

Geographical differences in information use and the evolution of mating strategies in *Argiope bruennichi*

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Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

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Zusammenfassung

Die Besiedlung neuer Umwelten kann zu Änderungen in der Phänologie und in der Variation von Fitness-Merkmalen führen und so den sexuellen Selektionsdruck verändern. In diesem Zusammenhang kann der Gebrauch von Informationen über die lokale Umwelt adaptive Paarungsentscheidungen erleichtern und helfen in neuen Habitaten erfolgreich zu sein. Ich habe die Prozesse für informierte Entscheidungsfindungen bei Männchen untersucht und dabei geographische Unterschiede im Genotyp und der sozialen Umwelt mit einbezogen. Zur Erkundung dieses Themengebiets habe ich ein monogynes Paarungssystem gewählt. Hier sollten männliche Paarungsentscheidungen stark auf lokalen Informationen beruhen, da Männchen nur wenige Paarungsmöglichkeiten haben und deshalb Fehlentscheidungen nur schwer kompensieren können.

Als Studiensystem habe ich die Radnetzspinne *Argiope bruennichi* gewählt. Männchen dieser Art sind einem hohen Risiko an sexuellem Kannibalismus ausgesetzt und verstopfen weibliche Geschlechtsöffnungen mit abgebrochenen Teilen des männlichen Paarungsapparates. Durch das Verstümmeln ihrer paarigen Genitalien können Männchen die ebenfalls paarigen weiblichen Genitalöffnungen vor Spermien anderer Männchen schützen, sich dafür aber nur zweimal in ihrem Leben verpaaren. Daraus folgt, dass sich Männchen entweder zweimal mit demselben Weibchen verpaaren können und sich damit 100 % der Vaterschaft sichern (Monogynie) oder die beiden möglichen Kopulationen auf zwei unterschiedliche Weibchen verteilen (Bigynie). Eine bigyne Paarungstaktik kann die Anzahl der Nachkommen erhöhen, hinterlässt aber bei beiden Weibchen jeweils eine Öffnung ohne Paarungspfropfen. Beide Taktiken setzen voraus, dass Männchen die erste Verpaarung überleben. Allerdings überleben nur 50-80 % der Männchen die erste Verpaarung und das Risiko kannibalisiert zu werden steigt mit zunehmender Kopulationsdauer. Männchen können demnach ihre Überlebenschancen verbessern, wenn sie frühzeitig vom Weibchen abspringen. Da die Kopulationsdauer gleichzeitig mit dem Transfer von Spermien korreliert, kann man die Modulierung der Kopulation inklusive des verbundenen Mortalitätsrisikos als extrem hohes Paarungsinvestment verstehen.

A. bruennichi hat ihr Ausbreitungsgebiet vor relativ kurzer Zeit von mediterranen (südlichen) und asiatischen Gebieten nach Nordeuropa ausgeweitet. Im Vergleich zu südlichen Populationen sind nördliche Spinnen kleiner und an eine sehr kurze Paarungssaison angepasst. Während das Paarungssystem von nördlichen Spinnen relativ gut untersucht ist, ist das Wissen über südliche Spinnen noch sehr unvollständig. Um diese Wissenlücke zu schließen, habe ich die *Life History* und männliche Paarungsentscheidungen zwischen beiden Populationen verglichen. Ich habe untersucht inwieweit lokale Information (1) den Zeitpunkt der Geschlechtsreife, (2) das Paarungsinvestment während der ersten Verpaarung und (3) den Gebrauch von monogynen und bigynen Paarungstaktiken beeinflusst. Ich fand über alle Versuche hinweg, dass Männchen beider Populationen überwiegend gleiche Entscheidungsregeln evolviert haben. Männchen wurden in einem ähnlichen Zeitfenster geschlechtsreif, wurden mit gleicher Wahrscheinlichkeit kannibalisiert und haben monogyne und bigyne Paarungstaktiken zu ähnlichen Anteilen benutzt. Im Folgenden erkläre ich, wie Männchen auf unterschiedliche Information reagiert haben.

Erstens fand ich Hinweise darauf, dass Männchen ihren internen Zustand (interne Information) als Indikator zur Evaluierung zukünftiger Paarungschancen nutzen. In nördlichen Populationen, wenden Männchen eine terminale Investmentstrategie an, wenn sie alt geworden sind. Das ist adaptiv, weil mit zunehmenden Alter Männchen häufiger Opfer sexuellen Kannibalismus werden. Ich konnte zudem

zeigen, dass Männchen beider Populationen eher eine bigyne Paarungstaktik gewählt haben, wenn sie eine vergleichsweise gute Körperkondition hatten. Da die Körperkondition und das Männchenalter miteinander korrelieren, habe ich Paarungstests durchgeführt, die beide Effekte entflechten sollten. Die Ergebnisse weisen darauf hin, dass das Männchenalter bei Paarungsentscheidungen eher eine Rolle spielt als die Körperkondition.

Zweitens habe ich getestet, ob Männchen Informationen über die Verfügbarkeit von adulten, virginen Weibchen nutzen, welche sie entweder vor oder während der Paarungssuche erhalten haben. Frühere Studien haben gezeigt, dass Männchen adulte, virgine Weibchen präferieren. Daher habe ich vorhergesagt, dass Männchen ihre Entwicklung als Reaktion auf Weibchenreize verkürzen, um nicht den Zeitpunkt des höchsten Weibchenvorkommens zu verpassen. Darüberhinaus habe ich erwartet, dass eine niedrige Weibchenverfügbarkeit zu einem hohen Paarungsinvestment und zur Nutzung einer monogynen Paarungstaktik führen sollte, da zukünftige Paarungsoptionen knapp sein könnten. In beiden Populationen haben Männchen ihre Entwicklung angepasst, wenn Weibchenreize vorhanden waren. Ansonsten haben Männchen Information über Weibchen nicht in ihre Paarungsentscheidungen integriert. Vermutlich ändern sich die Populationsdynamiken zu schnell, wodurch Informationen über Weibchenverfügbarkeit nicht zuverlässig genug sind um Paarungstaktiken anzupassen.

Drittens habe ich Entscheidungsregeln erforscht, die mit Weibchengröße im Zusammenhang stehen. Vorangegangene Studien fanden, dass nördliche Männchen Weibchen eher monopolisiert haben (monogyne Paarungstaktik), die groß und sehr fekund waren. Ich habe diese Ergebnisse aufgegriffen und untersucht, ob die männliche Partnerwahl auf einen fixen Schwellenwert für Weibchengröße zurückzuführen ist. Dabei fand ich heraus, dass nördliche Männchen eher eine vergleichende Bewertung der Weibchen vorgenommen haben und hierbei vermutlich chemische Reize von unterschiedlichen Weibchen genutzt haben. Ich konnte allerdings nicht endgültig klären können, ob die vergleichende Bewertung einer *Best-of-n*-Taktik oder einem anpassbaren Schwellenwert (*adjustable threshold*) zugrundeliegt. Desweiteren habe ich getestet, ob für südliche Männchen die Weibchengröße ebenfalls für die Partnerwahl relevant ist. Ich fand jedoch keine Hinweise darauf.

Im letzten Schritt habe ich versucht die Ergebnisse der Paarungstests in Zusammenhang mit der *Life history* von nördlichen und südlichen Männchen zu bringen. Ich habe Laboraten von zwei Jahren zusammengesucht, in denen Individuen in juvenilen Lebensstadien gesammelt wurden und unterschiedlichen Fütterungsbedingungen ausgesetzt waren. Ich fand, dass südliche Männchen mehr Unterschiede in Körpergröße und Entwicklungsdauer zwischen den Jahren und abhängig der Fütterungsbedingung aufwiesen als nördliche Tiere. Das lässt vermuten, dass nördliche Individuen Mechanismen evolviert haben, um Umweltbedingungen auszuhalten, die ansonsten die eh schon reduzierte Körpergröße negativ beeinflussen würden. In diesem Zusammenhang, haben Männchen vielleicht auch die Präferenz zum Monopolisieren großer Weibchen entwickelt. Die hohe Plastizität bei der Körpergröße südlicher Spinnen bot vermutlich eine ideale Vorbedingung für die Evolution der Partnerwahl und der Kolonisierung nördlicher Habitate.

Schlussfolgernd lässt sich sagen, dass ökologische Bedingungen einen wesentlichen Einfluss auf *Life-history*-Merkmale haben können, die auch eine Rolle für das Paarungssystem spielen. In einigen Paarungssystemen werden Paarungsentscheidungen an lokale Bedingungen angepasst and führen so zu geographischen Unterschieden in Entscheidungsregeln. Andere Paarungssysteme werden jedoch von stark sexuell selektierten Merkmalen dominiert (z. B. hohes Risiko an Spermienkonkurrenz und sexuellem Kannibalismus), die über ökologische Kontexte hinweg die gleichen Entscheidungsregeln benötigen.

Summary

The colonization of new environments can alter phenology and variation in fitness traits and lead to changes in sexual selection regime. The use of local information facilitates adaptive mating decisions and could also help an animal to prevail in new habitats. I examined informed decision-making processes in males while incorporating geographical differences in genotype and social environment. To explore this topic, I selected a monogynous mating system where male mating decisions should strongly rely on local information as males have only a few mating chances and can hardly compensate bad mating decisions.

I worked with the mating system of the orb-web spider *Argiope bruennichi*, which is characterized by a high risk of sexual cannibalism for males and mate plugging through genital mutilation. By mutilating their paired genitalia, males can secure the paired female openings against rival sperm, but can experience two copulations at most. Males have the option to allocate the two copulations to either one female and secure 100 % paternity (monopolization) or two females (bigyny). Bigyny may increase offspring numbers, but leaves both females with one unsecured genital opening. Both tactics imply that males survive the first copulation, which only applies to 50-80 % of males. The risk of getting cannibalized increases with the copulation duration and males enhance survival chances by jumping off the female at the right moment. Since the copulation duration is connected to sperm transfer, the modulation of copulation and the associated mortality risk can be understood as extreme mating investment.

A. bruennichi had recently expanded its range from Mediterranean (Southern) and Asian habitats to Northern Europe. In comparison to Southern populations, Northern individuals are smaller, and they are adapted to a very short mating season. While the mating system of Northern spiders is well studied, there is only little knowledge about Southern spiders. To close this gap, I compared the life history and male mating decisions between both populations. I investigated how much local information influences (1) the timing of sexual maturity, (2) mating investment during the first copulation and (3) the use of monogynous and bigynous mating tactics. Throughout the experiments, I found that males from both populations evolved mostly the same decision rules. Males matured in a similar time window, were cannibalized with the same probability and used monogynous and bigynous mating tactics in similar proportions. In the following, I explain how males responded to different information.

First, I found evidence that males use their internal state (internal information) as an indicator to evaluate future mating chances. In Northern populations, males applied a terminal investment strategy if they grew old. This was adaptive because as males age, they become more vulnerable to sexual cannibalism. I also showed that males from both populations more likely chose a bigynous mating tactic if they had a relatively good body condition. Since body condition and age are negatively correlated, I conducted mating tests to disentangle both effects. The results indicate that male age is more relevant for mating decisions than body condition.

Second, I tested whether males use information about the availability of adult, virgin females gained before or during mate search. Previous studies found that males prefer to mate with adult, virgin females. Therefore, I predicted that males would shorten their development time in response to female cues so as not to miss the peak of female occurrence. Moreover, I expected that low mate availability would favor high mating investment and monopolization since future mating options may be rare. I found that in both populations, males adjusted their development time to the presence of

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female cues. Otherwise, they did not integrate female information into their mating decisions. It may be that population dynamics change too quickly and make information about mate availability not reliable enough for males to adjust their mating tactics in a reliably adaptive way (or something along these lines).

Third, I explored decision rules related to female size. Previous studies found that Northern males were more likely to monopolize large and highly fecund females. I took up these results and investigated whether male mate choice was based on a fixed threshold for female size. I found that Northern males instead made a comparative assessment for which they probably used chemical cues of various females. However, I could not determine whether the comparative assessment was underlain by a best-of-n strategy or an adjustable threshold rule. I also tested whether female size is a relevant trait for mate choice in Southern males, but found no indication that it was.

In a last step, I tried to relate the results from the mating tests to the life history of Northern and Southern spiders. I gathered laboratory data from two years in which individuals were collected as juveniles and were exposed to different feeding treatments. I found that Southern individuals showed more differences in body size and developmental time between years and dependent on feedings treatments than Northern individuals. This indicates that Northern individuals may have evolved mechanisms to endure environmental changes that would negatively affect their already reduced body size. In this context, Northern males may also have evolved a preference for monopolizing large females. The high plasticity in Southern body sizes may have been ideal preconditions for the evolution of mate choice and the colonization of northern habitats.

In conclusion, ecological conditions often influence life history traits relevant for mating systems. In some mating systems, mating decisions are adjusted to local conditions and lead to geographical differences in decision rules. Other mating systems are dominated by strong sexually selected traits (e. g. high risk of sperm competition and sexual cannibalism) that require the same decision rules throughout ecological contexts.

General introduction

Introduction:

The role of phenotypic plasticity has been widely discussed in the field of evolutionary ecology (Baldwin, 1896; DeWitt, Sih, & Wilson, 1998; Pigliucci, 2010; Waddington, 1953; West-Eberhard, 2003). The expression of alternative phenotypes in different environments is an important condition that permits rapid adjustments and facilitates adaptations to novel conditions including the colonization of new habitats (Ghalambor, McKay, Carroll, & Reznick, 2007; Hendry, Farrugia, & Kinnison, 2008; Lande, 2009; Sih, Ferrari, & Harris, 2011). In this context, behavior has been identified as particularly plastic because it allows immediate responses to prevailing conditions, but can also lead to developmental changes that are sometimes irreversible (S. A. Foster, 2013; Snell-Rood, 2013). The reaction norm of behavior can be relatively broad, but often behavioral plasticity appears in the form of distinct conditional tactics in which, in certain environments or depending on the internal state, one tactic has a higher fitness value than the others (Gross, 1996). While the own internal state (e. g. age, body condition or mating status) is easy to assess, animals need to collect enough and reliable information about external conditions before choosing an appropriate tactic (Schmidt, Dall, & van Gils, 2010). In my thesis, I investigate the basis of informed decision-making processes in a mating context and thereby incorporate geographic differences in genotype and social environment. I selected the sexually cannibalistic orb-web spider *Argiope bruennichi* as a study species. *A. bruennichi* is as an ideal model system because its mating system is well studied and it had a recent range expansion from Mediterranean and Asian habitats into Northern Europe that allows a geographical comparison between original and new populations that experience

different environmental pressures (Krehenwinkel & Tautz, 2013; Schneider, Uhl, & Herberstein, 2015). Moreover, *A. bruennichi* has only evolved a few behavioral variants, which makes the analysis of this complex topic manageable (Fromhage & Schneider, 2012; Welke & Schneider, 2010). In the following, I outline the relevant theory underlying my research question, clarify the suitability of *A. bruennichi* in more detail and close the introduction with my study objectives.

Information use in mating decisions:

To make informed decisions, animals can either collect public information by observing the behavior of others and copying their decisions or use personal information that is gathered by direct interaction with the animals' environment (Dall, Giraldeau, Olsson, McNamara, & Stephens, 2005; Danchin, 2013; Schmidt et al., 2010). In a mating context, information about mate quality, the availability of mating partners as well as the risk of competition is the most relevant because such factors are likely to cause condition-dependent differences in reproductive success (Andersson, 1994; Jennions & Petrie, 1997). Hence, selection should favor the use of cues that provide information about the social environment.

While some mating decisions follow close upon the receipt of information, other plastic responses rely on experience during development and affect future mating decisions (Jennions & Petrie, 1997; Snell-Rood, 2013; Verzijden et al., 2012; West-Eberhard, 2003; West, King, & White, 2003). For instance, "socially cued anticipatory plasticity" is relatively common and describes the special case in which individuals determine the density of males and females by cues of conspecifics

and adjust their development accordingly (Kasumovic & Brooks, 2011). The use of information will always pay off in terms of fitness, but becomes more important the higher the degree of uncertainty (McNamara & Dall, 2010; Sih, 2013). Therefore, even animals with relatively simple neuronal systems make informed decisions based on public or personal information (Johansson & Jones, 2007; Kasumovic & Brooks, 2011; Mery et al., 2009).

Information use in mate-searching male spiders:

Web-spiders have a poorly developed visual system and perceive most of their environment by mechano- and chemo-receptive sensilla that are densely distributed over their whole body, especially on their limbs (Foelix, 2011; Ganske & Uhl, 2018; Tichy, Gingl, Ehn, Papke, & Schulz, 2001). Chemical information can be received through air or physical contact with the medium (Gaskett, 2007; Schulz, 2013). In many spiders, females produce chemical cues or sex pheromones which are directly emitted via the body cuticle or bound to the spider silk (Schulz, 2013). Both the female body and the silk can contain volatile cues that allow males to gather information about a female from a distance (Cory & Schneider, 2016; Gaskett et al., 2004). Volatile cues are involved in the localization of females and can inform males about female receptivity or mating status (Blanke, 1973; Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010; Papke, Riechert, & Schulz, 2001; Uhl & Elias, 2011). Several behavioral studies found that males prefer the chemical profile of unmated over mated females, and chemical assays confirmed that potent sex pheromones are often exclusively found in unmated females (Blanke, 1973; Gaskett et al., 2004; Herberstein, Schneider, & Elgar, 2002; Watson, 1986). In many spiders, males have an advantage in sperm competition if they can mate with a female before rival males (e. g. via mate plugging, first sperm precedence) (T. C.

Jones & Parker, 2008; Uhl, Nessler, & Schneider, 2010). Moreover, some studies found that male spiders altered their development or mating behavior if they were exposed to female cues (Kasumovic & Andrade, 2006; Nessler, Uhl, & Schneider, 2009; Neumann & Schneider, 2016). Hence, the underlying mechanism of several informed mating decisions seem to rely solely on the absence or presence of chemical cues. However, it is less understood whether pheromone emission per se can be actively or passively modulated (e.g. by adjusting quantity) and reveal further information about a female's condition.

Mating decisions in males:

It is commonly accepted that anisogamy (many small sperm cells and few large oocytes) was the driving force in the evolution of sex roles and the asymmetrical distribution of reproductive costs (Kokko & Jennions, 2008; Parker, Smith, & Baker, 1972). While males invest little in parental investment and can improve their fitness by increasing their mating rates, females have to bear a higher parental investment during which they are unavailable for mating (A. J. Bateman, 1948; Trivers, 1972). Thus, sexually active males are often in the majority and have to compete for fertilizations, permitting females to be choosy and to mate with males that promise good genes for their offspring (Andersson, 1994; Fisher, 1930). To convince choosy females, males have to pay high costs, including physical male competition, nuptial gifts and the display of costly traits that honestly signal their quality (Andersson, 1994; Olson & Owens, 1998; Vahed, 1998; Zahavi, 1975). In polyandrous mating systems, male contests are not necessarily decided at the time of copulation, but can continue through sperm competition (Birkhead & Møller, 1998). Here, males compete with sperm numbers and the amount of seminal fluids or special compounds that influence the female milieu (Parker & Pizzari,

2010). The chance to win pre- or postcopulatory competition can be determined by genetic preconditions and flexible mating decisions that are likely if mating conditions are uncertain (Andersson, 1994; Bretman, Gage, & Chapman, 2011).

Mating decisions of males are diverse and can comprise a trade-off between two or more relevant fitness traits. A classic example is the positive relationship between body size and time to grow. A large body size takes time but is associated with advantages in direct male competition (Hunt, Breuker, Sadowski, & Moore, 2009; Jennions & Petrie, 1997). However, if males have an advantage in mating with unmated females, the timing of maturation may be more important than large body size because males must scramble to reach unmated females before rivals do (Blanckenhorn, Preziosi, & Fairbairn, 1995; Danielson-Francois, Hou, Cole, & Tso, 2012). Here, information about mate availability may be valuable for making an optimal decision.

Another common trade-off arises from the limitation in resources (e. g. time, energy, sperm supplies) that males can allocate either to multiple females or, in larger proportions, to a few or even only one female (Harts & Kokko, 2013; Magrath & Komdeur, 2003; Trivers, 1972). By increasing their reproductive investment, males can enhance their paternity share with their current mating partner but may forfeit further mating chances and risk depleting reserves for future mates. The strategy that males should choose often depends on the male's internal state (e. g. male age, body condition or the infection status) (Cluttonbrock, 1984; Duffield, Bowers, Sakaluk, & Sadd, 2017). Males that are old, in poor condition or sick must expect decreased chances to enhance their future fitness, which makes it adaptive to put all resources in the current female (terminal investment strategy) (Candolin, 1999; Duffield et al., 2017; Engqvist & Sauer, 2001).

Although more common in females, it has been recognized that mate choice also occurs in males of several species throughout the animal kingdom (e. g. Amundsen, 2000; Byrne & Rice, 2006; Olsson, 1993; K. L. Reading & Backwell, 2007; Sargent, Gross, & Vandenberghe, 1986; Schwagmeyer & Parker, 1990). Males can express choosy behavior either by mating with preferred females or by asymmetrical distribution of reproductive investment to different females (Bonduriansky, 2001). As an explanation for the evolution of male mate choice, it has been suggested that choosiness is reserved for the sex with the higher parental investment (Trivers, 1972). However, there is also considerable evidence that even in the absence of parental investment, male mate choice can evolve (e. g. Gaskett, 2007; Hoysak & Godin, 2007; Verrell, 1982). Hence, the conditions facilitating the evolution of male mate choice seem to be broader than initially thought (Fawcett & Johnstone, 2003; Johnstone, Reynolds, & Deutsch, 1996; Kokko & Monaghan, 2001). In a previous review, Edward and Chapman (2011) synthesized the conditions predicted for the evolution of male mate choice. They emphasized three conditions in particular.

First, females need to show enough variation in quality traits so that choosy males improve their paternity share in comparison to a random choice. For instance, males can optimize offspring numbers by preferring fecund females, which they can often recognize by the female's body size (Honek, 1993; Marshall & Gittleman, 1994; Olsson, 1993; C. J. Reading, 2007). In systems with sperm competition and an effect of mating order on paternity, female mating status is another relevant choice criterion (Thomas, 2011). While the choice between unmated or mated females is relatively simple, female fecundity may be more challenging to assess and compare.

Therefore, secondly, male mate choice should only evolve if the benefits of choosing exceed

the costs of mate assessment. Costs include energy expenditure and mortality during mate search, lost mating opportunities, and the cognitive investment necessary to discriminate mates of different quality and to make a mating decision (Andrade, 2003; Jennions & Petrie, 1997; Reynolds & Gross, 1990). The assessment of females is further constrained by the spatial distribution of females. In leks or group-living animals, males can simultaneously assess and compare females, which enables males to choose the best female among a group of potential mating partners (best of n rule) (Janetos, 1980; Real, 1990). In contrast, sequentially searching males have to gather information stepwise. Here, a comparative assessment is unlikely to evolve because it demands more time and the ability to remember the position and quality of all visited females (M. L. Head, Jacomb, Vega-Trejo, & Jennions, 2015; Real, 1990). As an alternative, sequentially searching males often use a less costly threshold rule by which they stop mate searching when a female exceeds a fixed quality threshold (Dombrovsky & Perrin, 1994; Mazalov, Perrin, & Dombrovsky, 1996; Real, 1990).

Third, the availability of females needs to be higher than the male's capacity to mate. Herein lies the assumption that males will not evolve choosiness if they have the ability to mate with all females that are available as mating partners (Edward & Chapman, 2011). Several conditions play a role in this equation. While the mate availability is a function of density and operational sex ratio, the male's capacity to mate can be extremely restricted by costs of reproduction such as parental investment or high mating effort (e. g. courtship, ejaculate size, mate guarding, nuptial gifts) (Carroll & Corneli, 1995; Kvarnemo & Ahnesjö, 1996; Parker & Pizzari, 2010; Stoltz, Elias, & Andrade, 2009; Vahed, 1998). In turn, a high mating effort often results from extreme risks of competition. Hence, even in species where males are competitive and females care for

offspring, the mating costs may be high enough to allow the evolution of male mate choice (Fawcett & Johnstone, 2003). Similar conditions apply to several spiders including species where the mating investment of males is so high that males can only mate with a single female during their lifetime (Johnson, Trubl, Blackmore, & Miles, 2011; Waner, Motro, Lubin, & Harari, 2018; Welke, Zimmer, & Schneider, 2012).

The occurrence and evolution of monogyny in spiders:

Monogyny evolved multiple times in the animal kingdom, including in several spider species (Boomsma & Ratnieks, 1996; Pietsch, 1976; Schneider & Fromhage, 2010). Monogynous spiders have in common that females are larger and may cannibalize males at any time during mating, while males mutilate their genitalia in order to plug the female genitalia with broken-off parts of their sperm-transferring organs (Uhl et al., 2010; Wilder & Rypstra, 2008b). Mate plugging is adaptive because the genitalia of female spiders offer a good environment for sperm competition (Elgar, 1998). Females can store sperm of multiple males and additionally, in entelegyne spiders, females possess two genital openings, each of which leads to an independent spermatheca (Uhl, 2000). Males also have paired genitalia (pedipalps) that are secondary and only filled with sperm when males are sexually mature (William G. Eberhard & Huber, 2010). However, after the pedipalps are mutilated, they lose their function. Hence, males are restricted to two copulations during their lifetime (Fromhage & Schneider, 2006; Nessler, Uhl, & Schneider, 2007; but see Snow, Abdel-Mesih, & Andrade, 2006).

Monogynous males copulate once or twice with the same female. Single-mated monogyny is often a result of a successful attack by a cannibalistic female and hence beyond the

male's control (Wilder & Rypstra, 2008b). However, some studies suggest a sacrificial strategy in which a male willingly increases the risk of getting killed (Miller, 2007). Males can increase the risk by either copulating longer and thereby increasing the chance of the female successfully capturing the male or by showing no attempt to escape the female attack (Elgar, Schneider, & Herberstein, 2000; Miller, 2007; Schneider, Gilberg, Fromhage, & Uhl, 2006). In some species, male death after one copulation is obligate and may not even involve a female attack (Knoflach & van Harten, 2001; S. K. Schwartz, Wagner, & Hebets, 2013). Although this phenomenon is well described, it is poorly understood why males should forfeit one of their mating chances and thereby cut their reproductive success in half (but see Knoflach & van Harten, 2001).

Males who survive their first copulation can decide whether they want to stay with the current female (monogyny) or to continue mate searching (bigyny). When males opt for monogyny, they have the chance to plug both female openings and may thereby avoid the risk of sperm competition (Herberstein, Wignall, Nessler, Harmer, & Schneider, 2012; Nessler et al., 2007; Schneider, Thomas, & Elgar, 2001). In contrast, bigynous males can double their mating rates, but at the cost of leaving each female with one genital opening unprotected against rival sperm. Moreover, males that intend a bigynous tactic must also find a second female that is, ideally, unmated because mated females may already be monopolized. In mating systems with a fixed copulatory pattern, even once-mated females involve the risk that the unused and functional pedipalp does not match the virgin opening of the female (Snow et al., 2006; Uhl, Nessler, & Schneider, 2007). Hence, a bigynous mating tactic involves many risks and may make a monogynous mating tactic more adaptive.

A previous game theory model predicted that monogyny could evolve under a male-biased sex ratio because a monogynous tactic can

secure a higher paternity share than a bigynous tactic (Fromhage, McNamara, & Houston, 2008). Bigynous males will have to share paternity and may not find a second mating option, whereas monogynous males have a considerable chance to secure 100 % paternity (Fromhage et al., 2008). Such a scenario may apply to seasonal spiders where males are protandrous and more abundant at the beginning of the mating season than adult females (Elias, Andrade, & Kasumovic, 2011).

The model also predicted that monogynous and bigynous tactics might co-exist, either under negative frequency-dependent selection, or if the use of tactics is condition-dependent (Fromhage et al., 2008). However, only a few empirical studies show evidence for a bigynous mating tactic (Herberstein et al., 2005; Welke et al., 2012). For instance, males of *Argiope keyserlingi* never mate twice with the same female, which they leave after a period of mate guarding (Herberstein et al., 2005; Zimmer, Schneider, & Herberstein, 2014). Another example is the closely-related species *Argiope bruennichi*, where monogynous and bigynous mating tactics co-exist and are adjusted to environmental conditions (Welke et al., 2012). Thus, the mating system of *A. bruennichi* may offer a good opportunity to investigate decision rules for the use of conditional mating tactics.

Study subject:

Argiope bruennichi is widespread in Europe, but it is only in the past 100 years that it has expanded from Mediterranean and Asian regions to the colder countries in Northern Europe (Krehenwinkel & Tautz, 2013; Kumschick, Fronzek, Entling, & Nentwig, 2011). Presumably, the rapid range expansion was promoted by introgression of Asian alleles into the Mediterranean genotype that made rapid adaptations to Northern conditions possible (Krehenwinkel & Tautz, 2013). As a result, individuals from original Southern and recent

Northern populations show significant genetic and phenotypic differences, and some new adaptations in the north possibly altered the mating system (Krehenwinkel, Rodder, & Tautz, 2015; Krehenwinkel & Tautz, 2013). For instance, Northern females are much smaller than Southern females, and body size is correlated with female fecundity in spiders (Calisi & Bentley, 2009; Fritz & Morse, 1985; Kessler, 1971; Krehenwinkel & Tautz, 2013; Simpson, 1995). Furthermore, Northern conditions resulted in high seasonal constraints on reproduction and demanded high synchronicity of maturation (Zimmer, Welke, & Schneider, 2012). The locally-adapted variation in female body size and seasonality may have altered the selection regimes and favored different decisions rules for mating tactics and mate choice in Northern and Southern populations.

While the mating system of Northern populations is well studied, Southern populations are hardly investigated. In the north, the mating season only lasts for 3-4 weeks and shows a strong variation of population dynamics throughout the season (Zimmer et al., 2012). In the beginning, the operational sex ratio is extremely male-biased because males are protandrous (Zimmer et al., 2012). However, sexual cannibalism and *spontaneous male death* constantly take males from the mating pool and cause a female-biased shift towards the end of the season (Welke et al., 2012). Also, late in the season, most females have mated with one or two males and contain the risk of sharing paternity with rival males or even gaining no paternity at all (Zimmer et al., 2012). If males want to secure 100 % of paternity, they need to find an unmated female and block both female openings with mating plugs (Nessler et al., 2009). Monopolization by mate plugging is very efficient but requires that males survive the first copulation and have the chance to block the other side during a second copulation (Nessler et al., 2009). Only 20-50 % of males

have this chance, whereas the remaining males are cannibalized after the first copulation (Fromhage, Uhl, & Schneider, 2003; Nessler et al., 2009; Schneider et al., 2006; Welke & Schneider, 2010). Males can mitigate the risk of dying if they terminate copulations early, which will also reduce the relative number of transferred sperm (Schneider et al., 2006; Schneider & Lesmono, 2009; Welke & Schneider, 2010; Welke et al., 2012). Vice versa, copulations longer than 10 seconds usually end with sexual cannibalism, but ensure that males can allocate half of their sperm load (Schneider et al., 2006). Long copulations are interpreted as a male sacrificial strategy that incurs less fitness loss than if males copulate for a short time and die anyway. On the other hand, males that are successful in surviving can use their whole reproductive potential and either monopolize the current female or search for a second one.

