

**Phenotypic plasticity of life history and mating strategies
in African golden-silk spiders,
Nephila senegalensis and *N. fenestrata***

Dissertation

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Summary

The phenomenon of sexual size dimorphism (SSD) has received much attention in evolutionary biology, and concepts of natural and sexual selection have been convincingly applied to various sexually size dimorphic taxa. However, the evolution of particularly strong degrees of SSD with 'dwarf males' and 'giant females' remains challenging to explain in view of large male benefits that have been shown in a wealth of studies. Golden-silk spiders (genus *Nephila*) exhibit the most extreme cases of female-biased SSD among terrestrial animals and represent an established model lineage to study selective mechanisms promoting extreme size differences between the sexes. In addition, several species show an extraordinary within-sex variation of body size, particularly in males. Male-male competition is intense in most species and several studies have reported benefits of large size in contest competition. However, consistent large male advantages should generate directional selection, which would narrow the existing variation. Thus, to explain the evolution of extreme SSD as well as size variation still requires identifying the modes of sex-specific size selection, as well as selection dynamics that facilitate intrasexual size variation.

Phenotypic plasticity enables animals to adjust fitness-relevant traits to the prevalent conditions. Adaptive plastic responses can be used to adjust morphology and life history, but also the behavioural phenotype. I investigated the role of phenotypic plasticity in shaping mating strategies and life history traits in African golden-silk spiders, *N. senegalensis* and *N. fenestrata*. The study animals were expected to adjust these traits to different reproductive environments, potentially increasing their fitness beyond the level of baseline assumptions.

In order to address extreme male size variation in *N. senegalensis*, I evaluated reproductive success in size-mismatched males forced into long-term contest competition (**chapter 1**). The goal of this experiment was to assess whether differently-sized males are able to achieve similar average paternity under these conditions. My analyses show that competing males implemented strategies in relation to their own size, integrating their competitive ability, female reproductive value, and the degree of sperm competition into their mating decisions. Differential mating investment resulted in equivalent paternity success in each size class. My results exemplify how behavioural plasticity in mating strategies can dissolve principal large size benefits and thus help to explain the evolutionary maintenance of extreme size variation as well as SSD.

I studied a special case of life history plasticity, termed socially cued anticipatory plasticity, using a comparative approach (**chapter 2**). This mechanism has been proposed as a common element of male mating strategies. Particularly short lived males that specialize in terminal mating investment are expected to adjust their timing of maturation to social cues indicating the availability of receptive females. Males abbreviating their development to accomplish early maturation may reach a smaller adult size; hence this form of plasticity has also been suggested to increase male size variation. Male *N. fenestrata* follow a highly specialized terminal investment strategy, whereas male *N. senegalensis* are less specialized to monopolize females. Based on these differences, *N. fenestrata* males reared in the presence of cues simulating the presence of virgin females were expected to show a distinct developmental response. Contrary to predictions, however, female cues had no effect in *N. fenestrata*, whereas male *N. senegalensis* matured several days earlier when receiving cues of receptive females. While my findings confirm that socially cued plasticity occurs in *Nephila*, my observations contradict theoretical expectations with respect to specific mating strategies. Furthermore, the adjustment

of maturation did not affect male size; hence my results do not support this form of plasticity as a source of male size variation in *Nephila*.

Body size in *Nephila* is assumed to have evolved independently in each sex; thus divergent sex-specific selection is expected to have caused the evolution of extreme reversed SSD in these spiders. As these differences with respect to size selection should also be reflected in adaptive plasticity, the comparative study of plastic responses in males and females can help to identify the respective selection pressures. I studied sex-specific developmental plasticity in *N. senegalensis* (**chapter 3**). Animal growth is often constrained by unfavourable conditions, but growth restriction in early juvenile stages can later be compensated by adaptive catch-up growth (the compensation of growth deficits through delayed development). Natural selection should favour optimization of fecundity in growth-restricted females, whereas sexually selected benefits of early maturation should generate a stronger trade-off between size-related benefits and costs of a delayed maturation in males. Based on these differences, I analysed the development of study animals reared in different feeding treatments. My results confirm efficient growth and fecundity compensation in females, while males did not fully compensate growth deficits. These findings are consistent with divergent sex-specific size selection and thus add to our understanding of extreme female-biased SSD in *Nephila*.

Considering male mating investment in the presence of sexual conflict, I expected behavioural plasticity in male *N. fenestrata*. Males in this species often attempt to monopolize paternity with a single female, but females often attack and sometimes cannibalize males during copulation. Attacked males regularly eject (autotomize) legs during copulation, which the female then feeds on. Thus, male leg sacrifice may serve to pacify females and prevent further attacks, while from the female's perspective, the loss of legs may reduce a male's postcopulatory mate guarding ability. Based on the assumption that males sacrifice their legs to pacify females; losing increasing numbers of legs with stronger mating investment, I investigated whether males vary their mating investment according to female quality (**chapter 4**). My results show that female attacks were related to prolonged copulation duration and a higher number of legs ejected. Male mating success was also modulated by male size; further interrelated with age, size, and body mass of the respective female. These findings point towards condition-dependent strategies in male *N. fenestrata*, but it remains to be studied how these differences translate into individual fitness, and whether they may balance fitness payoffs between phenotypes as shown in *N. senegalensis*.

In conclusion, I show that adaptive phenotypic plasticity in *Nephila* spiders enables individuals to modify general patterns of life history and behavioural strategies; thus optimizing the adaptive value of the respective traits in response to specific environmental conditions. As these mechanisms are likely to facilitate size variation and extreme female-biased SSD over evolutionary time, my studies contribute to our understanding of these intriguing systems.

Zusammenfassung

Sexueller Größendimorphismus ist im Tierreich weit verbreitet. Die Evolution extremer Größenunterschiede mit so genannten Zwergmännchen und vergleichsweise riesigen Weibchen entspricht nicht den üblichen Mustern und ist noch immer weitgehend unverstanden. Radnetzspinnen der Gattung *Nephila* stellen ein wichtiges Modelltaxon zur Erforschung von extremem Größendimorphismus dar. Viele Arten zeigen darüber hinaus eine erhebliche Größenvariation innerhalb der Geschlechter, insbesondere bei den Männchen. Männchen konkurrieren häufig um Paarungsgelegenheiten und mehrere Studien konnten Vorteile für große Männchen zeigen. Da beständige Vorteile von Größe jedoch zu gerichteter Selektion und damit zur Einschränkung der existierenden Variation führen sollten, müssen andere selektive Mechanismen, die zur Erklärung der Evolution von extremem Größendimorphismus sowie der Aufrechterhaltung der Größenvariation in Betracht kommen, identifiziert werden.

