$	0.1
Pedipalp used in 1 <sup>st</sup> cop	59.4% right (19 of 32)	40% right (6 of 15)	46.8% right (22 of 47)	$X^2_2=1.93$	0.38
Genital damage 1 <sup>st</sup> cop	Big:12.5% Small:87.5% None: 0% (32)	Big:20% Small: 73.3% None:6.7% (15)	Big: 13% Small:78.3% None: 8.7% (23)	$X^2=4.41$	0.35
Genital damage 2 <sup>nd</sup> cop	-	Big:13.3% Small:80% None:6.7% (15)	Big:8.7% Small:65.2% None: 26.1% (23)	$X^2_2=2.6$	0.27
♂♂ tibia-patella-length (mm)	4.0 $\pm$ 0.45 (20)	3.95 $\pm$ 0.36 (14)	4.1 $\pm$ 0.56 (18)	$F_{2,49}=0.39$	0.68
♂♂ prosoma length (mm)	2.4 $\pm$ 0.2 (31)	2.4 $\pm$ 0.13 (16)	2.45 $\pm$ 0.25 (24)	Welch-Anova: $F_{2,68}=0.77$	0.47
♂♂ no. of legs before 1 <sup>st</sup> cop	7.13 $\pm$ 1.06 (23)	7.23 $\pm$ 1.17 (13)	7.29 $\pm$ 0.98 (28)	$X^2=0.29$	0.87
1 <sup>st</sup> ♀♀ adult weight (mg)	149.9 $\pm$ 65.7 (32)	130.1 $\pm$ 48.8 (16)	103.8 $\pm$ 40.1 (34)	$X^2=9.43$	<b>0.009</b>
2 <sup>nd</sup> ♀♀ adult weight (mg)	-	128.9 $\pm$ 50.27 (15)	133.3 $\pm$ 50.6 (24)	$z=-0.07$	0.94
1 <sup>st</sup> ♀♀ age at cop (days)	2.8 $\pm$ 2.6 (32)	1.4 $\pm$ 1.2 (16)	1.0 $\pm$ 0.8 (34)	$X^2=20.28$	<.0001
2 <sup>nd</sup> ♀♀ weight at cop (mg)	-	1.4 $\pm$ 1.2 (15)	2.1 $\pm$ 2.8 (24)	$z=1.16$	0.25
1 <sup>st</sup> ♀♀ mating status	21.9% mated (7 of 32)	6.3% mated (1 of 16)	17.7% mated (6 of 34)	$X^2=2.17$	0.34

Sample sizes are given in parentheses. Mean  $\pm$  standard deviation is shown for all data.  $F$ =ANOVA,  $X^2$ =Kruskal-Wallis-test,  $z$ =Mann-Whitney-U test,  $X^2_2$ =Chi-square test. Significant differences ( $P < 0.05$ ) are given in bold

those that were voluntarily monogynous with two copulations (M2) and those whose behavioral decision was unclear because they were cannibalized after a single copulation (M1).

#### Male features

A male may base his decision whether to continue mate search or to re-mate with the same female on estimations about his own phenotype and physical condition. However, no measurement of male size (tibia-patella length, prosoma length) predicted the mating tactic (Table 1). Which pedipalp he used in the first copulation and whether he damaged his genitalia or not was also not relevant for his mating decision (Table 1).

Interestingly, males with legs missing at their first copulation were found more frequently early in the season than later (linear regression:  $r^2=0.12$ ,  $F_{1,74}=10.54$ ,  $P=0.002$ ). The decline in injuries with progressing season coincides with a decline in male competition inferred from the changes in the OSR. Indeed, there is a significant negative relationship between the male bias in the OSR and the number of legs males lost at their first copulation ( $r^2=0.12$ ,  $F_{1,74}=9.9$ ,  $P=0.002$ ). The duration of a male's first copulation is directly and positively related to the probability of sexual cannibalism ( $t_{71}=2.06$ ,  $P=0.047$ ) and thereby influences a male's future mating opportunities. However, the differences in copulation durations are not significant if we compare the three mating categories (Table 1). Only within the monogynous males, we found that M1 males copulated slightly longer than M2 males ( $t_{39}=-2.09$ ,  $P=0.04$ ). Overall we observed a rather low frequency of cannibalism in the first copulation of 31.4% (32 of 102) if compared to previous studies (Fromhage et al. 2003; Schneider et al. 2006).

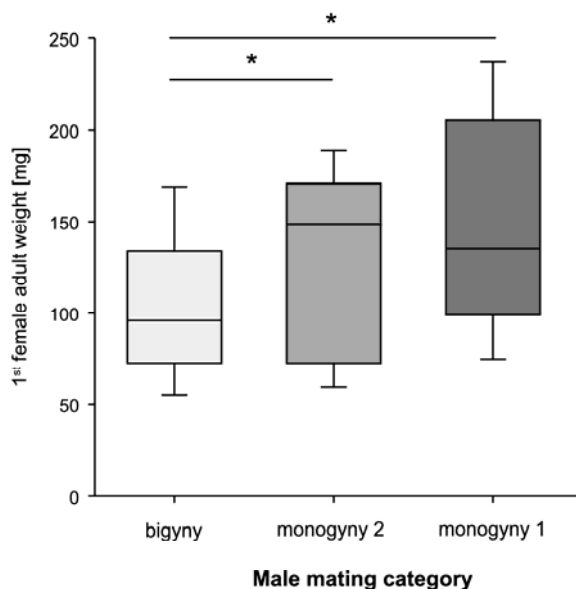
#### Female features

Most first copulations of males (90%) occurred with virgin females ( $\chi^2_2=38.7$ ,  $N=82$ ,  $P < .0001$ ) and this preference was found in all three mating categories ( $\chi^2_2=2.17$ ,  $N=82$ ,  $P=0.34$ ). Interestingly, first copulations of males with mated females were least frequent in the M2 category (Table 1). The frequency of copulations with mated females of bigynous males increased non-significantly from 17.7% in first matings to

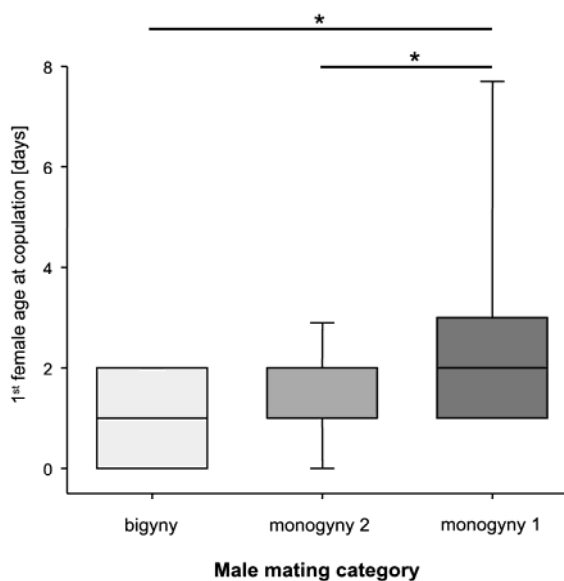
29% in the second matings (7 of 24,  $\chi^2_2=0.67$ ,  $P=0.41$ ).

A mated female will have at least one genital opening plugged and a male that copulates with a mated female risks to copulate into an already plugged genital opening and to gain no paternity share. In 10 out of 14 cases in which males mated with non-virgin females we were able to determine whether they copulated into a used or a virgin genital opening. Three of those males mated with a double-mated female and therefore had no chance to insert their pedipalp into a virgin genital opening. The remaining 7 males mated with single-mated females and only 4 of them successfully avoided the used genital opening. This finding corroborates earlier observations that males are not able to determine the unused genital opening in a half-sided virgin (Nessler et al. 2007).

The weight of a female had significant influence on the male mating categories (Table 1; Figure 2) and post-hoc pairwise comparisons of the categories revealed significant differences between bigynous males and both monogynous categories (M1 and B:  $Z=3.03$ ,  $N=66$ ,  $P=0.003$ ; M2 and B:  $t_{50}=2.02$ ,  $P=0.049$ ; Figure 2). Furthermore, 2<sup>nd</sup> mates of bigynous males were significantly heavier than 1<sup>st</sup> mates ( $t_{58}=2.48$ ,  $P=0.016$ ) but did not differ in age ( $Z=1.16$ ,  $N=58$ ,  $P=0.25$ ). But the age of a female influenced the likelihood of sexual cannibalism: females that



**Figure 2:** Female adult weight of first mating partners of males. Males were more likely to be monogynous when mating with a heavy female first



**Figure 3:** Female adult age of first mating partners of males. Single-mated monogynous males mated with the oldest females.

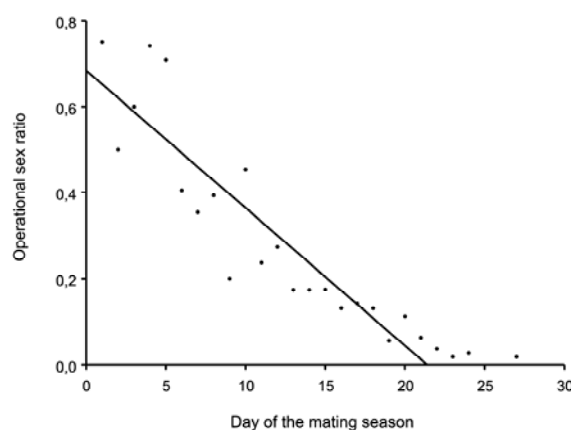
had cannibalized single-mated M1 males were significantly older than females of males that survived (M2, B; Table 1, Figure 3). First females of M2 and B males were not different in age (M2 and B:  $Z=0.87$ ,  $N=50$ ,  $P=0.39$ ).

#### Temporal and spatial selection regimes

The time of season had no significant influence on male mating tactics (date of a male's first copulation: ANOVA:  $F_{2,79}=1.19$ ,  $P=0.31$ ; adult date of the first female:  $\chi^2=2.63$ ,  $N=82$ ,  $P=0.27$ ). Although we found a change in the operational sex ratio during the course of the mating season (Figure 4;  $r^2=0.82$ ,  $F_{1,23}=104.4$ ,  $P < 0.0001$ ) this had no influence on male mating tactics (nominal-logistic regression:  $r^2=0.01$ ,  $\chi^2=1.86$ ,  $N=82$ ,  $P=0.39$ ). Only time of day had an effect. The first copulation of M2 males happened at an earlier time of day than those of M1 and B males ( $\chi^2=6.17$ ,  $N=81$ ,  $P=0.05$ ). This difference is more pronounced when comparing the M2 and the B males only ( $Z=-2.44$ ,  $N=49$ ,  $P=0.01$ ).

Also the spatial distribution of females had no influence on male mating decisions. A multivariate nominal logistic model revealed that measurements of female availability in a 3 meter radius around the 1<sup>st</sup> mating partner of a male, explained none of the variation in male mating tactics (nominal-logistic regression:  $r^2=0.04$ ,  $\chi^2=7.35$ ,  $P=0.29$ ; individual factors: number of sub-adult females:  $\chi^2=3.22$ ,  $P=0.2$ , number of virgin females:  $\chi^2=2.21$ ,  $P=0.33$ ; number of mated females:  $\chi^2=1.75$ ,  $P=0.42$ ).

Additionally, the number of potential mating partners (irrespective of their developmental status) surrounding the 1<sup>st</sup> female of a male had no influence on its mating category (2 m radius:  $X^2=0.17$ ,  $N=83$ ,  $P=0.92$ ; 5 m radius:  $X^2=3.58$ ,  $N=83$ ,  $P=0.17$ ); neither did the distance of the 1<sup>st</sup> mate to its nearest female neighbor ( $X^2=1.14$ ,  $N=80$ ,  $P=0.57$ ). Interestingly, the developmental status of the nearest neighbor differed between categories ( $X^2_{2}=12.27$ ,  $N=67$ ,  $P=0.02$ ) and revealed that the 1<sup>st</sup> mate of B males was mostly neighbored by mated females (75%) while 60% of the 1<sup>st</sup> females of M2 males were neighbored by sub-adult females ( $X^2_{2}=4.36$ ,  $N=39$ ,  $P=0.04$ ).



**Figure 4:** The operational sex ratio given as the ratio of adult males and adult females regardless of their mating status during the course of the season.

#### Reproductive success

M1 males monopolize only one copulatory opening of a female but leave the other one available for rivals. Only 3 of 22 M1 males shared a female with a successor and thereby lost between 10-90% of the paternity. The remaining 19 M1 males secured 100% paternity. The average paternity of M1 males was  $90.8 \pm 25.3\%$ .

M2 males secure 100% paternity if they mate with a virgin. But their paternity share is reduced if they mate with an already mated female which occurred in one case, or if a successor copulated with their female which happened in 5 out of the 15 cases. The average paternity of M2 males was  $90.7 \pm 20.5\%$ .

B males ideally monopolize both of their females but may also lose paternity shares with both of them. Estimating reproductive success in this category is difficult because of missing data for either the first or the second mating

partner of a male. The combined data of those males that provided us with paternity shares of either their first or second female average paternity with the first female of  $65.1 \pm 42.4\%$  ( $N=8$ ) and with the second females  $53.2 \pm 42.7\%$  ( $N=8$ ) in sum they fertilized the equivalent of 118.3% of a single female's eggs.

Only 4 of the B males provided us with data for both mating partners and their paternity share ranged from 20%, when the male invested both his copulations into already plugged genital openings, up to a maximum of 200% where both females were virgin and did not remate. The average paternity of those 4 bigynous males was equivalent to  $150.1 \pm 87\%$  of a single female's eggs. Hence, we have good indication that the bigynous mating tactic has the highest variance in paternity share.