Variation in population dynamics and risks inherent in their unusual mating system might be the driving forces that led to the evolution of conditional male mating tactics and male mate choice in *A. bruennichi* (Edward & Chapman, 2011). Previous studies found that males perceive female mating status and female age (Cory & Schneider, 2016; Schulte, Uhl, & Schneider, 2010). Males prefer virgin, adult females over mated females, and are more likely to visit females close to oviposition, which is a function of female age (Cory & Schneider, 2016; Schulte et al., 2010). Both decisions probably depend on chemical information from the female (Gaskett, 2007). While the preference for virgins can be explained by a discovered sex pheromone that is only produced by adult, virgin females, the mechanisms behind the age-dependent choice are unknown (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010). Males also express their choosiness by adjusting their mating effort to female quality. For instance, it was found that males copulated for a short time and were more likely to adopt a bigynous tactic if the first

mating partner was a sister or small (Welke & Schneider, 2010; Welke et al., 2012).

The conditions that influence the relative frequencies of conditional mono- and bigyny were explored in a state-dependent dynamic game model based on *Argiope bruennichi*'s mating system (Fromhage & Schneider, 2012). It was found that the relative frequency of tactics should depend on variation in female quality, seasonality and the operational sex ratio (Fromhage & Schneider, 2012). More precisely, a bigynous mating tactic should be prevalent early in the mating season when protandry is high and the mating pool largely consists of unmated females (Fromhage & Schneider, 2012). These conditions give males the opportunity to reject a second mating with a small female and to find another mating option. Since original and recent populations differ exactly in traits influencing the frequency of mating tactics, it is worth investigating whether these changes affect the mating decision rules of males.

Study objectives:

The aims of my thesis were (1) to investigate the underlying rules guiding informed mating decisions and (2) to trace whether differences in selection regime resulting from the colonization of different habitats influence those decision-making processes. Thereby, I focused on adjustments in life history traits that are relevant to reproduction and plastic male mating tactics. I explored two different mating tactics. First, I asked which conditions influence the length of the first copulation, including the connected risk of sexual cannibalism. Second, I investigated which conditions prompt males to choose monogyny versus bigyny.

- (1) On what basis do males make decisions that are relevant to their reproductive success?

Internal state:

In **Chapter I**, I tested whether *A. bruennichi* males apply a terminal investment strategy if they become old. For this purpose, I conducted mating tests with males of different adult ages and monitored the courtship duration, the copulation duration and the probability of sexual cannibalism. I predicted that old males would increase courtship duration and prolong the copulation that increases the risk of sexual cannibalism.

Mate availability:

In **Chapters II and III**, I tested whether male mating decisions are based on information about mate availability that males gain prior to or during mate searching. I predicted that high mate availability should favor bigyny, whereas low mate availability should lead to a higher probability of male sacrifice or female monopolization. In **Chapter II**, I manipulated the information about females available to males during mate searching and conducted mating tests in large test arenas in which naïve adult males were exposed to either a high or low availability of adult, virgin females. The high mate availability treatment consisted of four adult, virgin females and the low availability treatment contained only one adult, virgin female and three subadult females that should be chemically invisible for males. I traced mate-searching activity, reproductive investment during the first copulation and the chosen mating tactic of surviving males. In **Chapter III**, I investigated whether experience prior to sexual maturity affects the development and later mating decisions of males. Shortly after males became subadult, I divided them into two treatments in which half constantly received female cues in the form of silk from adult, virgin females, while the other half remained naïve. I expected that the presence of female cues should signal the start of the mating season and prompt males to shorten the developmental time at the cost of

smaller body sizes. After males became adult, I conducted no choice tests, in which I determined the mating tactic of males.

Female quality:

In **Chapter IV**, I investigated whether, in Northern populations, male mate choice is based on a locally adapted threshold for female size. To test this, I made use of the size differences between Northern and Southern populations and expected that males should have evolved a threshold adjusted to the size variation of Northern females, while Southern females should usually lie above the locally adapted threshold. In two treatments, I presented males with three Northern or three Southern females (never mixed) and tracked which of the three females males chose to copulate with first, as well as the male's chosen mating tactic.

- (2) Do males from original and recently-established populations differ in the use of informed mating decisions and phenotypic variation?

Geographical differences in the use of mating tactics:

For my second objective, I explored whether differences in selection regime of Northern and Southern populations led to genetic determinations of decision rules that are adaptive for the population-specific environment. In **Chapter III**, I compared the use of monogynous and bigynous mating tactics in no-choice mating tests, where males had the chance to copulate twice with the offered female or to leave her after one copulation. I expected that Northern males would more often use a bigynous mating tactic because the comparably small body size and the synchronized appearance of adult females offer a good opportunity for males to choose. Also in **Chapter III**, I tested whether Southern males respond to female cues, as has already been found in previous studies with Northern

males (Nessler et al., 2009). Moreover, I compared whether males from different populations respond differently to female cues, although I expected similar adaptations.

In **Chapter V**, I investigated whether female size is a relevant trait for mate choice decisions in Southern males. I used the experimental set-up explained in Chapter IV, but exposed Southern males to three differently-sized females from the same (Southern) population. I expected that Southern males should behave like Northern males and be more likely to monopolize large females. Experiments of Chapter IV and V occurred in parallel, allowing me to compare results from both populations.

Geographical differences in the variance of life history traits:

In **Chapter V**, I looked for differences in developmental plasticity of life-history traits between Southern and Northern populations. During the range expansion, *A. bruennichi* should have undergone adaptations to environmental changes that facilitated their establishment in Northern Europe. I assumed that Northern individuals should all mature at a given time of the year, but simultaneously evolve mechanisms to endure environmental changes that might negatively affect their already reduced body size. In contrast, Mediterranean conditions may allow more variation in phenology and body size, resulting in a higher environmental influence on life history traits. To test this, I gathered laboratory data from two years and compared whether Northern and Southern individuals differed in maturation time and body size between years. Moreover, I introduced a feeding treatment, in which one group received food ad libitum, while the other group was extremely limited in food supply. This was done to test in how Northern and Southern individuals can tolerate unfavorable conditions. I expected that Northern individuals would be more stable in maturation time and body size over the two

years of the experiment, and that this would be independent of feeding treatment. In comparison, I expected that Southern individuals would show more variation overall.

Note:

All chapters should be individually comprehensible, which led to some overlaps of information between chapters. Moreover, the individual chapters may differ in the use of language (British or American) and structure according to specific guidelines of different scientific journals.

CHAPTER I

Influence of male age on risk-taking behavior during copulation in a sexually cannibalistic spider

Anna-Lena Cory, Michelle Metzinger & Jutta M. Schneider

Abstract:

Monogynous mating systems are characterized by a high mating investment of males into a single female and are particularly common in spiders. Here we test whether reproductive investment strategies change with increasing adult age in males of the sexually cannibalistic spider *Argiope bruennichi*. More specifically, we ask whether predictions from aging theory also apply to mating systems where adult males are short-lived and extremely constrained in their mating rate. Male *A. bruennichi* mature before females and do not hunt as adults but search for the webs of virgin females. Males can maximally mate twice, but many fall victim to the aggressive females already during their first mating. Males can reduce but not eliminate this mortality risk by terminating copulation after a few seconds at the cost of less sperm transfer. Alternatively, males could opt for a single long, self-sacrificial copulation and secure a minimum paternity of 50% that can reach 100% because many females do not remate. The latter, terminal tactic is less risky compared to the alternative, in which males have a chance to inseminate both female spermathecae and apply mating plugs that securely protect them against rivals. We tested the hypothesis that old males favor the low-risk, terminal tactic if the chances to survive a first copulation decrease with age. Since age negatively correlates with body condition, we built the same age classes in males that derived either from the field or from our laboratory cultures. Field-collected males have a lower body condition at maturation than laboratory-bred males allowing us to disentangle both effects. Unlike expected, laboratory-bred males with the better body condition were more often cannibalized than field-collected males. Independent of the rearing conditions, young males had a higher probability to survive their first copulation than older ones. As predicted, the duration of the first copulation increase with age and old males less often attempted to escape from female attacks. However, this only applied to old males that had reached an age that hardly exists in nature. Our results agree with theory by showing that the probability of a terminal investment strategy in *A. bruennichi* males increases with age. This is more likely an adaptation to a higher vulnerability during old age than to a poor body condition.

Keywords

Body condition, male sacrifice, monogyny, rearing condition

Introduction:

Life history theory predicts an increased or even terminal reproductive investment once the residual reproductive value approaches zero (Cluttonbrock, 1984; Duffield et al., 2017). A reduction in reproductive value can occur for many reasons, one of which is aging (Cluttonbrock, 1984; Duffield et al., 2017; Ivy, Weddle, & Sakaluk, 2005; Pianka & Parker, 1975). Accordingly, males of species with a high male mating effort should increase their mating investment in the current mate with increasing age. In agreement, many studies found that senescent males intensify sexual signaling, increase parental care, produce larger nuptial gifts or accept higher risks (e. g. Benowitz, Head, Williams, Moore, & Royle, 2013; Duffield et al., 2018; Engqvist & Sauer, 2002; Lafaille, Bimbard, & Greenfield, 2010; Wedell & Ritchie, 2004; Win, Kojima, & Ishikawa, 2013). In contrast, other studies found the opposite namely that senescence leads to a decline in mating performance (Billing, Rosenqvist, & Berglund, 2007; Richard, Lecomte, de Fraipont, & Clobert, 2005). Aim of our study was to test how mating performance differs in young and old males in a mating system in which males can maximally mate twice and are regularly cannibalized by females.

Sexual cannibalism frequently occurs in mantises and spiders where males are significantly smaller than females (Buskirk, Frohlich, & Ross, 1984; Elgar & Schneider, 2004; Wilder & Rypstra, 2008b). Theories about the evolution of sexual cannibalism are diverse and range from a pure foraging strategy of females to a mechanism by which females can control the duration of copulation (Andrade, 1998; Barry, Holwell, & Herberstein, 2008; Elgar et al., 2000; Schneider, 2014). Monogynous mating systems in spiders are generally associated with sexual cannibalism although the contribution of the sexes to male death can vary between stereotyped female attacks and active male sacrifice (Andrade,

1996; Foellmer & Fairbairn, 2004; Steven K. Schwartz, Wagner, & Hebets, 2014; Snow & Andrade, 2004; Welke & Schneider, 2010). Males can mate maximally twice, once with each of their paired mating organs, thereby inseminating both female sperm storage organs (Miller, 2007; Schneider & Fromhage, 2010). The probability with which males survive their first copulation varies among species and under a high risk of sexual cannibalism, survival may be traded off against a prolonged copulation that is often linked to benefits under sperm competition (Andrade, 1996; Fromhage & Schneider, 2005a; Schneider & Elgar, 2001; Wilder & Rypstra, 2008b).

Only a few studies investigated the relationship between male age and sexual cannibalism and found that old males were indeed more often cannibalized (Morse & Hu, 2004; Wilder & Rypstra, 2007). However, in these studies, sexual cannibalism was unrelated to the duration of copulation because it occurred prior to or after copulation (Morse & Hu, 2004; Wilder & Rypstra, 2007). Here, we used a different and unexplored approach and asked whether old males actively increase the risk of sexual cannibalism after their first copulation as a terminal investment strategy. To test this, we used the orb-web spider *Argiope bruennichi*, where males co-determine the time of copulation and thereby influence the probability of getting caught and killed by the female (Nessler et al., 2009; Welke & Schneider, 2010).

In *A. bruennichi*, males are extremely limited in mating rates because the risk of sexual cannibalism is very high and males break off parts of their paired mating organs to plug the genital openings of females (Fromhage et al., 2003; Nessler et al., 2007). After mutilating both genitalia, males become functionally sterile and reach their fitness optimum after two copulations, although most males are already cannibalized after the first copulation (50-80 %) (Fromhage et al., 2003; Nessler et al.,

2009; Schneider, Fromhage, & Uhl, 2005; Schneider et al., 2006; Welke & Schneider, 2010). The risk of getting cannibalized increases with copulation duration. Males that copulate more than 10 seconds will most certainly die but transfer at least half of their sperm load (Fromhage et al., 2003; Schneider et al., 2005; Schneider et al., 2006). Males seem to influence the trade-off between the risk of dying and the copulation duration since males copulate shorter and more likely escape sexual cannibalism if they mate with a sister (Welke & Schneider, 2010). However, even when males copulate short, some risk of dying is still given and will leave these males with smaller paternity chances than sacrificial males.

Besides their copulatory investment, *A. bruennichi* males can influence their fertilization success by the duration of courtship (Schneider & Lesmono, 2009). Schneider and Lesmono (2009) found that double-mated females more likely chose the sperm of males that initiated longer courtship bouts. Courtship behavior can be energetically demanding for spider males, and it may be adaptive to expend the energy on hand for courtship if it is not needed for future reproduction (Cady, Delaney, & Uetz, 2011; Hoefler, 2008; Kotiaho et al., 1998). Therefore, we also tested whether old males attempted longer courtship bouts as a terminal investment.

To test whether males of the species *A. bruennichi* are more likely to opt for a long, sacrificial single copulation (terminal investment strategy) with increasing age, we conducted mating tests in which we determined the courtship duration, the copulation duration and the occurrence of cannibalism of young and old males. Inherent in many aging studies is the problem that age and body condition are often correlated. This is also true for web spiders because males stop to build webs shortly after they became adult and hardly eat (Foelix, 2011). Thus, males will

lose weight with time, and old males generally have a poorer body condition than young males (Foellmer & Fairbairn, 2005b; Nessler et al., 2009). As a method to disentangle both traits, we tested age-dependent mating decisions in males from two origins (laboratory and field-collected) that are known to differ in body condition (Kaae & Shorey, 1972; Rosenthal & Stuart-Fox, 2012; Wilder & Rypstra, 2008a). This is because, in nature, individuals often deal with uncertain food availability, while laboratory-bred individuals are regularly fed with high-premium food leading to a better body condition (Calisi & Bentley, 2009; Pasquet, Toscani, & Anotaux, 2018; Rosenthal & Stuart-Fox, 2012).

In summary, we predicted that old males would invest more time in courtship behavior and more frequently risk their life. The latter should become apparent by a longer duration of copulation resulting in a higher probability of sexual cannibalism. Alternatively, old males may unintentionally die because they are more vulnerable to female attacks compared to young males. Here, we would expect that old males show a higher likelihood of sexual cannibalism independent of how long males copulate. If the general body condition of males has an impact on male mating decisions, we would expect a higher rate of sexual cannibalism and stronger effects of age in field-collected males.

Material and Methods:

Collection:

We tested *Argiope bruennichi* males that were either bred in the laboratory from egg to adults or collected in the field shortly before they were sexually mature. Sexually mature and penultimate males can be easily recognized and distinguished by their secondary genitalia, the pedipalps. While the pedipalp of penultimate males shows an undifferentiated genital bulb, the genital bulb of adult males

consists of distinct sclerite structures (Uhl et al., 2007). Field-collected males originated from four meadows in Lower Saxony and Schleswig Holstein, Germany (Wedel, Lunenburg Heath, Reinstorf, H  hbeck) and were collected in the time between the end of June and the middle of July 2017. Laboratory-bred males came from 36 egg sacs and were the F2 generation of spiders collected in 2015 in Hamburg and its surroundings. All females were laboratory-bred and originated from the same F2 generation.

Maintenance:

Spiders from the laboratory and field-collected males were solitary kept in cups that were upside down and roughened from the inside to facilitate walking for spiders. Depending on the spiders' size, we used cups with a volume between 250 ml and 1 000 l. In the bottom of the cups was a hole that we stuffed with cotton wool. To ensure humidity, we moistened the cotton wool on two days a week. On three more days the cups were sprayed with water from the inside. Twice a week, juveniles and penultimate males were ad libitum fed with

Drosophila spp. that were bred on a special culture medium (Dr. D. Bretz, Birlin-M  hle GmbH, Grenzacher Str. 9, 79618 Rheinfelden). Instead of *Drosophila*, large juvenile and penultimate females received three *Calliphora* spp. To simulate the natural condition, that adult males stop building webs and capturing prey, we removed the food and gave adult males nothing to feed. We checked each day whether individuals molted to sexual maturity and put adult females in Perspex frames (35 x 35 x 6 cm), in which the mating tests took place.

Test groups:

In a fully factorial design, field-collected males and laboratory-bred males were each separated in the age categories "young" and "old". Laboratory studies revealed that most males reach sexual maturity within a period of approximately 13-14 days (Cory & Schneider, 2018a) and we considered males as still young if they became adult within this time frame. Thus, young males had an adult age between 3 and 12 days (laboratory-bred: 7.7+2.3 days; field-collected: 7.5+2.2 days), while old males

Table 1.1: Comparison of test conditions for young (3-12 days) and old (13-22 days) males collected in the field or bred in the laboratory. Two sample t-tests and Two-sample Welch t-tests were used if the data were normally distributed; otherwise, we used a Mann-Whitney U test (MWU).

Field-collected males	N	Test	Test statistics	p
♂ body size	57	MWU	W=458	0.411
♂ condition at maturation	57	Welch t-test	t=0.63	0.532
♀ size	60	t-test	t=0.81	0.419
♀ condition on test day	60	t-test	t=0.26	0.795
♀ adult age	60	t-test	t=0	1.000

Laboratory-bred males	N	Test	Test statistics	p
♂ size	62	t-test	t=0.60	0.553
♂ condition at maturation	62	t-test	t=1.01	0.317
♀ size	62	t-test	t=1.06	0.295
♀ condition on test day	62	t-test	t=0.04	0.968
♀ adult age	62	MWU	W= 550	0.319

were between 13 and 22 days adult (laboratory-bred: 17.5 ± 2.2 days; field-collected: 17.5 ± 2.2). To exhaust the limits of age effects, we extended the age range of laboratory-bred males and introduced a third and independent test group. The group was composed of 17 laboratory-bred males with an age between 24-33 days (median=26 days) that should generally not occur in nature. We tested these males at the same time when we tested field-collected males.

Old and young males were tested under the same conditions (Table 1.1) and on the same day. However, tests with laboratory-bred (excluding males older than 23 days) and field-collected males had to occur at different times because field-collected males became later adult than laboratory-bred males. As a result, laboratory-bred males were mated with slightly younger females (~ 2 days, Mann-Whitney U test, $N=122$, $W=1263.5$, $p=0.0020$), but who had the same body size (Two sample t-test, $N=122$, $t=1.56$, $p=0.1214$) and body condition (Two sample t-test, $N=122$, $t=0.56$, $p=0.5761$) as females that were mated with field-collected males. Nevertheless, we initially included female age into our statistical analysis but found that including female age did not

improve our statistical models. Therefore, we omitted female age in our final analysis.

Male condition in test groups:

To verify that the body condition of old and field-collected males decreased, we determined the residual body condition of all males (residuals of linear regression between weight and fixed body size; Jakob, Marshall, & Uetz, 1996). To do this, we assessed the weight shortly after sexual maturity and on the test day. As an approximation for fixed body size, we used the tibia-patella length of the first leg pair. To obtain the leg length, we dissected the first leg pair of frozen individuals, photographed them under a binocular and measured them with the software Leica Application Suite V4.6 (Leica Microsystems (Switzerland) Limited).

We found that the body condition at maturation (Two sample t-test: $N=119$, $t=5.9738$, $p<0.0001$) as well as the body condition on the test day (Two sample t-test: $N=119$, $t=4.1871$, $p<0.0001$) was better in laboratory-bred males than in field-collected males (Fig. 1.1a). Independent of whether males were reared in the laboratory or collected in the field, young males (3-12 days)

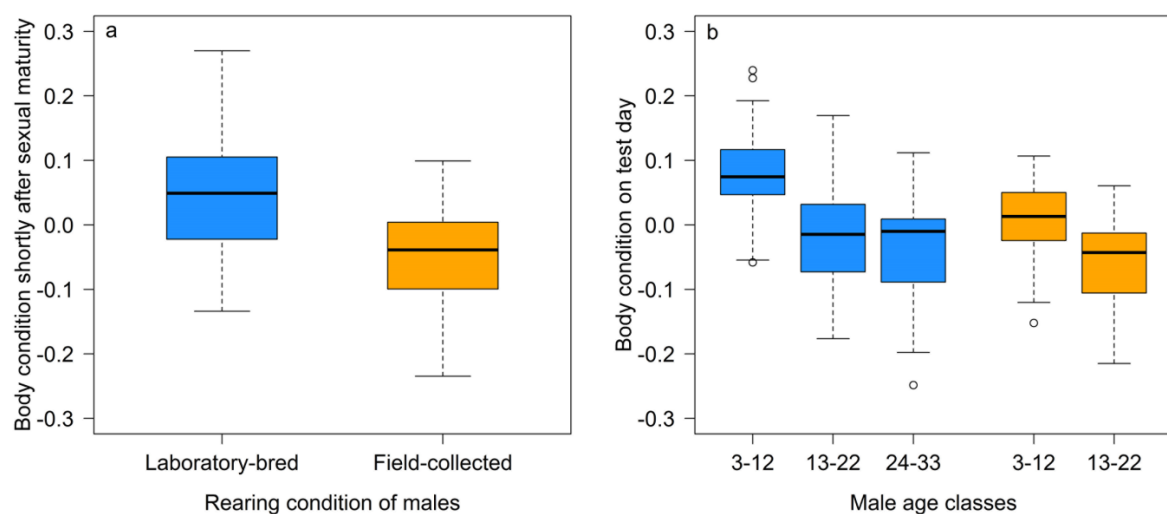


Figure 1.1: Residual body condition of laboratory-bred (blue) and field-collected males (orange). a) Male condition shortly after sexual maturity. b) Male condition on test day separated by male age classes.

had a significantly better body condition than old males (13-22 days) (Two sample t-test: laboratory-bred: $N=62$, $t=-4.6061$, $p<0.0001$; field-collected: $N=57$, $t=-3.4865$, $p=0.0010$). Unexpectedly, the body condition did not further decrease in males older than 23 days (Comparison between age classes “13-22 days” and “24-33 days”: Mann-Whitney U test: $N=48$, $W=241$, $p=0.6387$; Fig.1.1b).

Conduction of mating experiments:

We only used virgin and unrelated males and females for the experiments. The experiment started, when we transferred the test male onto a brush and gently put him in one of the upper corners of the female web. From here on, we noted the courtship duration, the copulation duration, and the occurrence of sexual cannibalism and whether males showed escaping behavior. Escaping behavior can be easily recognized by male movements that often result in the attempt to jump off the female. Only if females removed the copulating male from her genital organs without any male action, we concluded that males were complicit and did not attempt to escape. To measure the copulation duration and the courtship duration we used a stopwatch. Courtship started when the male had contact with the female and ended with the start of the copulation. We stopped the clock if the male did not move for one minute and continued the time measurement when the male moved again towards the female. We terminated experiments if males did not move for one hour. On the same day of the experiment, we froze all test individuals by -80°C for later measurements.

Statistics

We used R (R Core Team, 2018) for data analyses. We performed parametric tests for normally distributed data and otherwise non-parametric tests. For most of the data, we

applied statistical modeling. We generated multivariate models to test effects on courtship duration, copulation duration and the occurrence of sexual cannibalism. As explanatory variables we used the two main male age classes (young: 3-12 days; old: 13-22 days), rearing conditions (field-collected vs. laboratory-bred) and the interaction between both variables. We detected significant effects by a stepwise simplification of the full model (Brabec & Pekàr, 2016).

To test effects on courtship duration and copulation duration, we applied generalized linear models each with a Gamma distribution and used “log” as link function. Testing effects on the copulation duration, we detected one outlier (Outlier Test: $N=121$, studentized residuals=5.5242, Bonferroni $p<0.0001$) and removed a male that copulated for long 98 s (which is a very long copulation in *A. bruennichi*) from the final model. To test effects on the probability of sexual cannibalism, we applied a binary logistic regression.

Separately from the two age classes (young and old), we tested the effects of male age as continuous variable in males older than 23 days. To test in how far courtship duration and copulation duration are influenced by male age, we applied linear regressions for which we had to log-transform both response variables. The analysis of copulation duration revealed inconclusive results because of one influential data point. Therefore, we tested additionally for a potential positive correlation between male age and copulation duration by applying a Kendall rank correlation, which is more stable against outliers.

Results:

Courtship duration and copulation duration:

We compared the pre-copulatory investment of males with an adult age between 3-12 days

Table 1.2: Summary of results including males that were between 3 and 12 days (young) or 13 and 22 days (old). Effects on courtship and copulation duration were tested with a generalized linear model with a Gamma error structure with a log link function; effects on the occurrence of sexual cannibalism were tested with a logistic regression. Estimates and standard errors of the full model are in brackets and the estimates of the minimal adequate model are without brackets.

Courtship duration (N=120)						
Effect		Estimates \pm SE*	F	Deviance	Df	p
Intercept		(6.09 \pm 0.15)				
Lab/field	(field)	(-0.27 \pm 0.21)	0.004	0.004	1	0.9450
♂ age class	(old)	(-0.23 \pm 0.21)	1.255	1.073	1	0.2649
Lab/field x ♂ age class	(field x old)	(0.29 \pm 0.29)	0.055	0.048	1	0.8159
Copulation duration (N=120)						
Effect		Estimates \pm SE*	F	Deviance	Df	p
Intercept		(2.02 \pm 0.13)				
Lab/field	(field)	(-0.11 \pm 0.19)	0.001	0.001	1	0.9711
♂ age class	(old)	(-0.10 \pm 0.19)	0.006	0.003	1	0.9394
Lab/field x ♂ age class	(field x old)	(0.21 \pm 0.27)	0.652	0.345	1	0.4209
Occurrence of sexual cannibalism (N=122)						
Effect		Estimates \pm SE**		Deviance	Df	p
Intercept		0.31 \pm 0.32				
Lab/field	(field)	-0.99 \pm 0.38		6.805	1	0.0091**
♂ age class	(old)	0.93 \pm 0.39		6.015	1	0.0142*
Lab/field x ♂ age class	(field x old)	(0.66 \pm 0.77)		0.716	1	0.3975

*log-transformed; **logit-transformed

or 13-22 days. Males started courtship immediately after they had physical contact with females. Females responded indiscriminately and quickly to male courtship and usually adopted a copulatory position within a minute (34 s median; interquartile range: 13.5-83 s). Males courted for ca. 299.7 s (median; interquartile range: 174-499.0 s) until they copulated between 0.58-98.7 s (median: 6.5 s; interquartile range: 4.8-8.0 s). Neither the courtship duration nor the copulation duration could be explained by male age class, rearing condition or the interaction of both (Table 1.2, Fig. 1.2).

Since we found no evidence for age-dependent pre-copulatory investment in males younger than 23 days, we further assessed the mating investment in males that were between 24 and 33 days adult. Still, male age did not influence

the duration of courtship (linear regression: $N=17$, $F_{1,15}=1.547$, $p=0.2327$), but we found a positive relationship between male age and the duration of copulation (Fig.1.2), at least after excluding a male that copulated 65 seconds (linear regression: $N=16$, $F_{1,14}=4.671$, $p=0.0485$; linear regression including the outlier: $N=17$, $F_{1,15}=2.223$, $p=0.1567$). Due to the inconclusive result, we also applied a Kendall rank correlation and could confirm that the copulation duration increased with male age (including outlier male: $N=17$, Kendall's $\tau=0.391$, $p=0.0360$; excluding outlier male: $N=16$, Kendall's $\tau=0.450$, $p=0.0196$).

Copulation duration and sexual cannibalism:

In the male age range between 3 and 22 days, more than half of the males (55.8 %) were

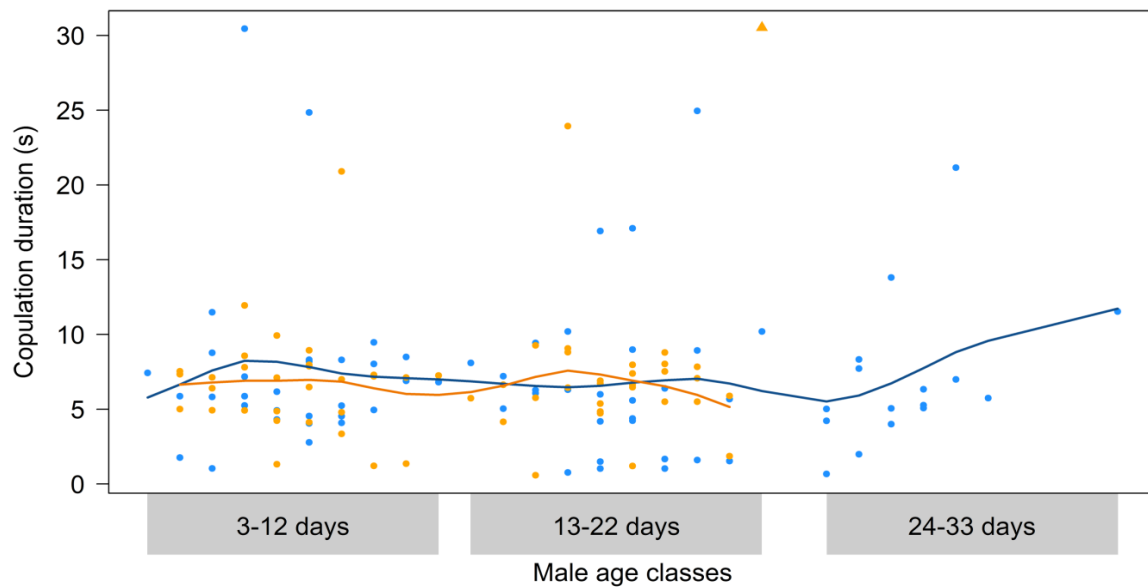


Figure 1.2: The relationship between copulation duration and male age in laboratory-bred (blue) and field-collected males (orange). For the smoothing splines, we used the within R implemented command “smooth.spline” and chose a smoothing parameter of 0.5. We excluded the oldest field-collected male (orange triangle) because his very long copulation led to an extreme increase in the smoothing spline, although there was no statistical effect of male age on copulation duration in males with an age between 3 and 22 days.

cannibalized. With one exception, males always died if they copulated longer than 10 seconds and even if males copulated shorter, the probability of surviving was only 47.3 %. The previously found relationship between copulation duration and sexual cannibalism was, however, only found in laboratory-bred males (Mann-Whitney U test: laboratory-bred: $N=61$, $W=235$, $p=0.0073$; field-collected males: $N=60$, $W=415$, $p=0.6558$). Also, laboratory-bred males were more often cannibalized than field-collected males (laboratory-bred: 67.7 %; field-collected: 45 %; Table 1.2, Fig. 1.3a). In addition to the significant impact of rearing conditions, we found that young males (3-12 days) were less likely cannibalized than old males (13-22 days) (Table 1.2, Fig. 1.3a). However, the probability of cannibalism could not be explained by the interaction between male age class and rearing conditions (Table 1.2).

Among males older than 23 days, all but two were cannibalized, and of the 15 males that died, only three had tried to end the copulation by jumping off the female. We conducted a logistic regression with the occurrence of

escape attempts as response variable and male age as continuous explanatory variable and found that older males were less likely to attempt escapes (Fig. 1.3b, logistic regression: $N=17$, deviance=5.0892, $p=0.0241$).