Natürliche Umwelten sind selten konstant, und phänotypische Plastizität ermöglicht die flexible Anpassung fitnessrelevanter Merkmale an die vorherrschenden Bedingungen. Plastizität ermöglicht die Anpassung von Morphologie und Life history-Merkmalen, aber auch die des Verhaltensphänotyps, z. B. innerhalb konditionaler Paarungsstrategien. In dieser Arbeit studierte ich die Bedeutung von adaptiver phänotypischer Plastizität im Hinblick auf Paarungsstrategien und Life history-Merkmale bei den afrikanischen Arten *N. senegalensis* und *N. fenestrata*.

Im Hinblick auf die extreme Größenvariation der Männchen habe ich die Hypothese überprüft, dass Männchen verschiedener Größenklassen im Mittel einen äquivalenten Reproduktionserfolg erreichen können; auch wenn diese unmittelbar gegeneinander um ein Weibchen konkurrieren (**Kapitel 1**). Meine Analysen zeigen, dass *N. senegalensis*-Männchen unterschiedliche Paarungsstrategien in Relation zur eigenen Größe einsetzen. Dabei integrieren sie ihre Konkurrenzfähigkeit, den reproduktiven Wert des Weibchens sowie den Grad von Spermienkonkurrenz in ihre Paarungsentscheidungen. Dies resultierte in einem äquivalenten Reproduktionserfolg verschiedener Größenklassen. Plastische Verhaltensstrategien können daher generelle Selektionsvorteile von gesteigerter Körpergröße auflösen und so zum Erhalt der Variation beitragen.

In einem vergleichenden Forschungsansatz habe ich eine spezielle Form von Life history-Plastizität (Socially cued anticipatory plasticity) untersucht, die als verbreitete Anpassung innerhalb männlicher Paarungsstrategien sowie als ein potenzieller Mechanismus zur Erhaltung von Größenvariation bei *Nephila*-Männchen vorgeschlagen wurde (**Kapitel 2**). Demnach sollten insbesondere kurzlebige Männchen, die auf die Paarungsinvestition mit einem einzigen Weibchen spezialisiert sind, plastisch auf Hinweise bezüglich der Verfügbarkeit virginer Weibchen reagieren. Sie sollten durch Anpassung ihrer Entwicklung den Zeitpunkt ihrer Geschlechtsreife daraufhin abstimmen. Diese Modifizierung könnte jedoch zu einer geringeren Körpergröße durch eine verkürzte Entwicklungsdauer führen. Meine Untersuchungsarten unterscheiden sich maßgeblich in ihrem Grad der Spezialisierung auf die Monopolisierung eines einzigen Weibchens. Den theoretischen Erwartungen folgend, sollten Männchen beider Arten entsprechend unterschiedliche plastische Reaktionen zeigen. Meine Ergebnisse zeigen jedoch, dass der erwartete Effekt bei stark spezialisierten, meist monogynen Männchen (*N. fenestrata*) ausblieb, während stärker polyandrische Männchen (*N. senegalensis*) unter Einfluss der simulierten Präsenz virginer Weibchen mehrere Tage früher geschlechtsreif wurden. Dies hatte jedoch keinen Einfluss auf die Größe der Männchen. Während meine Ergebnisse die Existenz

dieser Form von Plastizität in der Gattung *Nephila* prinzipiell bestätigen, weichen sie von den postulierten Erwartungen im Zusammenhang mit den jeweiligen Paarungsstrategien ab. Auch können meine Ergebnisse die Annahme, dass diese plastische Anpassung einen Teil der Größenvariation erklären kann, nicht untermauern.

Es wird angenommen, dass die Körpergröße in der Gattung *Nephila* in beiden Geschlechtern unabhängig voneinander evolviert ist. Daher kann gegenläufige geschlechtsspezifische Selektion als Ursache für den sexuellen Größendimorphismus gelten, wobei die selektiven Mechanismen, insbesondere im Hinblick auf die geringe Größe der Männchen, nicht abschließend aufgeklärt sind. Geschlechtsspezifisch unterschiedliche Selektionsregime, die zur Erklärung des extremen Größendimorphismus beitragen können, sollten sich auch in Unterschieden im Hinblick auf adaptive Plastizität ausprägen. Basierend auf dieser Annahme habe ich geschlechtsspezifische Plastizität der Entwicklung bei *N. senegalensis* untersucht (**Kapitel 3**). Das Wachstum von Tieren wird oft durch ungünstige Bedingungen limitiert, aber durch kompensatorisches Wachstum können Wachstumsdefizite früher juveniler Stadien wieder ausgeglichen werden; z. B. durch Verlängerung der Gesamtentwicklungsdauer (Catch-up growth). In Bezug auf selektive Faktoren, die mit dem Größenunterschied der Geschlechter vereinbar wären, untersuchte ich inwiefern natürliche Selektion bei wachstumsdefizitären Weibchen die plastische Optimierung ihrer Fekundität bewirkt, während sexuell selektierte Vorteile früher Geschlechtsreife bei Männchen einen stärkeren Trade-off zwischen Größenvorteilen und Kosten der Entwicklungsverzögerung hervorrufen. Meine Analysen der Entwicklung bei unterschiedlichem Nahrungsangebot zeigen die vollständige Kompensation früher Wachstumsdefizite bei den Weibchen, während diese bei den Männchen unvollständig blieb. Diese Ergebnisse bestätigen die zugrunde gelegten geschlechtsspezifischen Selektionsfaktoren, die zum Verständnis der bei Männchen und Weibchen unterschiedlich evolvierten Körpergröße beitragen.