## Discussion

We determined the frequency of monogynous and bigynous males in a natural population of *A. bruennichi* and those factors that influenced males in their choice for the best mating tactic. Monogyny and bigyny occurred in almost equal proportions in the study population and we found no temporal pattern. Hence, the hypothesis that males adapt their mating tactic to the changes of local competition was not supported. In contrast, males made state-dependent decisions based on female mating status, age, adult weight, local availability of further mating partners, and the time of day. Males were more likely to mate twice with the same female (monogyny) if it was early in the day, if the female was heavy and the next neighboring female was sub-adult. Males kept on searching for a second female (bigyny) if it was late in the day, the first female was light and the neighboring female was already mated. We found that bigynous males traded up to heavier females as second mates but showed an increased tendency to copulate with already mated females in these copulations. Interestingly, males that died after their first copulation had copulated with heavy females similar to the monogynists that copulated twice although mates of the former were older. These findings imply that male mating tactics are the result of behavioral plasticity and state-dependent decisions rather than being alternative reproductive tactics of a genetically fixed strategy.

Surprisingly we found both monogynous and bigynous males in similar frequencies throughout the season and detected no shift with the hypothesized changes in levels of male-male-competition. Due to protandry, the operational sex ratio (OSR) changes over the course of the season as males are removed from the mating pool via sexual cannibalism. Early in the season most males compete for the first maturing females while this can shift to the opposite pattern late in the season. Therefore we expected frequency shifts in male mating tactics over the course of the mating season.

The only temporal effect on male mating tactic we found was that first copulations of double-mated monogynists happened earlier during the day than those of bigynists. The time of the day could have an influence on a male mating decision because males that find a mating partner during the end of the day may delay their second copulation to one of the following days. This can only be true if males of *A. bruennichi* avoid matings during the night time which is in accordance with recent 24-hour field observations in which all but one copulation was observed during the day (SMZ, personal communication).

Body size can have a large impact on a male's reproductive tactic and generally influences his ability to compete for mating partners. Often the expression of different mating tactics such as sneakers and territorial males are determined by male size (Gross 1985; Zatz et al. 2011). In *A. bruennichi* we found no size differences between monogynous and bigynous males. Interestingly the number of legs a male had left was directly related to changes in the OSR during the mating season. While in the beginning of the season with a male biased OSR competition among males was high and males were frequently found with less than eight legs, this changed towards the end of the season when the OSR became female biased. Here males were more likely found with all their legs left. This supports theory that the OSR is a good indicator of intra-sexual competition within a population (Kvarnemo 1996) and that males in our study lost their legs during fights with rivals. However, the number of legs a male had left did not influence his choice of mating tactic.

Males in our study were mainly influenced by female quality and showed a clear preference for virgin over mated females. This preference is highly adaptive especially in a mating system



with effective mating plugs and limited male mating opportunities (Gaskett et al. 2004; Andrade and Kasumovic 2005; Huber 2005; Schulte et al. 2010) and has also been shown for males of the congener *A. keyserlingi* (Gaskett et al. 2004) as well as for several other species (Miller and Miller 1986; Toft 1989; Watson 1990; Eberhard et al. 1993; Fahey and Elgar 1997). A recent field study on *A. bruennichi* (Schulte et al. 2010) revealed that females lose their attractiveness for males after mating once. Given the high risk of gaining no or low fertilization success with a mated and possibly plugged female this selectivity seems adaptive. The presence of a mating plug decreases a male's copulation duration (Nessler et al. 2007) and severely limits paternity (Schneider and Lesmono 2009). Therefore the value of a virgin female is generally high because it enables males to avoid sperm competition and to monopolize a female by plugging both genital openings. Usually the roving males sense the presence of virgin females via airborne and web-bound pheromones (Chinta et al. 2010; Jerhot et al. 2010) but due to protandry adult males also secure their access to virgin females by lingering around the webs of sub-adults waiting for their final molt (Zimmer et al. 2012). Some males even court sub-adults, risking to get cannibalized prior to copulation (as observed in one case in the present study). Thus males seem to be eager to approach freshly molted females that are still immobile and defenseless while their exoskeleton hardens. Those opportunistic or "soft-matings" enable males to monopolize a female without the risk of sexual cannibalism. In the congener *A. aurantia* the majority of matings occur while the female is molting (Foellmer and Fairbairn 2005) but for *A. bruennichi* the natural frequency is yet unknown but investigations are in progress. In our study we excluded this option because females molted in the laboratory, but we observed one soft-mating in the field and saw a male hanging dead but not cannibalized or wrapped in silk next to the female with both of his pedipalps mutilated (KWW, personal observation). Despite the advantages of mating with virgins, copulations with mated females occurred in our study although at moderate frequencies. We had expected a high variance in the degree of polyandry in *A. bruennichi* due to protandry and high competition for females in the beginning of the season, however, Zimmer et al. (2012) found that females mated on

average with 1.3 different males and all but one female had at least one mating partner.

Besides female mating status, males were mainly influenced by female weight in their choice for the best mating tactic. Weight positively predicts female fecundity and thus consequently impacts on male reproductive success. Similar male mate choice preferences have been found in several insect and spider species (Vincent and Lailvaux 2006; Hoefler 2007; Barry 2010; Johnson et al. 2011). In our study, males that mated with relatively heavy females first were more likely to follow a monogynous than a bigynous mating tactic. Bigynous males may compensate this disadvantage by selecting a relatively heavy female as their second mating partner and may follow a tactic of first securing a successful insemination regardless of female quality before they move on to search for a high quality second partner. This interpretation may explain results from an earlier field study on *A. bruennichi* in which a small proportion of males inspected two given females and mated with the heavier one, while the majority of males mated with the first female they encountered regardless of whether a heavier female sat right next to her (Schulte et al. 2010). In a natural setting, virgin females of *A. bruennichi* are regularly rejected by some males (Zimmer et al. 2012) while others stay to mate; an observation that is difficult to explain given that males compete for access to virgins (see above) (Andrade and Kasumovic 2005). These counterintuitive male mating decisions may be explained by the coexistence of alternative mating tactics: one tactic is to mate with the first available female while the alternative is to accept only females with particular characteristics. Alternatively or additionally, at least a proportion of the selective males observed in the field may have been already mated bigynists in search for a second mating partner (male mating status was not assessed in Schulte et al. 2010).

Some of the monogynous males were cannibalized after their first copulation while others used both of their mating options with the same female. While the latter can be interpreted as a clear-cut male decision, interpreting the decisions of single-mated males is more difficult. At least some of these males may have been forced into monogyny by aggressive females while others may have chosen to sacrifice themselves to their mating

partner already during their first copulation. Whether a male falls victim to sexual cannibalism during the first copulation is a direct function of its duration; any copulation longer than 10 seconds will most likely end with the death of the males while males that jump off before 10 seconds have a relatively high chance of surviving (Schneider et al. 2006). Indeed, copulation durations of single-mated males show a high variance which suggests that some of them chose to copulate for longer in spite of sexual cannibalism being the likely consequence, while others attempted to escape but failed. Single-mated monogynists mated with the heaviest and oldest females of all male behavioral categories. High adult weight indicates high fecundity, and adult age indicates that oviposition will occur soon. Both traits benefit male reproductive success and may hence favor a monogynous strategy with a safe option of monopolizing a single spermatheca only but with the maximal sperm transfer (Nessler et al. 2009; Rittschof 2011). Alternatively, older females may be more aggressive and more successful in capturing and cannibalizing a male that attempts to escape.

The high frequency of single-mated males increased the success of bigynous males in the study population. Bigyny would not have evolved if all males copulated twice with a female especially in a species with such a highly effective paternity protecting mechanism. Males in *A. bruennichi* place their plug in about 85% of the copulations into unused genital openings and thus reduce the risk of direct sperm competition (Nessler et al. 2007, 2009). However, single-mated females (as well as virgin females) are still available for insemination, offering sufficient mating opportunities on which bigynous males can capitalize. A bigynous mating strategy should only be viable if a large enough proportion of females mate only once supporting the theoretical prediction of frequency dependent co-occurrence of monogynous and bigynous mating strategies: with increasing numbers of bigynists, more females will receive multiple copulations so that mean reproductive success as well as its variance will drop (Fromhage et al. 2008).

Based on a male's copulation duration and genital damage we were able to calculate the paternity share of many observed males. This calculation revealed that both monogynous tactics, single and double mated, gained equal

paternity shares (about 90%) while the bigynous males had a higher overall paternity share with their females (about 130%). But the variance in paternity share for bigynous males was higher than for monogynous ones and they risked copulating with two already mated females because they showed an increased affection to mated females. Thus bigynous males opt for the mating tactic with the highest paternity share but also with the highest variance. These results imply that males use a conditional strategy with three different alternative tactics. Conditional, or state-dependent, reproductive strategies are defined by different reproductive success of their alternative tactics (Gross 1996). This seems to be the case in *A. bruennichi* where bigynous males gained on average a higher paternity share than monogynous ones. Theoretical models on the evolution of monogyny in spiders have shown that the alternative reproductive tactics (monogyny and bigyny) can coexist under certain conditions (Fromhage et al. 2008). Fromhage et al. (2008) proposed that the two strategies may not be genetically determined but conditional reproductive tactics enabling spider males a plastic response to local selection regimes. According to our results we suggest that the occurrence of mono- and bigyny is due to conditional male mating decisions.

## Conclusions

In conclusion, our study shows that mono- and bigynous mating tactics coexist in a population of spiders with one-shot genitalia. Males appear to make individual decisions based on the value of the first female they happen to encounter and environmental factors such as the mating status of the next available female and the time of day of the first copulation. Temporal fluctuation of operational sex ratios as well as larger scale spatial factors such as female availability within the population did not play a role. Predictions derived from demography alone are likely not sufficient to explain mating tactics. Thus our results imply that males are plastic in their mating decision and can choose simultaneously between different mating tactics. The mating tactics differ in their fitness and are state-dependent suggesting that they are part of a conditional strategy.

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## Chapter 3: Sexual cannibalism benefits offspring survival

Klaas W. Welke • Jutta M. Schneider

### Abstract

Polyandry may provide females with benefits that directly affect their condition and fecundity and/or that enhance the quality of their offspring through receiving better or more compatible paternal genes. In polyandrous species with sexual cannibalism, females may gain considerable nutritional benefits through consuming a mating partner. However, in many spiders with high rates of sexual cannibalism, males are very small compared to the females and nutritional gains through sexual cannibalism are considered small or absent. While this is widely accepted, no study has tested for effects of multiple mating with and without sexual cannibalism on female and offspring fitness. We designed an experiment that simultaneously investigated direct and indirect benefits of polyandry and sexual cannibalism in the orb-web spider *Argiope bruennichi*. We used monandrous and polyandrous females that were either allowed to consume their mating partner or not and recorded fecundity traits and offspring survival under simulated overwintering conditions. We found that female mating rate did not affect fecundity or offspring survival. But independent of female mating rate and the number of males consumed, cannibalistic females produced bigger clutches with heavier eggs. Cannibalistic females produced offspring with a prolonged survival time compared to the offspring of females that were prevented from eating males. This prolonged offspring survival was independent of female mating rate and the number of males consumed.

*Keywords:* *Argiope bruennichi*, genetic benefits, material benefits, mating effort, orb-web spider, paternal investment, polyandry, sexual cannibalism

### Introduction

Polyandry, defined as females mating with multiple males during a breeding season, is widespread in sexually reproducing animals and, since mating is costly, theory predicts that benefits should exist that are large enough to compensate for these costs (Arnqvist and Nilsson 2000). Analogous to the benefits of female choice, benefits of polyandry can either be indirect (genetic) or direct (material; Jennions and Petrie 2000).

Additive genetic benefits arise whenever paternal genes increase offspring viability or attractiveness (e.g. sexy sons, sexy sperm; Neff and Pitcher 2005) and non-additive genetic benefits arise from intragenomic interactions between parental genomes (Zeh and Zeh 1996, 1997). These interactions may result in incompatibilities that reduce the reproductive success of a female. Incompatibilities originate from inbreeding or selfish genetic elements, for example, and females should avoid mating with incompatible males in the first place or, if that is not possible, may reduce negative effects via polyandry. Polyandry enables females to favour

superior males cryptically based on additive or non-additive genetic benefits (Halliday 1983; Eberhard 1996; Gabor and Halliday 1997; Herberstein et al. 2011) or to increase the genetic diversity among their offspring (Ivy and Sakaluk 2005) and thereby reduce the costs of mating with inferior or incompatible males.

Direct material benefits gained through reproduction are more conspicuous than indirect benefits and consist of non-genetic male contributions that enhance a female's fitness (Arnqvist and Nilsson 2000). These material contributions include, for example, nuptial gifts such as small prey items, fecundity-enhancing substances delivered within the ejaculate, access to additional breeding territories, and additional paternal care (see Arnqvist and Nilsson 2000). Direct benefits are considered the main cause of polyandry in insects and have also been demonstrated in other animal taxa (Ojanguren et al. 2005; Engqvist 2006; Gershman 2010). However, even if polyandrous females in nuptial gift-giving species gain material benefits in every mating, this does not exclude the

possibility that genetic benefits might exist as well (Jennions and Petrie 2000).

From the male perspective, material donations provided to the female during or before mating may represent a paternal investment by which males increase female fitness or the fitness of their offspring (Gwynne 2008). However, when males mate with polyandrous females this male investment may also benefit rival males (Vahed 1998). Alternatively, males may use material donations as a mating effort to gain access to otherwise aggressive females or to prolong their copulation duration and thereby improve their paternity share under sperm competition (Simmons and Parker 1989).

Self-sacrifice of the male via sexual cannibalism may be considered an extreme form of nuptial gift giving (Vahed 1998), but obviously only if it does not occur before mating (Elgar and Schneider 2004). Pre-insemination cannibalism leaves males without any reproductive success and hence is nonadaptive for them (Rabaneda-Bueno et al. 2008) unless genital contact is still possible for males after the onset of cannibalism, as reported for praying mantids (Barry et al. 2008). However, the latter would rather fall in the category of post-copulatory sexual cannibalism, which is defined as the act of killing and consuming a mating partner during or after copulation and mostly occurs in species with a high sexual size dimorphism, with the bigger female being the cannibalistic sex (Vahed 1998). Post-copulatory cannibalism, on the one hand, may function as a male mating effort to prolong copulation duration and to increase their paternity share. On the other hand, it may be a paternal investment strategy by which males increase female fitness (Elgar and Schneider 2004). But again in polyandrous species, the latter explanation is questionable and there is a high risk of investing in the offspring of a rival male if mechanisms to monopolise a female are lacking. Alternatively, sexual cannibalism can be a female mating strategy to avoid male monopolisation attempts or females can use sexual cannibalism to bias paternity towards preferred mating partners by manipulating male copulation durations (Elgar et al. 2000; Prenter et al. 2006).