Discussion:

We hypothesized that old males of the species *Argiope bruennichi* would apply a terminal investment strategy and increase the duration of courtship as well as the time they copulate, which is also associated with a higher risk of sexual cannibalism. While we found no effect of male age on the duration of courtship, older males were indeed more often cannibalized. However, only if males exceeded an adult age of 23 days, the likelihood of cannibalism could be considered a terminal investment strategy and actively provoked by male behavior. Only then, long copulations and fewer escaping attempts could be explained by age and suggest strategic adjustments. Before that, we classify successful cannibalism of old males as a consequence of increased vulnerability. A higher vulnerability can also be a result of a

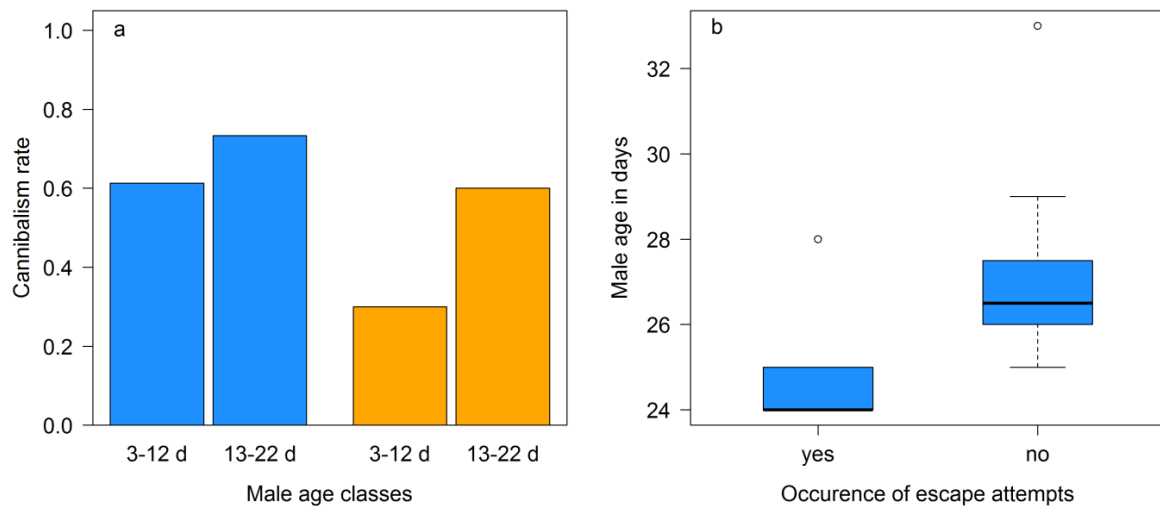


Figure 1.3: The effect of male adult age on the occurrence of sexual cannibalism and escape attempts. (a) Effects of male age on the cannibalism rate in laboratory-bred (blue) and field-collected males (orange). (b) Effects of male age between 24 and 33 days on escaping attempts during copulation.

poor body condition. Indeed male age and body condition were negatively correlated. We attempted to disentangle both variables by using males reared under different conditions. Although field-collected males had a poorer body condition than laboratory-bred males, they courted and copulated similar long and were less often cannibalized. This indicates that the effects we detected are more likely explained by terminal adjustments to senescence than to a poor body condition alone.

In agreement with our hypothesis, considerably old males showed a terminal investment strategy and did not try to escape sexual cannibalism (male sacrifice). By doing this, males were able to copulate longer and could, therefore, increase sperm transfer and paternity (Schneider et al., 2006). Copulations longer than 10 seconds always ended in male death, which is in agreement with previous studies (e. g. Fromhage et al., 2003; Schneider et al., 2005). However, even if males copulated shorter than 10 seconds, the chance of survival was less than 50 %. Thus, males that tried to survive followed a comparably risky strategy as they copulated short but were killed regardless, forfeiting most of their reproductive potential. Our results suggest

that this strategy becomes even riskier with age as males were more vulnerable to female attacks when they grew old. Therefore, it is adaptive if from a certain age on, males switch to a terminal investment and at least use the chance to invest more sperm.

A higher vulnerability of old males was also suggested in the crab spider *Misumena vatia*, where old males were more likely attacked by females and victim to pre-copulatory cannibalism (Morse & Hu, 2004). Similar results were found in the spider *Micaria sociabilis*, in which reversed sexual cannibalism occurs (Sentenska & Pekar, 2013). Here, large males killed old females presumably as response to low prey availability and only under a female-biased sex ratio (Sentenska & Pekar, 2013, 2014). Different to our study, old males of *M. vatia* and *M. sociabilis* were cannibalized as a form of mate rejection, while in *A. bruennichi*, females made no difference between males and always attacked (Fromhage et al., 2003; Morse & Hu, 2004; Sentenska & Pekar, 2013). Nevertheless, these and our results show that old males face an increased risk of getting killed late in life and that the evolution of age-related mating strategies can be adaptive. Unfortunately, only a few studies investigated age-dependent mating decisions in male

spiders. For instance, it was found that males use different courtship displays and are less choosy if they are old (O'Hanlon, Wignall, & Herberstein, 2018; Rundus, Biemüller, DeLong, Fitzgerald, & Nyandwi, 2015). The latter is often explained by the risk that costs of continuing mate searching may exceed the benefits of being choosy (Edward & Chapman, 2011). The same explanation should apply to the question of whether males should increase their reproductive investment or not.

Surviving the first copulation is particularly beneficial if males have the opportunity to copulate with another female that ideally is unmated and signals her location (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010; Schulte et al., 2010). Such a bigynous mating tactic should be favored by young males that are sufficiently fit and have enough time to continue mate searching. In our experiment, young males experienced only a brief period since maturation without encountering any female, while old males had to wait longer which might give the impression of low mate availability. This effect might be reinforced by the short mating season that only lasts for 3-4 weeks (Zimmer et al., 2012). By the end of the mating season, unmated and signaling females are rare and unmated males are likely relatively old (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010; Zimmer et al., 2012). However, the latter explanation should only apply to our test males older than 23 days. Since most *A. bruennichi* males are protandrous, they are usually older than females at the start of the mating season (Zimmer et al., 2012; Chapter V). Hence, males that were between 12 and 22 days may experience the peak of the mating season, whereas males older than 23 days may be indeed too old to find a further mating partner and should apply a terminal investment strategy. Whether the terminal investment of old males is a consequence of old age or a strategic decision needs to be tested in the future.

In web-building spiders, male age and body condition are strongly correlated (Foellmer & Fairbairn, 2005b). Hence, any effects of old age could also be a result of a poor male condition. Several studies found that independent of male age, a poor body condition can have a negative impact on fertility and mating success and may decrease mating effort and mate choosiness (Albo, Toft, & Bilde, 2011; J. S. Gibson & Uetz, 2012; Holzer, Jacot, & Brinkhof, 2003). Since a poor condition reduces longevity and future mating opportunities (Kasumovic, Brooks, & Andrade, 2009), a terminal investment strategy would be an adaptive response to poor condition independent of age (Candolin, 1999; Engqvist & Sauer, 2001). To disentangle effects of male age and body condition, we tested males that were reared under natural and artificial food conditions. Many studies have shown that food quality and quantity affect growth, survival, and reproduction in spiders (Hawley, Simpson, & Wilder, 2014; Kleinteich, Wilder, & Schneider, 2015; Toft & Wise, 1999; Wilder & Rypstra, 2008a). We found that field-collected males had a worse body condition than laboratory-bred males. This is not unusual because food availability is highly variable and uncertain (Anotaux et al., 2012; Anotaux, Toscani, Leborgne, Chaline, & Pasquet, 2014; Rosenthal & Stuart-Fox, 2012). Despite their poorer body condition, field-collected males were less vulnerable to female attacks than laboratory-bred males. A reason could be that those males we collected in the field were particularly fit as they underwent a natural selection process whereas our laboratory-bred males were protected from predation and fed ad libitum. The observed differences between collected and laboratory-bred males suggest that body condition per se is unlikely to be the main factor that explains survival of cannibalism.

In conclusion, our results confirm that even in species where males have very low mating chances, male age is a factor that can influence lifetime reproductive success. In *A. bruennichi*,

the maximal reproductive success is achieved after two copulations, which few males reach particularly if they are older and more vulnerable to female attacks. Hence, it is adaptive for ageing males to adopt a terminal investment strategy that may secure more paternity as a short copulation that still contains a high risk of dying. Our findings could further reveal that we need to be very cautious when we choose our test individuals because

their origin influences the life history and may have a strong impact on behavioural traits including mating decisions.

Acknowledgements

We thank Tomma Dirks and Angelika Taebel-Hellwig for their help with animal husbandry.

CHAPTER II:

Mate availability does not influence mating strategies in males of the sexually cannibalistic spider *Argiope bruennichi*

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Abstract

Background. Sexual selection theory predicts that male investment in a current female should be a function of female density and male competition. While many studies have focused on male competition, the impact of female density on male mating investment has been widely neglected. Here, we aimed to close this gap and tested effects of mate density on male mating decisions in the orb-web spider *Argiope bruennichi*. Males of this species mutilate their genitalia during copulation, which reduces sperm competition and limits their mating rate to a maximum of two females (bigyny). The mating rate is frequently further reduced by female aggression and cannibalization. Males can reduce the risk of cannibalism if they jump off the female in time but will then transfer fewer sperm. An alternative solution of this trade-off is to copulate longer, commit self-sacrifice and secure higher minimal paternity. The self-sacrificial strategy may be adaptive if prospective mating chances are uncertain. In *A. bruennichi*, this uncertainty may arise from quick changes in population dynamics. Therefore, we expected that males would immediately respond to information about low or high mate availability and opt for self-sacrifice after a single copulation under low mate availability. If male survival depends on information about prospective mating chances, we further predicted that under high mate availability, we would find a higher rate of males that leave the first mating partner to follow a bigynous mating strategy.

Method. We used naïve males and compared their mating decisions among two treatments that differed in the number of signalling females. In the high mate availability treatment, males perceived pheromone signals from four adult, virgin females, while in the low mate availability treatment only one of four females was adult and virgin and the other three were penultimate and unreceptive.

Results. Males took more time to start mate searching if mate availability was low. However, a self-sacrificial strategy was not more likely under low mate availability. We found no effects of treatment on the duration of copulation, the probability to survive the first copulation or the probability of bigyny. Interestingly, survival chances depended on male size and were higher in small males.

Discussion. Our results do not support the hypothesis that mate density variation affects male mating investment, although they clearly perceived mate density, which they presumably assessed by pheromone quantity. One reason for the absence of male adjustments to mating tactics could be that adaptations to survive female attacks veil adaptations that facilitate mating decisions.

Keywords

female density, male mating effort, male sacrifice, sex pheromones, sexual cannibalism

Introduction:

Mating decisions of males often reflect the general trade-off between increasing either mating rates or the investment in the current mate (Magrath & Komdeur, 2003; Scharf, Peter, & Martin, 2013; Trivers, 1972). The trade-off results from the risk of losing paternity to rival males, since females are often choosy and polyandrous (Arnqvist & Nilsson, 2000; Jennions & Petrie, 1997, 2000; Parker, 1970). Strategies that secure or optimize paternity gains by increasing their reproductive effort are mate guarding, nuptial feeding, mate plugging, courtship behaviour, male fighting, and ejaculate expenditure (Alatalo, Höglund, & Lundberg, 1991; Andersson, 1994; W. G. Eberhard, 1994; Uhl et al., 2010; Vahed, 1998; Wedell, Gage, & Parker, 2002). However, increased reproductive effort has costs such as the depletion of resources (e. g. the amount of sperm), or the consumption of time and energy (Cordts & Partridge, 1996; Hughes, Chang, Wagner, & Pierce, 2000; Saeki, Kruse, & Switzer, 2005; Sparkes, Keogh, & Pary, 1996). Accordingly, males that allocate more time, energy or resources to one mating partner, risk depleting reserves for future mates and missing further mating opportunities. Hence, the decision whether males should invest in the current or future mating partners should strongly depend on mating opportunities (Kokko & Rankin, 2006; Kvarnemo & Ahnesjö, 1996).

Mating opportunities for males are a function of female density and male competition (Kvarnemo & Ahnesjö, 1996), although many studies focus on the latter. There is ample evidence that males invest more in mate guarding, nuptial gifts, and/or ejaculates in response to a strong risk of male competition (Bailey, Gray, & Zuk, 2010; Carroll & Corneli, 1995; DelBarco-Trillo, 2011; Weir, Grant, & Hutchings, 2011). In contrast, the effects of female density on male mating investment are rarely investigated, and if considered they are often veiled in studies regarding population

densities or sex ratios (Barrett, Evans, & Gasparini, 2014; Gage, 1995; Kokko & Rankin, 2006; Lauer, Sih, & Krupa, 1996; Linklater, Wertheim, Wigby, & Chapman, 2007; Oku, 2009). Studies about sex ratios indeed depict the actual mating opportunities but disregard that under natural conditions, gathering information about mating opportunities may be constrained by the male's inability to perceive social cues. For instance, in mating systems where males are adapted to locate females by female sex pheromones or sex-specific chemical cues (Wyatt, 2014), males may not necessarily perceive sex-specific chemical cues of conspecific males. In this scenario, males are not able to determine the current sex ratio but can assess female density with some accuracy.

Female localization through chemical cues (or sex pheromones) is particularly common in moths, mantises, and spiders (Gaskett, 2007; Greenfield, 1981; Holwell, Barry, & Herberstein, 2007; Maxwell, Barry, & Johns, 2010; Schulz, 2013). Some members of these taxa are known to assess female traits such as body condition or mating status by using chemical cues (e. g. Barry, 2010; S. P. Foster & Johnson, 2011; Stoltz, McNeil, & Andrade, 2007). Furthermore, in several spider species males developed faster if they received volatile or silk-based pheromonal cues produced by females (Cory & Schneider, 2018a; Kasumovic & Andrade, 2006; Neumann & Schneider, 2016). These findings imply that chemical cues are also involved in the assessment of female availability. Other studies suggest that males adjust their mating investment in response to experience with chemical cues produced by females. For example, it was found that males of the cellar spider *Pholcus phalangioides* courted longer if they had experienced silk cues of non-virgin, but receptive females (Hoefler, 2007). However, it is unknown whether males also adjust their mating investment in the current mate immediately on the receipt of chemical information about

female availability. Immediate responses to social information may be particularly relevant in species where the availability of mating partners can be very dynamic throughout the mating season, as is the case in some well-studied orb-web spiders (Foellmer & Fairbairn, 2005a; Zimmer et al., 2012).

In many spiders, males show an extremely high limitation in mating rates because, with each mating encounter, males risk being cannibalized by the female (Elgar & Crespi 1992). For instance, in the orb-web spider *Argiope bruennichi*, 50-80 % of males die after a single copulation (Fromhage et al., 2003; Welke et al., 2012). The causes of sexual cannibalism are still debated and are diverse (Elgar & Schneider, 2004; Schneider, 2014; Wilder, Rypstra, & Elgar, 2009). Some studies have found that sexual cannibalism depends on the hunger state of females and may be an adaptation to food limitation in nature (Berning et al., 2012; Moskalik & Uetz, 2011). Apart from ecological reasons, sexual cannibalism may also be considered a manifestation of sexual conflict (Schneider, 2014). While some studies suggest that sexual cannibalism give females control over mating (Anderson & Hebets, 2017; Schneider et al., 2006), other studies indicate that males actively sacrifice their life (Andrade, 1996; Schneider et al., 2006; S. K. Schwartz et al., 2013). A male benefits from self-sacrifice if his body contributes to the female's fecundity or offspring survival and if paternity security is high (Elgar & Nash, 1988; Welke & Schneider, 2012). However, as with any reproductive effort, self-sacrifice has to be traded off against future mating opportunities and should, therefore, depend on female availability (Fromhage & Jennions, 2016).

Extreme male mating investment including self-sacrifice has evolved independently in some spider taxa limiting males to maximally two mating chances (Schneider & Fromhage, 2010). The limitation arises because their paired genitalia (pedipalps) also function as

mating-plugs in the equally paired female genital openings (Uhl et al., 2010). Mate plugging by genital mutilation may indeed prevent insemination by rivals but limits males to a single use of each pedipalp (Uhl et al., 2010). Accordingly, males can only invest in one (monogyny) or two females (bigyny). A mating system, in which bigyny and male sacrifice behaviour coexist, provides a good opportunity to test effects of female availability because poor mating decisions have severe fitness consequences.

To test predictions about the density-dependent reproductive investment of males, we used the highly cannibalistic *A. bruennichi*, in which males show monogynous and bigynous mating tactics (Fromhage et al., 2003; Welke et al., 2012). Females stereotypically attack males during copulation, but males can reduce the risk of cannibalization if they copulate briefly and try to jump off in time (Fromhage et al., 2003; Schneider et al., 2005; Welke & Schneider, 2010). By decreasing copulation duration, males also reduce the amount of sperm they transfer (Schneider et al., 2006). The trade-off between sperm transfer and mortality risk is further complicated because a considerable risk of mortality remains even after a short copulation (Fromhage et al., 2003; Welke & Schneider, 2010). Accordingly, self-sacrifice after a long copulation (more than 10 seconds) can be considered a comparably low-risk strategy because the outcome is predictable and 50% of the life-time sperm load can be transferred (Fromhage et al., 2003; Welke & Schneider, 2010). On the other hand, surviving males have the chance to either minimize the risk of sperm competition in the first female by plugging the second genital opening of the same female or to continue mate searching (bigyny) (Nessler et al., 2007; Welke et al., 2012). A successful bigynous strategy can enhance the overall fitness of a male, but a single copulation leaves one female genital opening unsecured (Nessler et al., 2007; Welke et al., 2012). Moreover,

bigynous males also need to find another mating partner, which should strongly depend on female availability (Fromhage & Schneider, 2012). Thus, under a low density of females, it may be better to increase the investment in the current mating partner particularly if male competition is high. Strong male competition is generally expected in such mating systems because they are associated with extreme female-biased sexual size dimorphism (females more than twice as large as males; Hormiga, Scharff, & Coddington, 2000; Scharff & Coddington, 1997) and reduced mortality of males during development, leading to a male-biased sex ratio (Danielson-Francois et al., 2012; Foellmer & Fairbairn, 2005a; Fromhage, Elgar, & Schneider, 2005; Fromhage & Schneider, 2005b).

For our experiments, we used males that had no experience with female cues until we tested their mating decisions in response to different levels of female density. To manipulate information about female density, we made use of the fact that in *A. bruennichi* only adult, virgin females produce a sex pheromone that attracts males (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010). We conducted mating tests in which males were exposed to either four adult, virgin females (high mate availability) or one adult, virgin and three penultimate females (low mate availability) that do not produce the sex pheromone. We chose the term “mate availability” instead of “female density” because we used the same total female density for both treatments. We predicted that under low mate availability, copulation duration and the likelihood of sexual cannibalism would be higher than under high mate availability. Since our predictions are based on the assumption that males adjust their mating investment to the chance of finding prospective mates, we also predicted that if mate availability and thus prospective mating chances are low, males would rather stay with the current mate than continue mate searching.

Material and Methods:

Spider husbandry:

We used laboratory-bred *Argiope bruennichi* that derived from 37 egg sacs and whose parents originated from Southern France (Cory & Schneider, 2018a). All spiders were kept in cups that were turned upside down and had a hole filled with cotton wool. Also, the cups were roughened from the inside to facilitate web-building inside the cups. Cup volume (from 50 ml to 1 l) and food provision was adjusted to the spiders' body size. Small spiders received pollen and approximately three dead *Drosophila melanogaster* three times a week. Two days a week, larger individuals received ca. 20 *Drosophila* spp. and large subadult and adult females were fed with three *Calliphora* sp. Spiders were provisioned with water six times a week. Generally, we sprayed water inside the cups; but on days after food provision, we only watered the cotton wool to prevent the prey from escaping. During water provision, we also checked for moults of penultimate males and females and registered the day of maturation. Penultimate and adult spiders can be recognized and distinguished by their genitalia. Males develop a genital bulb on the distal part of each of their pedipalps (Uhl et al., 2007). The genital bulb is undifferentiated in penultimate males whereas it shows discrete sclerotized structures in adult males. Females can be identified by the scape, which is a sclerotized structure covering the epigyne (Uhl et al., 2007). The scape already starts to develop during the penultimate stage but is shorter and less differentiated than in adult females. Furthermore, the scape of penultimate females is covered by a thin membrane and is only exposed after females moult to sexual maturity. Females of both maturation states were put in frames (trapezoidal shape: upper width 14.5 cm, lower width 9 cm, length 13 cm) that could be fixed in our test arenas (see experimental design), which offered males access to the females during the mating tests.

Spiders either died a natural death in the laboratory and/or were frozen by -80°C. Some males were sexually cannibalized during the mating tests, which conforms to the natural situation. We used the tibia-patella length as an index for body size. To measure leg length, we photographed the legs under a binocular and used the measuring tools in the software Leica Application Suite V4.6 (Leica Microsystems ((Switzerland) Limited). In addition to body size, which is fixed after maturation, we also determined the weight shortly after maturation and on the test day with a calibrated scale (Mettler Toledo AB54-S; accuracy 0.1 mg).

Treatment groups:

We tested mating decisions of naïve males with no prior experience with female cues in two experimental set-ups with either a low or high availability of mating partners. In both treatments, males were exposed to four females, but we manipulated the number of pheromone-emitting females.

The low mate availability treatment was composed of one adult, virgin and three penultimate females. We know from previous studies in *A. bruennichi* that adult, virgin females are highly receptive whereas penultimate females are not (Schneider et al., 2006, personal obs A. Cory 2014). Furthermore, subadult females do not produce the sex pheromone that was found on the body and silk of adult, virgin females and that attracted males (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010).

In the high mate availability treatment all females were adult and virgin. The four females in each high mate availability treatment were matched for adult age (we only allowed an age difference of two days) because previous studies found that mate attraction depends on female age (Cory & Schneider, 2016). We ensured that the mean ages of the four females in the high mate

availability treatment did not differ from the ages of the adult, virgin females in the low mate availability treatment (Mann Whitney U test: $N=50$, $W=288$, $p=0.8167$).

While we controlled for the adult age, we were unable to match for body size, which is a common measure of female quality. To minimize potential effects of female quality, we used individuals from Southern France for which previous studies found that female body size had no effect on male mating decisions (Cory & Schneider, 2018a). Males generally mated with the first female they encountered. Body sizes of the females that copulated in both treatments revealed no difference (t test: $N=50$, $df=48$, $t=-0.7504$, $p=0.4567$).

Completion of the treatments required a considerable number of penultimate and virgin adults of similar age so that we had to use some females multiple times. We ensured that we only re-used females that had no contact with males in previous tests. Males and females were always unrelated, but in some tests, two of the four females were siblings.

Test arenas:

The tests were conducted in test arenas (Fig. 2.1) simulating semi-natural conditions in which females were randomly distributed and males could move freely. The floor of the arenas was covered with fresh packing paper, on which males could easily walk. The backend of the arena had an air permeable window through which a ventilator produced a slight airflow to ensure pheromone flow within the arena. The starting point for males was at the

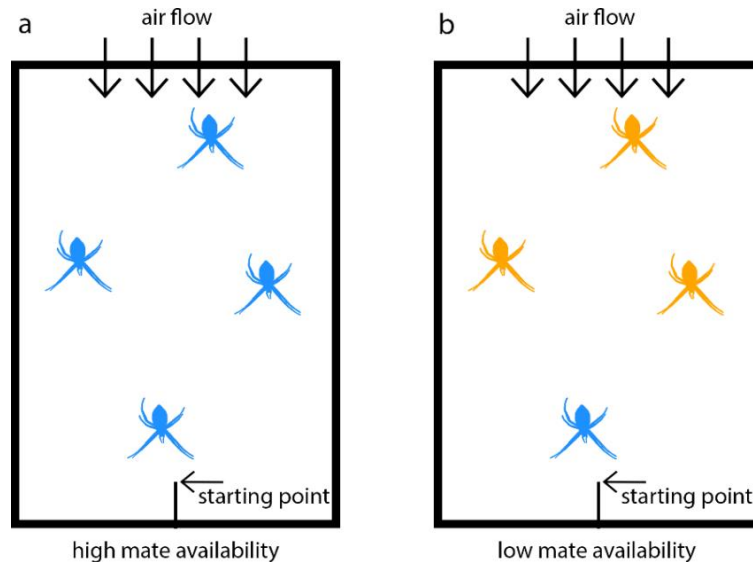


Figure 2.1: Scheme of experimental set-up. Males were placed in test arenas (100 x 70 x 50 cm) and exposed either exposed to (a) four virgin adult females (high mate availability) or to (b) one adult, virgin female and three penultimate females (low mate availability). Adult virgin females are blue and penultimate females orange-colored.

front end in a central position and consisted of a roughened plastic stick positioned with an inclination of approximately 45 °. Each test arena was fitted with the same four applications to fix the frames with the females in identical positions (front, back and two in the middle). In the high mate availability treatment, the four females were randomly positioned on the four spots. In the low mate availability tests, the single adult, virgin female was always located in the front position closest to the male starting point, and the three penultimate females were randomly distributed to the remaining positions.

Test procedure:

Between July 14 and August 16 2016, the experiments took place outside on the roof of the Institute of Zoology of the Universität Hamburg. We monitored the mating tests in up to eight test arenas that were placed underneath a waterproof pavilion roof. In clean and prepared arenas, males were gently placed on the top of the roughened plastic stick (starting point). The time males left the plastic stick was defined as the start of mate searching. Males that did not start their mate

search within 45 minutes were replaced. Males that were caught at the edges of the arena were reintroduced to the starting point and the clock was restarted. Males that ended up in the web of a penultimate female in the low mate availability treatment were given the chance to leave this female for 45 minutes until they were reintroduced to the starting point. However, males that visited penultimate females might also choose an opportunistic mating strategy, in which they mate with females while they moult to sexual maturity and are defenceless (Uhl, Zimmer, Renner, & Schneider, 2015). To take this into account, we analysed our data with and without males that had contact to penultimate females. Since the results did not deviate from each other, we only present the analysis with the total sample size and provide the other results as supplemental material (S1).

During the mating tests, we monitored which female was visited, the duration of copulation and whether the male was cannibalized or not. We also scored whether surviving males copulated a second time with the first mating partner (monopolization) or whether they left the female. We defined that males left the female when there was no contact with the

web or frame of the female and a clear absence of male dragline silk attachments for at least one hour. In such cases, we presumed an intention to continue mate searching and follow a bigynous tactic.

Statistical analysis:

We used R (R Developmental Core Team, 2014) for data analyses. We performed parametric tests for normally distributed data and otherwise non-parametric tests.

Mate searching and localization:

Our predictions rely on the assumption that males estimate the density of adult virgin females via sex pheromone quantity. Thus, mate searching and localization should also be influenced by the number of signalling females. We tested whether latencies until mate search started and the probability of reintroduction after 45 minutes differed between treatments. Several previous studies from our lab found no evidence for sequential male mate choice in *A. bruennichi* (Schulte et al., 2010). We nevertheless explored with binomial tests whether male mate search was directed towards the largest, most attractive female, or to the closest female.

Copulation duration and sexual cannibalism:

We generated multivariate models to test influences of main effects and covariates on different dependent variables. We simplified the model by removing one variable at a time. Afterwards, we compared each simplified model with the original model using likelihood ratio tests. One by one, we omitted all variables that led to non-significant changes of deviance and removed the least-significant variable first. In the end, we kept the model that successfully explained more variation in the data than the other models.

To test whether copulation duration depended on mate availability, we applied a Cox regression model using the package “survival” (Therneau, 2014). As further explanatory variables, we included female size (tibia-patella length), female condition (residuals of the linear regression between female test weight and female size; see Jakob et al., 1996) and the relative weight change of males between adulthood and test day. We used the relative weight change rather than the residual-based male condition that we used for females because earlier studies found a significant effect on male mating decisions (Cory & Schneider, 2018a). In a separate Cox regression model with a reduced sample size (we could not measure the legs of four males), we also tested whether male size affected copulation duration.

To test whether mate availability affected the probability of sexual cannibalism, we applied a binary logistic regression. We used the same female and male traits as above.

Mating decisions in surviving males:

The sample size of surviving males was fairly small, which was why we only conducted a Fisher exact test to explore whether males of the low mate availability treatment would rather stay with the first mating partner than to continue mate searching.

Results:

Mate searching and localization:

In the low mate availability treatment, males started mate searching significantly later than in the high mate availability treatment (high mate availability: median=32 s, interquartile range=184 s; low mate availability: median: 266 s, interquartile range=580 s; Mann-Whitney U test: N=50, W=202, p=0.0328).

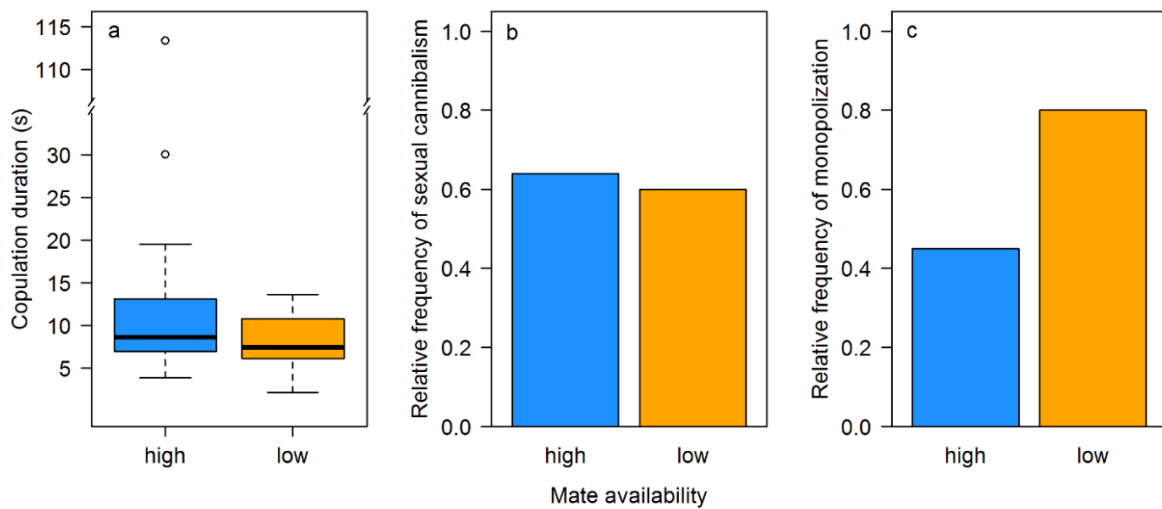


Figure 2.2: Effects of mate availability on mating behavior. Effects of mate availability (high, low) on (a) the copulation duration (Cox regression: $N=50$, $\text{Chi}^2=3.6061$, $p=0.0576$); (b) the relative frequency of sexual cannibalism (logistic regression: $N=50$, $\text{df-deviance}=0.3082$, $p=0.5788$). and (c) the relative frequency of males that survived the first copulation and copulated a 2nd time with the same female (monopolization) (Fisher exact test: $N=19$, $p=0.1698$).

Moreover, we found a tendency that males were more disoriented in the low mate-availability treatments, so that males had to be reintroduced to the starting point after 45 minutes more frequently than males in the high mate availability treatment (high mate availability: 16 %; low mate availability: 56 %; Pearson Chi^2 test: $N=50$, $\text{Chi}^2=3.4286$, $p=0.0641$).

In the low mate availability treatment, 7 of 25 males visited a penultimate female first. A prior visit of a penultimate female had no effect on the rate of cannibalism (Fisher exact test: $N=25$, $p=0.6592$) or on the duration of copulation (Mann-Whitney U test: $N=25$, $W=76$, $p=0.4584$) with the receptive female. In the high mate availability treatment, 48 % of males mated with the closest female in the arena, which deviates significantly from a 25 % chance (Binomial test: $N=25$, $p=0.0178$). We found no evidence that males were more likely to visit the heaviest female (Binomial test: $N=25$, $p=0.4872$).

Copulation duration and cannibalism rate:

Most males (90 %) copulated less than 15 seconds (Fig. 2.2a) and one outlier male from the high mate availability treatment copulated for 113 seconds. The duration of copulation was not affected by mate availability or any other effect that we tested (Table 2.1) regardless of whether the outlier was included or not. Therefore, we kept this male in the final analysis. Unlike in previous studies using different populations from the same species (Fromhage et al., 2003; Welke & Schneider, 2010), copulation duration was not significantly associated with the probability of sexual cannibalism (Mann-Whitney U test: $N=49$, $W=327$, $p=0.3944$). The overall rate of cannibalism was ca. 60 % and did not significantly vary with mate availability (Fig 2.2b; Table 2.2). The only significant predictor of sexual cannibalism was a large body size of males (Table 2.2, Fig. 2.3).