Auch für Männchen von *N. fenestrata* habe ich die Annahme plastischer Verhaltensstrategien in Bezug auf ihr Paarungsinvestment zugrunde gelegt. Bei dieser Art gibt es deutliche Anzeichen eines sexuellen Konflikts zwischen dem Bestreben der Männchen, Weibchen zu monopolisieren, und Gegenanpassungen der Weibchen, die sich in Attacken während der Kopulation und häufigem sexuellen Kannibalismus zeigen. Attackierte Männchen autotomieren oft Beine, die dann vom Weibchen gefressen werden. Dieses „Beinopfer“ könnte aus Sicht der Männchen dazu dienen, weitere Attacken und sexuellen Kannibalismus zu verhindern, während der Beinverlust aus Sicht der Weibchen die Fähigkeit des Männchens, spätere Paarungsversuche von Rivalen abzuwehren, reduziert. Ich studierte die Paarungsstrategie der Männchen unter der Annahme, dass sich die Anzahl der autotomierten Beine mit zunehmendem Paarungsinvestment erhöht (**Kapitel 4**). Meine Ergebnisse zeigen, dass Attacken der Weibchen mit längeren Kopulationen und einer höheren Anzahl verlorener Beine assoziiert waren. Der Paarungserfolg der Männchen wurde auch durch Vor- und Nachteile ihrer jeweiligen Größe moduliert, deren Bedeutung für den Reproduktionserfolg unterschiedlicher Phänotypen jedoch noch offen bleiben muss.

In dieser Arbeit konnte ich zeigen, wie durch adaptive phänotypische Plastizität grundsätzliche Muster von Life history-Merkmalen und Verhaltensstrategien modifiziert werden können. Dadurch kann der adaptive Wert des jeweiligen Merkmals optimiert und an die vorherrschenden Bedingungen angepasst werden. Diese Mechanismen tragen dazu bei, Größenvariation und extremen Größendimorphismus über evolutionäre Zeiträume zu ermöglichen und zu erhalten.

General introduction

Phenotypic plasticity

Natural environments are rarely constant, but usually undergo changes with respect to abiotic parameters, which also entail changes in the existing biotic communities. As most of this variation takes place on timescales sufficiently short to affect individual organisms during their lifetime, selection should favour short-term adaptation to environmental change in the vast majority of species.

Phenotypic plasticity, defined as the capacity of a genotype to express different phenotypes under different extrinsic conditions, enables animals to adjust their phenotype to recurrent environmental changes, as well as irregular fluctuations (reviewed in Agrawal 2001; Pigliucci 2001; West-Eberhard 2003). Animals are able to respond plastically to large-scale environmental parameters, such as temperature, humidity, and light regime, but may also adjust their phenotype to spatially and temporally limited conditions. For example, plastic responses can be used to adapt to the social environment, including mate availability and reproductive prospects (reviewed in Kasumovic & Brooks 2011). The capacity for phenotypic plasticity, however, is of course limited by boundaries of the genotype's reaction norm, which can only span degrees of trait expression allowing an organism to maintain its physiological functioning (Stearns 1989). Furthermore, adaptive plastic responses may involve other potential costs of plasticity that constrain its evolution (DeWitt et al. 1998; Relyea 2002). Importantly, although phenotypic plasticity may mitigate direct effects of potential selection pressures on the genotype, between-genotype variation with respect to the capacity for adaptive plastic responses

makes plasticity itself a trait subject to selection (reviewed in Stearns 1989). Phenotypic plasticity is an important factor in shaping an animal's morphology (e.g. regarding the expression of ornaments and weapons), life history (e.g. regarding the duration of development), and behaviour (e.g. regarding mating tactics). Plasticity of behaviour differs from plasticity of morphology and life history in that the behavioural phenotype expressed in an adult individual does not necessarily need to be induced and shaped during juvenile development. Furthermore, plastic behavioural responses are reversible; hence the ability for flexible responses is maintained. Often several plastic traits are interrelated; for example, an individual's adult size usually depends on the duration of pre-maturation development and the size increase achieved in this period of time (i.e. the specific growth rate) (Via & Lande 1985).

Body size in the context of life history and mating strategies

The biological concept of life history evolution is based on the observation that in all organisms, optimization of survival and growth by natural and sexual selection is limited by evolutionary constraints regarding the allocation of available resources (reviewed in Bell 1980; Roff 1992). These constraints are inevitable, because organisms cannot evolve to perfection in terms of fitness maximization, which would mean to start reproduction at the time of birth and to produce infinite numbers of offspring over unlimited time. Such an organism (a so-called 'Darwinian Demon') cannot exist because resources for growth and reproduction are always limited and the realized body mass limits number and size of offspring. Thus, efficient allocation of available resources between development and growth, as well as

the maintenance of basic vital functions and reproduction, is essential (Law 1979; Leimar 2002).

An animal's life cycle comprises two major phases; the developmental period taken to reach sexual maturity and the reproductive phase (Charlesworth 1994; Roff 2002; Stearns 1992). Age and body size at the time of the first reproduction, number and size of offspring, the specific reproductive strategy, and lifespan, are considered the most important life history traits, which are particularly important to understand life history evolution (reviewed in Stearns 1976). The basic preconditions for life history selection to act on are determined by taxon-specific qualities and associated intrinsic trade-offs and constraints (phylogenetic effects) on the one hand, and by extrinsic ecological challenges on the other hand (reviewed in Blomberg & Garland 2002; Flatt & Heyland 2011). These preconditions generate strikingly different ranges for individual life history traits in higher level taxa, and divergent selection between and within species further enhances the diversity of existing life history strategies (reviewed in Stearns 1989).

Body size, in general, is an important determinant of an animal's fitness and particularly advantages of large size have been supported by a wealth of studies, whereas findings corroborating small size benefits are scarce (reviewed in Blanckenhorn 2005). Males in many species are selected to grow large, as the increase in physical strength and power associated with increased size and body mass elevates their chances to succeed against rivals in contest competition. This relationship has led to the evolution of male-biased sexual size dimorphism (SSD) in various taxa. However, females in many animal groups benefit from large adult size as well, and female-biased (or

reversed) SSD is common in several taxa (reviewed in Fairbairn et al. 2007). Large size advantages in females mainly relate to the capacity to produce higher numbers of offspring or larger, more viable offspring than smaller females (Andersson 1994; Trivers 1972).

Adult size usually increases with the duration of development taken to reach sexual maturity; thus, early maturation generally bears the cost of relatively small adult size. However, this cost can be charged against several benefits: a shorter developmental period increases the probability to survive until maturation, because of a lower risk of developmental errors and a generally reduced risk of mortality through predation, disease, or environmental extremes. Furthermore, a shorter generation time results in offspring that can in turn reproduce early in life. Conversely, large size benefits, in general, have to be traded off against exactly those costs that can be avoided by fast maturation (Roff 1992; Stearns 1992).