Mating systems with polyandrous and sexually cannibalistic females are common in orb-web spiders with a high sexual size

dimorphism (Wilder and Rypstra 2008; Schneider and Andrade 2011). Several studies have searched for nutritional benefits of cannibalising a small male with differing results (Barry et al. 2008). In *Argiope bruennichi* no increase in fecundity of cannibalistic females was found, while in *Argiope keyserlingi* cannibalism led to an increased investment in egg energy density (Fromhage et al. 2003; Blamires 2011). However, no study has separated potential direct benefits through the consumption of several males from indirect benefits of multiple mating. Here we chose *A. bruennichi* to disentangle the effects of polyandry and mate consumption. The polyandrous females of *A. bruennichi* are sedentary while the males leave their webs at maturation to start mate search. Females attract males via pheromonal cues (Chinta et al. 2010) and both males and females possess paired genitalia: males have two sperm-transferring pedipalps, and females two sperm-storing spermathecae that are filled by males via two separate sperm ducts (Foelix 1996). During copulation males damage their pedipalps and the broken-off parts remain inside the female genital tract to serve as highly effective mating plugs (Nessler et al. 2007). Because of this genital mutilation the pedipalps are useless after copulation, limiting the male to mating with at most two females. The male mating rate is further limited by cannibalistic behaviour of the females, which try to grab and wrap up the males at the onset of copulation. In laboratory experiments only about 30% of the males survive their first copulation (Schneider et al. 2006). If they survive, males have the choice between mating with the same female a second time or continuing mate search and opting for a bigynous mating strategy. A recent field study on *A. bruennichi* revealed that 50% of the males continued their mate search after they survived their first copulation (K. W. Welke, S. M. Zimmer and J. M. Schneider, unpublished data). Since all males lose their chance for future reproduction after their second copulation because of the functional sterility, self-sacrifice has smaller costs at this point.

In an experimental study we created four treatments and mated females either to a single male or to two or three different males. Additionally, we divided these groups into two subcategories with one being induced to consume their mating partner(s) while the other



was prevented from male consumption. We recorded traits indicating female fecundity as well as offspring survival under climate and food stress. Should polyandry constitute a direct benefit, we expected higher fecundity and higher investment per egg in the polyandrous treatment and if this effect is coupled to sexual cannibalism increased fecundity is expected only in the cannibalism subtreatment. In contrast, indirect benefits of polyandry should result in differences between the singly and multiply mated females regardless of male consumption. While increased compatibility should affect hatching rate, the option of sexual selection of male quality through polyandry should manifest itself in improved offspring survival in the polyandrous treatment irrespective of male consumption. A combination of direct and indirect benefits can also be revealed by the design, namely by consistent differences between polyandrous and monandrous females in both fecundity and offspring performance.

## Methods

### *Study Animals*

We collected subadult males and females of *A. bruennichi* between June and August 2007 from different populations around Hamburg, Germany. Spiders were brought to our laboratory at the University of Hamburg where they were housed individually in plastic cups (males: 250 ml; females: 330 ml). Conditions at our laboratory were held constant at 25 °C with a relative humidity of 45% and with natural light conditions. Spiders were watered 5 days of the week and were fed twice per week. We fed males with *Drosophila* sp. ad libitum throughout the experiment and subadult females were given three *Calliphora* sp. flies. Adult females received four *Calliphora* flies twice per week throughout the experiment. All animals were weighed at least one day after the final moult. Adult females were transferred into Perspex frames (36 x 36 cm and 6 cm high) where they built their typical orb-webs and where the mating trials took place.

### *Mating Procedure*

Virgin females and males were randomly assigned to one of four mating treatments: monandry with male consumption (M+C, N=21), monandry without male consumption

(M-C, N=22), polyandry with male consumption (P+C, N=24), and polyandry without male consumption (P-C, N=21). In the monandry treatment females were mated once or twice to the same male and in the polyandry treatment they were mated once to two or three different males. Therefore polyandrous females had on average more copulations than monandrous ones (mean  $\pm$  SD: polyandry: 2.24  $\pm$  0.43 copulations; monandry: 1.65  $\pm$  0.48 copulations; Mann-Whitney U test:  $Z=-5.12$ ,  $N=88$ ,  $P<0.0001$ ).

All animals we used were initially virgin and the mating trials began as we introduced males with a soft brush into the female's web. All males immediately started to court and none of them refused the assigned female. We recorded female attempts at cannibalism (here defined as female attack and the action of wrapping up a male, but not its consumption) and measured copulation durations with a stopwatch. Copulation starts with the male inserting his pedipalp and the female reacting with immediate aggression and it ends when the male pedipalp is detached from the female genital opening. Captured males that were silk wrapped by the females were rescued by pulling them out of the female's fangs or out of the web with soft forceps. If males in the monandry treatments survived their first copulation they were allowed to copulate a second time with the same female. After a male's second copulation we removed him from the female's web and measured tibia-patella length of the first pair of legs using a digital calliper. Males that were assigned to the male consumption treatment (M+C, P+C) were subsequently fed to the female. We introduced the males into the females' webs and simulated vibrations manually. All males that we offered were readily fed upon by the females. Males from the treatments without male consumption (M-C, P-C) were not returned to the web but stored in 70% ethanol. After the mating trials the mated females were transferred into plastic cups (330 ml) to build eggsacs.

### *Fecundity*

To assess the nutritional impact of male consumption on female body mass we calculated their daily weight gain by weighing the experimental females on the day of copulation and 8 days later. We used this interval to ensure that females were weighed before they built their first eggsac as this can

occur 10 days after maturation. As a control group for the influence of copulation on resource allocation to eggs we used 14 unmated females and weighed them on the day of their final moult and 7-12 days later and compared daily weight gain to that in our experimental females. Eggsacs from all four treatments were weighed on the day of their construction and were left for an incubation period of 28 days inside a small gauze-covered plastic vial. Within this period spiderlings hatch and in nature they hibernate inside the eggsac. Twenty of our experimental females (22.5%) died before oviposition but mortality was evenly distributed across our mating treatments (M+C=4, M-C=7, P+C=5, P-C=4, Control=2 dead females; chi-square test:  $\chi^2_2=1.9$ ,  $N=102$ ,  $P=0.76$ ). After a female provided us with at least three intact eggsacs it was killed by hypothermia and we measured tibia-patella length by using a dissecting microscope and the Leica IM500 measuring tool.

#### Offspring Survival

Offspring of the first eggsac were intended for a growth experiment and were separated after hatching. Unfortunately, the spiderlings did not start feeding and died very quickly. We suspect that a period of low temperatures is required that triggers active foraging of the spiderlings.

Second eggsacs were opened after 28 days and the freshly hatched spiderlings were placed in family groups in a petri dish covered with a paper towel and were placed in a climate chamber at 8 °C and 60% humidity to simulate winter temperatures at a 12:12 h dark:light cycle. We did not feed them and their survival was monitored weekly for 20 weeks. We observed no cases of cannibalism between spiderlings.

The number of spiderlings observed per eggsac ranged from 8 to 30.

#### Statistical Analysis

Statistical analyses were performed using JMP 7.0.2. for Windows (SAS Institute Inc., Cary, NC, U.S.A.) or R version 2.13.0 (R Development Core Team 2011). All summary statistics are reported as mean  $\pm$  SE. Data were tested for normal distribution and equal variances and we used parametric tests whenever possible. For the analysis of offspring survival we used the Kaplan-Meier survival analysis. To analyse offspring survival in detail we fitted generalised linear mixed models (GLMM) with gamma error distribution and inverse-link functions (Crawley 2007) using the glmmPQL function implemented in the package MASS for R (Venables and Ripley 2010). We added mother ID to all GLMMs as a random effect to account for the repeated use of the same female.

## Results

#### General Measurements

Table 1 shows that females in the four treatments did not differ in their weight gain after maturation and the following interval between copulation and oviposition. Duration of the second copulation and male killing differed as a natural consequence of the experimental design: we used virgin males as second mates of females in the polyandry treatments and males generally attempt to survive their first copulation by terminating copulation and jumping off early while they copulate as long as they can in their second copulation. Therefore first copulations were very similar in all treatments (first copulation duration: M-C:  $10.97 \pm 3.04$  s; M+C:  $7.46 \pm 1.7$  s;

**Table 1:** Female fecundity traits with respect to female mating rates and male consumption

Parameters	Monandry - male consumption	Monandry + male consumption	Polyandry - male consumption	Polyandry + male consumption	Test statistic	P
♀ Weight gain after copulation (mg)	17.24 $\pm$ 2.73 (15)	22.34 $\pm$ 2.74 (15)	18.32 $\pm$ 2.56 (12)	16.76 $\pm$ 3.15 (15)	$F_{3,53}=0.84$	0.48
♀ Weight gain after final moult (mg)	17.54 $\pm$ 3.55 (10)	14.6 $\pm$ 3.04 (10)	23.68 $\pm$ 1.54 (11)	17.83 $\pm$ 3.29 (10)	$F_{3,37}=1.78$	0.17
First egg sac						
Weight (mg)	134.23 $\pm$ 12.23 (15)	140.8 $\pm$ 10.54 (17)	143.74 $\pm$ 11.24 (17)	141.3 $\pm$ 14.26 (19)	$F_{3,64}=0.1$	0.96
Average egg weight (mg)	0.52 $\pm$ 0.03 (15)	0.55 $\pm$ 0.06 (16)	0.5 $\pm$ 0.02 (17)	0.54 $\pm$ 0.03(15)	Welch ANOVA: $F_{3,59}=0.49$	0.69
Clutch size	219.33 $\pm$ 21.97 (15)	229.06 $\pm$ 18.37 (16)	247.88 $\pm$ 18.37 (17)	253.81 $\pm$ 27.1 (16)	$F_{3,60}=0.54$	0.66
Hatching rate (%)	78.15 $\pm$ 7.52 (15)	58.02 $\pm$ 9.49 (15)	81.43 $\pm$ 6.9 (17)	74.3 $\pm$ 7.52 (15)	$\chi^2=5.47$	0.14
Second egg sac						
Weight (mg)	125.43 $\pm$ 8.07 (13)	122.03 $\pm$ 10.4 (15)	106.09 $\pm$ 13.87 (10)	114.69 $\pm$ 12.77 (18)	$F_{3,52}=0.44$	0.72
Average egg weight (mg)	0.46 $\pm$ 0.03 (4)	0.69 $\pm$ 0.06 (7)	0.48 $\pm$ 0.07 (3)	0.62 $\pm$ 0.05 (6)	$F_{3,16}=3.9$	0.03
Clutch size	214.91 $\pm$ 15.12 (11)	176.73 $\pm$ 17.29 (15)	175.3 $\pm$ 23.46 (10)	205.42 $\pm$ 14.66 (12)	$F_{3,44}=1.26$	0.3
Hatching rate (%)	73.84 $\pm$ 9.45 (10)	79.02 $\pm$ 9.06 (14)	65.22 $\pm$ 12.82 (10)	74.9 $\pm$ 7.98 (12)	$\chi^2=1.77$	0.62

Sample sizes are given in parentheses and vary because not all data were available for all animals. Mean  $\pm$  SE are shown for all data. F=ANOVA,  $\chi^2$ =Kruskal-Wallis test. Significant differences ( $P<0.05$ ) are given in bold.

P-C:  $6.51 \pm 0.35$  s; P+C:  $28.52 \pm 15.07$  s; Kruskal-Wallis test:  $\chi^2=4.09$ ,  $N=75$ ,  $P=0.25$ ; first copulation cannibalism: M-C: 76.19%; M+C: 61.9%; P-C: 72.43%; P+C: 66.67%; chi-square test:  $\chi^2=1.12$ ,  $N=87$ ,  $P=0.77$ ) while second copulations of polyandrous females performed by virgin males were shorter than second copulations of monandrous females which were performed by a previously mated male (M-C:  $37.95 \pm 7.47$  s; M+C:  $66.6 \pm 35.25$  s; P-C:  $6.34 \pm 1.49$  s; P+C:  $9.18 \pm 2.26$  s; Kruskal-Wallis test:  $\chi^2=28.81$ ,  $N=62$ ,  $P < 0.0001$ ). This difference is also reflected in the reduced rate of male killing in second copulations of polyandrous females (M-C: 100%; M+C: 100%; P-C: 57.14%; P+C: 86.36%; chi-square test:  $\chi^2=16.78$ ,  $N=67$ ,  $P=0.001$ ). However, across all treatments the total duration of copulation did not differ (Kruskal-Wallis test:  $\chi^2=3.63$ ,  $N=67$ ,  $P=0.3$ ) so that these differences are likely to be of little relevance for the amount of ejaculate received, which is directly related to copulation duration (Schneider et al. 2006).