Mating decisions in surviving males:

63.2 % of the surviving males remated and monopolized the first mating partner. While nearly all males (8 of 10) stayed with the first

Table 2.1: Results of the Cox regression model testing effects of mate availability (low, high) on the copulation duration (N=50). The effect of male size was tested in a different model because the sample size was reduced (N=46). The coefficients (coef) and their standard errors (se), and the hazard ratios (exp(coef)) are taken from the full model.

Effect	coef ± se	exp(coef)	Chi ²	df	p
Availability of virgin ♀♀ (low)	0.5743 ± 0.3166	1.7759	3.6061	1	0.0576
♀ size	0.0703 ± 0.1177	1.0728	0.3567	1	0.5503
♀ condition	0.0052 ± 0.0026	1.0052	3.0991	1	0.0783
♂ relative weight change	-2.0859 ± 1.6427	0.1242	1.4882	1	0.2225
Effect	coef ± se	exp(coef)	Chi ²	df	p
♂ size	0.1032 ± 0.2499	1.1087	0.1683	1	0.6816

mating partner in the low mate availability treatment, only four of the nine surviving males monopolized the female under high mate availability. However, the likelihood of monopolization did not significantly differ between treatments (Fig. 2.2c; Fisher exact test: N=19, p=0.1698), which might be due to the very small sample size.

Discussion:

Theory predicts that mating opportunities should strongly influence mating investment (Kokko & Rankin, 2006; Trivers, 1972). In *Argiope bruennichi*, males can increase their mating investment by prolonging copulation duration, which also increases the risk of sexual cannibalism. Due to their short mating season (Zimmer et al., 2012), we expected that males would quickly respond to current information about mate availability. We predicted that under a low availability of adult, virgin females, males would copulate longer and be more often cannibalized than under a high availability of adult, virgin females. Under the latter condition, survival and a bigynous mating tactic would yield the largest fitness return. However, we found no evidence for a strategic adjustment of mating tactic to different levels of mate availability during mate searching.

Contrary to our predictions, neither the probability of sexual cannibalism nor the duration of copulation differed between high

or low mate availability. Also, there was no significant evidence that males would leave the first mating partner after the first copulation and presumably engage a bigynous mating tactic when additional adult virgins were nearby. This was unexpected because low mate availability renders a bigynous mating tactic risky and a self-sacrificial strategy with a single, long copulation should be the safer option to enhance male fitness. Even though frequency differences complied with the expected direction, sample size and power were likely too small to statistically verify the effect.

Despite our failure to detect any effect of mate availability on male mating investment, we suggest that variation in female availability is biologically relevant for *A. bruennichi* males. *Argiope bruennichi* is widespread in Europe and can occupy meadows in high densities (Bruggisser et al., 2012; Krehenwinkel & Tautz, 2013; Kumschick et al., 2011). While the spatial distribution of females may not be a problem, males have to deal with temporal changes in the availability of adult, virgin females (Zimmer et al., 2012). As in many spider species, the availability of adult, virgin females is very low, especially in the beginning of the mating season, and the protandrous males strongly compete for access to the early maturing females (Elias et al., 2011). Moreover, the combination of male scramble competition and low mate rejection in females (Schneider

Table 2.2: Results of the binary logistic regression testing effects of mate availability (low, high) on the cannibalism rate (N=50). The effect of male size was tested in a different model because the sample size was reduced (N=46). The estimates and standard errors (SE) of the estimates are logit-transformed. Brackets show estimates and standard error of the full model and results of the minimal adequate model are without brackets.

Effect		Estimates \pm SE	df-deviance	df	p
Intercept	(Reference level: high availability)	(-3.0123 \pm 2.2115) 0.4895 \pm 0.2914			
Availability of virgin ♀♀	(low availability)	(-0.3185 \pm 0.6160)	0.3082	1	0.5788
♀ size		(0.4500 \pm 0.2861)	2.2874	1	0.1304
♀ condition		(-0.0023 \pm 0.0056)	0.1676	1	0.6822
♂ relative weight change		(-2.6694 \pm 3.8577.)	0.3953	1	0.5295
Effect		Estimates \pm SE	df-deviance	df	p
Intercept		-5.0451 \pm 2.7963			
♂ size		1.2490 \pm 0.6213	4.9700	1	0.0258

et al., 2006) leads to a swift disappearance of virgin females from the mating pool. Thus, the density of adult, virgin females is very dynamic throughout the season, and it should be adaptive for males to integrate social information in their mating decisions. This might be most important for populations in Northern Europe where the mating season is very short. However, in this study, we used spiders derived from Mediterranean populations where seasonal constraints and competition may be less severe. We chose individuals from Southern Europe because unlike in Northern males, their mating decisions are not influenced by female body size (Cory & Schneider, 2018a), for which we could not control in our high mate availability treatment. In addition to this difference, Southern males also respond to female cues and shorten their developmental time to the presence of those (Cory & Schneider, 2018a).

In contrast to our experiment, most studies tested effects of social experience on mating decisions. Here, individuals received social information (e. g. absence and presence of social cues) for a much longer period of time than in our study. It is well established that social information during development can affect life history traits and mating decisions

(Kasumovic & Brooks, 2011; Rodriguez, Rebar, & Fowler-Finn, 2013) such as mating preferences (Hebets, 2003; Rutledge, Miller, & Uetz, 2010; Stoffer & Uetz, 2016). For instance, it was found that females of the wolf spider *Schizocosa ocreata* were more choosy as adults if they had a high encounter rate of males during development (Stoffer & Uetz, 2015). Hence, mate availability might have a strong impact on mating decisions, but individuals may need enough time to process social information.

We argue that male mating investment will strongly rely on information about adult, virgin female availability, particularly in mating systems where males are adapted to locate receptive females by sex pheromones. To account for this, we manipulated the number of adult, virgin females while leaving the density of webs with females constant. We used penultimate females in the low mate availability treatment because they do not produce the pheromone known for adult, virgin females (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010). However, absence and presence of a pheromone and the ability to distinguish do not necessarily mean that males cannot perceive the presence of penultimate females. In the field, males are often found in

the vicinity of penultimate females and are known to copulate with them during their final moult (Zimmer & Schneider, 2016). This is beneficial for males because there is no risk of sexual cannibalism from a freshly moulted mate (Uhl et al., 2015). Accordingly, penultimate females do indeed promise future mating opportunities.

Although many studies found that spider males encounter penultimate females (Bel-Venner & Venner, 2006; Biaggio, Sandomirsky, Lubin, Harari, & Andrade, 2016; Erez, Schneider, & Lubin, 2005; Johnson, 2005), there is ample evidence that males more easily detect adult virgins than immature or mated females (Gaskett, 2007). In many species, including *A. bruennichi*, males recognize adult, virgin females by sex pheromones that are absent in subadult and mated females (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010; Gaskett, 2007; Thomas, 2011). In line with this, behavioural choice tests with *A. bruennichi* confirmed a preference for virgin females (Schneider, Zimmer, Gatz, & Sauerland, 2016; Schulte et al., 2010). Similar results were found in other spider species (Stoltz et al., 2007; Tuni & Berger-Tal, 2012; Watson, 1986). For instance, males of *Stegodyphus lineatus* showed less searching behaviour when presented to immature females than to adult, virgin females (Tuni & Berger-Tal, 2012). Our results agree with this finding. Under the low mate availability treatment, males waited longer until they moved and needed more attempts to find a female compared to males under high mate availability. This suggests that male sensory organs respond to increased quantity of pheromones.

Our low mate availability treatment simulated the beginning of the mating season, in which males could expect future mating chances with adult, virgin females. As the mating season proceeds, future mating chances become increasingly scarce because most females have moulted to adulthood and mated (Zimmer et al., 2012). Thus, we suggest that future studies

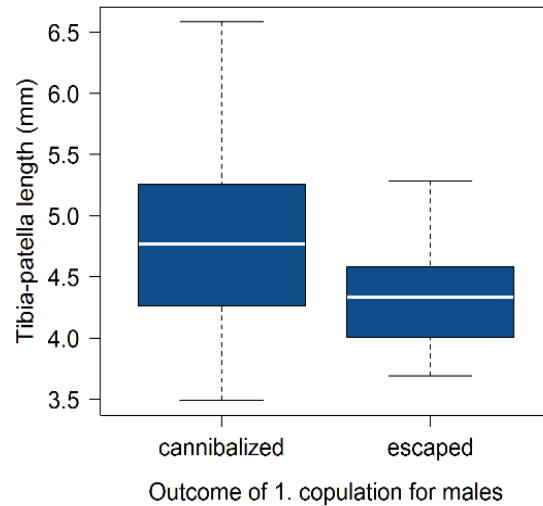


Figure 2.3: Male size effect on sexual cannibalism. Males that were cannibalized by the female during their first copulation were larger in body size than males that escaped the female attack (logistic regression: $N = 50$, df -deviance=4.97, $p=0.0258$).

should introduce a control treatment with double-mated females instead of penultimate females to simulate conditions late in the season. While females that had copulated once still offer mating opportunities, double-mated females have both copulatory openings plugged and in *A. bruennichi*, copulations into plugged genital openings are unlikely to return paternity gains (Nessler et al., 2007). We further suggest a second control-group, which only consists of a single adult, virgin female to test whether males are generally able to assess the presence of females independent of their developmental and mating state.

Another reason for our negative results could be that information about mate availability is not a reliable predictor of fitness, particularly in the absence of information about the degree of male competition. While it seems likely that *A. bruennichi* males can estimate mate availability by female sex pheromones, it is unknown whether males receive cues from other males to make informed decisions. Simultaneous mate choice experiments showed that *A. bruennichi* males do not modify their mate choice decisions in the presence of

silk cues from rival males (Schneider et al., 2016). On the other hand, it was found that males decrease the duration of courtship if a rival male is present in the female web (Schneider & Lesmono, 2009). Similarly, males copulated longer and more likely plugged the female in the presence of a rival in the closely related species *Argiope lobata* (Hirt, Ruch, & Schneider, 2017). We, therefore, propose that future studies with *A. bruennichi* should integrate information on male density either by testing it as a single factor or in relation to the sex ratio.

Contrary to several previous studies on *A. bruennichi*, we could not confirm the well-established positive relationship between copulation duration and cannibalism risk (Fromhage et al., 2003; Nessler et al., 2009; Schneider et al., 2006; Schneider & Lesmono, 2009; Welke & Schneider, 2010). We suggest that this interesting difference may be explained by differences in body size between populations. All previous studies used Northern populations, while we used individuals from the Mediterranean area that are larger and more heterogeneous in body size (Cory & Schneider, 2018a; Krehenwinkel & Tautz, 2013). Since size differences between sexes matter in sexually cannibalistic spiders (Wilder & Rypstra, 2008b), such differences might have affected our results. In contrast to previous studies, we detected size dependent male survival and a benefit of small male size. Observations suggest that larger males were less likely to escape because females were more successful in grasping larger males. A small size advantage of males in avoiding sexual cannibalism was already shown in other studies with spiders (Elgar et al., 2000; Schneider & Elgar, 2001), although the opposite was also found (Elgar & Jones, 2008; Elgar et al., 2000; Persons & Uetz, 2005). Possibly, size related survival differences between males have obscured the relationship between copulation duration and sexual cannibalism.

To our knowledge, this study is the first that tested immediate effects of different levels of mate availability on male investment in a mating system that strongly relies on chemical communication. Although chemical communication is involved in mating interactions of many species, the use of chemical cues is poorly understood (Wyatt, 2014). In comparison, we find many studies on mating systems where males can assess female availability by vision (Barrett et al., 2014; Berglund, 1995; Lauer et al., 1996). For instance, it was found that pipefish males (*Syngnathus typhle*) were only choosy if they were exposed to a high density of females during decision-making (Berglund, 1995). In line with this, a study on social experience in guppies (*Poecilia reticulata*) exposed males visually to a low or high female availability and found that, thereafter, large males showed a higher sexual interest in the current female if other mating opportunities were absent (Barrett et al., 2014). In contrast, water strider males (*Aquarius remiges*), that were housed under different density treatments with either females or males, only adjusted their pre-copulatory mating effort if they had experienced a high density of males before (Lauer et al., 1996). Independent of whether social information was manipulated during or before male mating decisions were tested, this diversity of findings shows that future research should be based on an integrated framework of information about mate perception, mating constraints and ecological conditions.

Conclusion:

Life history theory predicts a trade-off between the current and the future mating effort (Stearns, 2000). Which balance of investment maximizes fitness of males should strongly depend on prospective mating opportunities (Kokko & Rankin, 2006). However, adaptations to adjust investment to mate availability may only evolve if males can gain sufficient information about their social environment.

Little is known about information use in a sensory world based on volatile chemicals particularly in the context of mating strategies. Because of their low maximal mating rate, males of sexually cannibalistic spiders are under strong selection to optimize their investment. More studies on such systems may prove useful to further our understanding of chemical information and how it is integrated into decision making. While several studies found that males gain social information through chemical cues by females (Gaskett, 2007; Greenfield, 1981; Holwell et al., 2007; Maxwell et al., 2010; Schulz, 2013), it is much less clear whether males can detect chemical cues of rival males. More studies are desired that address whether and how males can perceive rivals and integrate competition in their mating decision. For instance, male spiders may assess other males either by chemical cues or by silk threads that males leave behind during mate search. Besides proximate reasons, ecological and behavioural

constraints resulting from the mating system (e. g. mating systems with sexual cannibalism) may also influence adaptations and prevent that males will flexibly respond to mate availability. Here, empirical studies could test whether an interaction between mate availability and risk of sexual cannibalism influences male mating decisions.

Acknowledgements:

We thank Julia Becker, Tomma Dirks, Sebastian Franke, Onno Preik, Angelika Taebel-Hellwig and Leonie Schaum for their help with animal husbandry. We are particularly grateful to Tomma Dirks and Leonie Schaum for their support during the experimental phase. We thank Stefanie Zimmer, Brent Stoffer and two anonymous referees for valuable comments on our manuscript.

Supplemental material:

Results without males that had prior contact to penultimate females:

Table S2.1: Results of the cox regression model testing effects of mate availability (low, high) on the copulation duration (N=43). The effect of male size was tested in a different model because the sample size was reduced (N=40).

Effect	coef	exp(coef) ± se	Chi ²	df	p
Availability of virgin ♀♀ (low)	0.6070	1.8349 ± 0.3445	2.0413	1	0.1531
♀ size	0.1174	1.1246 ± 0.1306	0.8016	1	0.3706
♀ condition	0.0075	1.0075 ± 0.0042	2.3084	1	0.1287
♂ relative weight change	-2.0092	0.1341 ± 1.7564	0.9777	1	0.3228
Effect	coef	exp(coef) ± se	Chi ²	df	p
♂ size	0.1220	1.1130 ± 0.8852	0.1705	1	0.6796

The coefficients (coef) and the exponentiated coefficients (exp(coef)) and their standard errors (se) are taken from the full model.

Table S2.2: Results of the binary logistic regression testing effects of mate availability (low, high) on the cannibalism rate (N=43). The effect of male size was tested in a different model because the sample size was reduced (N=40).

Effect		Estimates ± SE	df-deviance	df	p
Intercept	(Reference level: high availability)	(-2.9879 ± 2.3826) 0.4895 ± 0.2914			
Availability of virgin ♀♀	(low availability)	(-0.3838 ± 0.6530)	0.3402	1	0.5597
♀ size		(0.4750 ± 0.3088)	2.5175	1	0.1126
♀ condition		(0.0003 ± 0.0075)	0.0019	1	0.9653
♂ relative weight change		(-0.8524 ± 4.0854)	0.0437	1	0.8345
Effect		Estimates ± SE	df-deviance	df	p
Intercept		-5.4241 ± 3.0503			
♂ size		1.3399 ± 0.6816	4.6050	1	0.0319

The estimates and standard errors (SE) of the estimates are logit-transformed. Brackets show estimates and standard error of the full model and results of the minimal adequate model are without brackets.

CHAPTER II

Author's contribution to Chapter II:

Chapter II was published in *Peerj*, 6. doi:10.7717/peerj.5360

I took part in conceiving the experimental design (30 %), and I prepared and conducted the experiments. For the manuscript, I made the complete statistical analysis, prepared figures and tables, and wrote the first draft. My supervisor Prof. Jutta Schneider revised the manuscript.

Special notes:

Julia Becker, Tomma Dirks, Sebastian Franke, Onno Preik, Angelika Taebel-Hellwig, and Leonie Schaum helped me to raise my test specimens.

During the experimental phase, I was supported by Tomma Dirks and Leonie Schaum.

Date: _____

Supervisor: _____

CHAPTER III

Effects of social information on life-history and mating tactics of males in the orb-web spider *Argiope bruennichi*

Anna-Lena Cory & Jutta M. Schneider

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Abstract:

Informed mating decisions are often based on social cues providing information about prospective mating opportunities. Social information early in life can trigger developmental modifications and influence later mating decisions. A high adaptive value of such adjustments is particularly obvious in systems where potential mating rates are extremely limited and have to be carried out in a short time window. Males of the sexually cannibalistic spider *Argiope bruennichi* can achieve maximally two copulations which they can use for one (monogyny) or two females (bigyny). The choice between these male mating tactics should rely on female availability that males might assess through volatile sex pheromones emitted by virgin females. We predict that in response to those female cues, males of *A. bruennichi* should mature earlier and at a smaller body size, and favour a bigynous mating tactic in comparison to controls. We sampled spiders from two areas close to the Southern and Northern species range to account for differences in mate quality and seasonality. In a fully factorial design, half of the sub-adult males from both areas obtained silk cues of females, while the other half remained without female exposure. Adult males were subjected to no-choice mating tests and could either monopolize the female or leave her (bigyny). We found that Southern males matured later and at a larger size than Northern males. Regardless of their origin, males also shortened the subadult stage in response to female cues, which, however, had no effects on male body mass. Contrary to our prediction, the frequencies of mating tactics were unaffected by the treatment. We conclude that while social cues during late development elicit adaptive life-history adjustments, they are less important for the adjustment of mating decisions. We suggest that male tactics mostly rely on local information at the time of mate search.

Keywords

monogyny; developmental plasticity; female availability; male mate choice

Introduction:

Plasticity in mating strategies is beneficial under unpredictable conditions, but it requires collecting and processing reliable information (Dall et al., 2005; Ghalambor et al., 2007; Gross, 1996; Schmidt et al., 2010; Sinervo & Lively, 1996; Taborsky & Brockmann, 2010). While some mating decisions immediately follow on the receipt of information, other plastic responses rely on experience during development and have a delayed effect on mate preferences or sexually selected traits (Guevara-Fiore, 2012; Hebets, 2003; Jennions & Petrie, 1997; Snell-Rood, 2013; Verzijden et al., 2012; West-Eberhard, 2003; West et al., 2003). Recent evidence shows that adult phenotypes including mating strategies are affected by the early social environment and suggest that social cues from conspecifics can provide young individuals with information about population densities or prospective dynamics of mates and rivals (Bailey et al., 2010; Clark et al., 2015; Guevara-Fiore, 2012; Kasumovic & Brooks, 2011; Kodric-Brown, 1986; Stoffer & Uetz, 2015).

Estimating the degree of future competition is particularly relevant for males in early life stages if morphological or physiological traits are plastically adjusted. Some studies showed that males reared under high densities needed more time to mature but grew larger or even developed larger testes (Gage, 1995; Kasumovic, Bruce, Herberstein, & Andrade, 2009; Kasumovic, Hall, Try, & Brooks, 2011; Stockley & Seal, 2001). Both traits can improve reproductive success through advantages in either sperm competition or physical male competition (Nylin & Gotthard, 1998). However, the trade-off between time and size at maturation should be balanced in favour of developmental speed in scramble competition mating systems, where more time to grow will be traded against the risk of missing mating opportunities. Here, pheromonal cues produced by females that inform males about the availability of mature females can be highly



Figure 3.1: Picture of a female (large) and male (small) *Argiope bruennichi*, illustrating the extreme sexual size dimorphism. Photo by Anna-Lena Cory

fitness relevant for the timing of maturation and mating strategies (Kasumovic & Andrade, 2006; Neumann & Schneider, 2016). For instance, studies on pipefish and guppies showed that male choosiness vanishes under low female availability (Barrett et al., 2014; Berglund, 1994). Accordingly, we expect that female cues may not only affect the ontogeny but also later mating decisions such as mating rates and male mate choice.

Here we are concerned with male responses to social cues related to female availability in mating systems with extreme male mating investment. Mating systems in which males invest in monopolizing paternity with one female at the cost of future mating have been shown to evolve under a male biased sex ratio (Schneider & Fromhage, 2010). Such monogynous mating systems are rare but evolved several times independently in spiders (Schneider & Fromhage, 2010). Monogynous spider males plug female genital openings with parts of their genitalia (pedipalps) which

cannot be reused and thereby limit their mating frequencies drastically (Fromhage & Schneider, 2006; Knoflach & van Harten, 2001; Masumoto, 1993; Nessler et al., 2007; Schneider et al., 2001; S. K. Schwartz et al., 2013). Since males have two pedipalps, they can achieve a maximum of two copulations. Females have two independent genital openings each connected to a different spermathecae but a male can only inseminate and plug one during any copulation. Accordingly, a virgin female that receives only one copulation retains her virginity on the unmated side. While some species practice obligate monogyny and always invest maximally in a single female, males of other species may also mate with two females (bigynous mating tactic) (Fromhage et al., 2008). Fromhage and Schneider (2012) investigated the conditions under which each mating tactic is preferred using a state-dependent dynamic game model. Model assumptions derived from the well-studied spider *Argiope bruennichi*, a sexually dimorphic species (Fig. 3.1) with high rates of sexual cannibalism and very effective mate plugging by genital mutilation (Fromhage et al., 2003; Nessler et al., 2007). The model revealed that both mating tactics can stably co-exist and that bigyny should increase in frequency under a high density of virgin females that vary in quality, which may be the case early in the mating season (Fromhage & Schneider, 2012). Thus, monogyny versus bigyny is also a form of mate choice whereby bigynous males do not maximally invest in females of low quality but search for a second mating partner. The bigynous tactic involves the risk of losing paternity to rivals that inseminate the still virgin sides of both females. Despite high densities, bigynous males risk failure of finding a second virgin mating partner because mated females that are already plugged offer no paternity gains anymore. Hence, adult males will benefit from information about the availability of virgin females rather than general female density, and the perception of

mating partners should affect their mating tactic.

In this study, we specifically address whether the perception of virgin female availability before maturation affects male life-history and mating decisions. Spiders, including *A. bruennichi*, are an ideal system to test responses to cues because females produce volatile sex pheromones that are emitted from the female body and their silk (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010; Gaskett, 2007; Schulz, 2013). These chemical cues can be easily applied, and males perceive them from a distance. In many spiders, females stop signalling and lose receptivity after they have mated (Thomas, 2011). Also in *A. bruennichi*, the sex pheromone is only found in virgin females (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010) and in line with this, males prefer virgin over mated females (Schulte et al., 2010). Thus, males benefit from anticipating the period when the density of virgin females is highest. The mating season lasts for nearly a month, although most mating events occur within two weeks when the majority of females moult to maturity (Zimmer et al., 2012). Males start maturing earlier than females and soon leave their webs to actively search for females. They can maximally copulate twice and generally die during their second copulation, although many do not survive their first copulation due to female attacks (Schneider et al., 2005). These spiders can occur in high densities and virgin females access quickly to mating. Accordingly, population dynamics can quickly change during the mating season and males should be very sensitive to female cues to not miss the optimal timing for mate search.

Argiope bruennichi experienced a recent range expansion from Mediterranean into Northern Europe. To account for potential differences in ecology and life-history that may have led to local adaptations and also may have affected decision rules underlying mating tactics, we compared individuals from Northern and Southern Europe representing the edges of the

species distribution (Krehenwinkel et al., 2015; Krehenwinkel & Tautz, 2013). Northern males experience a shorter season, which presumably synchronises maturation and makes female availability more predictable, but restricts the time for growth in both sexes. Indeed, Northern individuals are smaller than individuals from the south (Krehenwinkel & Tautz, 2013), and the females produce fewer eggs because fecundity is a direct function of body size (Marshall & Gittleman, 1994; Simpson, 1995). The reduced female quality, as well as the higher seasonality in the north, should have selected for higher rates of bigyny in Northern males. During a field study in Northern Germany, the frequency of bigyny was estimated to be 50 % (Welke et al., 2012). Should bigyny be affected by female size and by unpredictable female availability, we expect lower rates of bigyny in Southern males.

To test our predictions, we sampled egg-sacs or recently hatched spiderlings from different locations in Europe and raised them under standardized conditions. Half of the male spiders received silk from virgin females as cues, while the other half got no information. To receive quantifiable life history traits, we assessed the time that males needed for their last instar and measured the body size of males shortly after maturation. After reaching sexual maturity, males were allowed either to mate twice with the same female (monogyny) or to leave the female after the first mating (bigyny). To sum up our hypotheses, we predict that without information through pheromonal cues produced by females (1a) Northern males need less time to develop because they are smaller, and that (1b) they are more often bigynous than Southern males because females are of lower quality and the high seasonality should reduce uncertainty about female availability. In the presence of female cues during development, we hypothesize that (2a) both, Northern and Southern males, have a shorter developmental time compared to uninformed males. Furthermore, we hypothesize that (2b) informed males engage more often in a

bigynous tactic. If the frequency of bigyny is influenced by female cues, it might also inform males about the opportunity to choose. Thus, we predict that (3) informed males are choosier, which should become apparent through an interaction between the exposure of female cues and female quality such that there is an effect of female size on the mating tactic.

Material and Methods:

Collection and maintenance:

We collected spiders from two different geographical areas in Northern and Southern Europe between April 25th and May 18th, 2015. In the Northern area, we collected 26 egg sacs shortly before the spiderlings left their egg sac at four different locations (Northern Germany: Dahlenburg, Wedel, Lüneburg, Buxtehude). In the Southern area, we collected young juvenile spiders having had one or two moults outside their egg sacs. The Southern spiders also came from four different locations (Belfou, Carcassonne, Villefloure, Gardie/Saint Hillaire). *Argiope bruennichi* is abundant in Europe and the collection of these spiders required no permits.

In the laboratory, spiders were raised under ambient temperatures in upturned plastic cups of different volumes depending on the spiders' size. We provided them with water six times a week. Twice per week, juvenile spiders and males received approximately 20 *Drosophila* spec. and large females received three *Calliphora* spec. We checked for moults to subadult and adult stage at least six times a week. The subadult stage is the last developmental stage before the final moult. Subadult and adult males can be recognized by their secondary genital organs, the pedipalps. In subadult males, the distal part of the pedipalps is thickened and only shows

differentiated sclerite structures if males are sexually mature. Adult females can be recognized by inspecting their genitalia for the presence of the scape which covers the genital openings (Uhl et al., 2007). Adult females were transferred into Perspex frames (approx. 35 x 35 x 6 cm) for building orb webs in which the mating tests were conducted. About half of the males died during the mating tests because of sexual cannibalism or spontaneous male death, the others mated only once and died of old age. Females either died naturally in the laboratory or were frozen at -80 °C after they had laid two egg sacs which is the average number in the laboratory (Schneider et al., 2005).

Provision of female cues:

Shortly after reaching the subadult stage, males were split into two treatments of which one was sheltered from female pheromones inside a walk-in climate chamber (Southern males: N=44, Northern males: N=57). The other half was kept in an identical climate chamber and received female cues (Southern males: N=45, Northern males: N=59). Both climate chambers had a photoperiod of 14:10 LD, a temperature of 23 °C and 50 % humidity at daytime and 20 °C and 70 % humidity at night. Female cues were given in the form of silk taken from the web of a virgin female. Silk was wrapped around a self-made construction (*silk carrier*) consisting of a raw plug and an adjustable nut (Fig. 3.2). With the head of the *silk carrier*, we wrapped silk of the sticky spiral that was located between two adjacent radii. Each male cup was equipped with a *silk carrier* including the males without female silk to control for undesired effects caused by the *silk carriers* themselves. We ensured that males had regular contact with the silk, at least with their forelegs and pedipalps, which are known to be important for the reception of sex pheromones (Jiao, Chen, Du, Chen, & Liu, 2011). Depending on environmental conditions pheromones lose their activity which can be

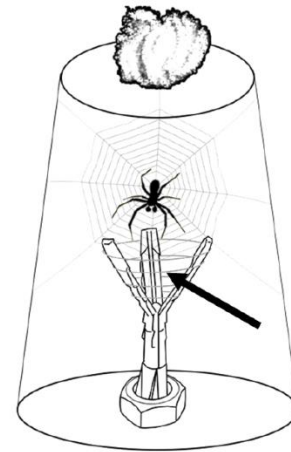


Figure 3.2: Scheme of a juvenile male in a typical upturned plastic cup and a silk carrier. Silk from a virgin female's web is wrapped around the top of the silk carrier (dark grey lines, arrow). The cups had a hole in the bottom fitted with cotton wool which was regularly moistened with water.

after a few weeks but also after a few days (Baruffaldi, Costa, Rodriguez, & Gonzalez, 2010). To ensure that males had constant access to female pheromones, we replaced the *silk carriers* of both treatment groups three times per week with 1-2 days between replacements. All males received female cues from both Northern and Southern females. Females were virgins and not older than 16 days post maturation, and males received cues of the same female maximally twice. After reaching maturity, males received female cues or empty *silk carriers* for six more days. After that, males from the treatment with cues of females were transferred to the climate chamber with the males without female cues and stayed there until they were used for mating tests.

We used silk of virgin females that were born and raised in our laboratory. The parental lineage of these females was collected in 2014, mostly at the same locations as in 2015. Females that provided silk were not used for mating tests. Since females are larger than males, they need more time to develop. To constantly have adult females for silk provisioning at hand, we opened some egg sacs already in March (instead of waiting for natural

hatching in May) and transferred large juveniles to a cabinet with a temperature of 27° C at daytime and 20° C at night and a photoperiod of 14:10 LD with long days to speed up their development. Adult females were kept in the same climate chamber, in which the males receiving female cues were raised.

Mating tests:

The mating tests were conducted in spatially divided shelf compartments (55 x 75 x 67 cm) that were only open at the front to keep potential pheromone transmission between mating tests as low as possible. The front had to be open to be able to interfere during copulation (see below). The experimental set-up consisted of three Perspex frames. The test-frame with a female in her web was positioned in the front, and two frames with empty webs were positioned next to the test frame at an angle of 90 degrees, forming a U-shape. Thus, males could escape from the test frame into an empty and safe frame in two directions.