In many animal species, growth does not cease with sexual maturity; e.g. in many species of fishes, reptiles, and mammals, but also in invertebrates, such as phylogenetically ancient lineages of spiders (Foelix 2011) and several groups of crustaceans (Duffy et al. 2007). In many others, however, determinate growth results in a fixed body size at the time of maturation (e.g. in most arthropods and mammals, but universally in birds, Fairbairn et al. 2007; Szekely et al. 2007).

The evolution of these different growth strategies is related, in part, to a fundamental difference between reproductive strategies: both determinate and indeterminate growth is common in animal species that normally reproduce repeatedly in recurring reproductive cycles (iteroparous species). As large size is often associated with higher

reproductive success, those species in which size and mass still increase after sexual maturity draw significant fitness benefits from this growth strategy. In contrast, many short-lived species experience only a single reproductive period in their lifetime (semelparous species). These species are therefore expected to optimize adult body size for a single reproductive event, without the chance to increase lifetime fitness in the future; hence these species generally show determinate growth (Horn 1978).

As age and body size at maturation result from the specific mode of pre-maturation development, this period is strongly selected to integrate the above-mentioned trade-offs to implement the most efficient life history strategy possible. It is commonly recognized that an animal's adult size is strongly influenced by basic environmental conditions experienced during development and growth; most notably the quantity and quality of food (Hector et al. 2012; Kleinteich & Schneider 2010), as well as ambient temperature in poikilothermic animals (Angilletta Jr et al. 2004). Thus, the realized adult size is almost always modulated by trade-offs that preclude maximal growth, and the capacity to plastically adjust body size enables animals to optimize this important life history trait (West-Eberhard 2003).

Female-biased sexual size dimorphism and associated mating systems

Sexual size dimorphism is common in various taxa across the animal kingdom (Darwin 1871). For example, males are usually larger than females in mammals (Andersson 1994), while birds of prey (Falconiformes) present a well-known example for female-biased SSD in higher vertebrates (Szekely et al. 2007). Likewise, in those poikilothermic vertebrates that exhibit sexual size dimorphism, females are typically the larger sex, and the same is

true for SSD in invertebrates (Fairbairn et al. 2007).

The mating systems of many animal taxa showing male-biased SSD conform to the 'traditional sex roles', which have been first described by Charles Darwin (Darwin 1871). These mating systems are characterized by indiscriminate males that strive to mate with many females, and females choosing a single or very few mates. However, as Darwin already noted (Darwin 1854), several types of mating systems deviate from the baseline model of classical sex roles (Arnold 1994; Emlen & Oring 1977; Schneider & Fromhage 2010).

In a number of poikilothermic taxa, particularly pronounced female-biased sexual size dimorphism, with a degree of size differences between the sexes exceeding all cases of SSD in higher vertebrates, has evolved (Blanckenhorn 2005). Dimensions of reversed SSD where females are at least twice as large as males, but may grow to several hundred times the male size in some aquatic species, are referred to as extreme sexual size dimorphism (Scharff & Coddington 1997), which has been discovered in fishes (Evans et al. 2011; Regan 1925), molluscs (Vollrath 1998a), crustaceans (Darwin 1854), insects (Boomsma & Franks 2006), and arachnids (Fairbairn et al. 2007; Vollrath 1998a). This phenomenon has received much attention from evolutionary biologists since Darwin, mainly because the very small size in males relative to female size (often described as 'male dwarfism') has been challenging to explain within the framework of sexual selection theory (Fairbairn et al. 2007; Foellmer & Moya-Laraño 2007; Vollrath 1998a).

Extreme between-sex size differences are also associated with relatively rare and unusual mating systems, and often involve further distinct morphological modifications that reflect a high degree of specialization

with respect to reproductive strategies (Cheng & Kuntner 2015; Evans et al. 2011). Very low mating rates are characteristic of the tiny males in these species, and males in many species are monogynous; they mate with only one female in their lifetime. Females, in contrast, are often polyandrous and accept several mates (Jennions & Petrie 2000). Males frequently have evolved adaptations to monopolize fertilization of a single female, e.g. by producing genital plugs to block rival males' insemination attempts (Uhl et al. 2009), or by defending the female against competing males (Holdsworth & Morse 2000). Others apply condition-dependent alternative mating strategies to succeed in male-male competition (Pilastro et al. 1997). Extreme female-biased SSD is particularly common in several groups of web-building spiders, e.g. in widow spiders (genus *Latrodectus*, family Theridiidae), crab spiders (genera *Misumena* and *Misumenoides*, family Thomisidae), and a number of orb-web spider genera, such as *Cyrtophora*, *Argiope* (Cheng & Kuntner 2014), and *Nephila*; the latter including the most extreme cases of female-biased SSD in terrestrial animals (Kuntner et al. 2013).

Reproductive strategies in *Nephila* spiders

Golden-silk spiders (genus *Nephila* Leach, 1815; Araneidae, Nephilinae; Dimitrov et al. 2017) are primarily known for their large, colourful and conspicuous females that build enormous orb-webs, and for 'dwarf-males'. The genus has become an established model lineage to study extreme reversed SSD, but in addition, these spiders show a range of uncommon behavioural and morphological adaptations in the context of reproduction and have been used to investigate male-male competition (Miyashita 1993; Fromhage &

Schneider 2005b), sexual cannibalism (Schneider 2014), male mate choice (Elgar et al. 2003), and adaptive developmental plasticity (Kasumovic et al. 2009).

Extreme reversed SSD in *Nephila* is likely facilitated by the genetic decoupling of size determinant hereditary factors in early nepheline evolution (Kuntner & Coddington 2009). Female size has been suggested to be driven mainly by fecundity selection (Higgins 1992; Kuntner & Elgar 2014; Rittschof 2010) and the notion has been put forward that the result is rather 'female gigantism' than male dwarfism (Coddington et al. 1997; Higgins et al. 2011; Kuntner & Coddington 2009). Female body mass in *Nephila* spiders can exceed male body mass more than a hundred times (Coddington et al. 1997), and females of *N. pilipes* (Robinson & Robinson 1980) and *N. constricta* (sub *N. pilipes*; Higgins 2002) are the largest of all web-building spiders. Small male size in *N. pilipes* has been related to benefits of protandry and relaxed size selection under scramble competition (Danielson-Francois et al. 2012), but these arguments do not apply to *Nephila* in general, as intense male-male competition is common in several species. Other studies suggested reduced nutritional requirements (Higgins & Goodnight 2010) and cannibalism avoidance (Elgar 1991; Elgar & Fahey 1996) to favour small male size. However, experimental evidence in support of small male size advantages is scant, and selective mechanisms limiting male size remain largely unsolved (Higgins et al. 2011; Kuntner & Elgar 2014).