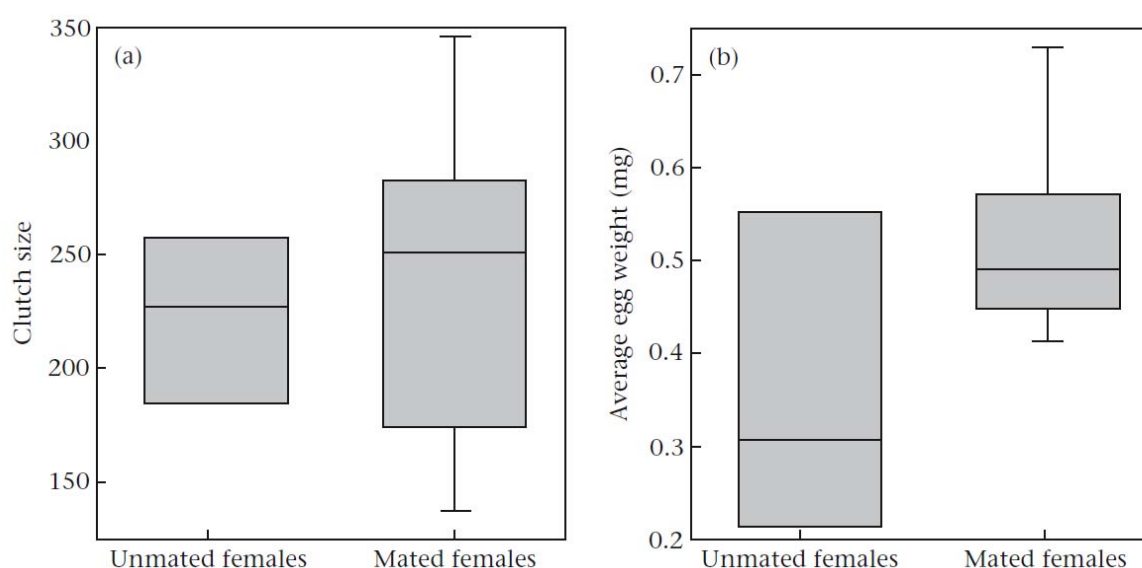
#### *Influence of Copulation on Resource Allocation to Clutches*

Our study species produces eggsacs without mating and we used this opportunity to explore female allocation to eggs with and without mating. Unmated females were slightly although not significantly lighter than mated females but did not differ in size from them (adult weight: unmated:  $118.68 \pm 16.11$  mg;

mated:  $155.87 \pm 7.32$  mg; Mann-Whitney U test:  $Z=-1.89$ ,  $N=91$ ,  $P=0.06$ ; leg length: unmated:  $6.79 \pm 0.31$  mm; mated:  $6.84 \pm 0.1$  mm; ANOVA:  $F_{1,97}=0.02$ ,  $P=0.88$ ). Unmated and mated females produced first eggsacs of similar clutch size (unmated:  $207.13 \pm 33.04$ ; mated:  $237.97 \pm 10.71$ ; ANOVA:  $F_{1,70}=0.9$ ,  $P=0.34$ ; Figure 1) but different weights (unmated:  $81.86 \pm 12.62$  mg; mated:  $118.33 \pm 5.6$  mg; ANOVA:  $F_{1,76}=6.14$ ,  $P=0.02$ ). Thus, on average, unfertilised eggs were lighter than fertilised eggs suggesting that copulation directly affects maternal allocation (Figure 1).

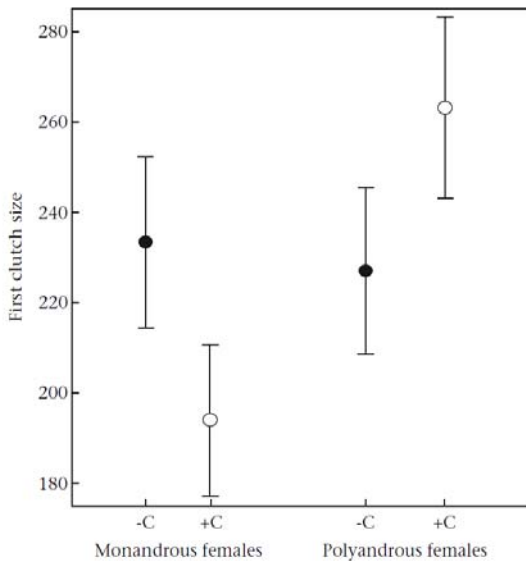
#### *Fecundity*

We were interested in how polyandry, male consumption and their interaction affected female fecundity and reproductive decisions. We computed several linear regressions (standard least squares) with clutch size (CS), egg mass (EM) and average egg weight (AEW) as the dependent variables. Female size and weight strongly affect reproduction in spiders (Fritz and Morse 1985; Simpson 1995) and we therefore added female leg length and female weight gain as fixed effects to the models. We analysed first and second eggsacs separately. As expected, female size (CS:  $F_{1,38}=5.74$ ,  $P=0.02$ ; EM:  $F_{1,38}=13.07$ ,  $P=0.001$ ; AEW:  $F_{1,38}=6$ ,  $P=0.02$ ) and mass gain (CS:  $F_{1,38}=61.43$ ,  $P<0.0001$ ; EM:  $F_{1,38}=17.38$ ,  $P=0.0002$ ; AEW:  $F_{1,38}=9.61$ ,  $P=0.004$ ) were significant predictors in all models concerned with first eggsacs (whole model: CS:



**Figure 1:** (a) Clutch size and (b) average egg weight of the first egg sacs of unmated and mated females. Box plots show the median (line), quartiles (box limits) and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (error bars).

## Sexual cannibalism benefits offspring survival



**Figure 2:** Clutch size of the first eggsac of females with one or more mates and with (open circles) or without (closed circles) male consumption (+/-C). Values are mean  $\pm$  SE corrected for female size and weight gain after copulation.

$r^2=0.7$ ,  $F_{5,33}=15.34$ ,  $P<0.0001$ ; EM:  $r^2=0.53$ ,  $F_{5,33}=7.5$ ,  $P<0.0001$ ; AEW:  $r^2=0.32$ ,  $F_{5,33}=3.14$ ,  $P=0.02$ ). The treatments alone had no significant influence in any model (polyandry: CS:  $F_{1,38}=2.84$ ,  $P=0.1$ ; EM:  $F_{1,38}=1.67$ ,  $P=0.2$ ; AEW:  $F_{1,38}=0.1$ ,  $P=0.76$ ; male consumption: CS:  $F_{1,38}=0.01$ ,  $P=0.93$ ; EM:  $F_{1,38}=0.01$ ,  $P=0.92$ ; AEW:  $F_{1,38}=0.85$ ,  $P=0.36$ ) but the interaction significantly affected clutch size of the first eggsac (polyandry\*male consumption:  $F_{1,38}=4.23$ ,  $P=0.047$ ) with polyandrous and cannibalistic females having the biggest clutches and monandrous cannibalistic ones the smallest (Figure 2).

In the second eggsacs none of our factors explained the variation in clutch size and average egg weight (whole models: CS:  $r^2=0.34$ ,  $F_{5,24}=2.48$ ,  $P=0.06$ ; EM:  $r^2=0.57$ ,  $F_{5,7}=1.82$ ,  $P=0.23$ ; AEW:  $r^2=0.46$ ,  $F_{5,7}=1.17$ ,  $P=0.41$ ). But females that were allowed to consume males produced

**Table 2:** Results of the generalized linear mixed model to explain offspring survival time including mother ID as random factor ( $N=530$  spiders, 23 families)

Variable	Estimate	SE	df	t	P
Intercept	0.19	0.09	507	2.17	0.031
Clutch size	<0.001	<0.001	18	1.87	0.078
Male consumption	-0.18	0.06	18	-3.15	0.006
Polyandry	-0.11	0.06	18	-2.01	0.06
Polyandry * male consumption	0.12	0.08	18	1.61	0.125

Significant differences ( $P < 0.05$ ) are given in bold.

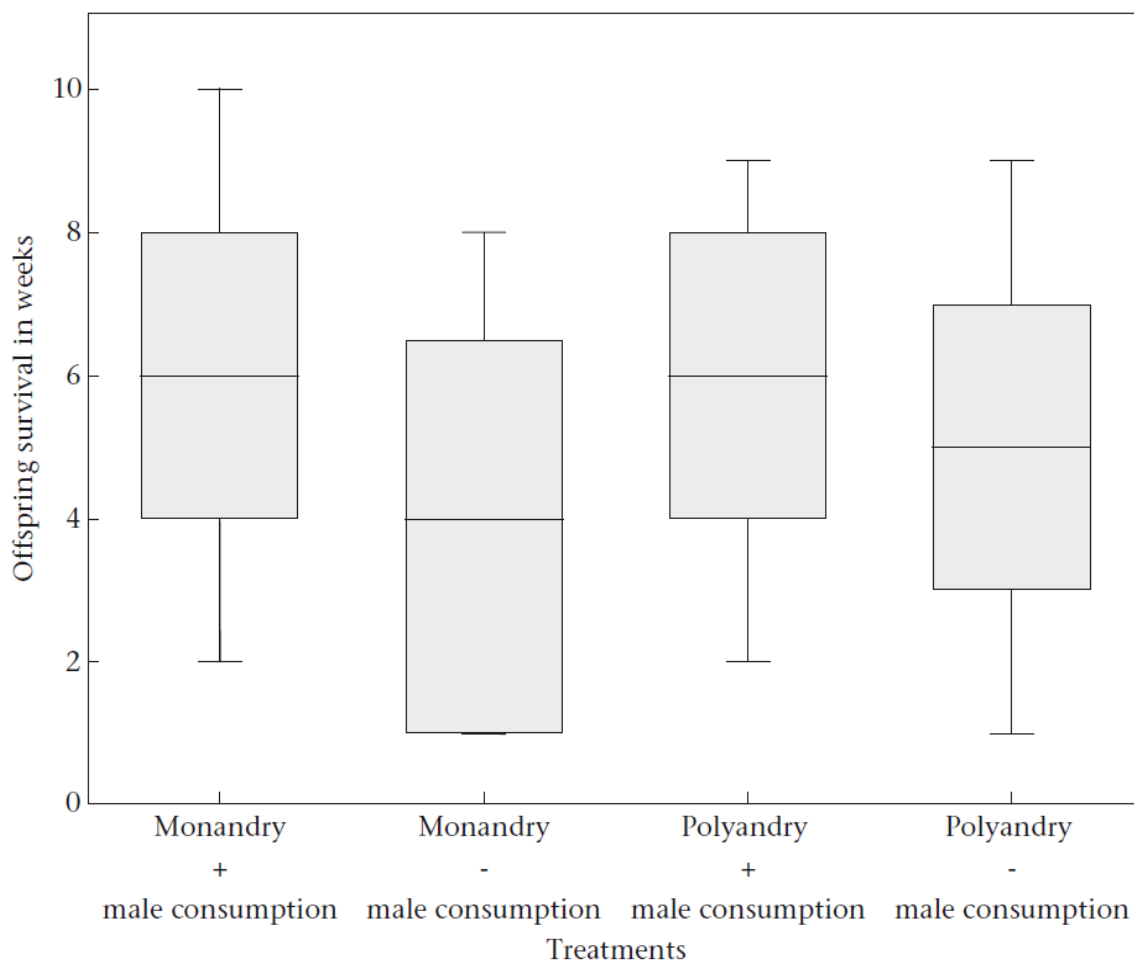
larger eggs in their second eggsac (pooled  $t$  test:  $t_{20}=3.32$ ,  $P=0.004$ ; Table 1) while the female mating rate had no influence on average egg weight (pooled  $t$  test:  $t_{20}=-0.51$ ,  $P=0.62$ ). These results suggest that females become nutritionally limited after they produced a first eggsac and that consuming one or more males will only make a difference at that stage.

### Offspring Survival

A survival analysis revealed that offspring derived from the male consumption treatments survived significantly longer than offspring derived from mothers that were prevented from consuming males (survival in weeks: M+C=6.45  $\pm$  0.26; M-C=4.61  $\pm$  0.35; P+C=5.87  $\pm$  0.19; P-C=5.1  $\pm$  0.26; Kaplan-Meier analysis: log-rank  $\chi^2_3=21.77$ ,  $N=590$ ,  $P<0.0001$ ).

To explore this variation in offspring survival in more detail we ran a GLMM on offspring survival in weeks including male consumption, polyandry and their interaction as fixed effects and mother ID as a random factor. Irrespective of the treatments, larger females lay more eggs and within this size variation, the physical state of a female will affect investment in individual eggs. As we were interested solely in the effects of male consumption we added clutch size of the second eggsac as covariate to remove the effect of female size on female investment. The model revealed a significant influence of male consumption on offspring survival while the influence of polyandry, the interaction between polyandry and male consumption, and clutch size were not significant (Table 2, Figure 3).

Since male consumption positively influenced offspring survival, we tested for a dosage effect in the male consumption treatments and replaced the category of male consumption by the weight of males consumed by females. The model revealed no effect of the weight of males consumed on offspring survival suggesting that a dosage effect is absent (GLMM: intercept:  $t=1.24$ ,  $P=0.21$ ; clutch size:  $t=2.2$ ,  $P=0.07$ ; weight of males consumed:  $t=-0.63$ ,  $P=0.55$ ; polyandry:  $t=-2.17$ ,  $P=0.07$ ; weight of males consumed\*polyandry:  $t=1.75$ ,  $P=0.13$ ;  $N=326$ , families=11).



**Figure 3:** Offspring survival time of all four experimental treatments. Box plots show the median (line), quartiles (box limits) and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (error bars).

## Discussion

The number of mating partners had no influence on female fecundity or on female investment and did not affect offspring survival. Hence our results lend no support to the hypothesis that polyandry is associated with indirect genetic benefits to the female. Our study revealed multiple effects of male consumption: female fecundity measured as size of the first clutch was highest in polyandrous, cannibalistic females. Furthermore, females that consumed one or more males laid heavier eggs in their second eggsacs suggesting that male consumption influenced resource allocation by females. We found that females that consumed a male produced offspring with an improved tolerance to starvation under simulated overwintering conditions compared to those that were prevented from male consumption. This effect was not enhanced in the polyandry treatment in which females consumed several

males and hence it does not select for multiple mating.

Our finding that cannibalistic females showed an increase in fecundity accords with a recent study on the congener *A. keyserlingi* where cannibalistic females increased their fecundity by producing eggs with a higher yolk density measured as egg energy density (kJ/g) than females that were fed with low protein-high energy prey (Blamires 2011). Blamires (2011) suggested that a physiological trigger is initiated when feeding on protein-rich prey resulting in the increased egg energy densities.