Males of both geographical origins were randomly chosen from both treatments to mate with females of the same geographical origin. A mating trial began by placing a male gently into the left or right upper corner of the female web with a paintbrush. From this corner position, males would enter the web and signal their presence and reveal themselves as a mating partner (Wignall & Herberstein, 2013). Our main interest was to determine whether males would use a monogynous or bigynous mating tactic. However, male mating decisions are often veiled due to female aggression and sexual cannibalism. If males died after the first copulation, we could not be certain whether the males sacrificed themselves or whether they were not fast enough to escape the female attack. We, therefore, prevented sexual cannibalism after the first copulation following a standardized scheme. In *A. bruennichi*, the

copulation is short but easy to recognize because the female bends over and pulls silk out of her spinnerets within the second of genital contact. During the tests, we intervened two seconds after this and quickly placed a toothpick between the female chelicerae, giving the males a chance to escape. Although we blocked the female chelicerae, females still tried to wrap males into silk. In the process of freeing themselves from the silk wrapping males regularly lost legs as common in undisturbed copulations. Males that lose 1-3 legs can still accomplish further mate search and a second copulation, but we excluded four tests in which males had lost all four forelegs. We further excluded three Southern males and five Northern males that did not try to escape from the female and died during the first copulation. All other males survived and did not copulate longer than known from other studies: 91.5 % of males copulated less than 10 seconds (N=106), which is the threshold after which males are generally cannibalised (Fromhage et al., 2003; Nessler et al., 2009). Note that we measured the copulation duration while we disturbed the female attack. Since these measures might not be accurate, we did not use them in the final statistics, although exploratory statistics showed no influence. After the first copulation, we monitored whether the males copulated again (monogyny) or whether they left the female (bigyny). We defined 'leaving a female' when a male left the test frame, moved a distance of at least 18 cm (half frame length) and stayed away for at least one hour. We also checked that the male had no contact-thread to the female web. Some males left the shelf compartment, forcing us to interrupt the trial to recover the male. Experiments were conducted between 9 am and 5 pm after which the shelf-compartment was sealed until the next morning. The mating strategy of single-mated males that had not re-mated during the first day could be assessed by inspecting the set-up on the next morning: A male was considered monogynous if his body remains

were found underneath the female web which is good evidence for sexual cannibalism after copulation. A male was considered bigynous if he had not made contact to the test-female's web (no draglines visible) but was sitting in one of the other frames. We excluded one male because he died the next day, which might be a sign of injury.

Measurements:

Adult males and females were weighed to an accuracy of 0.1 mg on the day of sexual maturity and again on the test day, which was between 13-25 days (median=18 days) after reaching maturity. We measured the tibia-patella length of both forelegs from frozen females and used leg size as an approximation for the overall spider size. To estimate female condition, we used leg size as an approximation for the overall body size (e. g. Higgins, 1992) and computed the residuals of the regression between leg size and test weight. We refrained from measuring leg length in males because some monogynous males were cannibalised over night by the female so that no body parts were left to measure which would have led to a reduced sample size. Male body size and body weight at maturation are highly correlated (Schulte et al., 2010) and we, therefore, used weight as a proxy for size at maturity. Without the leg length of males, we could not estimate the male condition in the same way as we estimated the female condition. Instead, we used an approximation for changes in male energy reserves and calculated the relative weight difference between adult weight and test weight, which was generally negative because males rarely feed after reaching sexual maturity.

Statistical analysis:

We analysed our data within the R environment (R Developmental Core Team,

2014). To test for differences in the homogeneity of variance between groups, we performed F tests and Levene tests (part of the package "car")(Fox & Weisberg, 2011). Since we had to ignore potential dependence structures these results should be interpreted with some caution. Moreover, we performed statistical modelling to test for population differences and for effects of female cues on developmental time and male mating tactics. Effects of female cues were analysed for both geographical origins separately. We used the R-package "fitdistrplus" to evaluate error structures and log-transformed the data to have a normal error structure if necessary (Delignette-Muller & Dutang, 2015). To test our models we used the R-package "nlme" (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2013). We simplified the models by removing fixed variables using the anova command (maximum likelihood methods) and compared the prior model with the simplified model.

Developmental differences between individuals of different geographical origin:

We compared the duration of the subadult stage (in days) and the male adult weight of Northern (N=116) and Southern males (N=89) by performing linear mixed effects models with "geographical origin" as fixed factor. We included "collection site" as a random factor because we found differences in the duration of the subadult stage between the four collection sites in each geographical origin (Kruskal-Wallis test: Southern males: N=89, $\chi^2=12.44$, $df=3$, $p=0.006$; Northern males: N=116, $\chi^2=10.47$, $df=3$, $p=0.015$).

If Southern males experience a more variable mating season as we assumed, the timing of male maturation might also be more variable and less synchronous in Southern compared to Northern populations. To test this, we conducted a linear mixed effect model and used as response variable the date of the first adult Northern or Southern male as the

starting point of the mating season and counted the days that passed between the starting points and the adult dates of all males. As explanatory variables, we included the “*geographical origin*” as fixed factor and the “*collection site*” as random factor.

Effects of female cues on developmental time:

We constructed models in which we tested whether the reception of female cues had a significant effect on male developmental time (here duration of the subadult stage). In addition to the reception of females cues (yes, no), we used the adult weight of males as a continuous explanatory variable because a prolonged subadult stage may lead to a larger body size. For the analysis of Southern males, we included “*collection site*” as a random factor. Remember that we collected egg sacs in Northern populations and, thus, had to use siblings. Therefore, we included the “*family lineage*” as a random factor in the model with Northern spider data. Since we analysed both populations separately, we also checked whether in Northern males the “*family lineage*” had an effect on the duration of the subadult stage and indeed found differences in the developmental time between families (only families with at least three replicates were included: Kruskal-Wallis test: $N=105$, $df=17$, $\chi^2=41.904$, $p=0.001$).

Effects of geographical origin and female cues on male mating tactics:

To test whether the addition of female cues during the subadult stage would influence later mating decisions, we conducted binary logistic models with the two mating tactics (bigyny, monogyny) as the response variable. We did not use “*collection site*” or “*family lineage*” as a random factor because, in both populations, the estimate of the variance explained by these random factors was 0. As explanatory variables, we included reception of female

cues, female size and body condition, the relative weight change of males, female and male post-mature age, and the duration of the subadult stage. Moreover, we included into the model the interaction term “*female cues : female size*” to test whether the reception of female cues would influence male choosiness. Additionally, we considered the interaction between *female cues* and *relative weight change in males* because we found a significant influence of male condition as a single variable on mating decisions.

We had to exclude three tests because we could not measure the legs of two females and found a measuring error in one male. In the end, we had a sample size of 50 Northern males with 25 males in each treatment group and 56 Southern males of which 26 males received female silk.

To test whether males of both treatment groups had similar conditions during the mating tests, we tested for differences in the explanatory variables between the treatment groups within the model. After we had to reduce the sample size, we found in Southern males, that the males receiving female cues had a shorter subadult stage (Mann-Whitney U Test: $N=56$, $W=511$, $p=0.046$) and were younger (Mann-Whitney U Test: $N=56$, $W=561.5$, $p=0.004$). We tested whether this would have an impact on our analysis, but found no evidence.

Results:

Developmental differences between individuals of different geographical origins:

Southern males matured at a larger size than Northern males (Linear mixed effect model: $N=204$, $W=99.5$, $p<0.001$; Fig. 3.3a) and needed more time to reach sexual maturity (Linear mixed effect model: $N=204$, L -Ratio=16.37, $p<0.0001$; Fig. 3b). The variation

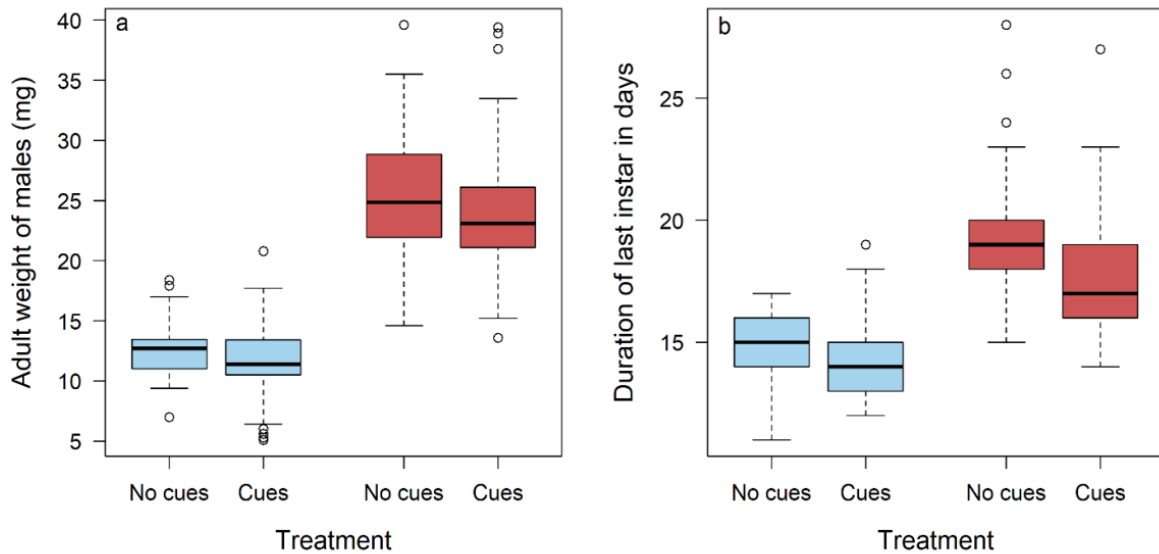


Figure 3.3: Treatment effects (no cues or cues) on adult weight (a) and the duration of the subadult instar (b) in Northern (blue) and Southern (red) males.

in adult weight (Levene test: $N=204$, $df=1$, $F=31.472$, $p<0.001$) and in developmental time (Levene test: $N=204$, $df=1$, $F=16.806$, $p<0.001$) was heterogeneous between geographical origins with the range and interquartile range of both traits being larger and more uneven in Southern males than in Northern males (Fig. 3.3a, b).

We presumed that Southern males are adapted to a longer mating season, which should be revealed by a larger variation in the timing of maturation than in Northern males. However, Southern (15.4 ± 5.8 days; range: 32 days) and Northern males (13.3 ± 6.6 days; range=30 days) matured within a similar timeframe (Linear mixed effect model: $N=204$, $df=1$, $L\text{-ratio}=1.871$, $p=0.171$) and variances did not differ significantly (F test: $N=204$, $F_{114,88}=1.3046$, $p=0.1924$) even though the first Southern male had his final moult six days before the first Northern male.

Effects of female cues on developmental time:

Analysing both geographical origins separately, we found that the reception of female cues consistently led to a significant reduction of the developmental time (Table 3.1) with homogeneous variation between males of both treatment groups (*female cues/no female cues*) (Northern males: Levene test: $N=116$, $df=1$, $F=0.571$, $p=0.452$; Southern males: Levene test: $N=89$, $df=1$, $F=0.301$, $p=0.585$; Fig. 3.3b). However, we found no support for the prediction that a reduction in developmental time led to a reduced adult weight in males. In line with this finding, there was no relationship between the adult weight and developmental time (Table 3.1) nor did the reception of cues lead to different variance in adult weight between treatment groups (Northern males: Levene test: $N=116$, $df=1$, $F=2.175$, $p=0.143$; Southern males: Levene test: $N=89$, $df=1$, $F=0.497$, $p=0.483$; Fig 3a).

Table 3.1: Results from linear mixed effect models on the developmental time (duration of the subadult instar) of Northern and Southern males.

Northern ♂♂ (N=116)					
Variable		Estimate ± SE	L ratio	df	p
Intercept	Reference level: no ♀ cues	(15.097 ± 1.068) 14.978 ± 0.233			
Cues (♀ cues; no ♀ cues)	♀ cues	(-1.348 ± 1.246) -0.566 ± 0.254	4.929	1	0.026*
♂ adult weight		(-0.009 ± 0.082)	0.488	1	0.485
♀ cues : ♂ size		(0.065 ± 0.099)	0.428	1	0.513
Southern ♂♂ (N=89)					
Variable		Estimate ± SE	L ratio	df	p
Intercept	Reference level: no ♀ cues	(3.011 ± 0.096) 2.943 ± 0.032			
Cues (♀ cues; no ♀ cues)	♀ cues	(0.020 ± 0.128) -0.058 ± 0.028	4.284	1	0.039*
♂ adult weight		(-0.003 ± 0.004)	2.853	1	0.091
Cues : ♂ adult weight		(-0.003 ± 0.005)	0.442	1	0.506

Data from Southern males (estimates and SE) were log-transformed. In Northern males, we used family lineage as a random factor (26 groups) and in Southern males the collection site (4 groups). Estimates and SEs of the full models are in brackets; those of the minimal adequate model are without brackets. The L ratio is the abbreviation for the likelihood ratio. Significant effects are indicated by asterisks.

Effects of geographical origin and female cues on male mating tactics:

The frequency of the bigynous mating tactic was 56 % and did not differ between Northern and Southern males (binary logistic regression: N=109, DF-deviance=0.34807, $p=0.2369$; Fig. 3.4). The treatment (*female cues/no female cues*) as factor or in the interaction with female size did not affect the frequency of bigyny (Table 3.2, Fig. 3.4). Instead, we found a significant interaction between the treatment and the relative weight change in Southern males (Table 3.2). Southern males with a relatively low weight change were more likely bigynous but only if they had received female cues (Fig. 3.5). Northern males that lost relatively less weight also favoured the bigynous tactic (Fig. 3.5), but this was independent of the treatment (Table 3.2). The second significant effect on the probability of bigyny was female size. Corroborating findings from a field study (Welke et al., 2012) males were more likely bigynous when the female was small (Table 3.2; Fig. 3.6), although this

was not significant ($p=0.056$) in Southern males. Neither the female post-maturation age nor female condition had a significant effect (Table 3.2).

Discussion:

Plastic traits, as for example life-history strategies or alternative mating tactics, are known to respond to cues that provide social information (Engqvist & Taborsky, 2017; Kasumovic & Brooks, 2011; Kodric-Brown, 1986; Rodriguez et al., 2013). Upon maturation, females of *Argiope bruennichi* emit a volatile sex pheromone that attracts males (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010). We tested whether the reception of female pheromones during the subadult stage influences life-history and mating decisions in males. In a common garden situation, we compared males that came from the Northern and Southern edges of the species' distribution. These regions differ in climatic factors that likely affect the available

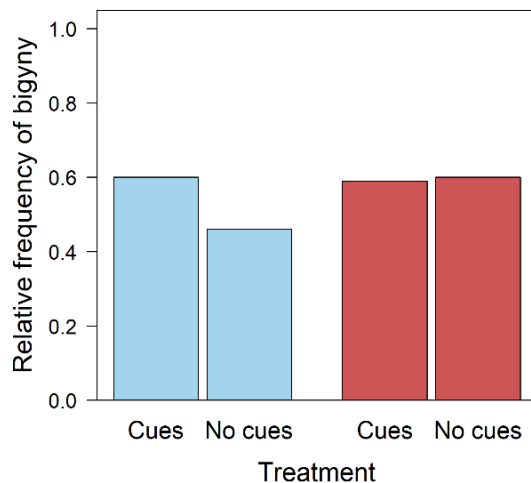


Figure 3.4: Relative frequency of bigyny depending on the reception of female cues by Northern (blue bars) and Southern (red bars) males.

duration for growth and reproduction, and indeed, males from Northern populations had a shorter developmental time and were smaller than males from Southern populations. Males from both regions matured earlier if they received female cues during the subadult stage. While this provides evidence that males indeed perceived the silk cues we found no corresponding differences in the frequencies of monogynous and bigynous mating tactics. Whether or not a Southern male selected the bigynous tactic depended on his body condition in interaction with the treatment whereas for Northern males female quality was more important than the presence of cues.

Ecological differences may lead to local adaptations in traits under natural and sexual selection (Jennions & Petrie, 1997; Stearns, 2000). During their northwards range expansion (Krehenwinkel et al., 2015; Krehenwinkel & Tautz, 2013), *A. bruennichi* had to adapt to a shorter spring and summer and a shortening of the mating season. As expected, we found that Southern males had a longer developmental time and matured at a larger body size compared to Northern males even though they developed under the same conditions in the laboratory. We had also expected a larger variation in these traits in Southern compared to Northern males, but

males from both regions showed a similar maturation window of about 30 days. This pattern might suggest that the trade-off between age and size at maturation is generally shifted towards time matching the notion that it is of paramount importance for male reproductive success to hit the peak of female maturation (Zimmer et al., 2012). Across all populations, the length of the subadult stage is found to be a plastic trait that responded to the presence of silk from webs of virgin females.

In agreement with two earlier studies on two other spider species (Kasumovic & Andrade, 2006; Neumann & Schneider, 2016), male *A. bruennichi* shortened their subadult stage if they were exposed to the silk of virgin females. The silk contains a specific sex pheromone that attracts males to their web and induces male courtship (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010). Our results indicate that males make use of this sex pheromone to assess the availability of virgin females and plastically adjust their developmental time to this social information. This is adaptive because mating chances are limited and male reproductive success relies on finding virgin females as they cannot gain paternity with double-mated and plugged females (Nessler et al., 2007). A strong preference for virgin versus mated females was confirmed in simultaneous mate choice experiments (Schneider et al., 2016; Schulte et al., 2010). Differentiation between virgin and mated females likely depends on the sex pheromone which virgin but not mated females produce (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010). Our results are consistent with the mate opportunity hypothesis (Morbey & Ydenberg, 2001) that males benefit from maturing in time and scramble for opportunities to be first in finding a female that has just matured in the vicinity. Indeed, the local availability of virgin females can be very heterogeneous as females mature over a period of a few weeks (Zimmer et al., 2012). Even if early virgins are taken by rivals, males

Table 3.2: Summary of results about effects on the probability of monogynous and bigynous mating tactics.

Northern ♂♂ (N=50)					
Effect		Estimates ± SE	df-deviance	df	p
Intercept	(Reference level: no ♀ cues)	(-17.008 ± 8.285)			
		-6.589 ± 2.620			
Cues (♀ cues; no ♀ cues)	♀ cues	(0.180 ± 6.197)	0.323	1	0.570
♀ size		(0.867 ± 0.724)	6.101	1	0.014*
		0.924 ± 0.411			
♀ condition		(1.471 ± 1.834)	1.379	1	0.240
♀ post-mature age		(0.091 ± 0.273)	0.151	1	0.698
Rel. ♂ weight change		(-6.674 ± 6.258)	5.944	1	0.015*
		-8.193 ± 3.711			
♂ post-mature age		(0.196 ± 0.131)	1.974	1	0.160
Duration of subadult stage		(0.485 ± 0.317)	2.109	1	0.147
Cues : ♀ size		(-0.149 ± 0.979)	0.038	1	0.845
Cues : rel. ♂ weight change		(-1.963 ± 8.146)	0.023	1	0.879
Southern ♂♂ (N=56)					
Effect		Estimates ± SE	df-deviance	df	p
Intercept	(Reference level: no ♀ cues)	(0.834 ± 5.495)			
		-0.920 ± 0.756			
Cues (♀ cues; no ♀ cues)	♀ cues	(-17.622 ± 12.433)	-	-	-
		-3.369 ± 1.903			
♀ size		(0.949 ± 0.743)	3.651	1	0.056
♀ condition		(-0.470 ± 2.027)	0.039	1	0.843
♀ post-mature age		(0.120 ± 0.237)	0.606	1	0.436
Rel. ♂ weight change		(-3.264 ± 4.663)	-	-	-
		-3.231 ± 4.033			
♂ post-mature age		(-0.066 ± 0.119)	0.275	1	0.600
Duration of subadult stage		(-0.314 ± 0.190)	3.466	1	0.063
Cues : ♀ size		(1.482 ± 1.419)	1.308	1	0.253
Cues : rel. ♂ weight change		(-37.578 ± 20.851)	7.748	1	0.005*
		-19.875 ± 10.392			

The estimates and standard errors of the estimates are logit-transformed because they arose from a binary logistic regression. The estimates and the standard errors of the full model are in brackets, and the results of the minimal adequate model are without brackets. Significant effects are indicated by asterisks.

benefit from catching the onset of female maturation as they may encounter and guard subadult females that mature within days (Zimmer & Schneider, 2016). These males mate with the female during the moulting process when her exoskeleton is still soft and thereby avoid sexual cannibalism (Uhl et al., 2015). Even though it is unknown how males locate subadult females that are not known to produce sex pheromones, a proportion of 56.45 % of all females has been found guarded by a male (Zimmer & Schneider, 2016).

According to life history theory, early maturation should come at a cost, which is

generally presumed to be body size (Abrams, Leimar, Nylin, & Wiklund, 1996; Nylin & Gotthard, 1998) following the classical trade-off that males either mature early and are small or mature later and are large. Compromising large body size is expected to have high fitness costs if male mating success depends on physical strength (Hunt et al., 2009; Jennions, Moller, & Petrie, 2001). However, in species that scramble for access to females, negative fitness consequences of reduced body size due to earlier maturation should be less severe. Contrary to life history theory the body size of *A. bruennichi* males was unaffected by earlier maturation. Previous

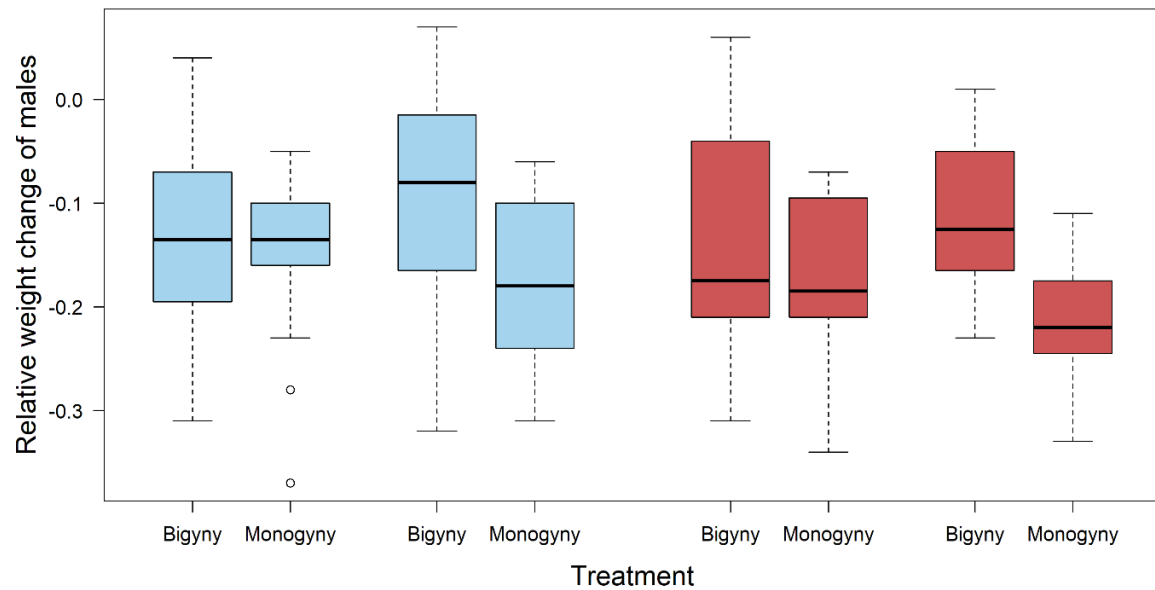


Figure 3.5: Descriptive statistics of the relative weight changes between adult moult and mating separated for males that selected the bigynous and monogynous mating tactic and received female cues or not. Black bars depict data from Northern males and white bars from Southern males.

studies on effects of pheromonal cues produced by females on maturation are inconsistent in that no effect on body size was detected in the orb-web spider *Nephila senegalensis* (Neumann & Schneider, 2016), whereas male red-back spiders *Latrodectus hasselti*, had a smaller body size when they matured earlier in response to female cues (Kasumovic & Andrade, 2006). A difference between the studies was the feeding regime that was ad libitum in *A. bruennichi* and *N. senegalensis* but variable in red-back spiders (Kasumovic & Andrade, 2006; Neumann & Schneider, 2016). The size effect in red-back spiders was primarily apparent in males that received a low diet (Kasumovic & Andrade, 2006). Future experiments should evaluate if the costs of early maturation vary depending on the food regime in the laboratory and compare the finding to responses of males collected in the field.

The plasticity in the duration of the subadult instar appears to be a highly adaptive trait in species with extremely low mating rates that crucially depend on mating with virgin females. Finding a similar response in three spider species from different families (*L. hasselti*, *N.*

senegalensis and *A. bruennichi*) suggest that the length of the subadult instar might be another trait that evolved independently in monogynous mating systems, next to genital damage and permanent sperm depletion as adults (Michalik & Rittschof, 2011; Uhl et al., 2010). Its presence supports the general hypothesis that protandry is strongly favoured by selection in mating systems with a first-male advantage in sperm competition (Simmons, Llorens, Schinzig, Hosken, & Craig, 1994; Uhl et al., 2010; Wedell, 1992; Wiklund & Forsberg, 1991).

Alternative mating tactics can be conditionally adjusted to prevailing conditions, or they might be fixed due to genetic adaptations or experience during development (Taborsky & Brockmann, 2010; West-Eberhard, 2003). Using information e.g. about female availability minimizes uncertainty about the success of a bigynous tactic which depends on the chance of finding a second female. However, we have to reject our hypothesis that males adjust their mating tactic to social information received during pre-adult stages. A previous study found that cues that males received as adults did modify mating behaviour

(Nessler et al., 2009). This might suggest that information about female availability during mate search is more reliable, and it might be adaptive to adjust mating tactics then. This is in accordance with the study of Swanger and Zuk (2015), who found that in the Pacific field crickets (*Teleogryllus oceanicus*), only the adult social environment shape female responses to sexual signals, but not experience during development. However, in the closely related species *T. commodus* the quantity of male calls prior to maturity affected how quickly females choose males (Kasumovic, Hall, & Brooks, 2012). Thus, whether and how early social information affects the adult life of individuals differs between species and as shown here, even within species suggesting that local ecology plays an important role.

The success of the bigynous mating tactic should depend on the energy reserves a male has left to continue mate searching. Correspondingly, males of both populations were more likely bigynous if their relative weight loss was small. The reduction in body weight occurs because adult males search for females soon after they reach sexual maturity. From then on, males no longer build capture webs and barely eat (Foellmer & Fairbairn, 2005b). Searching for a second mating partner involves additional risks and costs. Males not only need to find a second female, the search itself is risky because it is energy and time consuming and bears the risk of predation (Andrade, 2003; Kasumovic, Bruce, Herberstein, & Andrade, 2007). Moreover, if males have found a second female they need to perform courtship display again, which is energetically costly (Cady et al., 2011; Stoltz et al., 2009). Therefore, it may only be adaptive to engage in a bigynous tactic if the male has enough energy reserves to master a second mating. It is, therefore, puzzling that uninformed Southern males did not show this flexibility as the factor of body weight change was only significant in combination with the presence of cues. In the absence of female

cues, these males may have followed a behavioural program that makes sense very early in the season when most females are not yet mature (Nessler et al., 2009; Zimmer et al., 2012).

Bigyny versus monogyny can be viewed as a form of male mate choice (Fromhage & Schneider, 2012; Welke et al., 2012). Male mate choice is generally rare but might evolve in systems with high male mating investment (Barry & Kokko, 2010). Males should only invest in mate choice if female availability provides opportunities to choose and if variation in female quality justifies the costs of choosiness (Bonduriansky, 2001; Edward & Chapman, 2011). For example male pipefish *Syngnathus typhle* were only choosy if the operational sex ratio was female-biased (Berglund, 1994). In our study, males were more likely monogynous if the female was relatively large although the correlation was only significant in Northern males. This finding corroborates results from a field study in Northern Germany (Welke et al., 2012). In Southern males, female size had no influence on male mating decisions. Perhaps male selectivity according to female size only

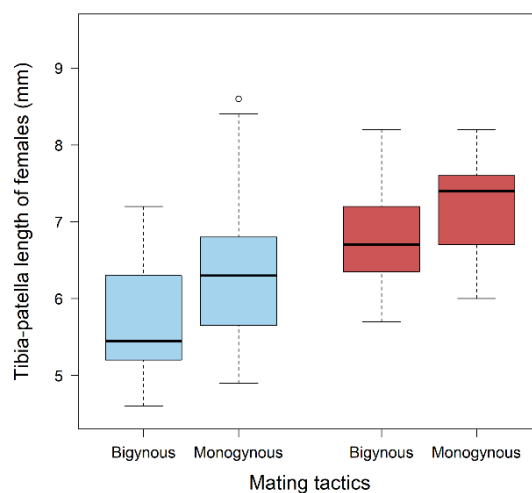


Figure 3.6: Males were more likely monogynous if the female had a larger body size. Tibia-patella length was used as an approximation for overall female size. Black bars depict data from Northern males and white bars show Southern males.

emerged in the course of the colonization of Northern Europe. A decrease in female body size and thus fecundity during the range expansion may make female quality a more relevant trait. However, since our experiments did not focus on female quality and results are correlational, we need further studies that investigate the mechanisms of quality-dependent male mate choice and address questions concerning the ability of males to determine female size or condition by female chemical cues.

Less than 40% of males survive their first copulation making it impossible to score the mating tactic of the majority of males. Therefore, we prevented sexual cannibalism by blocking the female chelicerae with a paintbrush allowing males to escape the wrapping. We found no indications (see Material and Methods) that the handling affected male behaviour or the frequency of the observed tactics. Therefore we assume that the decision to sacrifice their life during the first copulation or to attempt an escape was made before our interference.

Our findings support the idea that the use of social information is a fitness relevant trait in variable environments because it minimizes uncertainty. The adaptation to local environmental conditions should take place in different stages of life history including the mating season. The perception of social cues can give information about the availability of mating partners and competitive conditions but also about the start of the mating or breeding season. Several studies could show that mismatches in sexual maturity and the start of the breeding season lead to reduced fitness or even to extinction (T. Jones & Cresswell, 2010; Reed, Jenouvrier, & Visser, 2013). The successful colonization of new habitats and the adaptation to rapidly changing climate conditions requires processing a combination of information from the abiotic and biotic environment. Our findings support this idea and additionally show that

independent of climatic conditions gathering and processing information by social cues is important in sexual selection.

In conclusion, we show that *A. bruennichi* males plastically respond to their social environment through the reception of chemical cues produced by virgin females. These cues are used to make information-based decisions on the timing of maturation, but they are less relevant for later mating decisions. This may be adaptive because population dynamics and thus female availability may change rapidly, which is why the availability during the male's subadult stage may not be the same than during mate search. A flexible use of mating tactics and the use of local information may be beneficial to respond to environmental conditions during the mating season. Perhaps this plasticity has facilitated swift adaptation to novel conditions during the recent range expansion of the species.

Behavioural and life-history responses to social cues have been investigated in several insect and spider species and revealed intriguing similarities and differences between and within species. More studies are desired to explore variation in costs and benefits of life-history adjustments and elucidate the selection pressures favouring the evolution of plastic responses in development and behaviour to social cues.

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Author's contribution to Chapter III:

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I took part in conceiving the experimental design (30 %). I prepared and conducted the experiments with the assistance of Neele Heinbockel (former bachelor student). For the manuscript, I made the complete statistical analysis, prepared figures and tables, and wrote the first draft. My supervisor Prof. Jutta Schneider revised the manuscript.

Special notes:

Corinna Adrian, Julia Becker, Tomma Dirks and Angelika Taebel-Hellwig helped me to collect and raise my test specimens.

Date: _____

Supervisor: _____

CHAPTER IV

Males of a sexually cannibalistic spider chemically assess relative female quality

Anna-Lena Cory & Jutta M. Schneider

Abstract:

Background: Male mate choice is expected in taxa with high male reproductive investment, which is characteristic of several monogynous mating systems. Monogyny is common among sexually cannibalistic spiders in which males terminally invest into monopolising a single female. To prevent sperm competition, males of *Argiope bruennichi* break off pieces of their paired mating organs and plug one of the female's equally paired genital openings. As a result, males are limited to two copulations, which they can allocate to one or two females. Male *A. bruennichi* preferentially monopolise large, more fecund females, while they may leave smaller females after a single copulation and resume mate search. Here, we test the hypothesis that males of *A. bruennichi* apply a locally adapted threshold rule to mate choice.

We used mating arenas and gave males a choice between three different-sized females of the same age that were matched for origin: all three females originated either from the same Northern European population as the males or from Southern populations where females grow significantly larger. Since the smallest Southern female was about the same size as the largest Northern female, males were expected to be choosy among Northern females, but to accept all Southern females since they would be above their locally-adapted threshold.

Results: Males copulated with the first female they encountered, which was independent of her size. Regardless of the females' origins, males always chose a monogynous tactic with the largest female, while they left the smallest female after one copulation. Unexpectedly, males left Southern females who were of a similar size as monopolised Northern females. Since males have very poor eyesight and did not actively sample all females, they must have gained information about relative size differences between females based on volatile chemical cues only.