The genus has a pantropical distribution and comprises 14 species according to recent taxonomic research (Kuntner et al. 2013). Females build their webs in bushes and trees, often aggregating in clusters (Elgar 1989). A single cluster may comprise over 20 webs in close proximity (pers. observations) and

Nephila spiders are important insect predators in various ecosystems (Kuntner et al. 2013). Immature males build regular webs, whereas adult males cohabit with females and feed on the females' prey. Often several males accumulate on a female's web and compete for mating opportunities. Female webs are long-lasting structures in most species, and only the capture area is regularly rebuilt. In some species, males use connective frame threads to travel from one female to another (pers. observations).

Mature spider males possess secondary copulatory organs (copulatory bulbs) located at the end of their pedipalps, which are inserted into the female's paired copulatory openings. Sperm are transferred to the female's sperm storage organs (spermathecae) via independent copulatory ducts; thus two copulations are required to inseminate both of a female's sperm stores. In most nephiline genera (e.g. *Herennia*, *Nephilingis* and *Nephilengys*), male terminal investment strategies associated with copulatory mate plugging have been observed; thus specialization to monopolize females is considered ancestral in the lineage (Kuntner et al. 2013). Morphological, molecular, and behavioural data, however, suggest an evolutionary reversal towards less specialized mating strategies in *Nephila*, although several traits associated with monogyny persist in the genus (e.g. spermatogenesis ceases with sexual maturation; Michalik & Rittschof 2011; Schneider & Michalik 2011). Males in several species have evolved slender copulatory organs that do not serve to produce effective mating plugs anymore. As paternity monopolization opposes female interests, as well as those of rivals, antagonistic coevolution between the sexes may have caused this reversal. Slender male copulatory organs allow bypassing existing mating plugs, while modification of female genital

morphology probably corresponded to render plugs ineffective and facilitate multiple mating (Kuntner et al. 2009). Females may benefit from polyandry by avoiding inbreeding (e.g. Tregenza & Wedell 2002; Welke & Schneider 2009) and reproductive failure after mating with an infertile male (Andrade & Banta 2002). Furthermore, multiple mating permits sequential mate choice (Elgar et al. 2000; Snow & Andrade 2005) and helps securing sufficient sperm to fertilize large numbers of eggs (Kuntner et al. 2012a).

While copulatory genital damage associated with ineffective rudimentary mating plugs still occurs in several species (e.g. *N. komaci*, *N. inaurata*, *N. turneri*, *N. constricta*, and *N. pilipes*), male copulatory organs in other species (e.g. *N. clavipes*, *N. edulis*, and *N. senegalensis*) remain intact during copulation (Kuntner et al. 2009). Hence, in the latter, there is no mate plugging and repeated copulations with the same organ are possible (e.g. Schneider & Michalik 2011).

My study species, *N. senegalensis* (Walkenaer, 1841) and *N. fenestrata* Thorell, 1859, are moderately-sized species, which occur in large parts of sub-Saharan Africa. Females attain a maximum body length of approximately 25 mm in *N. fenestrata* and up to 30 mm in *N. senegalensis* (pers. observations). Males in both species reach approximately 10 mm body length, but especially in males, there is remarkable variation in size (Higgins et al. 2011; pers. observations). Both species are similar regarding general biology and ecology, but differ distinctly with respect to mating strategies. *Nephila fenestrata* is the only species in the genus in which males produce functional mating plugs that reduce copulation success in subsequent males (Fromhage & Schneider 2006). This mating strategy probably represents a secondary

evolutionary reversal towards monogyny (Kuntner et al. 2013). Males in this species often attempt to monopolize paternity with a female through mate plugging and postcopulatory mate guarding; frequently impeded by sexual cannibalism (Fromhage & Schneider 2005b). In contrast, male *N. senegalensis* do not produce mating plugs; they are able to mate several times and do not face a high risk of sexual cannibalism. Based on these different combinations of traits, *N. senegalensis* and *N. fenestrata* are suitable model species to investigate sex-specific life history selection, within-sex size variation, and phenotypic plasticity of development and mating strategies.

Research goals

Extreme male size variation and SSD;
alternative mating strategies and
behavioural plasticity

In **chapter 1**, I study fitness implications of male-male competition in *Nephila senegalensis*, which shows female-biased SSD, as well as remarkable size variation in males. Previous studies investigating male mating strategies and reproductive success in *Nephila* have mainly reported large male advantages (Christenson & Goist 1979; Rittschof 2010). However, extreme male size variation suggests absence of directional selection, which would narrow the existing size range. Large males' dominance in male-male contests thus raises the question how selection could generate and maintain extreme male size variation over evolutionary time.

I investigate size-related mating strategies and associated fitness consequences in dyads of size-mismatched males competing for a single female. For this purpose, I conducted a long-term mating experiment and assessed

the paternity success of individual males, using the sterile male technique (Parker 1970). I predicted differently-sized males to achieve similar paternity, potentially by implementing alternative mating strategies that balance reproductive success.

Naturally and sexually selected life history plasticity

In **chapter 2**, I comparatively assess socially cued anticipatory plasticity (SCAP) in males of *Nephila senegalensis* and *N. fenestrata*, which differ in the degree of specialization to monopolize single females. Short-lived males in species with terminal male mating investment are expected to adjust the timing of maturation to social cues indicating the availability of receptive females, but this has rarely been experimentally demonstrated. In both of my study species, male-male competition is intense, and male reproductive success should critically depend on timely maturation and rapid location of a receptive female.

Using climate-control chambers, I reared split broods of both species either in the presence or absence of virgin female silk cues. Based on potentially strong selection in favour of socially cued plasticity in *N. fenestrata*, in which males follow a highly specialized terminal investment strategy, I predicted a distinct developmental response in this species, but a weaker response in *N. senegalensis* in which males are less specialized to monopolize females.