The hypothesis that sexual cannibalism evolved because of the nutritional value of consuming one or more males (Newman and Elgar 1991) has received much attention. While studies on species with relatively large males in comparison to the females have provided some support for the hypothesis (Birkhead 1988; Elgar and Nash 1988; Barry et al. 2008), research on species with a large sexual size dimorphism

(SSD) generally failed to find an effect of nutritional benefit through male consumption that was large enough to justify it being a strong selective agent (Elgar and Schneider 2004). A recent study on wolf spiders with a moderate SSD suggests that males are prey of low nutritional value in comparison to crickets mainly because the male body contains fewer lipids, which are essential for egg production (Wilder and Rypstra 2010). However, lipids and protein quantity may be only part of the value of a food item. In vertebrates it has been proposed that conspecifics form high-quality prey for females owing to a similar composition of proteins, while in spiders the existence of amino acids that are essential for silk production may benefit cannibalistic females (Meffe and Crump 1987; Crump 1990; Craig et al. 2000). This accumulation of essential substances contained in the male body may be a possible explanation for the prolonged offspring survival time in cannibalistic females. However, detailed analyses of the components of male bodies may shed light on the mechanism behind this effect.

Our study adds a previously undetected benefit to the consumption of males during copulation in species with small males. *Argiope bruennichi* females may have problems accumulating sufficient resources for their second clutch since the interval between the production of successive eggsacs is only 10 days. Our spiders were fed in the laboratory and while they received plenty of food, it is possible that the quality of prey differed from what they capture in nature. Hence, field studies need to determine whether the food limitation in our study reflects what happens in nature and whether the reported benefit of cannibalism is also present under natural conditions.

The entire phenomenon of sexual cannibalism and its benefits may be relevant for both sexes. The female will clearly benefit if her offspring have improved resistance to starvation and this benefit of eating a male may only be counteracted by contents in the male soma that may have negative impacts on female fitness. However, the possibility that female physiology is manipulated by eating a male in order to enhance a male's success in sperm competition has not yet been investigated in spiders. The potential benefit for a male depends on the probability of whether his contribution is advantageous to his own offspring or to the offspring of rival males. However, a moderate

risk of shared paternity would only be relevant if self-sacrifice is costly for the male so that the nuptial gift is traded off against alternatives that may contribute to male fitness (Buskirk et al. 1984). Such an alternative to sexual cannibalism can be mate guarding, which is known for other species with similar mating strategies. Males of *Nephila fenestrata*, for example, attempt to survive two copulations and even though they are rendered sterile by genital damage, they benefit from surviving copulation because they can protect their paternity by vigorous mate guarding (Fromhage and Schneider 2005). Even though both *N. fenestrata* and *A. bruennichi* males damage their genitalia to produce mechanical mating plugs, the species differ in the plug's effectiveness: low in *N. fenestrata* and high in *A. bruennichi* (Fromhage and Schneider 2006; Nessler et al. 2007). Hence the gain from post-copulatory mate guarding is rather low in *A. bruennichi*.

Multiple paternity occurs in *A. bruennichi*, although a recent field study observed that fewer than 30% of the females mated with multiple males (S. M. Zimmer, K. W. Welke and J. M. Schneider, unpublished data). Male *A. bruennichi* generally die during their second copulation and tolerance of cannibalism is considered a mating effort that prolongs copulation, increases sperm transfer and the paternity share (Schneider et al. 2006). The risk of sperm competition in *A. bruennichi* is high if a male encounters an already mated female or if the male is cannibalised before he can inseminate and plug both genital openings of a female. While both scenarios regularly occur in nature, a certain proportion of males succeed in completely monopolising a female and this is facilitated by the efficient mechanism of paternity protection in *A. bruennichi*. However, the probability of completely monopolising a female is about 70% ignoring the risk of sexual cannibalism after the first insertion (Nessler et al. 2007). If the chances of monopolising a female are high enough to balance the costs that result from death and sperm competition, provisioning the offspring through self-sacrifice may be maintained by selection.

*Argiope bruennichi* has only recently invaded most of northern Europe from southeastern Europe (Guttmann 1979). Females produce their eggsacs from August to September and the spiderlings hatch out of the eggs after about 4 weeks. Spiderlings remain inside the eggsac

until the following April when temperatures and daylength increase. Sudden cold snaps and prolonged winter seasons, which occur regularly in northern latitudes, may delay the development of prey and this may provide offspring of cannibalistic females with an advantage over non-cannibalistic ones, since they have a higher tolerance for these starvation periods. If females' cannibalistic tendencies are heritable, then any cold period during which most of the spiderlings of non-cannibalistic females die would lead to the maintenance of cannibalism. Such an advantage may also be relevant in southeastern Europe where winters are less cold. However, here spiderlings also remain inside eggsacs for a long time and food conditions at hatching may vary as well. A critical starvation threshold may even be reached sooner at higher temperatures. However, all of this is speculation and requires further research.

Here we have shown that consumption of a small male enhances offspring survival, presumably through an improved allocation of nutrients by cannibalistic females to their clutches. A possible mechanism could be an increased investment in yolk density, which is induced by male consumption (Blamires 2011). The male body as a mating effort or as a paternal investment is not a new proposal (Buskirk et al. 1984), but based on our findings we would argue that even if the latter benefits were more common they would still not be large enough to explain the entire phenomenon. Anyhow, our result adds an interesting twist to the current discussion of cannibalism in species with a high SSD. We predict that benefits of male consumption would be more common in species in which the male paternity protection mechanism is very efficient as in *A. bruennichi*, leading to a significant chance of monopolising a female. Detailed analysis of the nutritional value and composition of male body tissue as well as comparative studies with other cannibalistic and size-dimorphic species are needed to clarify which mechanisms are responsible for the fitness increase in cannibalistic females.

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## Chapter 4: Males of the orb-web spider *Argiope bruennichi* sacrifice themselves to unrelated females

Klaas W. Welke and Jutta M. Schneider

### Abstract

Costs of inbreeding can lead to total reproductive failure and inbreeding avoidance is, therefore, common. In classical sex roles with no paternal care, the selective pressure to avoid inbreeding is mostly on the female, which carries the higher costs. In some orb-web spiders, this situation is very different because females are polyandrous and males are monogynous or at most bigynous. Additionally, females of many entelegyne orb weavers are thought to bias paternity post-copulatorily towards a desired mate. This increases the selective pressure on males to adjust their investment in a mating with regard to the compatibility to a female.

Here, we examine whether genetic relatedness influences mating behaviour in the orb-web spider *Argiope bruennichi*. We mated either a sibling or a non-sibling male to a female in single copulation trials and compared copulation duration, cannibalism rate and female fecundity.

Our experiment revealed that males prolonged their copulation duration and were cannibalised more frequently when mating with a non-sibling female. Males mating with a sibling female were more likely to escape cannibalism by copulating briefly, thus presumably increasing their chances of re-mating with a more compatible female. This suggests that males can adaptively adjust their investment relating to the compatibility of a female.

*Keywords:* sexual cannibalism; male self-sacrifice; inbreeding avoidance; *Argiope bruennichi*

### Introduction

Inbreeding occurs if closely related individuals mate and increase homozygosity in their offspring (Pusey and Wolf 1996). This carries a number of risks that can reduce reproductive success up to the total loss of offspring (Charlesworth and Charlesworth 1987; Hoogland 1992).

Classical sex roles imply that females suffer most from inbreeding and that selection should favour mechanisms of inbreeding avoidance in females (Jennions and Petrie 2000). Pre-mating adaptations in this context include, for example, kin recognition through social learning (Dobson et al. 1997), olfactory imprinting (Gerlach et al. 2008) and sex-specific dispersal (Cockburn et al. 1985). Polyandry creates an opportunity for post-copulatory mechanisms to reduce the costs of inbreeding for females, even in the absence of kin recognition (Cornell and Tregenza 2007). This has been shown in a number of taxa, including mammals (Firman and Simmons 2008), birds (Foerster et al. 2003), insects (Arnqvist and Nilsson 2000) and spiders (Welke and Schneider 2009).

In spiders, sedentary species may be particularly prone to inbreeding as eggs are laid in large clutches and often hatch synchronously. While aerial dispersal (ballooning) will distribute the sexes widely, it is not an obligate strategy, as shown for *Argiope bruennichi*, and a proportion of young always stays near the egg sac (Walter et al. 2005). Additionally, silk-related dispersal is known to be reduced by inbreeding (Bonte 2009). While avoidance of kin-mating has not been demonstrated in spiders, polyandry can reduce its negative effects up to a total rescue (Bilde et al. 2007).

Whereas females avoid inbreeding or reduce its negative consequences, males with an unlimited sperm supply and no paternal investment experience low costs if they mate with a sister (Bateman 1948). This is different in mono- and bigynous mating systems, in which males invest heavily in any single mating and inbreeding may hence carry a large opportunity cost. Monogynous mating systems are taxonomically widespread but are particularly common among spiders (Schneider and

Fromhage 2010). Males possess paired mating organs, the pedipalps, and in many monogynous spiders, they are restricted to use each pedipalp only once, leading to a maximum of two copulations in their lifetime (Herberstein et al. 2005). Thus, males should be under strong selection to exercise mate choice.

In the cannibalistic and polyandrous orb-web spider *A. bruennichi*, the much smaller males are invariably attacked during their first copulation and cannibalised in up to 80 per cent of the cases (Fromhage et al. 2003; Fromhage and Schneider 2005; Schneider et al. 2006). All males die after their second copulation, a pattern also known for other *Argiope* species (Sasaki and Iwahashi 1995; Foellmer and Fairbairn 2003). Whether a male survives its first copulation or not depends on the duration of genital contact: males that jump off early (less than 5 s) have a chance of surviving while males that copulate longer (greater than 10 s) invariably die (Schneider et al. 2006). Cannibalism and its associated prolongation of copulation positively influence sperm transfer and relative paternity (Schneider et al. 2006).

In this study, we examine whether genetic relatedness between mates influences their mating strategies. We predict that males should be less inclined to sacrifice their life when copulating with a sister.

### Material and methods

#### *Study animals*

We collected egg sacs of *A. bruennichi* on meadows near Hamburg in the Nordheide and Buxtehude, Germany, in February and March 2008. To minimise the risk of collecting more than one egg sac per female, we chose a minimum distance of 50 m between individual egg sacs (females place their egg sac within a radius of 1–2 m).

Egg sacs were placed in plastic containers (15 x 12 x 7 cm) in which the spiderlings hatched. We fed them with *Drosophila* sp. once a week and sprayed with water daily. Once spiderlings built their own webs they were placed in individual plastic cups (250 ml). We watered them 5 days a week and fed them *Drosophila* sp. twice a week. Female and male spiders are easy to distinguish before their final moult because of the male's bulbous copulatory organs (pedipalps). As soon as we could sex females, they were transferred to bigger plastic cups (500

ml). Males stayed in their cups until we used them for mating experiments. Spiders were all weighed after their final moult. Adult females were transferred into Perspex frames (30 x 30 x 6 cm) in which they built normal-sized webs. Mating trials were conducted in those frames.

#### *Mating procedure*

Only virgin spiders were used for the experiment. We defined sibling spiders as individuals that originated from the same egg sac, even though they could be half-siblings. Non-siblings were derived from different egg sacs.

Ninety-one females from 14 families were mated with either one sibling ( $N=45$ ) or one non-sibling ( $N=46$ ) male after a preset schedule and a split design. Each trial consisted of a single copulation. Males were weighed before the start of a trial because of sexual cannibalism and the loss of legs during mating. We introduced males with a soft brush on one of the frame threads of the female's web. Males were given a time limit of 2 h to copulate, which none of them exceeded and the mean duration until copulation was equal between the treatments (non-siblings:  $22.4 \pm 2.9$  min; siblings:  $24.2 \pm 3.7$  min). All experimentally matched pairs copulated regardless of the treatment. During a mating trial we documented copulation duration and cannibalism attempts. Cannibalised males were removed from the female fangs; survivor males were killed by hypothermia.

We weighed females after copulation to minimise disturbance and to avoid web damage prior to the mating trials. After weighing, females were returned into their Perspex frames to build egg sacs. Egg sacs were weighed and then stored in gauze-covered plastic vials. Hatching rate, clutch weight and clutch size were determined for all first egg sacs.

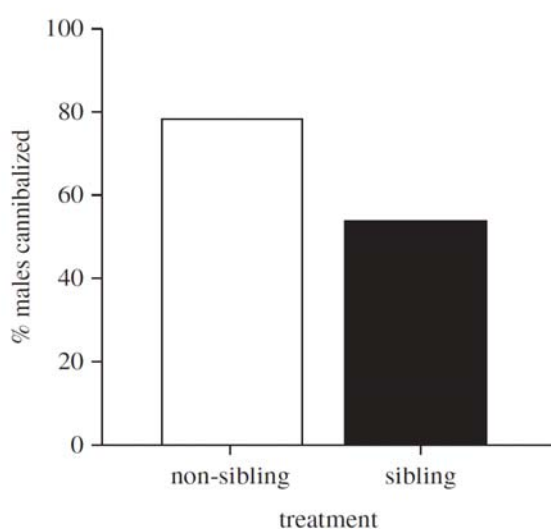
Statistical analyses were performed with JMP 7.0.2. Data were checked for normal distribution using the Kolmogorov–Smirnov test. We used parametric and non-parametric tests as appropriate. Sample sizes differ between analyses because not all data were available for all trials. All values are given as mean  $\pm$  s.e.

### Results

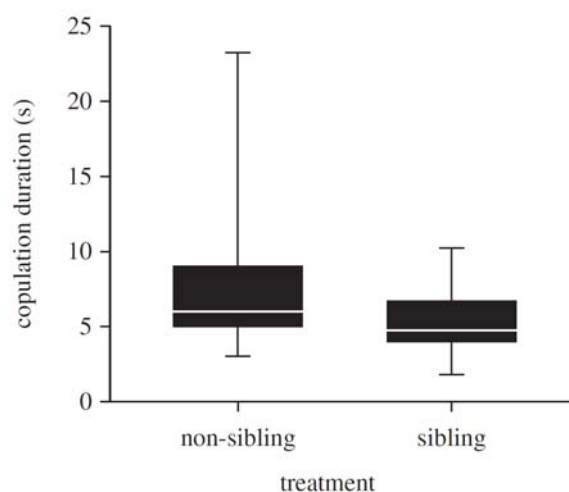
Siblings escaped sexual cannibalism more often than non-siblings (siblings: 46.7%; non-siblings:

21.7%;  $\chi^2=6.3$ ,  $P=0.01$ ,  $N=91$ ; Figure 1). Copulation duration was shorter for siblings ( $5.81 \pm 0.55$  s) than for non-siblings ( $9.03 \pm 1.27$  s; Mann-Whitney U test:  $Z=22.53$ ,  $P=0.01$ ,  $N=89$ ; Figure 2). Corroborating previous results, cannibalism significantly prolongs the duration of copulation duration (logistic regression:  $\chi^2=30.17$ ,  $P=0.0001$ ,  $N=89$ ). A logistic model (whole model:  $r^2=0.22$ ,  $\chi^2=25.52$ ,  $P=0.04$ ,  $N=91$ ) showed a significant influence of treatment ( $\chi^2=8.07$ ,  $P=0.005$ ) on cannibalism but no influence of female family ( $\chi^2=19.12$ ,  $P=0.16$ ).