Discussion: Our findings suggest that male *A. bruennichi* can assess relative differences in mate quality and adjust their mating tactic to the prevailing conditions. We reject the presence of a locally adapted threshold and suggest that our results are consistent with a best-of-n rule or an adjustable threshold, but do not allow us to distinguish between them.

Keywords

male mate choice, monogyny, sampling strategies, sex pheromones, sexual cannibalism male mate

Background:

In mating systems with traditional sex roles females are generally the choosy sex while male mate choice evolves under conditions of high male mating effort or paternal investment and considerable differences in quality between females (Andersson, 1994; Bonduriansky, 2001; Edward & Chapman, 2011; Trivers, 1972). Female traits that males consider are mainly related to fecundity and mating status under conditions of sperm competition particularly if sperm precedence patterns are present (Honek, 1993; Olsson, 1993; C. J. Reading, 2007; Thomas, 2011). Generally, the evolution of choosiness requires the opportunity and the ability of a comparative assessment of potential mates (Edward & Chapman, 2011; Wittenberger, 1983). However, the ability to compare mates and make a decision may demand different degrees of cognitive investment depending on the underlying mate choice rule.

Cognitive demands are especially high for a comparative assessment following the best-of-n strategy which describes that individuals simultaneously or sequentially compare and choose among a group of potential mates (R. M. Gibson & Langen, 1996; Janetos, 1980; Real, 1990; Wittenberger, 1983). Particularly a sequential assessment demands extended memory and virtual comparisons. Therefore, the latter should only evolve under very specific conditions such as a short latency between encounters (Milinski & Bakker, 1992; Real, 1990). In comparison, a threshold rule is less costly and requires no memory and is more likely to evolve even in small-brained animals (Dombrovsky & Perrin, 1994; Mazalov et al., 1996; Real, 1990). In line with this, several studies with invertebrates, mostly crustaceans and insects, signify that females compare male quality to a fixed standard (Backwell & Passmore, 1996; Cooley & Marshall, 2004; Ivy & Sakaluk, 2007). Other studies found that a threshold can also shift, allowing adjustments to prevailing conditions (Backwell & Passmore,

1996; Bakker & Milinski, 1991; Beckers & Wagner, 2011; Reid & Stamps, 1997). However, as with the best-of-n strategy, an adjustable threshold requires a comparative assessment. Here, the average quality of available mates has to be assessed first in order to adjust the internal standard.

Models of comparative mate choice rules assume an initial information sampling phase in which at least two potential mates are compared (Real, 1990; Wittenberger, 1983). While most mate choice experiments are indeed binary, sampling becomes more complex and costly with increasing options (Janetos, 1980; Real, 1990; Roff, 2015). The number of mates being assessed can be observed easily in animals where information is gathered through direct inspection visits that can be followed and counted (Forsgren, 1997; Uy, Patricelli, & Borgia, 2001). However, acoustic or chemical signals can be perceived from a distance and potentially compared without changing position. To evaluate the sampling, we need to know what the animal can detect and process and up to which distance information is received. Unfortunately, such information is only available for a limited number of species as for example for acoustic sexual signalling in crickets or frogs (e. g. Deb & Balakrishnan, 2014; Gerhardt & Klump, 1988; Murphy, 2012). In the context of mate choice, chemical communication is the least known modality, although it is relevant in various animal taxa (Johansson & Jones, 2007). Here we investigate how male spiders having very poor vision, but plenty of chemoreceptive sensilla decide on their mating tactic when confronted with several females of different quality (Foelix, 2011; Ganske & Uhl, 2018).

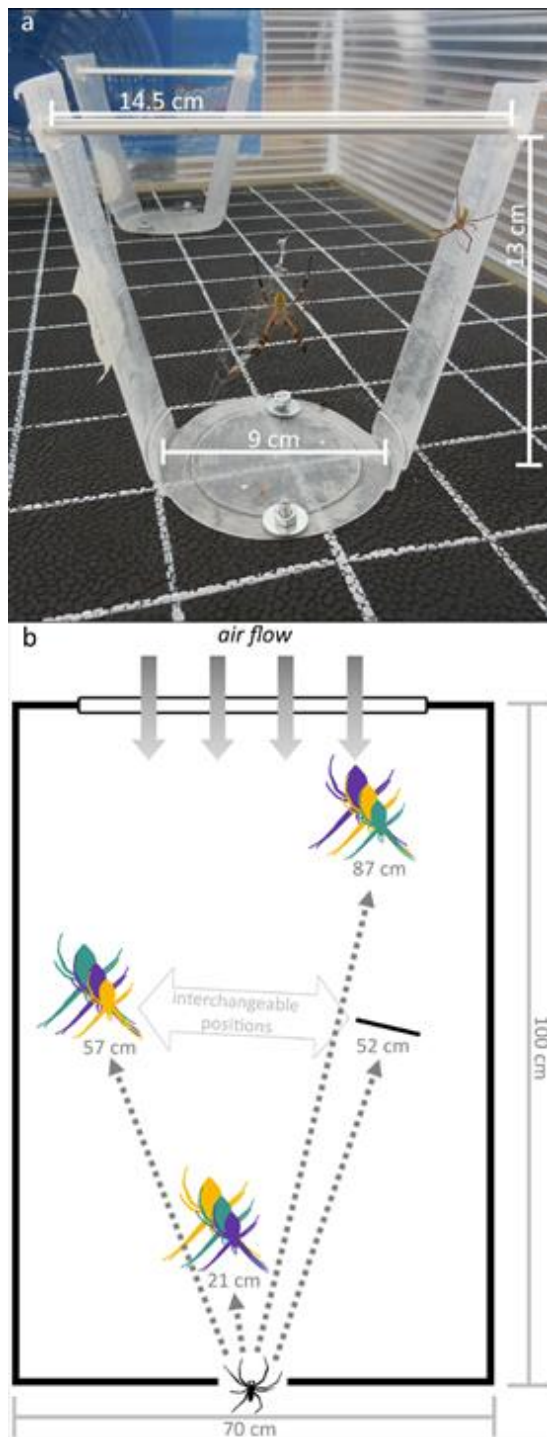
In web-spiders, males are the searching sex, and they find females through volatile sex pheromones emitted by the female and the web (Gaskett, 2007). While the chemical structures of pheromones have been analysed

in a few spider species, nearly nothing is known about the information contained in these semiochemicals (Schulz, 2013). Particularly in species, where males are regularly cannibalised by females, males could benefit from chemical information about female quality that they receive from a distance before entering female webs. Females of the sexually cannibalistic orb-web spider *Argiope bruennichi* (Fig. 4.1a) show a high variation in body size, of which fecundity is a direct function (Krehenwinkel & Tautz, 2013; Marshall & Gittleman, 1994; Simpson, 1995). Females attack every male during copulation and often kill males during their first copulation. Dying after one copulation reduces male mating rates by half (Fromhage et al., 2003) because males damage their paired genitalia during copulation and can maximally copulate twice (once with each mating organ) (Nessler et al., 2007). Males that survive their first copulation can then decide whether to mate a second time with the same female (monogyny) or to leave and inseminate a second female (bigyny). Accordingly, males are monogynous either because they are cannibalised by the female already after their first copulation, or because they survive and choose to copulate with the same female again. Survival of the first copulation is only possible if the male jumps off before 10 seconds have passed and we consider death after such a short copulation a consequence of an unsuccessful escape attempt (Schneider et al., 2006). Chances of survival rapidly approach zero after this, such that a single long copulation may mean that a male sacrificed his future reproduction in favour of transferring more sperm during his first copulation (Schneider et al., 2006). In any case, these males leave one genital opening free for a potential future sire. However, not all females remate, as one copulation is sufficient to fertilise all her eggs (Schneider et al., 2005).

Males that copulate twice with the same female minimise the risk of sperm competition

because both female genital openings are securely plugged, and further copulations are unlikely to result in paternity (monopolisation; Fromhage et al., 2003; Nessler et al., 2007). On the other hand, bigynous males can potentially sire more offspring if they successfully mate with two virgin females particularly if these females do not re-mate. Accordingly, males have the option to allocate their mating effort strategically. Field observations suggested that males chose a monogynous tactic preferentially with large and fecund females (Welke et al., 2012). A dynamic game-theoretical model based on data from *A. bruennichi* confirmed that male mate choice evolves under realistic conditions (Fromhage and Schneider, 2012). However, it is unknown how males find their first female to mate with and how the availability of alternative mating options influences their decisions.

In large test arenas, we offered naïve *A. bruennichi* males a choice between three virgin females of different size (small, medium, large) to explore their decision rules (Fig. 4.1b). To distinguish between the potential of a locally adapted threshold and a comparative assessment, we made use of geographical differences in female size and fecundity: females from populations in Southern Europe are significantly larger than females from populations in Northern Europe (Krehenwinkel & Tautz, 2013). We exposed males from Northern populations to three females either from the Northern (treatment 1) or Southern populations (treatment 2) that differ significantly in the size distribution of females. To simulate natural conditions, we randomly ordered the females at different distances relative to the male starting position (Fig. 4.1b). We monitored which female was encountered first, the duration of the first copulation, the occurrence of sexual cannibalism and the chosen mating tactic.



We predicted that the probability of monogyny (either by sexual cannibalism or monopolisation) should increase with the size of the female that the male encountered and inseminated first. If males have a fixed threshold, the threshold should be adapted to Northern conditions. Accordingly, we expected a higher probability of monogyny with

Figure 4.1: Design of test arenas. a) Photograph of *Argiope bruennichi* female (center) in her web with a male (right) inside a test frame (©AL Cory). The test frames were custom-made and allowed for stationing females on fixed positions within the arena. b) Scheme of the test arena. Males could choose between three females that were matched for origin (Southern or Northern) and differed in size and distance from the male's starting point (lowest spider silhouette), whereby there were two options for the middle-distant position. Three of six possible distribution patterns are illustrated by different colours of female silhouettes (from close to far distant: dark purple= small-medium-large, teal= medium-large-small, amber= large-small-medium). Note that the scheme does not depict that males were exposed to females from either Northern Europe or Southern Europe only.

Southern females because any locally adapted threshold in body size would be reached sooner. Alternatively, males may be able to assess and compare current female quality conditions in a patch. If they use a comparative assessment, we would expect that mating tactics are not affected by female origin but by female relative sizes. In the presence of a comparative assessment, we would further expect that males more likely avoid small females from the start and visit large females directly.

Results:

Choosing the first female:

Males started their search after raising their front legs where many sensory hairs are located. Half of the males took less than two minutes (interquartile range: 1-16 min) to arrive at their first web. All but two males copulated with the female that they encountered first (N=54). However, which female that was did not depend on distance or female size class. The first encounter was not more likely with the nearest female (close=33.3 %; medium=24.1 %, far=42.6 %; Chi² test: N=54, Chi²=1.444, df=2, p=0.49) nor

Table 4.1: Effects on mating tactics in males that survived the first copulation. We used a binary logistic regression (N=23) to test effects of female origin and female size class on the probability of monogyny. The estimates and standard errors (SE) of the estimates are Firth bias-corrected and logit-transformed. Results of the full model are in brackets, and the results of the minimal adequate model are without brackets. The final model only included female size class.

Effect		Estimates \pm SE	Chi ²	df	p
Intercept	Reference level: small ♀♀	-1.85 \pm 0.92			
♀ origin (Northern (N); Southern (S))	S ♀♀	(-0.48 \pm 1.10)	0.06	1	0.803
♀ size class (small, medium, large)	medium ♀♀	1.85 \pm 1.16	11.02	2	0.004*
	large ♀♀	4.24 \pm 1.86			
♀ origin : ♀ size class	S ♀♀ : medium ♀♀	(1.33 \pm 2.50)	0.46	2	0.793
	S ♀♀ : large ♀♀	(0.14 \pm 3.26)			

with the largest female in the arena (small=25.9 %, medium-sized=46.3 %; large=27.8 %; Chi² test: N=54, Chi²=2.11, df=2, p=0.35).

Copulation duration and occurrence of sexual cannibalism (monogyny after a single copulation):

Males had a median copulation duration of 6.2 s (interquartile range: 5.2-8.4 s). Female origin (Mann Whitney U test: N=54, W=316.5, p=0.41) or size class (Kruskal-Wallis test: N=54, Chi²=1.04, p=0.59) did not influence the duration of copulation, but corroborating earlier findings, a short copulation lowered the likelihood of sexual cannibalism (Mann-Whitney U test: N=54, W=490, p=0.02). Five males copulated longer than 10 seconds, and only one of these males (copulation duration: 10.67 s) survived. There was no evidence that a copulation longer than 10 seconds occurred due to female size class or female origin.

Southern females cannibalised more males (70.3 %) than Northern females (44.4 %), but the difference was only marginally significant (logistic regression: N=54, df-deviance=3.76, p=0.053). There was no significant influence of female relative size class (logistic regression: N=54, df-deviance=3.64, p=0.162) or the interaction of origin and female size class (logistic regression: N=54, df-deviance=0.35,

p=0.841) on the probability of sexual cannibalism.

Monogyny (=monopolisation) or bigyny:

We only considered male decisions as unambiguous after they survived the first copulation. We monitored whether males stayed and re-mated with their first mate (monogyny) or whether males left the first mate for one hour after they escaped from their first copulation. In total, 23 males survived the first copulation, of which 13 males left their first mate. Seven of them encountered a second female that was always larger even if the first female was in the medium size class. Only two males copulated with the second female that in both cases belonged to the size class “large” within the observation time of one hour. We classified the remaining males as “intended bigynous” if they did not return to the first female within an hour and if they showed a clear distance to the first mating partner without any contact (directly or by silk threads) to the female’s web or the test frame.

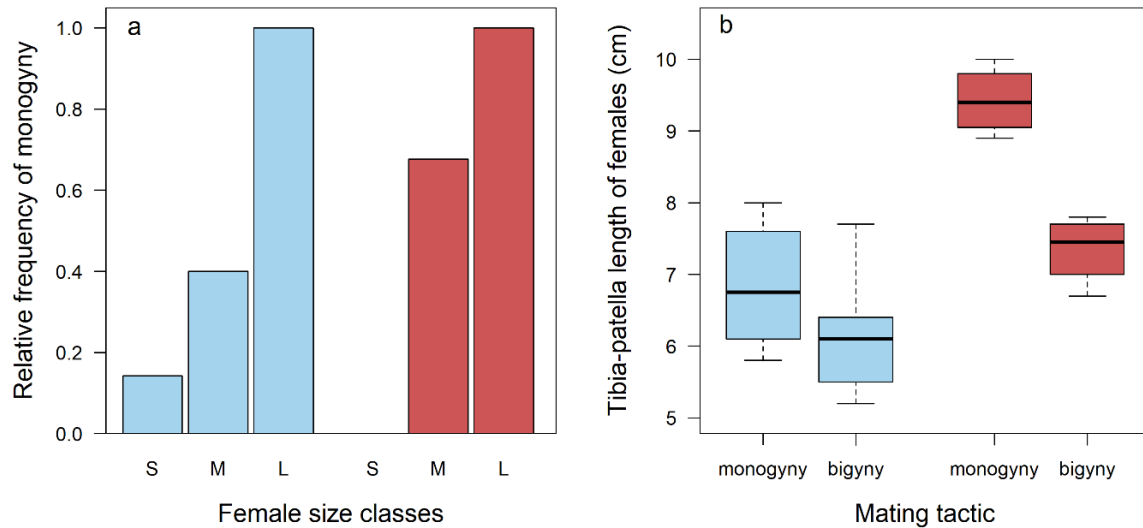


Figure 4.2: Effects of female body size on male mating tactics. The figures (a, b) depict the effects of body size of Northern (black) and Southern (white) females on mating tactics in males that survived the first copulation. a) Relative frequency of monogyny dependent on female size classes (S=small, M=medium-sized, L=large). b) Body size (leg length) of first female mates chosen from monogynous and bigynous males.

Among the 23 males that survived their first copulation, 43.5 % monopolised their first mate. The probability of monogyny could not be explained by an interaction between female origin and size class and was independent of female origin as single variable (Table 4.1). However, we found that with increasing female size class, males were more likely monogynous (Table 4.1). Interestingly, the five females from the size class “large” were all monopolised, but nine out of ten “small” females were left after the first copulation (Fig. 4.2a). The relationship between the likelihood of monogyny and female size was still present when we dropped the size classes and used the leg length as an index for the body size of females instead (logistic regression: $N=23$, df -deviance=1.36, $p=0.02$). The use of female size as a continuous variable further revealed that males left Southern females that were approximately as large as Northern females that males monopolised (Fig. 4.2b).

Reproductive success of monopolising males:

We also analysed the fitness consequences of the size-dependent choice of males by comparing their reproductive success. As an index for reproductive success, we approximated number of eggs contained in the first egg sac of the males’ first mating partner by translating its weight into the number of eggs by using the estimates derived from the regression between egg sac weight and egg-number using other females of the same cohorts (see Methods). Monogynous males mated with females that produced 331.2 ± 69.3 eggs while females of bigynous males produced on average 246.9 ± 53.4 eggs in their first egg-sac. The difference is statistically significant (logistic regression: $N=18$, DF -deviance=7.0014, $p=0.0081$).

Female leg length correlated positively with the estimated number of eggs contained in the first egg sac (Spearman rank correlation: $N=38$, $\rho=0.938$, $p<0.0001$) and Southern females produced more eggs than Northern females (Mann-Whitney U test: $N=38$, $W=49$, $p<0.0001$).

Discussion

Argiope bruennichi males adopted a monogynous mating tactic if the first female they encountered was relatively large and they were bigynous if they had encountered a relatively small female first. This was adaptive because females of monogynous males produced on average 25 % more eggs than females of bigynous males. Unexpectedly, absolute female sizes played no role, as bigynous males left larger Southern than Northern females. These findings indicate the absence of a fixed inherent size threshold as a basis for tactic choice. More likely, males applied a flexible standard based on information they must have gained about the relative quality of females nearby, even though males have not visited all females. Hence, our results suggest that males selected their first female independent of her quality and left if there were better options available. Our findings are consistent with an adjustable threshold or a best-of-n rule.

This study provides experimental confirmation of earlier observations that monogyny in *A. bruennichi* is a mating decision based on a trading-up mate choice mechanism which requires the assessment of relative female size differences (Edward & Chapman, 2011; Welke et al., 2012). Our findings suggest that males acquire and use information about the quality of females in the vicinity and adjust their behaviour without visiting the webs of females. Since males could not rely on their poor eye-sight, they could only have accomplished that by using volatile chemical cues or sex pheromones (Foelix, 2011; Gaskett, 2007; Johansson & Jones, 2007). Several studies on spiders have shown that chemical cues or sex pheromones offer males information about female quality traits (Cory & Schneider, 2016; Riechert & Singer, 1995; Stoltz et al., 2007; Tuni & Berger-Tal, 2012). In *A. bruennichi*, at least one sex pheromone was discovered in virgin females (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010). If males use

pheromones to distinguish females of different quality, either the quality or quantity of pheromones must differ between females of different sizes and fecundity. There is evidence in moths that the quantity of pheromones correlates with female quality (S. P. Foster & Johnson, 2011; Harari, Zahavi, & Thiery, 2011). Similarly, sex pheromone titres of male *Nasonia vitripennis* were found to correlate with sperm numbers and females were found to use pheromone titres to identify sperm-limited males (Ruther, Matschke, Garbe, & Steiner, 2009). Whether pheromone emission in spiders qualifies as an honest signal of quality and is used by males to choose their mating tactic, still needs to be tested.

While our data suggest the absence of a fixed threshold, we cannot distinguish whether males adjusted their threshold to the perceived conditions or whether they applied active mate choice underlying a best-of-n rule. Both strategies require a comparative assessment of multiple mates either by a direct comparison of mates (best-of-n rule) or by equalising the internal standard against the actual mate quality conditions in a patch (adjustable threshold). If males use an adjustable threshold, they might have raised their standard when they were exposed to chemical cues of Southern females. This explanation is in agreement with a study by Reid and Stamps (1997) who tested sampling tactics in female pine engravers (*Ips pini*). Here, male quality differed between patches visited by females and dependent on patch quality, females lowered or raised their threshold (Reid & Stamps, 1997). Such a flexible threshold rule is consistent with our results and a more parsimonious mechanism than mate choice based on the best-of-n rule, which requires that males simultaneously perceive distinct size differences between females. If males indeed perceive the quality of specific females via chemical cues, it seems curious that they do not use this information to select the best mates for their first choice. Our data and

previous studies show that *A. bruennichi* males never reject a first female based on her quality (Schulte et al., 2010). Rather, males appear to search randomly and mate with any female they happen to encounter first. Studies on other taxa also found that males never rejected the first female, but instead adjusted the investment in the second female depending on the quality of the first one (P. W. Bateman & Fleming, 2006; K. L. Reading & Backwell, 2007; Wong & Svensson, 2009). Thus, males often seem to use sampling strategies that allow for more flexibility than a fixed threshold would, and still entail fewer costs than active mate choice.

Although the use of an adjustable threshold seems more likely, we cannot definitively exclude that males evolved a mechanism allowing them to compare distinct females without high cognitive demands. For instance, female decorated crickets (*Gryllodes sigillatus*) avoid re-mating with the same male probably by comparing their own chemical profile with that of a male, a mechanism that does not involve learning and remembering a template (Ivy et al., 2005). A similar mechanism may explain that males visited the first female randomly and only applied the best-of-n strategy after securing the first copulation. Males may have to be close to a female to detect whether another female in the vicinity sends a different or a comparatively stronger signal than the present female so that their sensory system would be stimulated despite sitting in a pheromone cloud. This means, only relatively short distances between signalling females would enable the male to compare the chemical profile of his mate with that of another signalling female. A prediction that could be tested in future studies.

To date, we have no information over which distances spider males can perceive sex pheromones. Transmission of chemical or acoustic signals over long distances can be disturbed by many environmental factors (e. g. noise, pollutants, mate distribution), which

may make a direct comparison of mating partners difficult (Brumm & Slabbekoorn, 2005; Deb & Balakrishnan, 2014; Rosenthal & Stuart-Fox, 2012). Deb and Balakrishnan (2014) estimated the active spaces of calling male tree crickets (*Oecanthus henryi*) and found that most females would only hear calls of one male at a time and, hence, would be unable to compare males simultaneously. More research on olfaction of spiders is needed to elucidate how perception is related to behaviour.

So far, we discussed monogyny as a strategy to monopolise large females by plugging both female openings. However, less than 50% of the males survive to accomplish two copulations. Alternatively, males may invest in a single, long copulation and allow the female to cannibalise them during this first copulation. However, comparisons of copulation duration with small and large females do not indicate that males sacrificed themselves to large females. Also, males did not copulate longer with Southern females, although the cannibalism rate was higher than with Northern females. If anything, our findings indicate that a larger body size gives females an advantage in catching and cannibalising males.

Conclusions:

In conclusion, males use female size as reliable information about mate quality. Our results indicate that males can acquire such information via chemical cues even from a distance and use it to adjust their mate choice decisions to prevailing conditions. Such adaptations are particularly beneficial if female encounters are dangerous and occur sequentially. While we can reject the hypothesis of a fixed threshold, our results are consistent with a best-of-n strategy or an adjustable threshold rule. Therefore, we propose that future studies should examine the mechanisms behind size-dependent pheromone emission and link the mechanism to the decision-making process in males.

Methods:

Collection and Maintenance:

We collected juvenile *Argiope bruennichi* SCOPOLI 1772 from six locations in Southern France (Carcassonne and its surroundings) and nine locations in Northern Germany (Hamburg and its surroundings) between 1st until 22nd June 2014. Spiders were raised under laboratory conditions with a photoperiod of 16:8 LD and an ambient temperature of ca. 22 °C. They were held in cups of 250, 500 or 1,000 ml size depending on the spider's size. Each week, we provided water on five to six days and fed them twice with ca. 20 *Drosophila* spp. or three *Calliphora* spp. Very small individuals received three to five *Drosophila melanogaster* three times a week. We checked on at least six days a week for sexually mature males and females which can easily be determined by inspection of the genitalia (Uhl et al., 2007).

Measurements:

We took the weight of each female directly after the final moult and before they fed as an adult by using a calibrated scale (Mettler Toledo AB54-S; accuracy 0.1 mg). To avoid stress and harm to the animals, we measured the tibia-patella length only after their death. Left and right front legs were removed and photographed under a microscope, and the tibia-patella length was measured with the measuring tool in the software *Leica Application Suite V4.6* (Leica Microsystems (Switzerland) Limited). We used the adult weight to assign females to three size classes prior to the mating trials. We later confirmed that adult weight is an acceptable approximation of body size as it correlated strongly with leg length (Spearman correlation: $N=119$, $\rho=0.952$, $p<0.0001$). As a measure of fecundity, we took the weight of the first egg sac from each female.

Experimental set-up:

Adult females were placed in open plastic frames (Fig. 4.1a) scratched from the inside to facilitate silk attachment and web building. The frames were fitted with two holes in the bottom to fix them on the floor of the choice arenas. We kept each frame in a 3 l plastic box with air holes. To encourage females to build webs, we put one *Calliphora* in each plastic box. Females were frozen at -80° C after they died a natural death in the laboratory, or after they had produced two egg sacs. Males either died during the mating trials by sexual cannibalism, or they died naturally in the laboratory.

We designed choice arenas (Fig. 4.1b) to resemble a natural situation for a mate-searching male closely. Males entered the arena by walking down a stick attached to a platform at one of the shorter sides of the arena. A fan on the opposite end secured a constant weak airflow ensuring that the male perceived volatile pheromones from the three females that resided in their webs inside the arena. Those three females either originated from the Northern or the Southern population only. Since females of both origins significantly differed in adult weight (Mann-Whitney U test: $N=122$, $W=422$, $p<0.0001$) and leg length (Mann-Whitney U test: $N=119$, $W=279$, $p<0.0001$) we divided the three females into population-specific size classes (small, medium, large). The difference in adult weight between the small and large female to the medium one had to be at least 20 mg. A posteriori, we could confirm that in both treatments, the leg length of females differed significantly according to their size class (Kruskal-Wallis Test: Northern females: $N=76$, $\chi^2=47.71$, $p<0.0001$; Southern females: $N=80$, $\chi^2=50.14$, $p<0.0001$). Moreover, pairwise comparisons revealed that between treatments, only "large" Northern females and "small" Southern females had a similar size (Pairwise Wilcoxon Rank Sum test: $N=51$, $p=0.6442$), while in all other size classes,

Southern females were larger (Pairwise Wilcoxon Rank Sum test: all comparisons revealed a maximum $p < 0.0003$). In each test, the three females were closely matched for post-maturation age and did never differ in age for more than two days. Throughout the experiment, we used females that had a similar age that lay between four and eight days (median=5 days; interquartile range: 4-6 days). The positions of the three females in the arena were at different, fixed distances to the starting point (see Fig. 1b). By alternating the positions of the different size-classes, we achieved a balanced distribution of female size classes on all positions.

Males had one hour to find the first female before we replaced them. We observed the search and the mating outcome of each male. Once a male had found a female, we measured the copulation duration of the first mating, noted the occurrence of sexual cannibalism and determined his mating tactic: a male was defined as monogynous, if he copulated once and was cannibalised by the female or if he copulated twice with the same female. However, we only considered the latter option as a chosen mating tactic because we were unsure whether male death after the first copulation occurred willingly. A male was defined as bigynous, if he copulated once with the first female and then intended a bigynous tactic by leaving her web for at least one hour. In nature, it is extremely unlikely that males leave a female and then come back. Within this hour, we noted whether males visited and mated with one of the two other females. If males did not visit a second female, males had to show a clear distance to the first mating partner and were not allowed to have contact to the female's web or her test frame neither directly nor by silk threads. We planned 60 trials in total, 30 for each female origin but had to exclude three trials from each subset. We used six arenas in parallel, which were placed outdoors. Arenas were cleaned with ethanol between trials. To fulfil our conditions of age-

matched females and minimal adult weight differences of 20 mg, we re-used some females but only those that had no prior contact with males. In total, we used 34 of the 122 females in more than one test, but never more than three times (mean 2.2). Re-used females were evenly distributed across both origins and all size classes so that potential biases can be excluded.

Statistics:

We used R 3.0.3 (R Developmental Core Team, 2014) for the statistical analysis. Unless otherwise specified, we stated the mean and the standard error of continuous data. We checked whether data were normally distributed and chose parametric or non-parametric tests accordingly. We used single tests to analyse effects on the male's pre-copulatory choice and copulation duration, whereas effects on the probability of monogyny were tested with statistical models.

We used separate binary logistic models to test how female size affected the probability of cannibalism after the first copulation and the probability of monogyny and bigyny in surviving males. By restricting the latter analyses to males that survived their first copulation, we ensured that monogyny was an unambiguous male decision without potential influences of the females' cannibalistic attacks during the first copulation. As explanatory variables, we used the females' geographical origin (Northern, Southern), the female size class (small, medium, large) and their interaction. In previous models, we also included the adult age of males because it had a wide range between 8 and 34 days (20.7 ± 5.5 days) and could likely affect male decisions (Cluttonbrock, 1984). Since we did not find that male age improved any model fit, we decided to simplify the results and only present the effects of female quality.

Since our female size classes had no strict borders, we also dissolved the size classes and

used female size (leg length) as a continuous variable on the probability of monogyny. In another binary logistic regression, we explored the fitness outcome of monogynous and bigynous males. As an index for fitness, we used the number of eggs produced for the first egg sac. Counting eggs would have been destructive, which we had to avoid due to upcoming experiments. Instead, we used a mathematical equation to translate the weight of the first egg sac into an approximate number of produced eggs. To receive the function, we used laboratory data of 66 Northern females (see supplemental material) that did not participate in this study and of which we knew the weight of the egg sac plus how many eggs the egg sac contained. We found a strong positive relationship between weight and number of eggs per egg sac (linear regression: $N=66$, $F_{1,64}=127.06$, $p<0.0001$, adjusted $R^2=0.6598$). Therefore, we could use the estimates of the linear regression to create the mathematical equation ($y=1.3029x+74.9097$).

We applied stepwise simplification of the models by excluding effects that resulted in non-significant changes of deviance. When testing for the effect of the female size class on the probability of monogyny, standard errors of the estimates were very high (due to a quasi-complete separation). Therefore, we used the package “logistf” (Heinze, Ploner, Dunkler, & Southworth, 2013) and applied a Firth bias correction to the logistic regression. The model simplification was performed with penalised likelihood ratio tests using the *drop1* command.

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CHAPTER V

Males and females from low and high latitudes show different plasticity in life history and mate choice in the sexually size dimorphic spider *Argiope bruennichi*

Anna-Lena Cory & Jutta M. Schneider

Abstract:

There is strong evidence that the colonization of new habitats leads to changes in phenology and phenotype such as adult body size. Phenotypic plasticity may facilitate the adaptation to new environments, but only a few studies compared plastic responses to environmental conditions between colonizing and original phenotypes. The sexually cannibalistic spider *Argiope bruennichi* has recently expanded its range northwards and is now widespread all over Europe. The range expansion required adjustments to shorter growth and reproductive seasons and colder temperatures. Northern individuals are smaller than Southern individuals and more synchronous in their maturation. The northwards expansion is well-documented and still ongoing, and *A. bruennichi* attuned rapidly to northern conditions. Here we explore whether developmental plasticity contributed to the observed adjustments. Therefore, we used laboratory data from raising experiments in a common garden situation and compared the timing of and the body size at maturation for both sexes between years and between two feeding treatments. We found that adult body sizes were particularly highly plastic in Southern individuals facilitating rapid adaptation to novel climatic conditions and different resource availability.