In **chapter 3**, I investigate sex-specific catch-up growth (a compensatory developmental mechanism to balance growth deficits) in *N. senegalensis*. Extreme reversed SSD provides a model system with 'dwarf males' that differ so much from females as if belonging to a different species. Divergent selection pressures are assumed to have initiated and

promoted the evolution of SSD, and these differences are expected to modulate adaptive compensatory development as well. Unfavourable growth conditions can prevent animals from reaching an optimal body size, and such limitations may severely affect an individual's fitness, particularly in short-lived species with determinate growth and a narrow time-frame for reproduction. Adaptive catch-up growth enables animals to compensate growth restriction in early juvenile stages by delaying development under improved conditions. The resulting delay of sexual maturation, however, may also involve fitness costs that have to be charged against the benefits of growth compensation.

I analyse catch-up growth based on the study animals' development tracked in different feeding treatments, e.g. comprising a fixed period of early low feeding conditions followed by unrestricted feeding conditions, permanent unrestricted feeding conditions, or permanent low feeding conditions. A second experimental section was conducted to assess the adaptive value of growth compensation in terms of lifetime fecundity (LTF) in a subset of females. I predicted effective catch-up growth in early food-restricted females to result in full compensation of growth deficits and a lifetime fecundity equivalent to unrestricted females. Based on a stronger trade-off between size-related benefits and costs of a delayed maturation, I expected less effective catch-up growth in males.

Male mating investment and sexual conflict

In **chapter 4**, I study the peculiar behaviour of copulatory male leg ejection in *Nephila fenestrata* within the framework of male mating investment and sexual conflict. Males in this species have evolved an extreme

mating strategy, restricting them to copulate at most twice in their lifetime, and hence are expected to plastically adjust their mating investment to female quality. Increased male mating investment, however, may be severely constrained by female resistance behaviour. *Nephila fenestrata* males frequently eject their front legs during copulation as a reaction to female attacks, and females grasp these legs to feed on them. Males often attempt to monopolize females, performing two copulations followed by mate plugging and mate guarding (Fromhage & Schneider 2005b; Fromhage & Schneider 2006). As successful monopolization may decrease female fitness, female attacks and sexual cannibalism may represent counter-adaptations to prevent monopolization. However, females that are distracted with prey capture and feeding are less likely to cannibalize the male (Fromhage & Schneider 2005a); hence males may sacrifice their legs to pacify females in order to reduce the risk of attacks and cannibalism.

I first investigate whether males vary their mating investment (measured as the number of legs sacrificed) according to mate quality. I conducted mating trials in which males were presented with either unrelated or sibling females, and predicted higher male mating investment in unrelated females. In a second mating experiment, I study the function of male leg sacrifice. Male front legs were offered to females during copulations with males whose front legs had been removed. I predicted this simulation of copulatory leg ejection to prevent female attacks and sexual cannibalism. In addition, I study whether male legs serve as a nutritious donation by assessing their attractiveness as food items for females.

Note

Each chapter of this thesis constitutes an independent scientific article and must therefore be comprehensible in itself without reference to other sections; thus a certain degree of overlap between individual sections cannot be avoided. The use of American and British English as well as formatting details vary between chapters, conforming to the specific requirements of the journals where the articles have been published.

Note on a proposed new phylogeny and taxonomic changes

Throughout this thesis, I follow the most recent published phylogeny according to the time of finalization of each section. According to an upcoming new phylogeny, however, current state *Nephila* is diphyletic and most species, including *N. senegalensis* and *N. fenestrata*, will be placed in the genus *Trichonephila* Dahl, 1911. Furthermore, current Nephilinae will be elevated to family rank again and the inferred relationships between species of the African clade containing my study species will undergo some changes (Kuntner et al.; in prep.). The proposed changes do not affect the evolutionary scenario with respect to reproductive strategies as outlined in my thesis.

Chapter 1

Differential investment and size-related mating strategies facilitate extreme size variation in contesting male spiders

Rainer Neumann and Jutta M. Schneider

Abstract

Sexually selected variation in male body size is often associated with alternative reproductive strategies that may persist under frequency-dependent selection or result from ‘making the best of a bad job’ in inferior individuals. The spider genus *Nephila* is well known for female gigantism and male dwarfism, serving as an established model system to investigate selection on male size and the still enigmatic SSD. Less attention has been paid to the enormous size variation among males that exists in some *Nephila* species despite broad evidence for large male advantages in contest competition. As the existing variation contradicts strongly biased fitness returns among different phenotypes, detailed investigations of fitness consequences related to different body sizes and their associated mating strategies are required. We used the African golden-silk spider *N. senegalensis* to test whether size-dependent alternative mating strategies yield equivalent reproductive success in dyads of size-mismatched males competing for a single female. Our results confirm that differently-sized males achieve similar paternity and reveal complex interrelations between competing strategies, which mutually influence each other. Males integrate their own competitive ability, the intensity of sperm competition, and female reproductive value to adjust their mating investment accordingly. Size-related mating strategies combined with behavioural flexibility may thus promote the maintenance of male size variation by balancing fitness returns and exemplify how changes in the selective context can dissolve principal large size benefits. We suggest that flexible adaptive responses may significantly contribute to stabilizing SSD.

Introduction

Phenotypic variation between individuals is a basic premise in evolutionary biology, yet the degree of variation may range from subtle differences to strikingly distinct morphologies, even in members of the same sex. Pronounced sex-specific variation in phenotypes has been addressed in studies concerning, for example, female polymorphism (Svensson et al. 2009) or alternative male mating tactics (e.g. Taborsky 2001; reviewed in Taborsky & Brockmann 2010a). The existence and maintenance of extreme variation can still be challenging to explain in the face of selection acting to optimize specific traits toward maximum adaptiveness, potentially narrowing variation. In the context of sexual selection, an important trait may be the better promote an individual's reproductive success, the closer it resembles an ideal condition (e.g. Brooks et al. 2005; Luo et al. 2014; Reeve & Fairbairn 2001; Ryan & Wilczynski 1988).