Females in the two treatments did not differ in days from final moult until death (non-siblings:  $39.36 \pm 2.42$  days; siblings:  $40.95 \pm 2.54$  days; pooled t-test:  $t_{42}=0.45$ ,  $P=0.65$ ). Fifteen females died before they produced an egg sac but the mating treatment had no effect on female mortality ( $N=91$ ,  $\chi^2=2.13$ ,  $P=0.14$ ). Treatment did not influence any of the following parameters: number of egg sacs produced (Mann-Whitney U test:  $N=91$ ,  $Z=0.36$ ,  $P=0.72$ ); total weight of first egg sac (non-siblings:  $161.9 \pm 10.36$  mg; siblings:  $170.91 \pm 11.55$  mg; pooled t-test:  $t_{74}=0.58$ ,  $P=0.56$ ); silk weight of first egg sac (non-siblings:  $32.65 \pm 1.95$  mg; siblings:  $34.72 \pm 2.11$  mg; pooled t-test:  $t_{63}=0.72$ ;  $P=0.47$ ); combined weight of eggs in first egg sac (non-siblings:  $132.85 \pm 61.79$ ; siblings:  $129.93 \pm 53.04$ ; pooled t-test:  $t_{62}=20.2$ ,  $P=0.84$ ); hatching rate (nonsiblings:  $40.79 \pm 5.73\%$ ; siblings:  $41.64 \pm 6.45\%$ ; pooled t-test:  $t_{68}=0.1$ ,  $P=0.92$ ).



**Figure 1:** Males mated to a non-sibling female were cannibalized significantly more often than males mated to a sibling.



**Figure 2:** Copulations between unrelated pairs had a longer duration than copulations between siblings.

## Discussion

In accordance with our prediction, relatedness between mating partners influenced their mating behaviour: copulation duration and the risk of sexual cannibalism were significantly lower in sibling matings than in non-sibling matings.

One possible explanation for these results is that females react differently towards brothers, by interrupting copulations earlier to limit sperm uptake and/or by letting them survive because their brothers' future mating success would add to the females' inclusive fitness. However, this seems unlikely because females stereotypically attack any male independently of their degree of relatedness and independently of male courtship behaviour (Schneider and Lesmono 2009). Males cling to the genital opening and can actively modulate the timing of their escape (Nessler et al. 2009). The only reliable predictors of sexual cannibalism in *A. bruennichi* are copulation duration and male mating status. The female's influence, by contrast, appears to be limited (Fromhage and Schneider 2005).

The potential for polyandrous females to bias paternity cryptically towards a more compatible male (Welke and Schneider 2009) may reduce the value of a sister as a mating partner for a male. Male investment in a mating event is reflected by two alternative mating tactics. They can either be monogynists (first copulation: long, second copulation: none or first copulation: short and second copulation: long

with the same female) or bigynists (first copulation: short, second copulation: long, with another female (Fromhage et al. 2008)).

In our study, almost all of the non-sibling males were monogynous. Although re-mating with the same or another female would be possible, males sacrificed themselves to the female after a long first copulation. By prolonging their copulation, males increase their paternity share with a female but they also risk being cannibalised (Schneider et al. 2006). High mortality during mate search in addition to an increased paternity share and benefits for the offspring could make it beneficial for males to be monogynists (Buskirk et al. 1984; Andrade 2003). Thus, if a male has found a compatible female, his optimal strategy may be to perform a long first copulation and invest maximally instead of copulating briefly and risking death during further mate search. Half of the males mated to sibling females succeeded in cannibalism avoidance and thus in a natural setting would potentially be bigynous. But why do these males mate with their sisters at all? One possibility is that high male mortality during mate search selects for acceptance even of sub-optimal mating opportunities, but not at the cost of losing all other mating opportunities. This would explain why males tend to copulate briefly enough to survive (less than 5 s). Being monogynous with a sister would be disadvantageous because females might re-mate and post-copulatorily bias paternity towards a more compatible suitor and a sibling male would have sacrificed itself in vain.

In conclusion, our results support the idea that males adaptively modulate their mating tactic through selective self-sacrifice or escape behaviour.

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## Chapter 5: Inbreeding depression and indications for cryptic female choice in the orb-weaving spider *Argiope australis*

Klaas W. Welke and Jutta M. Schneider

### Abstract

Polyandry (multiple mating of females) may benefit females either directly (material) or indirectly (genetically). First, females may receive material donations or benefits from males like nuptial gifts, additional protection, or additional helpers for brood care. Gathering material benefits clearly is in the interest of the female and obviously increases her reproductive success. Without material benefits the existence of polyandry is puzzling but it is proposed that it evolved for genetic benefits. Genetic benefits of polyandry derive for example from genetic variance within the offspring, the possibility to trade up from inferior males by extra copulations, and the avoidance of incompatible genomes. By mating multiply females accumulate the ejaculates of different males inside their body which may enable post-copulatory selection processes (cryptic female choice).

In this study we examined the effects of inbreeding on the offspring of the orb-web spider *Argiope australis*. We were interested in the detrimental effects of incestuous matings on offspring quality. We staged sibling and non-sibling matings and we used body size and weight as well as lifespan of individuals as measure for inbreeding depression. Further we conducted double matings in which females were mated to both sibling and non-sibling males in varying order. The genital morphology and the mating behaviour of *A. australis* enabled us to experimentally induce copulations into different genital openings which allowed us to count sperm of individual males. We found that inbreeding depression is present in *A. australis* after a single generation of inbreeding and that inbred males and females suffered from a drastically reduced lifespan and additionally males were smaller than outbred conspecifics. The threat of inbreeding depression suggests that mechanisms to avoid incestuous matings would be useful for *A. australis*. Evidence for pre-copulatory inbreeding avoidance is lacking in all *Argiope* species and we found none either. However we find indications for post-copulatory avoidance mechanisms, namely cryptic female choice. Females stored less sperm of sibling males when these copulated after a non-sibling male. Polyandry seems to enable females in *Argiope* to trade up from poor quality males to superior mating partners and thereby lower the costs of inbreeding.

*Keywords:* *Argiope australis*, Araneidae, inbreeding depression, inbreeding avoidance, cryptic female choice

### Introduction

Mating with close relatives may reduce the fitness of an individual through the expression of deleterious recessive alleles and the loss of heterozygosity in inbred offspring (Charlesworth and Charlesworth 1987; Stockley et al. 1993). This inbreeding depression is suggested to be a main selective force for the evolution of polyandry (multiple mating by females; Zeh and Zeh 1996, 1997). Polyandry opens the stage for post-copulatory sperm interactions inside of the female such as sperm competition and cryptic female choice (Eberhard 1996). Through post-copulatory choice females may discriminate between the

ejaculates of several mating partners and compensate the costs of inbreeding (Zeh and Zeh 1996; Tregenza and Wedell 2000; Gasparini and Pilastro 2011).

For males this discrimination between ejaculates increases the costs of incestuous matings. By copulating with a sibling female males do not only risk producing inferior offspring but are also endangered to lose a substantial part of their paternity share with this female because of post-copulatory choice mechanisms. Especially for species with only a single or few mating opportunities in males (monogyny) this could result in a complete

reduction of a male's lifetime reproductive success.

Polyandry is quite common in the entelegyne spider genus *Argiope* and it has been shown that females are capable to partly compensate the costs of inbreeding post-copulatorily (Welke and Schneider 2009). The paired sperm storage organs (spermathecae) of entelegyne spider females form the perfect venue for post-copulatory choice mechanisms (Hellriegel and Ward 1998; Eberhard 2004; Herberstein et al. 2011). Only recently it has been shown that females of the wasp spider *A. lobata* use cryptic female choice to bias paternity towards non-siblings and thereby discriminate against inferior sibling males (Welke and Schneider 2009). But although females can decrease the costs of inbreeding by such mechanisms they may still be monopolised by males that break parts of their sperm transmitting organs (pedipalps) to plug up her genital openings (Nessler et al. 2007; Foellmer 2008). Due to this genital mutilation males are limited to a maximum of two copulations in their lifetime and their mating rate is further reduced by sexual cannibalism during/after copulation (Elgar and Schneider 2004; Herberstein et al. 2005; Nessler et al. 2009a). Because monogynous males run the risk of losing their complete reproductive success they should avoid inbreeding pre-copulatorily but no such strategies have been shown for *Argiope*. Quite the contrary, in the laboratory males of several *Argiope* species readily mate with sibling females although *A. bruennichi* males shorten their copulation duration with sibling females and thereby decrease the risk of sexual cannibalism (Welke and Schneider 2010). Males that survive their first copulation may continue their mate search for a more compatible female.

There is no information about inbreeding in the field but it is highly likely that siblings encounter each other frequently. After hatching from the egg sac spiderlings stay together on a communal web until they are large enough to build their own hunting webs. Since long distance dispersal is not obligate in *Argiope* (Walter et al. 2005) it is likely that incestuous matings occur.

Inbreeding depression is not well studied in spiders but one of the few existing studies on *Oedothorax apicatus* showed that inbreeding negatively affected hatching rate and fecundity

although only after two generations of inbreeding (Bilde et al. 2007). The same applies for group living spiders that are quite prone to inbreeding even with existing avoidance mechanisms such as pre-mature dispersal from the group (Bilde et al. 2005). However, the effects of inbreeding were rather low or absent in group living spiders (Bilde et al. 2005; Aviles and Bukowski 2006). These findings indicate that there is some tolerance towards inbreeding but it is unknown whether this can be generalised to other families such as orb-weaving spiders, Araneidae.

In order to quantify the degree of inbreeding depression and to investigate the existence of inbreeding avoidance mechanisms, prior, during and after copulation, we used the wasp spider *A. australis*. Therefore we mated the offspring of wild-caught pregnant *A. australis* females and created an inbred and an outbred F2 generation. We assessed the degree of inbreeding depression by using the adult body size, adult weight, lifespan and reproductive period of inbred and outbred offspring. To detect pre-copulatory inbreeding avoidance we observed matings between siblings and non-siblings both in the F1 and in the F2 generation. Further we investigated post-copulatory choice in females by using a subset of the inbred / outbred F2 generation for a sperm storage experiment. We mated females to both a sibling and a non-sibling male and counted the sperm females stored of each mating partner. We predict that inbreeding depression manifests in decreased body size and shortened survival in inbred individuals and expect that inbreeding is avoided either by pre-copulatory or by post-copulatory mechanisms.

## Methods

### *Study animals and rearing conditions*

For this study we used the Black and Yellow Garden Spider *Argiope australis* (Walckenaer 1805) which is found throughout the eastern part of Africa and is abundant in South Africa (Bjorn 1997). Commonly it is found in gardens and bushy vegetations. As characteristic for *Argiope* species males are much smaller than the females and often fall victim to sexual cannibalism during or after copulation (Sasaki and Iwahashi 1995; Elgar et al. 2000; Foellmer and Fairbairn 2004; Schneider et al. 2006). Males and females possess paired genitalia: males

have two secondary sperm transferring pedipalps (Foelix 1996) which can only be used once and often are damaged during copulation plugging up the female genital openings with the broken-off parts (Herberstein et al. 2005; Nessler et al. 2007; Foellmer 2008; Nessler et al. 2009a). The paired genital openings of females lead to two separate sperm storage organs, the spermathecae (Foelix 1996). The copulation pattern in the genus *Argiope* is fixed, meaning that a male's right pedipalp can only be inserted into the right genital opening of a female and vice versa allowing selective manipulation of the copulation scheme of males.

In 2008 we collected 6 pregnant *A. australis* females and one egg sac in Durban, South Africa. Distance between specimens was set appropriate to exclude direct relatedness. In our laboratory at the University of Hamburg we allowed females to build egg sacs. We placed those egg sacs in small plastic vials until the first spiderlings hatched. The complete egg sac, including already hatched spiderlings, was then transferred to plastic boxes (15cm x 11.5cm x 7.5cm) in which spiderlings build their communal hunting web. The plastic boxes were provided with *Drosophila* ad libitum twice a week and were sprayed with water on five days of the week. As soon as spiderlings build their own capture webs they were separated into upside down plastic cups (200 ml). When we were able to determine the sex of the spiders we transferred female spiders into bigger cups (400 ml) and fed them with *Calliphora* flies. Spiders were monitored daily for their final moult except on weekends. Spiders that moulted to adulthood were weighed within 24 hours and adult females were placed in Perspex frames (36 x 36 x 5cm) where they built their typical orb-web. Conditions in our laboratory were held constant at 25°C with a relative humidity of approx. 50% and a 12 hour light-dark cycle.

#### *Inbred and outbred F2 generation*

Offspring of 5 wild-caught females (F1) was used to create an inbred and an outbred line in the F2 generation. Therefore we mated a female either to a sibling (S, N=39) or to a non-sibling male (N, N=32) in single copulation bouts. The mating trials took place in the Perspex frames in which adult females had built their orb-webs. Each trial started with placing a male on

one of the upper frame-threads of the female's web. We monitored each copulation and measured copulation duration and the occurrence of sexual cannibalism. If a female caught the male after copulation we rescued him and stored him for morphological measures (tibia-patella length, carapace length and width). Offspring that derived from these single matings was reared in our laboratory as described above. Overall we reared offspring of 15 sibling matings and 16 non-sibling matings to measure the consequences of inbreeding and to conduct the sperm count experiment.