Given the large variation in size and fecundity of Southern females, we further asked whether Southern males show size-dependent mate choice, which is known for Northern males. To test this, we gave Southern males a choice between three different-sized females and monitored pre and post-copulatory male decisions. Unlike expected and unlike results from Northern males, Southern males did not show mate choice based on female size and fecundity. While reduction in female body size and fecundity in northern areas posed no hindrance for mating success of females, male selectivity might have evolved as a response to less favorable northern conditions.

Keywords

developmental plasticity, food availability, monogyny, seasonality, sexual cannibalism

Introduction:

Phenotypic plasticity plays a key role in colonizing new habitats and adapting to changing environmental conditions (Ghalambor et al., 2007; Lande, 2009; Pfennig et al., 2010). Environmental changes initially lead to behavioral adjustments followed by developmental modifications that may predetermine reproductive conditions (Angilletta & Dunham, 2003; Arendt, 1997; Gotthard & Nylin, 1995; Hector & Nakagawa, 2012; Xie et al., 2015). Body size is regarded as particularly sensitive to environmental changes and can significantly affect reproductive success (Andersson, 1994; Jennions et al., 2001). High reproductive success is associated with a large body size if it is positively correlated with female fecundity (Dickerson, Quinn, & Willson, 2002; Honek, 1993; C. J. Reading, 2007; Simpson, 1995) and success in male competition (Hunt et al., 2009; Jennions et al., 2001). How large an individual can become is restricted by the available time to grow that, in turn, depends on the start and duration of the breeding season (Blanckenhorn, 2000; Nylin & Gotthard, 1998; Stearns, 2000). Changes in phenology will likely affect the sexual selection regime if for example variation in fitness traits increases.

Most commonly, time for growth diminishes and breeding seasonality changes with increasing latitude (Blanckenhorn & Fairbairn, 1995; Morrison & Hero, 2003; Sniegula, Golab, Drobniak, & Johansson, 2016). With less time available, individuals have either to increase the growth rate or to remain small. Such adaptations can lead to considerable geographical differences in mean body size and body size variation and thereby, alter the selection regime (Abrams et al., 1996; Blanckenhorn & Demont, 2004; Masaki, 1967; Mousseau, 1997; Sniegula, Golab, & Johansson, 2016). For instance, female body size can be a trait under male mate choice particularly if it correlates with fecundity but whether it pays for males to be choosy will

depend on the degree of variation in female size (Bonduriansky, 2001; Edward & Chapman, 2011).

We used the sexually cannibalistic spider *Argiope bruennichi* that show considerable size differences between low and high latitudes and between sexes (female-biased size dimorphism) (Cory & Schneider, 2018a; Krehenwinkel & Tautz, 2013). Extreme differences in body size between sexes should generate sex-specific adaptations in growth rate because sexual maturation of males and females still need to be synchronized. Therefore, mating systems in such spiders may be particularly sensitive to changes in environmental conditions and offer a good opportunity to compare adaptations in life history and mate choice of males and females from different latitudes.

Argiope bruennichi is a European-cosmopolitan spider that originally occurred in the Mediterranean area but expanded to the north over the last 100 years (Krehenwinkel & Tautz, 2013; Kumschick et al., 2011). Northern spiders are smaller and tolerate lower temperatures than the Southern phenotype and have a mating season that only lasts for 3-4 weeks (Krehenwinkel & Tautz, 2013; Zimmer et al., 2012). Although suggested, it is unknown whether individuals from the north and south differ in seasonality because only field data from Northern populations exist. However, laboratory studies indicate that Southern males have more time to grow because they remain longer in the final instar (developmental state before adulthood) than males from the north (Cory & Schneider, 2018a). Although Northern males are time-constrained, they show a preference for large female size (Welke et al., 2012). Whether Southern males do this as well still awaits testing.

Here, we investigated whether *A. bruennichi* from Northern and Southern Europe differ in life history and male mate choice. First, we compared plasticity in timing of maturation

and body size between individuals from Northern and Southern populations. Second, we tested whether Southern males also show male mate choice adjusted to female size. Since our expectations about male mate choice behavior directly depend on the results of the first experiment, we will only present the mating experiment after the results of the life-history section.

Experiment I: Life history

In many spiders, life history significantly differs between sexes, especially in mating systems with extreme female-biased size dimorphism (Blanckenhorn et al., 1995; G. Head, 1995; Hormiga et al., 2000; Scharff & Coddington, 1997; Wilder & Rypstra, 2008b). The smaller males are mostly protandrous and under scramble competition to reach females before rivals (Elias et al., 2011). Hence, the timing of maturation may be more important for males than large body size (e. g. Kasumovic & Andrade, 2006). In contrast, large body size is very fitness relevant for females (Marshall & Gittleman, 1994; Simpson, 1995; Skow & Jakob, 2003), but can be limited by seasonal constraints. For instance, *Nephila clavipes* from highly seasonal habitats showed a negative relationship between body size and time of the mating season, while females from less seasonal habitats did not (Higgins, 2000). This suggests that females from less seasonal habitats are less restricted in development and can optimize body size even if it may take some time. Here, we tested the hypothesis that individuals that are already constrained in body size by time should be able to tolerate additional and possibly adverse environmental conditions that could further impede growth. The expansion to new habitats is often accompanied by several environmental changes (e. g. food availability, temperature) and high phenotypic plasticity may facilitate a rapid adjustment to novel stressors (Ghalambor et al., 2007; Kawecki & Ebert, 2004; Lee, 2002). Therefore, we assumed that

(ancient) Southern *A. bruennichi* respond plastically to changing conditions, while the (new) phenotype from the north is adapted to withstand unfavorable northern conditions and shows more uniformity in body size and timing of maturation.

To investigate phenotypic plasticity in body size and timing of maturation in *A. bruennichi* from Northern and Southern Europe, we gathered our laboratory data from two years and compared individuals that were collected as juveniles in different years and that were raised under different feeding conditions (low and high food availability). First, we looked at the time window in which all our individuals reached sexual maturity (first until the last spider that reached sexual maturity) and expected a larger variation in Southern individuals than Northern individuals. Second, we hypothesized that food shortage would prolong developmental time and decrease body size in Southern individuals but not in Northern individuals. Third, we assumed that Southern individuals would show more variation in body size between collection years while Northern individuals would show a similar mean body size between years. If males and females would differently cope with environmental conditions, we would expect more variation in body size in males than in females because body size should be more fitness relevant for females and hence, they should be adapted to maintain a large body size.

Methods: Life history

Collection:

In 2014 and 2015, we collected juvenile *Argiope bruennichi* SCOPOLI 1772 from eight sites in Southern France (Carcassonne and surroundings) and nine sites in Northern Germany (Hamburg and surroundings). In June 2014, we collected spiders around their third instar. The year after, the collection of spiders occurred earlier (between April 25th and May

Table 5.1: Birthplace, the developmental stage when individuals were brought to the laboratory and rearing conditions of individuals in the test years 2014 and 2015.

	2014	2015 (+)	2015 (-)
Birthplace	field	field	laboratory
Developmental state*	≥ third instar	≤ second instar	first instar
Food availability	high	high	low
Light conditions	16:8 LD	14:10 LD	14:10 LD

+ = high food availability; - = low food availability.

18th 2015) and we collected spiders that either were shortly before leaving the egg sac (Northern Germany) or had left their egg sac not long ago (Southern France). In the same year, we raised the F1 generation of spiders whose parents we collected in 2014. The different rearing conditions of spiders are summarized in Table 5.1.

Maintenance:

In the laboratory, we kept all spiders under room temperature and 16:8 LD conditions in 2014 and 14:10 LD conditions in 2015. We held spiders individually in upturned plastic cups (differently-sized depending on spider size: 50-1,000 ml) that had a hole at the bottom stuffed with cotton wool. On 5-6 days a week, we moistened the cotton wool or sprayed water inside the cup to provide the spiders with water. We checked on at least six days a week for sexually mature spiders by inspecting their genitalia (Uhl et al., 2007). Females were frozen at -80° C after they had produced egg sacs or died a natural death in the laboratory. Males also died naturally in the laboratory, or they were victims of sexual cannibalism during the mating tests (see 2. experiment). Spiders that participated in mating tests were stored at -80 °C after they died.

Food provision:

In the beginning, all very small individuals in 50 ml cups received three to five *Drosophila melanogaster* on three days a week. This was done to ensure that individuals reached an

instar in which they could easily catch prey. Only then, we introduced the food manipulation that ran until spiders reached sexual maturity.

In two treatments, we manipulated the availability of food by altering food quantity and duration of access to food. Under high food availability, we fed individuals with ca. 20-30 *Drosophila spec.* or three *Calliphora spec.* twice a week. After feeding, our food-flies usually survive for at least two or three days and, therefore, allowed ad libitum access to food. Under low food availability, the access to food as well as the food quantity was more limited. Here, spiders were also fed on two days but received less food, namely 7-20 *Drosophila melanogaster* between their third and sixth instar, and afterward, ca. 20 *Drosophila hydei*. The access to food was restricted to 48 hours a week because we removed the food whether dead or alive 24 h after each feeding.

While all field-collected spiders were reared under high food availability, the laboratory-bred F1 generation from 2015 were assigned to the low food availability treatment. We tested effects of low and high availability only in individuals that we reared in 2015 because only then we could introduce the feeding treatments from the third instar on. In 2014, some individuals already reached later developmental states when they were collected.

Measurements:

Following our standard lab protocol, we noted the date of sexual maturity and weighed each spider to an accuracy of 0.1 mg on the same day. Adult weight is highly related to the tibia-patella length (linear regression: males; $N=61$, $F_{1,59}=440.19$, $p<0.0001$; females: $N=276$, $F_{1,274}=4453.4$, $p<0.0001$) which is a preferable index for the fixed body size (e. g. Moya-Larano, Vinkovic, De Mas, Corcobado, & Moreno, 2008). Note that we measured the leg length of spiders that participated in mating tests after they died (see Cory & Schneider, 2018a, Chapter IV, the 2nd experiment of this Chapter). To determine the tibia-patella length, we detached first leg pairs, photographed the legs with a camera that was connected to a binocular and measured the length between tibia and patella with special software (Leica Application Suite V4.6; Leica Microsystems (Switzerland) Limited).

In 2015, we also noted the date on which males reached their penultimate state (preliminary instar) which we could recognize by the genitalia that were now visible but still undifferentiated. Ad-libitum fed males took part in a study testing effects of female silk cues on the developmental time of the final instar (Cory & Schneider, 2018a). In the current study, we only considered males that were raised without female cues.

Statistics:

We used R 3.5.1 for the statistical analyses (R Core Team, 2018). If we conducted single effect tests, we checked the data for normality and used non-parametric tests if we found a significant difference from a normal distribution. We mostly applied (generalized) linear models or (generalized) linear mixed effect models (package nlme; Pinheiro et al., 2013) if collection site or egg sac ID was entered as random terms. Before we chose the type of model, we tested whether the random effect would significantly enhance the model

and compared the AICs of the linear model and the linear mixed effect model using restricted maximum likelihood estimation. If necessary, we log-transformed the data to receive normally distributed residuals. Furthermore, we checked for data that may influence the distribution of residuals. We only removed data points, if removal did not change the significance of results but improved the model fit to receive better estimates. We tested the effect of single variables by model simplification. We removed the variable of interest from the full model and compared the original model with the simplified model with likelihood ratio tests. For model comparisons, mixed effect models were estimated with maximum likelihood.

We separated the analysis into three parts. First, we tested how the appearance of adult individuals depended on sex, geographical origin or food availability. Due to the platykurtic distribution of data, we decided to analyze the data with a binomial model. We split the time frame in which we found newly sexually matured individuals in half and tested effects of whether individuals reached sexual maturity early (first half) or late (second half) in the season (timing of maturation). We analyzed the data from 2014 and 2015 separately because only in 2015 we could test the effect of food availability. Furthermore, we could only consider the collection site as a random term in 2014, but not in 2015, where only some spiders were collected in the field, while the others were born in the laboratory (see Table 5.1). Second, we compared the adult weight of ad-libitum fed individuals between 2014 and 2015 and included the collection site as a random term. We analyzed data of males and females and Northern and Southern spiders separately because due to the natural difference in body size between sexes and populations (e.g. Cory & Schneider, 2018a; Fromhage et al., 2003; Krehenwinkel & Tautz, 2013) the data were bimodally distributed and as a result, statistical analyses including these

Table 5.2: Summary of results testing effects of sex, geographical origin and food availability (only in 2015) on the timing of maturation (early vs late). We conducted binary logistic regressions and included “collection site” as random effect for the data from 2014. If different, we present the estimates and standard errors (SE) of both the full model (in brackets) and the simplified model (without brackets).

	variable	Estimate + SE	Chi ²	p
2014 (N=780)	Intercept	-0.7119 ± 0.5399		
	Sex (male)	-0.9670 ± 0.5739	226.58	<0.0001***
	Geographical origin (Southern)	0.9237 ± 0.5636	29.13	<0.0001***
	variable	Estimate + SE	Df-deviance	p
2015 (N=461)	Intercept	0.9789 ± 0.5858		
	Sex (male)	-0.8387 ± 0.5823	26.89	<0.0001***
	Geographical origin (Southern)	(-0.6230 ± 0.5851)	2.20	0.1383
	Food availability (low)	-0.9014 ± 0.5835	49.58	<0.0001***

factors led to non-normally distributed model residuals. Finally, we tested effects of food availability on adult weight in both sexes and on the duration of the penultimate state in males. Besides food availability, we included into the latter analysis the timing of maturation as continuous variable plus the interaction between food availability and timing of maturation. We applied a linear mixed effect model with egg sac ID as a random factor to data from northern individuals (adult weight: 30 egg sacs; duration of final instar: 29 egg sacs (due to a removed outlier)). Data from Southern individuals were analyzed with linear models because we were unable to determine a random variable as the high-food-availability individuals were collected in the field when they had already left their egg sac while the low-food individuals were taken from egg sacs (Table 5.1).

Results: Life history

Males and females reached sexual maturity between May 11th to August 27th. Independent of test year, food availability or geographical origin, males matured earlier than females (Table 5.2, Fig. 5.1). Across both years, all spiders matured within a period of 55 ± 8 days. However, in 2014, Northern spiders matured significantly earlier than Southern spiders, but we found no difference in 2015 (Fig. 5.1a, b). While Southern individuals matured at the

same time in both years, Northern individuals were later adult in 2015 than in 2014 (Table 5.2) such that the phenology of both populations converged.

Despite their shorter developmental time in 2014, Northern females did not differ in maturation weight between years and males were even larger in 2014 despite having had more time to grow (Table 5.3, Fig. 5.2a, b). Southern males and females were both heavier in 2014 than in 2015 (Table 5.3, Fig. 5.2 a, b).

Focusing on the food treatments in 2015, the most obvious difference is the timing of maturation that is spread out over a much longer period under low food (Fig. 5.1 b, c).

Particularly the males started to mature much earlier under low than under high food availability. While maturing at an earlier date was associated with a longer penultimate instar in Southern males, no such effect was detected in Northern males (Table 5.4). In females, we found that low food availability led to lower maturation weights in Northern and Southern populations (Table 5.4, Fig. 5.2a). In contrast, males from the two populations differed in their responses in that low food availability resulted in a lower maturation weight in Southern males but not in Northern males (Fig. 5.3, Fig. 5.2b).

Instead, the maturation weight of Northern males decreased with progressing time of season, although the effect was only slightly

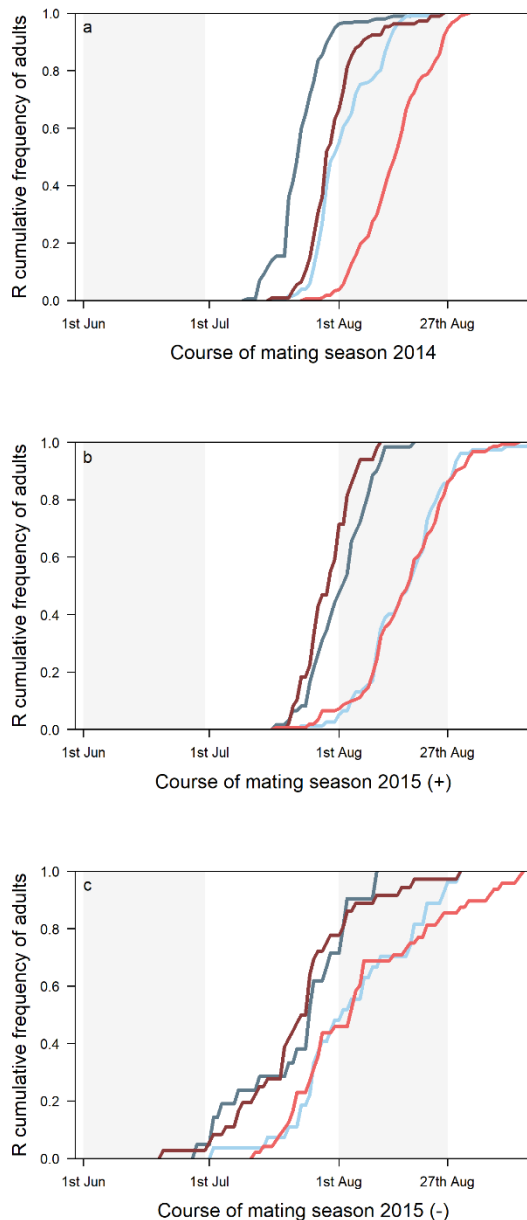


Figure 5.1: The relative cumulative frequency of northern (blue) and southern (red) sexually mature males (dark lines) and females (light lines) in different test years and under different rearing conditions (+)=high food availability; (-)=low food availability

significant (Table 5.4). Neither in Northern females nor in Southern males and females, the timing of maturation was associated with their maturation weight (Table 5.4).

Experiment II: Mating tests

Experiment I revealed that Southern females were particularly plastic in body size varying across a range of an order of magnitude. This size variation directly translates into variation in female fecundity (Marshall & Gittleman, 1994; Simpson, 1995). Since most males mate with only one female, males should benefit very strongly if they preferably mate with females from which they can expect a high reproductive output (Bonduriansky, 2001; Edward & Chapman, 2011). Northern males have already been shown to preferentially monopolise large females (Welke et al., 2012) but such data are lacking for Southern males.

Female monopolization can be achieved by mate plugging if males copulate twice with the same female (Nessler et al., 2007). That is because males and females have paired genitalia and per copulation, males only use one of their genital organs (pedipalps) to transfer sperm and to plug the compatible genital opening of the female. To plug the opening, males break off the tip of the pedipalp, which loses its functionality afterwards and leaves males with a maximal lifetime mating success of two copulations (Nessler et al., 2007). Thus, there are only limited possible mating outcomes for males that are well described for Northern males. In the first option, males only copulate once and die because they are cannibalised by the female that stereotypically attacks males. 50 to 80 % of males will not survive the first copulation, but males can decrease the risk of dying if they copulate short (Fromhage et al., 2003; Nessler et al., 2009; Schneider et al., 2006; Welke et al., 2012). Long copulations will likely end in male death but increase sperm transfer (Schneider et al., 2006). This option may be considered the most extreme form of reproductive investment. Exclusive paternity is not secured but even if the female remates, males will father at least half of the offspring. Since mating is very dangerous, it would be adaptive if males preferably visit large females

Table 5.3: Summary of results showing the differences in adult weight between the years 2014 and 2015 of individuals with high food availability in Northern and Southern males and females. We conducted linear mixed effect models with collection site as a random effect. Except for the data of Southern males, estimates and standard errors (SE) are log-transformed.

	variable	Estimates + SE	L ratio	p
Northern ♀♀				
(N ₂₀₁₄ =257; N ₂₀₁₅ =77)	Intercept (2014)	4.725 ± 0.064	0.1693	0.6807
	Year (2015)	-0.019 ± 0.055		
Northern ♂♂				
(N ₂₀₁₄ =201; N ₂₀₁₅ =57)	Intercept (2014)	2.919 ± 0.026	77.5507	<0.0001***
	Year (2015)	-0.386 ± 0.038		
Southern ♀♀				
(N ₂₀₁₄ =206; N ₂₀₁₅ =152)	Intercept (2014)	5.314 ± 0.050	131.8364	<0.0001***
	Year (2015)	-0.517 ± 0.040		
Southern ♂♂				
(N ₂₀₁₄ =108; N ₂₀₁₅ =44)	Intercept (2014)	28.72 ± 0.53	11.2335	<0.0001***
	Year (2015)	-3.43 ± 0.99		

or increase the copulation duration if the female is large. However, previous studies indicate that males neither search for large females nor adjust copulation duration to female quality (Schulte et al., 2010; Chapter IV). If males survive, they can leave the current mating partner and search for a second female (bigynous mating tactic). This option will leave one of the two female genital openings unplugged and give rival males the chance to occupy the virgin genital opening of the female. Therefore, the only safe option for the male to avoid sperm competition is to mate twice with the same female (monogynous mating tactic) and Northern males more likely

use this strategy if females are large (Welke et al., 2012; Chapter IV).

To test mate choice decisions in Southern males, we used the same experimental design as in Chapter IV and gave males a choice between three differently sized females. We monitored which female was encountered first (e.g. closest or largest), the duration of the first copulation, whether sexual cannibalism occurred and whether surviving males mated with the same female or left her to search for another mate. We hypothesised that Southern males would use the same decision rules as

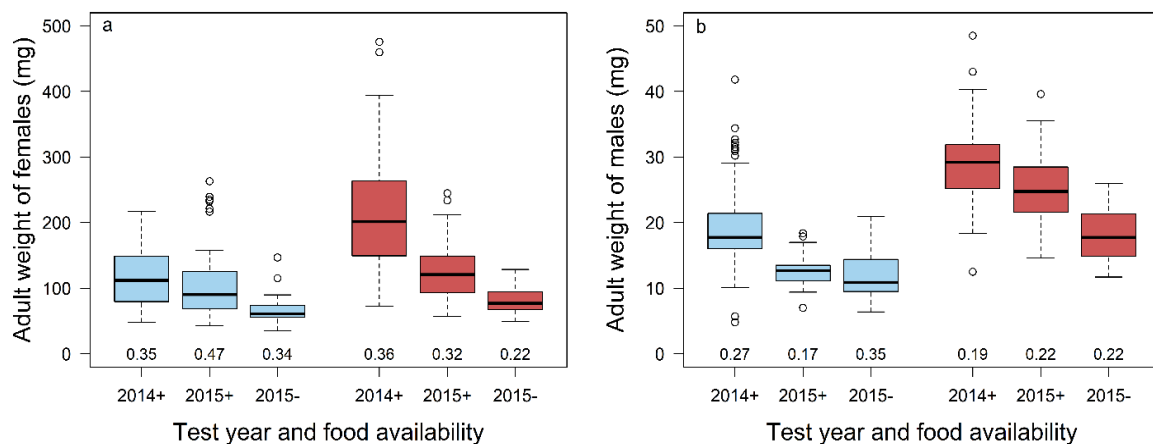


Figure 5.2: Adult weight of Northern (blue) and Southern (red) individuals in different test years and under different food availability (+=high; -=low). a) adult weight of females; b) adult weight of males. Numbers at the bottom indicate coefficients of variation.

Table 5.4: Summary of results testing effects of food availability on adult weight in males and females from Northern and Southern populations and the duration of the final instar in males. Besides food availability, it was also tested whether the timing of maturation and the interaction between both variables affect the adult weight. Only data from 2015 is included in the analysis. We conducted linear mixed effect models with collection site as a random effect in Northern individuals and linear models in Southern individuals. We took the estimates and standard errors (SE) of non-significant variables from the full model (in brackets), otherwise we present estimates and standard errors of the minimal adequate model (without brackets). [†] behind response variables indicate log-transformed data.

	variable	Estimate + SE	L ratio	p
Northern ♀♀				
Adult weight[†] (N _{high} =76; N _{low} =27)	Intercept	4.581 ± 0.064		
	Food availability (Low)	-0.434 ± 0.123	11.16	>0.0001***
	Timing of maturation	(0.004 ± 0.005)	1.14	0.2856
	Interaction	(0.001 ± 0.007)	0.01	0.9226
Northern ♂♂				
Adult weight[†] (N _{high} =57; N _{low} =20)	Intercept	2.726 ± 0.057		
	Food availability (Low)	(0.126 ± 0.139)	1.18	0.2775
	Timing of maturation	-0.013 ± 0.003	19.75	>0.0001***
	Interaction	(-0.002 ± 0.006)	0.17	0.6816
Duration of final instar (N _{high} =57; N _{low} =21)	Intercept	(14.330 ± 0.600)		
	Food availability (Low)	(-0.323 ± 1.095)	1.12	0.2697
	Timing of maturation	(0.051 ± 0.038)	2.06	0.1514
	Interaction	(-0.014 ± 0.049)	0.05	0.8198
	variable	Estimate + SE	F	p
Southern ♀♀				
Adult weight[†] (N _{high} =152; N _{low} =37)	Intercept	4.770 ± 0.025		
	Food availability (Low)	-0.413 ± 0.051	65.86	>0.0001***
	Timing of maturation	(0.005 ± 0.002)	2.75	0.0988
	Interaction	(-0.004 ± 0.004)	1.46	0.2280
Southern ♂♂				
Adult weight (N _{high} =43; N _{low} =36)	Intercept	25.29 ± 0.755		
	Food availability (Low)	-7.061 ± 1.126	39.35	>0.0001***
	Timing of maturation	(-0.103 ± 0.123)	2.19	0.1430
	Interaction	(0.028 ± 0.145)	0.04	0.8499
Duration of final instar (N _{high} =43; N _{low} =36)	Intercept	3.274 ± 0.047		
	Food availability (Low)	-0.228 ± 0.069	0.07	0.0014**
	Timing of maturation	-0.005 ± 0.002	4.27	0.0422*
	Interaction	(0.001 ± 0.006)	0.04	0.8493

males from the north. Therefore, we predicted (1) that males encounter females haphazardly and never reject the first female, (2) that the male's reproductive investment (copulation duration and sexual cannibalism) is independent of female size and (3) that once-mated males are more likely to monopolize the largest female.

Methods: Mating tests

Female size classes:

For the tests, we used spiders collected in 2014 and general methods are the same as described for experiment I.

We used a sequential search paradigm and gave 30 virgin males a choice between three differently-sized virgin females that we assigned to the size classes: small, medium and large. The size differences between the three

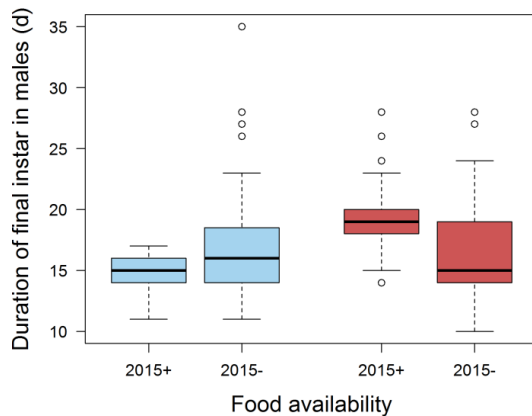


Figure 5.3: Duration of the final instar (penultimate state) in males from Northern (blue) and Southern populations (red) that were either reared under high food availability (+) or low food availability (-).

females were based on adult weight and the minimal difference had to be 20 mg (ANOVA: $N=90$, $F_{2,87}=173.14$, $p<0.0001$). Since weighing spiders after the final molt is a standard procedure in our laboratory, it is an easy way to estimate the overall female size without causing additional stress for the spiders. Later we confirmed a linear relationship between adult weight and the tibia-patella length of females (linear regression: $N=67$, $F_{1,65}=761.6$, $p<0.0001$; adjusted $R^2 = 0.9202$). Additionally, we weighed each first egg sac and used the weight as an index for female fecundity based on an existing calibration of weight and clutch size (see Chapter IV). Also, in the current sample we could confirm the correlation of body weight and egg sac weight (Kruskal Wallis test: $N=26$, $\chi^2=14.8295$, $p<0.0001$). We took care that the three females had a similar adult age (difference of 2 days the most). We confirmed post-hoc that they did not differ in body condition (ANOVA: $N=88$, $F_{2,82}=0.2981$, $p=0.743$). We estimated body condition by using the residuals of the linear regression between leg length and weight on test day (Jakob et al., 1996).

Test preparation:

At least one day before the mating tests were run, we transferred adult females into small frames (upper width 14.5 cm, lower width 9 cm, length 13 cm) that could be fixed on the floor of the test arenas (Fig. 5.4). and which were suitable for building webs. We encouraged web building in those frames by adding one *Calliphora*.

The experiments occurred parallel to the study presented in Chapter IV. They took place on the roof of the Institute of Zoology in Hamburg, Germany underneath an awning or a rain cover. In the morning before the tests started, we distributed the females into triple groups with one from each size-class. To create compatible groups at all times, we had to re-

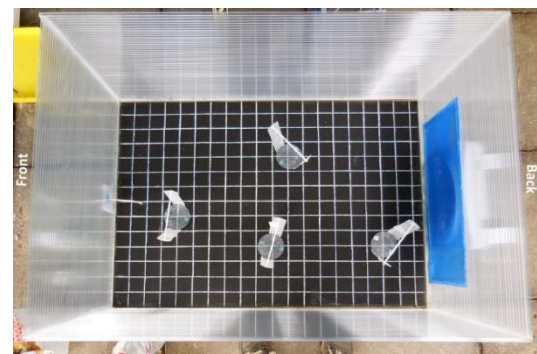


Figure 5.4: Picture of a test arena from above. The test arenas had a size of 100 x 70 x 50 cm. The front wall of the arena (4 x 4 cm) had an entrance that was used as starting point for the males. The entrance was 3 cm above the floor and was connected with the nubby floor by a plastic rod that males could walk along. On the back wall was a window of 50x20 cm that was sealed with blue gauze. Behind the window was a fan that gave a slight breeze into the arena that should ensure the flow of female sex pheromones or other chemical cues for all tests. This was done because in spiders communication strongly relies on olfactory cues (Gaskett, Herberstein, Downes, & Elgar, 2004; Schulz, 2013). On the floor were four different fixed positions, on which we could fix the frames of females. Since only three females participated in the tests one of the middle positions contained an empty frame (upper frame from the picture's perspective).

use 25 % of the females multiple times ($\sim 2.3 \pm 0.6$ times). Thereby, we took care to only re-use females that had no previous contact with males. The experiments took place in clean test arenas that had four fixed positions in three different distances to the starting point for males (1x close, 2x medium-distant, 1x far-distant). The three females were always positioned at three different distances, while the second middle-distant position was occupied by an empty frame. The order of the females was randomly determined before the experiment started which ensured that the six size-distance-constellations were equally often represented.

Procedure during mating tests:

The mating tests started, when we carefully put the male into the entrance of the arena by using a brush. Males had one hour to find the first female before we replaced them. If a male found a female, we timed the copulation duration by using a stopwatch and monitored whether the male was cannibalised after the first copulation and which mating strategy surviving males chose. A male was monogynous if he copulated twice with the same female. A male was bigynous, if he copulated twice with two different females or if he intended a bigynous strategy by leaving the web of the first mating partner for at least one hour.

Some males survived the copulation with the first female and remained in her web without remating until the end of the day and, hence, chose neither a monogynous nor bigynous tactic until then. We gave those males the chance to decide overnight and left them with the three females in the test arena. We marked the females individually with three *Pelikan* PLAKA® colors because females often build a new web in the morning. We covered the arenas with a plastic sheet from above. On the next day, we checked whether the male was alive or eaten by one of the females. Note that

males do not resist female attacks after two copulations and serve females as a meal. We only used such a date point if the remains of a male could be unambiguously assigned to a female. We also excluded tests if males were alive and still found in the web of the first mating partner.