In most animal species, the sex that is subject to particularly strong sexual selection pressures are the males (Bateman 1948; Darwin 1871). Alternative male phenotypes may persist through frequency-dependent selection, which is generally assumed to result in equal average fitness returns of different morphs under equilibrium (e.g. Maynard Smith 1982; Parker 1984; Shuster & Wade 1991). In many mating systems, however, a proportion of males represent a less competitive phenotype that is often regarded as inferior relative to high quality males (e.g. Cook et al. 1997). As these low quality males would be outcompeted in most contests, they may benefit more by adopting parasitic tactics, e.g. the behaviour of opportunistically sneaking copulations in the presence of dominant males (e.g. Gross 1985; Sato et al. 2004; reviewed in Taborsky &

Brockmann 2010b). In view of such male mating tactics, the primal assumption of equal fitness payoffs has been questioned, and low quality males have been suggested to make 'the best of a bad job', which yields lower fitness while still allowing for some reproductive success (e.g. Taborsky 1998). However, it has also been demonstrated that individual males may not be restricted to inflexible, status-dependent mating tactics, each of which is targeted on the associated fitness payoff, but may alternate tactics according to conditions (e.g. Carroll & Corneli 1995; Lee 2005). Such flexible strategies may allow otherwise inferior phenotypes to increase their fitness in specific settings and may thus be essential to understand male phenotypic variation in a range of mating systems (reviewed in Shuster 2010).

In many animals, body size is a crucial trait in male-male competition, hence sexual selection does not result in elaborate male-specific traits in those species, but merely favours large males over smaller rivals, which often leads to male-biased sexual size dimorphism (SSD; e.g. Lindenfors et al. 2007 2007). The reverse case of pronounced female-biased SSD is often associated with scramble competition and life history benefits of small male body size (e.g. Corcobado et al. 2010; Danielson-Francois et al. 2012; Foellmer & Fairbairn 2004).

The spider family Nephilidae is an established model system for extreme female-biased SSD (e.g. Higgins et al. 2011; Kuntner et al. 2013; Kuntner & Coddington 2009; Kuntner & Elgar 2014), but beyond that, males in the genus *Nephila* may vary drastically in body size, with size differences reaching an order of magnitude in some species (Elgar et al. 2003b). Therefore, *Nephila* species have been used in a range of empirical studies investigating potential costs and benefits of small male size, most of which

have reported benefits of large size (e.g. Christenson & Goist 1979; Elgar et al. 2003b; Miyashita 1993; Rittschof 2010; Schneider et al. 2000). Accordingly, large males physically dominate their smaller competitors and win most of all agonistic encounters (Constant et al. 2011; Elgar et al. 2003b; Fromhage & Schneider 2005b, this study), but previous studies have rarely related these behavioural observations to ultimate measures of fitness (but see Elgar & Jones 2008; Rittschof 2010). Distributions of male size in natural populations, as well as phylogenetic patterns (Higgins et al. 2011), clearly contradict strong directional selection on large male size. Rather, the existing variation implies balanced overall fitness of differently-sized males, indicating that physical strength may be less important in contest competition than previously assumed and other mechanisms mediating fitness returns have to be identified.

In the African *Nephila senegalensis*, often remarkably different-sized males compete for mating with a single female (pers. observations). Extreme male size variation in these spiders may require different developmental pathways, as arthropods can only increase body size by moulting, and may show polymorphism in the number of instars preceding sexual maturity. Adult size is a fixed trait in *Nephila* and, in laboratory-reared males, different numbers of developmental instars result in significantly different adult size (Neumann et al. n.d.). While directional selection on body size has received considerable attention (Blanckenhorn 2000; reviewed in Blanckenhorn 2005), fewer efforts have focused on systems in which size is a continuous trait. In such a case, measuring benefits of only upper and lower size extremes may not recognize selection dynamics in which the fitness of specific phenotypes will be affected by complex

interactions between small, intermediate, and large variants.

Males in *Nephila* are faced with the evolutionary constraint of sperm limitation (spermatogenesis ceases with sexual maturation; Michalik & Rittschof 2011a; Schneider & Michalik 2011) and frequently experience intense contest competition and/or sperm competition, as imposed by a male-biased operational sex ratio. These elements are part of an evolutionary scenario resulting in very low male mating rates (Fromhage et al. 2005; Fromhage et al. 2007) and males may benefit from conditional strategies involving variable mating investment, depending on the value of a female (Fromhage & Schneider 2012). Using a theoretical approach, Rittschof et al. (2012) have evaluated potential mating strategies in *N. clavipes*, a species similar in reproductive biology to our study species. Taking into consideration the potentially varying competition in natural mating systems, as well as seasonal effects, the model suggested that males adopt size-related mating strategies (e.g. more pronounced mate choice in large males and indiscriminate mating in small males) that result in equivalent fitness payoffs. However, empirical studies are required to evaluate the significance of conditional strategies in balancing fitness returns.

Here, we investigate extreme phenotypic variation by assessing paternity returns in dyads of males competing for a single female over their entire adult life-span. Males were assigned to three distinct size classes (small, medium-sized, and large) to generate competition between size-mismatched males in all combinations of size classes. We test the hypothesis that differently-sized males achieve balanced reproductive success and analyse how paternity returns depend on interrelations between particular male size classes and female traits. Males should

implement size-related mating strategies that could facilitate the evolutionary maintenance of extreme size variation. Specifically, we predict opportunistic unconditional mating behaviour in small males, while large males' investment in females should vary with conditions affecting potential fitness returns.

Methods

Study Species, origin and rearing of study animals

The African golden-silk spider *Nephila senegalensis* occurs in dry bush savannahs and semi-humid half-open woodlands. Adult females build large orb-webs between shrubs and trees that are usually maintained over relatively long periods of time (often > 2 weeks; Neumann & Schneider n.d.). Males cease web-building after reaching maturity and occasionally feed on prey caught by the female. Females are always receptive and accept several males for mating (Schneider et al. 2011; Schneider & Michalik 2011). Males transfer sperm to the females paired sperm stores (spermathecae) and eggs are not fertilized until they are laid.

We collected six egg sacs and 14 gravid females on the property of the Ongos game farm, Khomas-Hochland, Namibia (coordinates: 22°44'S; 016.97'E) in April 2011. Each of the egg sacs and spiders were collected at a different site with a minimum distance of approximately 30 m between them; thus representing 20 non-related family lines. The material was transferred to Hamburg, Germany (permit No. 84003; Ministry of Environment and Tourism, Namibia). We reared study animals from the wild-collected egg sacs and 14 egg sacs built in the lab. The hatchlings were separated after approximately two additional moults. Spiders were housed individually in plastic

cups and reared even-handedly until females grew larger than males. From then on, stocks of both sexes were maintained according to requirements. Male and female spiders were fed a standardized diet of *Drosophila* or *Calliphora* flies, respectively (see Schneider et al. 2011 for details).