#### *Consequences of inbreeding*

We quantified the effects of inbreeding by analysing the adult weight and adult size (tibia-patella length, carapace length and width) of inbred and outbred individuals. Furthermore we assessed the reproductive lifespan of an individual (time from maturity to death) and the total lifespan (time of egg sac construction to death).

#### *Sperm storage experiment*

For the sperm storage experiment we used individuals of the inbred and outbred F2 generation. For our treatments we double-mated females with a sibling and a non-sibling male (SN, N=12) or vice versa (NS, N=14). We amputated each male one of its pedipalps to assure that males ejaculated into opposite spermathecae. This was done by pressing the stem of the palp with tweezers until the male let go of it by autotomy. This inference is harmless for the males and occurs naturally during final moult (personal observations). The loss of one pedipalp has no influence on the male mating behaviour (Rovner 1967; Nessler et al. 2007). Mating trials followed the same procedure as described above and we assessed courtship, mating and cannibalistic behaviour. Females were allowed to catch males after copulation but were prevented from consuming them.

#### *Sperm counts*

We dissected the female opisthosoma and removed the two spermathecae. After we cleaned them from the attaching body tissue they were separated and placed individually in Eppendorf tubes in 100µl Schaerfe Casytone solution. The sclerotised spermathecae were carefully cracked open inside the Casytone

**Table 1:** Copulation behaviour of the single mating trials of sibling and non-sibling F1 individuals and data on the resulting egg sacs (mean  $\pm$  s.d.).

Copulation behaviour				
	Sibling	Non-sibling	Test statistic	P
Time until copulation (sec)	1874.74 $\pm$ 1605.83 (34)	1641.06 $\pm$ 1203.33 (28)	Z=-0.62	0.54
Courtship on opisthosoma (sec)	357.69 $\pm$ 287.49 (35)	365.08 $\pm$ 449.5 (25)	Z=-0.54	0.59
Cannibalism	10.3% (4 of 39)	16.7% (5 of 30)	X <sup>2</sup> =0.61	0.44
Copulation duration (sec)	8.79 $\pm$ 8.59 (35)	17.32 $\pm$ 55.7 (30)	Z=-0.21	0.83
Probability of ultra short copulations	20% (7 of 35)	26.67% (8 of 30)	X <sup>2</sup> =0.4	0.53
Genital damage	Big: 19 Small: 3 None: 13	Big: 15 Small: 2 None: 8	X <sup>2</sup> =0.2	0.9
Egg-sac data				
Failure in 1 <sup>st</sup> egg-sac production	17.4% (4 of 23)	18.2% (4 of 22)	X <sup>2</sup> =0.005	0.94
Failure in 2 <sup>nd</sup> egg sac production	50% (8 of 16)	20% (3 of 15)	X <sup>2</sup> =3.13	0.08
No. of egg sacs	2.65 $\pm$ 1.72 (23)	2.87 $\pm$ 1.84 (24)	Z=-0.3	0.75
1 <sup>st</sup> egg sac: days until hatching	67.83 $\pm$ 42.35 (18)	78.85 $\pm$ 48.37 (26)	Z=-1.09	0.28
Weight of 1 <sup>st</sup> egg sac	525.17 $\pm$ 117.33 (19)	501.12 $\pm$ 160.39 (18)	t <sub>37</sub> =0.52	0.6
Weight of 2 <sup>nd</sup> egg sac	462.48 $\pm$ 79.16 (8)	448.84 $\pm$ 114.87 (12)	t <sub>20</sub> =0.29	0.77

solution using a small dissecting scissor. Afterwards the probes were centrifuged in an Eppendorf Minispin for two minutes at 5000g. We sonified the probes three times for 40 seconds at 40% power (Bandelin Sonopuls HD 2070) to avoid sperm clumping. Between each cycle we paused for 40 seconds. Afterwards we centrifuged the probes again for 2 minutes at 5000g and Vortexed them for approx. 20 seconds. For the epifluorescence staining of the spermatozoa we brought five 0.2  $\mu$ l drops of each sample on a microscope slide and allowed them to dry completely. Afterwards the microscope slides were covered with methyl-acetic acid (3:1) and were left for 10 minutes to let it evaporate. Then we covered the samples with staining solution (Hoechst 33258) and left them for 10 minutes in darkness. The microscope slides were washed with 1xPBS buffer and kept in a bath of PBS buffer for 10 minutes in darkness. Finally the microscope slides were covered with Mowiol and a cover slip for conservation. We counted the number of spermatozoa under a fluorescence microscope and took pictures using the imaging software Olympus Cell<sup>^</sup>F.

## Results

### *Pre-mating inbreeding avoidance*

#### *Mating behaviour of the F1 generation*

We chose the time a male was mounted on the female opisthosoma prior to copulation as a measure for courtship investment because males perform an active drumming behaviour with their pedipalps before inseminating one of them. But both experimental groups (S / N) did not differ in their courtship duration as well as in total time elapsed until copulation took place (Table 1). Also the copulation duration was unaffected by inbreeding, as well as the cannibalism frequency (Table 1). Interestingly, we observed a mating behaviour we from now on refer to as ultra-short copulations (USC). These USC's were only about 1 second long and occurred equally frequent in both sibling (20%) and non-sibling copulations (26.7%;  $\chi^2=0.76$ ,  $P=0.38$ ). The probability of damaging their palps was low for males that performed USC's (3 of 13 males; regular copulations: 35 of 44;  $\chi^2=13.93$ ,  $P=0.0002$ ). The risk of being cannibalised was not reduced as a consequence of USC's (1 of 14; regular copulations: 7 of 43;  $\chi^2=90.53$ ,  $P=0.47$ ). Since we were not sure whether males transferred sperm in such copulations we included this question into the sperm storage experiment (see below).

### *Mating behaviour of the inbred and outbred F2 generation*

The frequency of USC's was higher than in the F1 generation with 46% (13 of 28) in virgin females and 38% (11 of 28) in mated females. Due to this high frequency of USC's only 9 of our 26 females were double-mated successfully. In 4 trials even both males performed USC's rendering the eggs of these females useless (see below). We found that USC's occurred equally frequent in the first copulations of our experimental groups (NS: 7 of 14, SN: 4 of 12;  $\chi^2=0.74$ ,  $P=0.39$ ), but in the second copulation significantly more sibling males copulated extremely short (NS: 7 of 14; SN: 1 of 12,  $\chi^2=5.8$ ,  $P=0.016$ ). Males that performed USC's had a lower probability of damaging their pedipalps (10 of 21 damaged; regular copulations: 31 of 32,  $\chi^2=18.74$ ,  $P<.0001$ ) while probability of being cannibalised during USC's was similar to regular copulations ( $\chi^2=1.91$ ,  $N=57$ ,  $P=0.17$ )

### *Post-mating inbreeding avoidance*

#### *Sperm Counts*

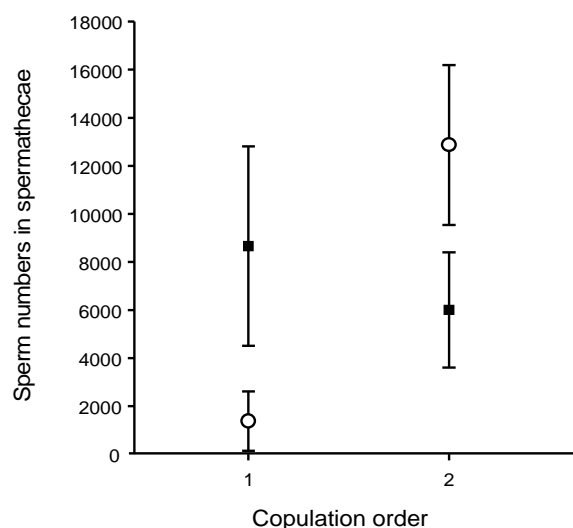
The sperm counts revealed that copulations shorter than 4 seconds did not result in a successful sperm transfer and one male that had copulated for 10 seconds did not transfer sperm successfully. Across all copulations we found that copulation duration and the number of sperm found inside the spermathecae were positively correlated (1<sup>st</sup> cop:  $r^2=0.28$ ,  $F_{1,23}=8.95$ ,  $P=0.007$ ; 2<sup>nd</sup> cop:  $r^2=0.4$ ,  $F_{1,24}=16.14$ ,  $P=0.0005$ ).

Sperm numbers stored in the spermathecae were not significantly different between treatments although in first copulations females had received more sperm of sibling males (NS:  $1376.92\pm4467.32$ , SN:  $8658\pm14369.95$ ;  $Z=1.68$ ,  $N=25$ ,  $P=0.09$ ) while in second copulations sperm numbers of non-sibling males that had copulated after a sibling male were slightly higher (NS:  $6007.14\pm8965.1$ ; SN:  $12866.67\pm11510.97$ ;  $Z=1.86$ ,  $N=26$ ,  $P=0.06$ , Figure).

### *Consequences of inbreeding*

#### *Egg sacs of the F1 generation*

Matings between siblings and non-siblings revealed no evidence for inbreeding depression in the F1 generation. Overall 46 of our 71 females (65%) built egg sacs and breeding failure was not related to inbreeding (likelihood ratio:  $\chi^2=1.3$ ,  $P=0.26$ ), but mainly due to USC's ( $\chi^2=6.49$ ,  $N=65$ ,  $P=0.01$ ). Inbreeding had no



**Figure 1:** sperm numbers counted in the female spermathecae after copulations with sibling/non-sibling and non-sibling/sibling males. The black squares represent sibling males and the clear circles non-siblings.

effect on the number of egg sacs females built and their egg sac weight (Table 1). Also the time until spiderlings emerged from the egg sac stayed unaffected from inbreeding (Table 1).

#### *Inbreeding effects in the F2 generation*

Offspring that derived from one generation of inbreeding showed detrimental effects. While in females inbred and outbred individuals did not differ in weight and size (Figure), inbred males were about 7% smaller and 8% lighter than outbred ones (Figure).

More blatant was the effect of inbreeding on individual lifespan. Both, males and females, suffered from a reduced lifespan and a shortened reproductive period as a consequence of inbreeding (table). Most apparent was the effect in adult females. Inbred females had a 46% shortened reproductive lifespan. Comparing individuals that had died as juveniles without reaching maturity we found the same effect (inbred:  $211.35\pm91.23$  days,  $N=29$ , outbred:  $316.99\pm102.39$ ,  $N=98$ ;  $Z=-4.59$ ,  $P<.0001$ ) although the number of individuals that reached adulthood at all did not depend on inbreeding (inbred: 257 of 286 (89.86%), outbred: 670 of 770 (87.01%),  $\chi^2=1.63$ ,  $P=0.2$ ).

### **Discussion**

Our experiments revealed negative effects of inbreeding in *Argiope australis* after only a single generation. The most apparent effect was

**Table 2:** Body parameters and lifespan of inbred and outbred males and females (mean  $\pm$  s.d.).

	Males				Females			
	Inbred	Outbred	Test	P	Inbred	Outbred	Test	P
<b>Adult weight (mg)</b>	21 $\pm$ 7.02 (118)	22.76 $\pm$ 7 (241)	Z=-2.4	0.016	423.65 $\pm$ 81.14 (139)	423.83 $\pm$ 108.72 (428)	t=0.02	0.98
<b>Mean leg length (mm)</b>	3.62 $\pm$ 0.42 (43)	3.86 $\pm$ 0.49 (47)	t=2.41	0.02	10.92 $\pm$ 0.73 (58)	10.96 $\pm$ 0.94 (61)	t=0.28	0.78
<b>Carapace length (mm)</b>	2.55 $\pm$ 0.24 (53)	2.69 $\pm$ 0.29 (62)	t=2.76	0.007	7.4 $\pm$ 0.47 (60)	7.51 $\pm$ 0.66 (60)	t=1.04	0.3
<b>Carapace width (mm)</b>	2.07 $\pm$ 0.18 (53)	2.15 $\pm$ 0.23 (62)	Z=-2.07	0.039	6.09 $\pm$ 0.39 (60)	6.16 $\pm$ 0.53 (60)	t=0.83	0.41
<b>Reproductive lifespan (days)</b>	129.35 $\pm$ 39.41 (68)	157.38 $\pm$ 55.12 (149)	Z=-4.09	<.0001	29.71 $\pm$ 25.25 (70)	54.79 $\pm$ 37.42 (195)	Z=-5.1	<.0001
<b>Lifespan (days)</b>	346.09 $\pm$ 99.76 (68)	428.97 $\pm$ 122.44 (147)	Z=-4.86	<.0001	264.34 $\pm$ 81.24 (70)	337.66 $\pm$ 105.76 (189)	Z=-5.46	<.0001

the reduction of adult lifespan of inbred females by almost 50% and a bit less for males. We found no effect on body parameters in females whereas inbred males were smaller and lighter than outbred ones. Our results suggest that inbreeding avoidance mechanisms are adaptive in *A. australis*. Contrary to our expectation, we did not find clear evidence for cryptic female choice but a slight trend towards female discrimination between sibling and non-sibling males in the number of sperm transferred during the second copulation. Additionally, we detected an increased occurrence of costly ultra short copulations without sperm transfer in sibling copulations that followed on a non-sibling pairing.

Inbred males were about 7% smaller and lighter than outbred males. Whether there are any fitness consequences of reduced size for inbred males in nature, however, remains speculative and ambiguous. So far it is not entirely clear how male size translates into reproductive success as small males may have a head start and win scramble competition while large males are better in contests (Blanckenhorn 2005; Johnson 2005; Foellmer and Moya-Larano 2007; Moya-Larano et al. 2007). Field observations on other *Argiope* species show that males often lure around webs of sub-adult females waiting for them to mature (Zimmer et al. 2012; Welke et al. in press). Often several males besiege the web of a single female and race to her as soon as she matures. Even if small size is beneficial in this context small males are disadvantaged in direct competition to large males. Additionally, large body size is advantageous if males invest in mate-guarding

their females. The absence of size differences in inbred and outbred females was surprising and both groups performed better than the F1 generation that originated from the wild-caught females (unpublished data). This may be due to an enhanced dietary provisioning of laboratory females compared to their wild-caught parents.