As in experiment I, we stored all spiders that participated in mating tests at -80°C and later measured the leg length. Moreover, we examined male pedipalps for damage blind to the treatment and results. We noted whether a small or large part of the embolus (sperm transferring genital part) was broken off.

Statistics:

We followed the same general procedure to handle data as explained in experiment I. First, we tested whether pre-copulatory mate choice depends on female size class or the distance to the male's starting position and applied Pearson χ^2 tests to see whether males encounter the largest or closest female with a higher probability than the other two females. Second, we analyzed whether the reproductive investment (copulation duration and probability of sexual cannibalism) was higher with large females. We used an ANOVA to analyse effects on the copulation duration, which we had to log-transform to achieve normality. To test effects of female size class on the cannibalism rate, we applied a binary logistic regression and included as a further explanatory variable the copulation duration to test whether it is related to the probability of cannibalism. We also used a binary logistic regression to test the effects of female size on post-copulatory mate choice (monogyny vs bigyny), which should become apparent by a higher probability of monopolising large females.

Results: Mating tests

Overall 95.6 % of the males copulated with the female, whose web they encountered first. The female they encountered first was not the consistently the closest female (χ^2 test: $N=30$, $\chi^2=2.4$, $df=2$, $p=0.3012$) nor the largest female in the arena (χ^2 test: $N=30$, $\chi^2=1.4$, $df=2$, $p=0.4966$; Fig. 5.5a) suggesting a random search. Furthermore, female size class had no influence on the duration of copulation (linear regression: $N=30$, $F=0.3618$, $p=0.6997$; Fig. 5.5b) or the cannibalism rate (binary logistic regression: $N=30$, $df\text{-deviance}=0.0733$, $p=0.9640$; Fig. 5.5c), which was 50 %. In agreement with the previous findings, cannibalised males copulated significantly longer than males that survived their first copulation (binary logistic regression: $N=30$, $df\text{-deviance}=4.7761$, $p=0.02886$). Of the 15

surviving males, seven males remated with the same female (monopolization). However, unlike in Northern males the probability of monopolizing a female did not depend on her size class (binary logistic regression: $N=15$, $df\text{-deviance}=2.1696$, $p=0.3380$; Fig. 5.5d).

We examined the genitalia of 27 males and found that the tip of the sperm transferring genital part (embolus) was broken off in 63 % of them. In the remaining males, the complete embolus was either missing or almost completely detached from the joint that connects the embolus to the rest of the genital organ. These emboli were further examined and also showed breakages on the tip suggesting that rather small than large breakages were intended for mate plugging.

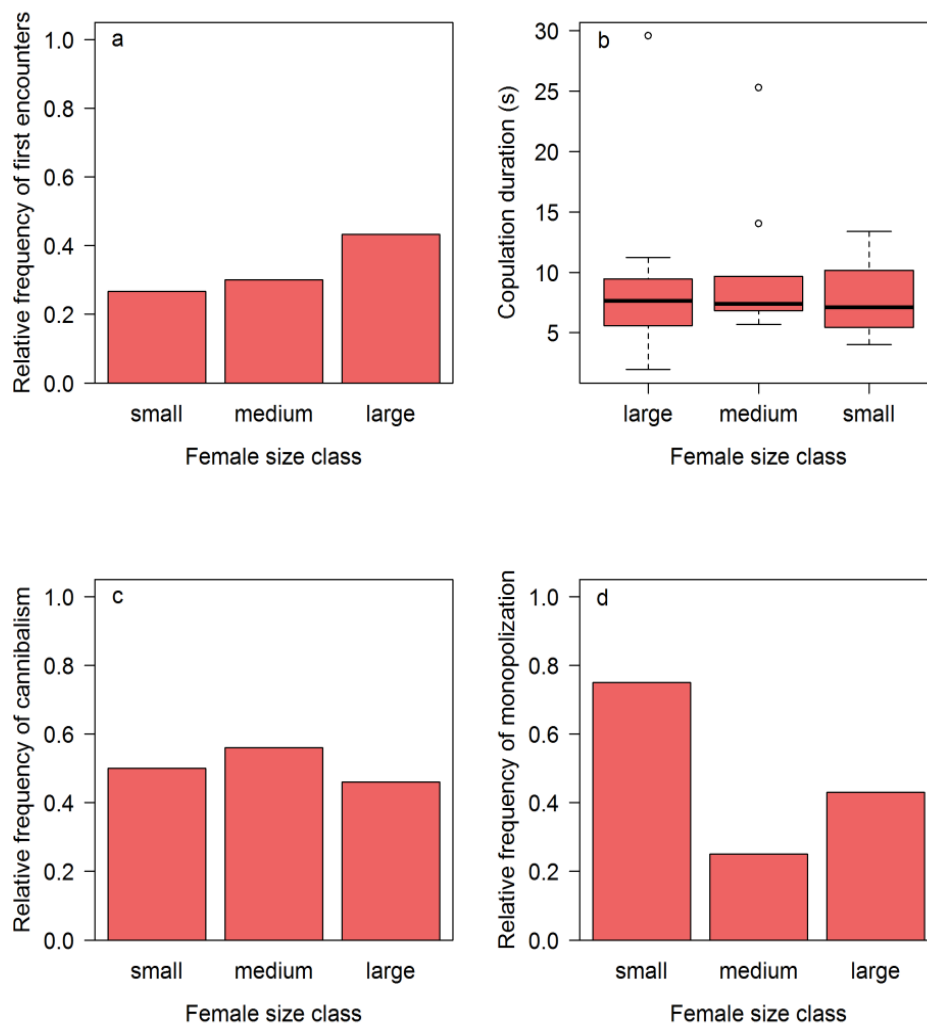


Figure 5.5: Effect of female size class on mating decisions.

Discussion:

We investigated differences in life history in the cannibalistic orb-web spider *Argiope bruennichi* that either came from Northern or Southern Europe. Both populations showed a similar phenology and similar responses to differences in feeding regime. The only difference between populations was that Southern individuals reached sexual maturity at a similar time in both test years, whereas Northern individuals matured earlier in 2014 than in 2015. However, we found considerable differences in the adjustment of adult weight between both populations. In Northern spiders, females had a robust adult weight between years but were lighter under low food availability, while males weighed less in 2015 but were not affected by the feeding conditions. In contrast, Southern males and females were both very plastic and showed significant differences in adult weight between years and feeding conditions. Despite the high plasticity in female adult weight, we found no indication that Southern males adjusted mate choice decisions.

Experiment I: Life history

Seasonality is assumed more variable in lower latitudes (Blanckenhorn & Fairbairn, 1995; Morrison & Hero, 2003; Sniegula, Golab, Drobniak, et al., 2016). We predicted that even under laboratory conditions, we could trace seasonal differences between Northern and Southern *A. bruennichi* and hypothesized that Southern individuals would show a longer and between years, a more flexible period in which we could observe freshly molted adults. On the contrary, our results indicate more variability in the emergence of Northern individuals. While Northern individuals became earlier adult in 2014, Southern individuals occurred at a similar time in both years. A reason could be that Northern *A. bruennichi* generally become earlier adult than Southern individuals, but since we collected all individuals in 2015 when

they were very small, laboratory conditions might have a stronger impact on development than in 2014. However, our results do not exclude that Southern spiders are adapted to a more flexible seasonality because we kept spiders under continuous conditions, whereas in nature, temperature and day length usually increases until the first adult *A. bruennichi* appear. Since Southern *A. bruennichi* showed high phenotypic plasticity in body size and developmental time, it would be likely that growth rates are plastically adjusted to increases in temperature and day length and lead to a more flexible breeding season.

It is only poorly understood how ectotherms synchronize the breeding season. The process of development is regulated by many interacting external variables such as photoperiod, temperature, food availability and social cues (Bradshaw & Holzapfel, 2007; Neumann & Schneider, 2016; Qiu, Gosselin, & Qian, 1997) and some variables are more important than others. For instance, larvae of the damselfly *Lestes congener* shortened their development and were smaller if only the light regime was changed to simulate the end of the breeding season (Rotheray, Goulson, & Bussiere, 2016). Unfortunately, the conditions between 2014 and 2015 not only differed in the light regime (16:8 LD and 14:10 LD) but also in the time when we collected spiders. Therefore, we cannot draw definite conclusions. Future studies should ascertain the natural population dynamics in Southern populations to verify seasonal conditions. Based on this, split-brood experiments simulating population-specific environmental conditions may help to understand the seasonal impact on the timing of maturation.

In agreement with our predictions, Southern populations showed a high variation in body size between test years and in response to the feeding conditions. In contrast, Northern females were similar large between years and the body size of Northern males was not affected by feeding conditions. This suggests

that Northern individuals are more capable of buffering unfavorable environmental conditions, which may have facilitated colonization of Northern habitats. Individuals from the north are a result of an admixture of Mediterranean and Asian genotypes (Krehenwinkel & Tautz, 2013). Presumably, this admixture led to a phenotype that was able to adjust rapidly to novel environments and to deal with the colder conditions in the north (Krehenwinkel et al., 2015; Krehenwinkel & Tautz, 2013). A study on the common frog *Rana temporalis* showed a similar pattern (Dahl, Orizaola, Nicieza, & Laurila, 2012) in that Northern tadpoles were more efficient in compensating a time of food shortage in comparison to Southern tadpoles that are less time-constrained and under a lower predation risk than Northern phenotypes (Dahl et al., 2012).

The sex-specific responses in Northern populations may be particularly explained by different selection pressures on growth strategies. While females should generally increase body size to enhance fecundity, males are under scramble competition and need to be sexually mature in time (Stillwell, Blanckenhorn, Teder, Davidowitz, & Fox, 2010), especially if the mating season is very short as it is in Northern populations (Zimmer et al., 2012). Hence, feeding conditions will generally have a severer impact on the fitness of females than on the fitness of males. Similar results were found in the wolf spider *Hygrolycosa rubrofasciata* (Vertainen, Alatalo, Mappes, & Parri, 2000). Here, females matured later and were smaller if the feeding regime was low, whereas feeding conditions had no impact on males (Vertainen et al., 2000). Another example is the cellar spider *Pholcus phalangoides*, where under unfavorable feeding conditions, females prolonged the duration of the final instar presumably to increase growth (Uhl, Schmitt, Schafer, & Blanckenhorn, 2004). In contrast, male cellar spiders had a similar duration of the final instar

independent of feeding conditions (Uhl et al., 2004). Why only Northern females had a similar adult weight between the years is difficult to understand. One reason could be that traits under high selection pressure should be under stabilising selection and therefore less plastic (Sniegula, Golab, Drobniak, et al., 2016; Stearns & Kawecki, 1994). This may be especially true for Northern females that are smaller and presumably experience higher seasonal time-constraints in development than Southern females (Krehenwinkel & Tautz, 2013). Indeed, our results suggest that Northern females were restricted in growth because they could not enhance their maturation weight by maturing later in the mating season. However, this was also true for Southern females. A reason could be that the development of Southern females strongly relies on information about current natural conditions from which they are cut off in the laboratory where conditions remain constant.

Different studies found that under unfavorable feeding conditions, individuals matured later and at smaller body size (Arendt, 1997; Bauerfeind & Fischer, 2005; Blanckenhorn, 1999; Kleinteich & Schneider, 2011; Uhl et al., 2004). Such a behavior can be adaptive if prolonging the development increases the body size at least to some extent or if a period of food shortage can be compensated when conditions become better (Blanckenhorn, 1999, 2006; Hector & Nakagawa, 2012; Metcalfe & Monaghan, 2001; Neumann, Ruppel, & Schneider, 2017). Therefore, it is curious that Southern males shortened the penultimate state. A reason could be that if males cannot expect better feeding conditions, it may be beneficial to become adult and start mate searching early. In *A. bruennichi*, males can use an opportunistic mating strategy in which they search for penultimate females and guard them until they molt to sexual maturity, during which they can safely mate with females. That is because females are defenseless during molting and cannot attack

(Fromhage et al., 2003; Uhl et al., 2015). However, our data also suggest that at least some males delayed development because the period in which final molting events occurred was very long. This further confirms a high flexibility in males.

Besides evolutionary thought, this study is also a reminder that slightly different conditions may change the outcome of an experiment because individuals can be sensitive and react plastically to slight differences. In 2014 and 2015, we collected individuals from the same regions but in different life stages and found significant differences in adult weight and the timing of maturation. Especially during development, individuals are known to react plastically and may develop non-reversible traits (Snell-Rood, 2013; West-Eberhard, 2003). Therefore, we highly recommend that different results with the same organism should be verified under consideration of effects that could have affected the organism's developmental plasticity.

Experiment II: Mating tactics

Variation in female quality is a key condition in the evolution of male mate choice (Edward & Chapman, 2011). Although female size and, thus, female quality seems to be very plastic in Southern *A. bruennichi*, we found no indication that Southern males adjusted mate choice decisions accordingly. Neither did males visit large females, nor did they increase their reproductive investment or use a monogynous mating strategy if females were large. This was surprising because males from the north preferentially monopolized large females and left smaller ones after the first copulation (Chapter IV). This suggests that size-dependent mate choice only evolved during the Northern colonisation (Krehenwinkel & Tautz, 2013).

Northern females are considerably smaller than Southern females and produce fewer eggs (Chapter IV). The reduced fecundity of Northern females might have favored fitness-

enhancing mating strategies. Furthermore, the evolution of male mate choice may have been facilitated by the synchronous occurrence of females providing numerous mate choice opportunities at the same time (Zimmer et al., 2012). In contrast, a low mate availability may prevent the evolution of male mate choice, even if other conditions such as variation in female quality should facilitate it (Barry & Kokko, 2010). Indeed, we assumed that Southern males would experience less seasonal constraints that may reduce synchronicity of maturing females and result in a lower temporal availability of females (Gotthard, 1998; Mousseau & Roff, 1989; Sniegula, Golab, Drobniak, et al., 2016). As mentioned above, only field data about population dynamics can resolve uncertainty about the spatial and temporal distribution of Southern males and females.

Males may assess female quality or female availability by chemical cues or female sex pheromones (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010; Gaskett, 2007). Only virgin females produce the sex pheromone that is found in their body and silk (Chinta, Goller, Lux, Funke, Uhl, & Schulz, 2010). Previous studies found that chemical cues produced by females are indeed involved in mate choice decisions of Northern males (Cory & Schneider, 2016; Chapter IV). In the field, Northern males more likely located virgin females that were close to oviposition (Cory & Schneider, 2016). This is adaptive because old females may lay eggs before they have time to mate with a second male. Moreover, Northern males seem to distinguish females of different body size by their chemical profile (Chapter IV). Female cues are also relevant for Southern males (Cory & Schneider, 2018a, 2018b). Earlier experiments could show that Southern males adjusted the developmental time to the presence and absence of silk cues of virgin females (Cory & Schneider, 2018a). Moreover, a previous study could show, that Southern males needed more time to locate females if the density of virgin

females was low (Cory & Schneider, 2018b). This may indicate that males did not evolve adaptations to female quality or availability, although they were presumably able to assess both.

Future studies should investigate which conditions might prevent the evolution of male mate choice in Southern populations. For instance, it was already found that *A. bruennichi* males in poor body condition more likely monopolized females than males that were in better shape and presumably more capable of continuing mate searching (Cory & Schneider, 2018a). However, this was true for males from both populations and did not prevent Northern males from adjusting mating decisions to female size. Another approach could be to investigate whether male mate choice occurs in Asian populations and if yes, whether this trait was transmitted during the admixture of Mediterranean and Asian populations.

Conclusion:

Adapting to new or changing environments can be very challenging. The sexually cannibalistic spider *A. bruennichi* was able to colonize almost whole Europe and had to cope with different environmental conditions. Genetic

analyses already suggested that Northern *A. bruennichi* were able to adjust rapidly to the new conditions. We aimed to expand the knowledge and investigated which conditions may have underlain the successful colonization and found geographical differences in life history and mating decisions. While Southern spiders showed a highly conditional plasticity in body size and no indication of male mate choice, Northern spiders had a comparatively stable body size, but adjusted male mate choice to female size (Welke et al., 2012; Chapter IV). Since Northern individuals are smaller, adaptations to life history and mate choice might have guaranteed the success of colonizing Northern Europe. We encourage future studies to combine different research disciplines (e. g. genetics, ecology, behaviour or morphology) to investigate the adaptive capacity of species to deal with unfavorable environmental conditions.

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General discussion

Summary of results:

I explored whether males make locally adapted mating decisions based on internal and external information. As model system, I used the sexually cannibalistic spider *Argiope bruennichi* and compared the life history and mating decisions between Southern and Northern individuals that differ in body size and seasonal conditions. I predicted that local conditions changed the selection regime and underlying male mating decisions. Common mating decisions concern maturation time, mating investment during the first copulation, and monogynous vs. bigynous mating tactics. Unexpectedly, I found that males from both populations follow similar rules for conditional mating strategies. Under the same conditions, Northern and Southern males reached sexual maturity within a similar time window and modulated the timing of sexual maturity in response to female cues. During mate search, males seem to find their first unmated mating partner randomly, whereby a high density of pheromone-producing females facilitates female localization. Information about mate availability, presented during development or mate search, led to no adjustments in male mating strategies. Rather, males considered their internal state to decide if further mate search was reasonable. In contrast to Southern males, Northern males adjusted their decision whether to continue mate searching (bigyny) or to monopolize the current mating partner according to female size. Thereby they considered relative size differences, suggesting a comparative assessment of females as their underlying mate choice rule. Presumably, the high plasticity of Southern populations and the flexible development adjusted to female cues were ideal pre-adaptations facilitating rapid adjustments to northern conditions. The northern feature, to assess relative differences in female size and to adjust mate choice

accordingly, may be a response to the smaller body size that is accompanied by a lower fecundity.

Information use in *Argiope bruennichi*:

The use of particular information can be relevant at different life stages, and the reliability of information depends on persistence, type and quality of a cue (Candolin, 2003; Dall et al., 2005; Kasumovic & Brooks, 2011). I found that males from both populations based their decisions on their internal state (internal information) and external information provided by females. In spiders, adult, virgin females often produce chemical cues or sex pheromones that are volatile or bound to silk, and I found that *A. bruennichi* males made use of both (Gaskett, 2007; Schulz, 2013). Female information was relevant during subadult and mature life stages, although not in every context. This shows that even in small animals, plasticity in life history mating decisions depends on various decision-making processes.

Behavioral plasticity can be divided into two major types (Snell-Rood, 2013). Activational plasticity enables individuals to respond rapidly to changing conditions and is often reversible (Snell-Rood, 2013). In contrast, developmental plasticity usually depends on experience or learning and may even induce irreversible adjustments (West-Eberhard, 2003). A common form of developmental plasticity is socially-cued anticipatory plasticity, in which plastic responses depend on cues from conspecifics (Kasumovic & Brooks, 2011). Socially-cued anticipatory plasticity was found in several spiders, including *A. bruennichi*, and is often associated with adjustments of body size and developmental time but also of mate preferences in female spiders (Hebets, 2003; Kasumovic & Andrade, 2006; Neumann &

Schneider, 2016; Stoffer & Uetz, 2015). However, it is still relatively unexplored whether male mating decisions are affected by juvenile or subadult experience with female cues. I aimed to close this gap in knowledge and gave males access to female cues during development and mate search. I found that early experience had no impact on later male mating decisions (developmental plasticity), whereas current local information led to adjustments in mating tactics (activational plasticity), at least to some extent. Presumably, current information is more reliable in mating systems where population dynamics constantly change. In sexually cannibalistic spiders, the operational sex ratio constantly changes because males are taken from the mating pool by sexual cannibalism and mated females stop producing chemical attractants (Elias et al., 2011; Thomas, 2011).

Several studies have found that males can recognize the mating status of females, which probably depends on the presence of potent attractants in adult, virgin females (Chinta, Goller, Lux, Funke, Uhl, & Schidz, 2010; Schulte et al., 2010; Thomas, 2011). Apart from this, the information content of volatile female sex pheromones or chemical cues is less understood (Gaskett, 2007). A previous study found that Northern *A. bruennichi* males monopolize relatively large females and otherwise continue mate searching after one copulation if females are relatively small (Welke et al., 2012). However, it was unknown how males assessed female size and whether the chosen mating tactic was based on a fixed threshold or a comparison of females. The results of Chapter IV indicate that males used volatile female cues to assess the quality of neighboring females and make a comparative assessment. While the results suggest the absence of a fixed threshold, they could not distinguish whether males used a best-of-n strategy or an adjustable threshold (Wittenberger, 1983). Using an adjustable threshold, males only need to perceive the

overall quality spectrum of females in the vicinity and compare the information with an internal threshold. Depending on whether all females in the vicinity fall below or exceed the locally adapted threshold for female size, males can lower or raise this threshold. An adjustable threshold should be less costly than a best-of-n strategy that requires that males are able to compare the quality of distinct females. I assume that males applied an adjustable threshold rule because even in no-choice tests where males had no opportunity to compare females, males monopolized relatively large females (Chapter III).

The importance of reaching sexual maturity in time:

Most organisms have to face a trade-off between growing large and synchronizing maturation with potential mating partners (Nylin & Gotthard, 1998). Particularly in seasonal habitats, the relevance of punctual maturation may come at the cost of body size since the breeding season often starts earlier in the year and may be shorter than in non-seasonal habitats (Blanckenhorn & Demont, 2004; Mousseau, 1997). In the course of the northern range expansion, *A. bruennichi* had to adapt to fewer warm months during which growth and mating can occur (Krehenwinkel & Tautz, 2013; Zimmer et al., 2012). Consistent with theory, Northern individuals are smaller than Southern spiders, and I also found that males had a shorter subadult stage (Chapter III, Chapter V). Apart from that, Northern and Southern individuals showed a similarly long maturation window at the same time of the year if they were reared in a common garden situation (Chapter III, Chapter V). Even under food restriction, most males and females matured in the same period as well-fed individuals (Chapter V), although their maturation window was prolonged. Thus, independent of environmental conditions and across populations, the timing of maturation seems to be extremely important.

As in many seasonal spiders and insects, *A. bruennichi* males are protandrous and start mate searching before females reach sexual maturity (Elias et al., 2011; Morbey & Ydenberg, 2001; Wiklund & Fagerstrom, 1977; Zimmer et al., 2012). The timing of maturation is crucial for males because they must scramble to be the first to mate with adult, virgin females. Mating with unmated females allows males to avoid sperm competition through mate plugging (Nessler et al., 2007). To fine-tune the timing of maturation, males shorten their developmental time in the presence of adult virgin female cues (Chapter III). Such developmental adjustments were also found in other spiders with pronounced sexual size dimorphism, constrained mating rates and a high risk of male competition (Kasumovic & Andrade, 2006; Neumann & Schneider, 2016). The occurrence of protandry in similar mating systems supports the mate opportunity hypothesis, predicting that strong protandry is adaptive when there is a first-male advantage in sperm competition (Morbey & Ydenberg, 2001; Simmons et al., 1994; Uhl et al., 2010; Wedell, 1992; Wiklund & Forsberg, 1991).

Often, males do not wait until they perceive female cues and instead mature much earlier than females (Zimmer et al., 2012). These males can search for subadult females and follow an opportunistic mating strategy (Uhl et al., 2015). When subadult females molt to sexual maturity, they are defenseless and offer males the chance to mate without the risk of sexual cannibalism (Uhl et al., 2015). Field observations have revealed that approximately half of all subadult females are guarded by at least one male (Zimmer & Schneider, 2016). It is, however, unknown whether males actively localize subadult females or whether encounters occur by coincidence. Previous studies suggest that spider males, including *A. bruennichi*, can detect adult virgin females more easily than immature females (Schneider et al., 2016; Searcy, Rypstra, & Persons, 1999; Tuni & Berger-Tal, 2012). Thus, early-maturing

males may be unsuccessful in finding subadult females and must wait until detectable adult, virgin females appear. When these males have their first mating chance, they will be relatively old, and in comparison to opportunistic mating with molting and defenseless females, they have to mate under the risk of sexual cannibalism. Since I found that the probability of sexual cannibalism is higher in old males (Chapter I; results are discussed below), early maturing males accept a high risk of forfeiting half their reproductive potential.

Under scramble competition, the timing of maturation seems to be under stronger sexual selection than body size. Nevertheless, body size is a significant predictor for reproductive success because female size correlates with fecundity (Honek, 1993; Kessler, 1971; Marshall & Gittleman, 1994; Skow & Jakob, 2003; Chapter IV). In *A. bruennichi*, Northern females are much smaller than Southern females. Therefore, a further decrease in body size may have severe fitness consequences (not only in females), and Northern individuals may require adaptations to tolerate detrimental conditions. Indeed, I found some evidence that Northern *A. bruennichi* have evolved mechanisms to maintain a mean body size, while the body size in Southern individuals varied remarkably between years and under different feeding treatments (Chapter V). However, the high plasticity in Southern individuals might have been optimal preconditions for the colonization of the north (Ghalambor et al., 2007).

No adjustments of mating tactics to mate availability:

While I found that males made developmental adjustments in response to female cues, male sacrifice and monogynous vs. bigynous mating tactics were altered neither by the presence nor absence of female cues during development (Chapter III), nor by the number of adult, virgin females available during mate

search (Chapter II). This was unexpected because information about mate availability decreases uncertainty about the chance of finding a second female, and hence, the success of a bigynous tactic. Mathematical models have predicted adjustments of mating tactics to the availability of adult, virgin females (Fromhage & Schneider, 2012). I predicted that males might be able to assess mate availability by chemically sensing the number of adult, virgin females in the vicinity, since such females are known to produce a potent sex pheromone (Chinta, Goller, Lux, Funke, Uhl, & Schidz, 2010). Indeed, in Chapter II, males localized females faster when exposed to a high number of adult, virgin females. A low number of adult, virgin females may indicate that it is early in the season and that more adult, virgin females will follow soon. Moreover, under scramble competition and a high risk of sexual cannibalism, the availability of adult virgin females and rival males may change continuously and prevent adaptive adjustments of mating tactics.

In my thesis, I only manipulated information about females and ignored effects of male competition. Mate availability is a function of both female density and male density and the use of only one information source may be an unreliable predictor of mate availability. This may also explain why I found no effect of mate availability in Chapters II and III. While it seems likely that *A. bruennichi* males can estimate mate availability by chemical cues produced by females, it is unknown whether males can perceive other males without direct contact. In many web spiders, spiderlings only stay with their siblings for a short time until they build their own web (Foelix, 2011). Hence, information about males must be gathered from a distance by chemical cues that are still unknown. A recent study on *A. bruennichi* found that the presence of silk cues from rival males caused no changes in mate choice decisions (Schneider et al., 2016). Therefore, it seems unlikely that males adjust their mating

decisions to male cues that do not involve direct contact.

Geographical differences in mating tactics concern female size:

Environmental differences may lead to local adaptations in mating tactics (Jennions & Petrie, 1997). I found that Southern and Northern males differed neither in the probability of sexual cannibalism nor in the probability of using a bigynous mating tactic (Chapter III, IV, V). However, Northern males were more likely to use a bigynous mating tactic if the first mating partner was small. Hence, Northern males applied monogynous and bigynous mating tactics as a form of male mate choice after they had visited their mating partners without a preference (Chapter IV). In Southern males, neither mate search nor mating tactics were predicted by variation in female size (Chapter IV, V). A reason could be that males' adjustments to female size only evolved in response to overall female size in the north. Alternatively, female size-dependent mate choice may have already evolved in Asian populations and was then transmitted to the Northern phenotype during the admixture of Mediterranean and Asian populations (Krehenwinkel & Tautz, 2013).

Why mating decisions of Southern males were unaffected by female size variation remains inconclusive. As with Northern males, Southern males risk their life with each mating (Chapter II, Chapter V), damage their genitalia (Chapter V), mate maximally twice (Chapter III, V), respond to cues of virgin females (Chapter III), and encounter females of various body sizes (Chapter V). Overall, female information appears to be less relevant to Southern males because both female size and information about female availability induced no changes in mating tactics. Instead, males based their decisions on their internal state that gave them information about their ability to bear the costs of a bigynous mating tactic.

Mating tactics adjusted to the male's internal state:

I found that males from both populations adjusted their mating decisions to their internal state (Chapter I, Chapter III). First, I found that in Northern populations, old males increase their mating investment (Chapter I). Second, I found that males from both populations used monogynous and bigynous mating tactics adjusted to their own relative weight loss from maturation to test day (Chapter III). Since in web spiders, adult males barely eat and lose weight with age (Foellmer & Fairbairn, 2005b), it is likely that I tested the same effect in both studies. In Chapter I, I was able to disentangle both effects and found that males adjusted their mating decisions not to their age, but to their own body condition.

Mating decisions of aging males seem to proceed as follows: Males usually try to survive the first copulation, but the risk of getting cannibalized increases with male age. Males that exceed an adult age of ca. 3.5-4 weeks will make a terminal investment and allow females to cannibalize them and, in return, males can copulate longer. This is adaptive because the substantial risk of sexual cannibalism for old males makes future reproduction very uncertain. Therefore, it is the safer option for old males to optimize paternity via a longer copulation with increased sperm transfer than an uncertain attempt to survive the female attack (Schneider et al., 2006). Younger males who survive the first copulation will more likely continue mate searching if they have a relatively low weight loss. Searching for a second mating partner involves additional risks and costs, including the required energy and time for mate searching and courtship plus the uncertainty to find a second, non-monopolized mating partner (Andrade, 2003; Cady et al., 2011; Kasumovic et al., 2007; Stoltz et al., 2009).

Outlook:

Previous studies and the results of my thesis could show that *A. bruennichi* is an ideal system for investigating conditions and constraints of recent colonization in animals. Therefore, I suggest that the work on *A. bruennichi* should be continued to close the existing gaps in knowledge. I recommend the following future research:

- Mating history in the field: I found that Northern and Southern males show relatively similar mating behavior, although my breeding experiments showed considerable differences in phenotypic plasticity. To understand these inconclusive results, I highly recommend collecting field data about the population dynamics of Southern individuals during the mating season. Here, it would be particularly relevant to collect similar information as has been gathered for Northern populations. This includes the operational sex ratio during the mating season, the number of male visitors per female, the probability of sexual cannibalism and the distribution of mating tactics (opportunistic mating, monopolization, bigyny). Moreover, it would be interesting to examine whether the broad reaction norm of life history traits found in the laboratory is also noticeable in the field.
- Cross-breeding experiments: To fully understand the rapid expansion into the north, we need to consider that the new phenotype is not only a result of new environmental conditions but also of admixture of Mediterranean and Asian populations. In my thesis, I only considered the Mediterranean origin and disregarded the Asian origin. Therefore, I suggest that future research should study decision rules for mate choice in the Asian phenotype and reconstruct the expansion to the north by cross-breeding experiments between Mediterranean and Asian individuals.

- Chemistry of pheromones: I could show that males use chemical cues to adjust mate choice decisions. However, the proximate mechanisms are still unknown. Males might have received information about female size either by differences in pheromone quantity or by differences in chemical profiles. Such information is important since chemical communication follows different rules of propagation in comparison to visual and acoustic cues (Bradbury & Vehrencamp, 2011).

Conclusion:

Ecological conditions can influence the life history of males and females and can, thereby, change the sexual selection regime. As a consequence, males from different geographical origins should evolve different

decision rules based on local information. However, in strongly sexually selected mating systems, the options to enhance fitness may remain the same across ecological contexts. In *A. bruennichi*, Northern and Southern populations differ in body size and seasonality, but show similar plasticity in information use and mating decisions. Presumably, the high risk of sperm competition and the associated first male advantage in mating has favored adaptations that help males win scramble competition. However, the conditions in the north required adjustments to the overall smaller body size of females and caused changes in mate choice rules. Given that *A. bruennichi* only recently expanded into Northern Europe, this study shows that decision rules can rapidly adapt to novel conditions and may facilitate the colonization of new habitats.

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