Inbreeding reduced lifespan of individuals severely and this difference directly translates into reduced lifetime fitness because *Argiope* females start egg production immediately after maturation and build their first egg sac within 15-20 days. Up to 3 or more successive egg sacs are build after intervals of 10-14 days. Thus the reduced adult lifespan means that females build fewer egg-sacs. For males the consequences of a shortened lifespan are not as obvious as for females. Maybe inbred males are not able to conduct prolonged mate search and suffer from reduced mate choice opportunities, however, this lacks empirical evidence.

Studies on inbreeding in spiders are scarce and mainly focus on its impact on the evolution of sociality in spiders (Bilde et al. 2005, Lubin and Bilde 2007, Lubin et al. 2009, Ruch et al. 2009). Very few studies have addressed the effects of inbreeding in solitary species yet. Bonte (2009) was able to show for three congeneric lyniphid species that inbred individuals were reduced in their dispersal modes and that inbreeding resulted in a decreased survivorship. In another lyniphid spider, *Oedothorax apicatus*, Bilde et al. (2007) demonstrated that inbreeding resulted in reduced egg numbers and decreased hatching success and that this effect was strongest after three generations of inbreeding. Interestingly,

females in *O. apicatus* were able to restore their reproductive output when they were given the opportunity to outbreed and it is suggested that post-copulatory selection processes are responsible for this rescue effect (Bilde et al. 2007). Complementing these studies we showed the fatal effects of inbreeding in *A. australis* after a single generation of inbreeding. We found that inbreeding effects were low on body size and adult weight while the lifespan of inbred individuals was drastically reduced. This is in accordance with results found in other taxa which generally show low effects of inbreeding on morphological traits, average effects on life-history traits, and a large effect on fitness related traits (DeRose and Roff 1999; Simmons 2011). In summary, our results prove the existence of inbreeding depression in *A. australis* and we demonstrated its effects on morphological and life history traits. Supposedly, these effects are much stronger in nature than under laboratory conditions (Jimenez et al. 1994, Cronkrak and Roff 1999). The presence of notable inbreeding depression after a single inbred generation in *A. australis* suggests that inbreeding avoidance would be highly adaptive, both for males and females.

Pre-copulatory mechanisms to avoid inbreeding are lacking in *Argiope* species and we could not detect any in our study either, although it would be very useful especially for males that face the risk of sexual cannibalism. Especially monogynous males carry high costs when it comes to inbreeding. First, they suffer from the consequences of inbreeding depression and second, males mating with a polyandrous sister run the risk of being discriminated against by cryptic female choice. Post-copulatory processes may lead to a dramatic reduction in a male's lifetime reproductive success. So, why do males mate with their sisters at all? Supposedly, the value of a virgin female is too high to reject the opportunity mating with her even with the risk of inbreeding. This may be adaptive if finding a mating partner is difficult but at the same time, the sister may not stay the only mating partner for a male. For example, if the risk of sexual cannibalism is low, males may copulate with only one of their pedipalps and use the opportunity to continue their mate search for a better 2<sup>nd</sup> female. In order to facilitate their ability to mate with two females, males of *A. bruennichi* manipulate their copulation duration

and thereby increase their survival chances after the 1<sup>st</sup> copulation (Nessler et al. 2009b; Welke and Schneider 2010). Males of *A. bruennichi* that mated with a sister shortened their copulations and increased their survival chances while males that mated with non-sibling females prolonged their copulations, faced sexual cannibalism and transferred more sperm (Welke and Schneider 2010). Whether this applies in *A. australis* has to be explored in a field study that determines male and female mating rates as well as the kin structure of populations to assess the risk of encountering siblings.

Although we did not find strong indications for cryptic female choice we found slight differences in sperm numbers transferred or stored between sibling and non-sibling pairs. However, these differences were only present in second copulations of females suggesting that females use polyandry to trade-up to superior mating partners while they are indiscriminate in their first copulations. Clearly we can only offer trends and a large variation in our sample. The lack of statistical significance in this study may be due to limited power of our data set rather than absence of the mechanism in *A. australis*. In fact, it has been shown in *A. lobata* that females avoid incompatibilities with their mating partners via cryptic female choice. Welke and Schneider (2009) found that females differentially stored the sperm of two males and traded up to more compatible males in a sperm competition situation between siblings and non-siblings. This difference in sperm storage also translated into increased P2 values of non-sibling males. Therefore we propose that *A. australis* also uses cryptic female choice as a mechanism to trade-up to more compatible mating partners. We tentatively suggest that cryptic female choice, or more general, polyandry is a widespread mechanism to avoid incompatible genomes in *Argiope*.

The occurrence of costly ultra short copulations in *A. australis* is puzzling. It is unclear if this mating behaviour is induced by the males or by the females. Why should an individual, either male or female, copulate without gaining reproductive success? Ultra short copulations occurred both in the F1 and F2 generation suggesting that it is a common event during mating in *A. australis*. For males such copulations can mean the loss of half of

their sperm reserve if their pedipalp gets mutilated and additionally they run the risk of getting cannibalised after only a single copulation. Conversely, the female may have a genital opening blocked without having collected sperm in the spermathecae and risks that eggs are not fertilised. The variability we found in the occurrence of USC's in the F2 generation suggests that it could function as a discrimination process between mates. However, from our results we cannot conclude whether USC's form a female reluctance strategy to male mating attempts or if it is simply a failure in genital coupling. The adaptive function of this peculiar mating behaviour requires further investigation.

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## General Discussion

In this thesis I focussed on the polyandrous and monogynous mating system of the spider genus *Argiope*. I found that natural mating rates of male and female *A. bruennichi* were not affected by temporal and spatial selection regimes and that multiple mating of females was largely explained by the frequency of male reproductive strategies (mainly the degree of bigyny) within the focal population. Further I showed that sexual cannibalism offers females a material benefit by increasing the survival abilities of their offspring. From the male perspective this may represent a small paternal investment. Within *A. australis* I found that inbreeding depression is a strong selective agent for the evolution of polyandry and that females use it to decrease these negative consequences of inbreeding.

### Polyandry in *Argiope*

#### a) Natural female mating rates

In Chapter 1 I showed that female mating rates were quite low (1.3) in *A. bruennichi* and that almost 75% of the females in the field mated with only one male. Interestingly, all females except one got mated in the population which differed from the expectation that late maturing females stay unmated. Neither spatial nor temporal patterns influenced female mating rates and the observed cases of polyandry were explained by male mating decisions, especially the occurrence of bigynous mating strategies and a strong male preference for virgin over mated females (Chapter 2, (Gaskett et al. 2004; Andrade and Kasumovic 2005; Schulte et al. 2010)). For *Argiope* females it would be ideal to mate at least with two males in order to enable post-copulatory selection processes (Eberhard et al. 1993; Hellriegel and Ward 1998). However, optimal mating rates of females often differ from what we find in nature because they are limited by male mating strategies and by ecological constraints (Holland and Rice 1998; Wiklund et al. 2001).

#### b) Sexual cannibalism

By experimentally separating the effects of polyandry and sexual cannibalism in *A. bruennichi* I showed that neither of them resulted in increased female fecundity (Chapter 3). This is in accordance with other studies on

sexual cannibalism in spiders high and moderate sexual size dimorphism (Fromhage et al. 2003; Wilder and Rypstra 2010). However, all previous studies focussed on fecundity traits only and did not illuminate differences in offspring quality. In Chapter 3 I showed that experimentally controlled cannibalism by females resulted in improved survival abilities of the offspring under climate and food stress. This finding complements previous results on the value of sexual cannibalism in spiders and is in accordance with a recent study on *A. keyserlingi* (Blamires 2011). Blamires showed that in *A. keyserlingi* cannibalistic females invest more into their egg production than non-cannibalistic ones. Cannibalism induced a higher yolk density in eggs and Blamires suggests that this increased investment may be caused by a physiological trigger. Alternatively it has been proposed that males deliver females essential nutrients and rare trace-elements that females do not receive from their usual prey (Meffe and Crump 1987; Craig et al. 2000). Apparently females gain material benefits through male consumption but the mechanisms remain unclear.

The positive effect of sexual cannibalism was not linked to polyandry and could not be enhanced by the consumption of several males. Contrary to expectations polyandry alone was not beneficial either. Informed by many studies in insects, I expected polyandrous females to benefit either from an increased genetic variation within their offspring (Ridley 1993) or from being able to discriminate between different males based on their genetic compatibility (Bretman et al. 2004; Simmons et al. 2006). Still genetic benefits of polyandry cannot be excluded because the matings we conducted were between non-siblings and supposedly incompatibility between mates was too low to have an impact on offspring fitness. Indeed there is strong indication for the evolution of polyandry as a mechanism of inbreeding avoidance (Chapter 5).

#### c) Inbreeding avoidance

In Chapter 5 I show for *A. australis* that inbreeding imposes large costs on individuals after only a single generation of inbreeding. Incestuous matings resulted in a drastic

reduction of offspring lifespan: inbred daughters had a 50% reduced adult life span while sons were affected less in terms of survival but matured at smaller size. In females a shortened reproductive period directly reduces her reproductive output. In *Argiope* egg production starts immediately after maturation and females built three or more successive egg sacs at intervals of 10-14 days. Inbred females that suffer from a reduced lifespan therefore produce less egg sacs.

The strong impact of inbreeding on female fitness will likely result in strong selection on inbreeding avoidance mechanisms and since pre-copulatory mechanisms are lacking in *Argiope* post-copulatory selection processes may act. Indeed sperm counts revealed a difference between the stored sperm numbers of sibling and non-sibling males at least in the second copulations of females (Chapter 5). Although the data showed only a non-significant trend this still implies the existence of cryptic female choice. It seems as if females discriminate against brothers but only after they secured a large enough sperm amount to fertilise all their eggs. A similar result was found in *A. lobata* where females stored more sperm of non-sibling males if these mated after a sibling male and this resulted in higher P2 values (paternity of the second male) of non-siblings (Welke and Schneider 2009). In conclusion, my results imply that inbreeding is an imminent threat in *Argiope* and that females are able to use post-copulatory selection to avoid the negative effects of inbreeding.

### Monogyny in *Argiope*

#### a) Natural mating rates of males

Theoretical framework on the evolution of monogyny predicts that it evolved in combination with a highly efficient paternity protection mechanism and under a strong male biased sex ratio (Fromhage et al. 2005; Fromhage et al. 2008). I found only a weak male bias in the effective sex ratio in the focal population of *A. bruennichi* (Chapter 1). However, this weak bias seems to be sufficient to favour monogyny in a species with such a highly efficient paternity protection as *A. bruennichi* (>80%, (Nessler et al. 2007; Fromhage et al. 2008)).

Further I found that a strong variation in the operational sex ratio (OSR) during the season

(Chapter 2). This resulted from the different developmental times of males and females and the strong urge of males to mature early in the season (protandry). Protandry enables males to be at the web of sub-adult females and besiege them before they mature. Thereby they increase their chances to mate with virgin females. Based on the assumption that a strong variation in male-male competition and mate availability shapes male mating strategies (Kvarnemo 1996) I expected that frequencies of monogyny and bigyny differ during the course of the mating season. But in contrast I found that both male strategies were present consistently during the season and that more than 50% of the males performed a bigynous strategy. The occurrence of male imposed monogyny (two copulations with a single female) was not influenced by the high degree of competition in the beginning of the season. Moreover, male mating decisions were explained by the quality of the first female they encountered and partly by their future mating opportunities.

Males of all strategies showed a strong preference for virgin females which is highly adaptive because they may completely monopolise her if they get the chance to copulate twice. Males were more likely to copulate twice with the same female if she was heavy, while bigynous males that mated with relatively light females traded-up to heavier females in their second copulation. The preference for heavy females has been found in many insect and spider species and males benefit from mating with heavy and more fecund females (Honek 1993; Vincent and Lailvaux 2006; Barry 2010).

Altogether my findings imply that males make state-dependent mating decisions and base this on a variety of conditions and that monogyny and bigyny can coexist within a population. Both male mating strategies seem to be part of alternative reproductive tactics rather than being genetically fixed.

#### b) Sexual cannibalism

As mentioned before the nutritional value of much smaller males supposedly is very low in highly size dimorphic species. However, in Chapter 3 I was able to show that males do contribute to the quality of their offspring by letting themselves be devoured by the female. Although male consumption enhanced offspring quality this effect was not large

enough to give an overall explanation for the evolution of male self-sacrifice. It is suggested that self-sacrifice has low costs for males if they are lacking future mating opportunities and if mate-guarding is not required to protect their paternity (Andrade and Banta 2002). Both assumptions apply in *A. bruennichi* because they mutilate their pedipalps and leave the broken-off parts as mating plugs inside the female. Thus self-sacrifice benefits monogynous males that successfully monopolised a female and since singly mated females are very common in *A. bruennichi*, paternal investment may be selected for.

### c) Inbreeding avoidance

As shown in Chapter 5 and in studies on *A. lobata* females have the ability to discriminate against incompatible males by the use of post-copulatory choice mechanisms. Therefore males should avoid mating with a sister because incestuous matings may not only result in inferior offspring but further they risk being discriminated against during post-copulatory selection processes (Chapter 5, (Welke and Schneider 2009)). This raises the question how males face the risk of inbreeding especially because of a lacking pre-copulatory inbreeding avoidance in *Argiope*.

In chapter 4 I was able to show that males of *A. bruennichi* that were mated to a sister shortened their copulation duration and thereby increased their survival probability to almost 50%. While males that mated with non-sibling females copulated longer and were more likely to get cannibalised (79%). It appears as if males adapted their copulation duration to the risk of inbreeding and aimed to increase their chances for a second copulation when mated to a sister. This allows males to compensate the costs of inbreeding by continuing their search for a more compatible female. For males that have found a compatible female it seems adaptive to invest everything into one long copulation and sacrifice themselves for the benefit of their offspring (Chapter 3).

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