Treatments and mating trials

Adult males (identified by fully developed copulatory organs on the pedipalps) were assigned to three distinct size classes (small = S; medium-sized = M; large = L) using the spiders' weight at the day of maturation as a reference of size, as male adult weight and size are highly correlated (see Schneider & Michalik 2011). The mean weight of small males was 28.51 ± 0.68 mg (range: 18-36.5 mg, $N = 45$); medium-sized males weighed 47.06 ± 0.39 mg (range: 43.1-54 mg, $N = 44$), and the mean weight of large males was 65.61 ± 1.07 mg (range: 59.3-91.4 mg, $N = 39$). Male sizes in nature cover the whole range of those used in our study (Neumann & Schneider n.d.). In each mating trial, two size-mismatched males competed for one female. We conducted mating trials so as to stage competition between males in all possible combinations of differing size classes, resulting in the following treatments: S versus M (one female; one small male, and one medium-sized male), S versus L (one female; one small male, and one large male), and M versus L (one female; one medium-sized male, and one large male). Spiders used in each mating trial were taken from unrelated family lines. Males and females were randomly chosen from the respective subsets.

Initial observation period

Prior to mating trials, each virgin female was placed inside a 40 x 40 x 12 cm sized air-vented Perspex frame that was tightly closed

with Perspex coverings on the front and back. These coverings were prepared with Vaseline in order to prevent spiders from attaching threads. The spiders built normal orb-webs inside the frames.

A mating trial was only initiated when the female had assumed her position at the hub of the web. We carefully removed the covering of the frame and placed both males on the web's supporting threads in the upper corners of the frame (left and right side were randomly assigned). The spiders were observed continuously during the first 3 h of each trial. Because males in *N. senegalensis* attempt to mate more readily when the female is feeding (see Schneider et al. 2011), we fed the female three *Calliphora* flies after 30 minutes and another two flies 75 minutes after starting the trial. The flies were carefully placed inside the web and the female always captured at least one prey item immediately. Feeding was delayed at the occurrence of courtship and mating to avoid disturbance of these behaviours.

During observations, we recorded copulations, agonistic male-male encounters, and cannibalism. Agonistic behaviour between males has been assessed in several *Nephila* species (e.g. Elgar et al., 2003; Fromhage and Schneider, 2005b; Constant et al., 2011) but corresponding data are lacking for *N. senegalensis*. We classified agonistic interactions as male-male chases and contact fights (i.e. males fight with their forelegs to displace each other). A male succeeded in an interaction when he caused the competitor to retreat, which often increased the distance to the female. We measured copulation duration as the period of time between the first inflation of the hematodocha (a pressure-generating organ securing sperm transfer) and the withdrawal of the male's copulatory organ from the female copulatory opening. After the 3 h initial observation period, the spiders were fed a portion (two

tablespoons) of *Drosophila* flies (anaesthetized with CO₂), especially to provide males with food. The spiders were kept inside the mating arenas for additional observations.

Additional observation periods

In order to capture effects of long-term competition and cohabitation, we conducted up to three additional 1 h observation periods in each trial; usually on day 3, 5, and 7 of the trial (in some cases observations had to be shifted due to female web renewal, as incomplete webs are insufficient for typical courtship and mating behaviour). The number of additional observation periods (mean: 2.39 ± 0.13) varied with male life-span. We fed the female three *Calliphora* flies at the beginning of 1 h observations and spiders were provided with *Drosophila* flies subsequent to each observation period as well. In-between and subsequent to behavioural observations, mating arenas were checked daily (on six days per week) for presence of study animals. Trials ended either with the production of an egg sac or when a male was no longer present. We were able to confirm cases of cannibalism without directly observing the female consuming a male when we found the male's remains inside the mating arena. Females that had not laid eggs until the end of a trial were transferred to rearing-cups again. When they had finally built an egg sac, they were killed by hypothermia (-80 °C) and preserved in alcohol. The length of patella and tibia of one foreleg was measured using a LEICA MZ 16 stereo microscope and LEICA IM500 imaging software (V.4.0).

Age, size, and weight of study animals

Newly matured males and females were weighed immediately, and females were weighed again before being used in the

experiment. We defined adult age as the number of days from day of maturity. Due to irregular availability of adult males, small males and medium-sized males were 2-3 days older, on average, than large males across treatments (small male age = 18.2 ± 1 days; medium-sized male age = 18.9 ± 0.8 days; large male age = 15.9 ± 1.2 days, Kruskal-Wallis-test: $\chi^2 = 7.747$, $N = 128$, $P = 0.021$). Female age at the beginning of trials was 17.45 ± 0.73 days (range: 6-29 days). Female size (given here as the length of patella and tibia of one foreleg) was 15.43 ± 0.12 mm (range: 13.57-17.43 mm; $N = 63$) and females weighed 1131.97 ± 23.56 mg (range: 731-1569.3 mg; $N = 64$) at the beginning of trials.

Cohabitation and cannibalism

Males cohabited with females in the mating arenas and 101 males were eventually cannibalized by the female (79.5 %; $N = 127$). Cannibalism in this species may not be classified as sexual cannibalism in the strict sense (reviewed in Elgar & Schneider 2004), as it often occurs independent of courtship and mating. Males could not be preserved and measured due to cannibalism. The mean male-female cohabitation duration was 9.54 ± 0.72 days (range: 1-29 days). In nature, we monitored 69 individually marked males of which 49 were observed with only one female. These males stayed with the female 3.1 ± 0.46 days (range 1-16 days) (Neumann & Schneider n.d.).

Paternity assignment

We used the sterile male technique (Parker 1970; see Schneider & Andrade 2011 for a detailed description) in order to assess paternity of both males; thus one irradiated and one non-irradiated male were used in each mating trial. Mature males of all size classes were randomly assigned to either the normal group (N-males) or the irradiated

group (R-males). R-males were irradiated with 40 Gy of X-rays (200 kVp with 0.8 mm and 0.5 mm Cu filtering, RS225; Gulmay Medical Ltd., Camberly, UK; dose rate 0.8 to 1.2 Gy/min) at the Laboratory of Radiobiology and Experimental Radio oncology, University Medical Center Hamburg-Eppendorf. This method of sterilization does not limit the ability of an R-male's sperm to fertilize eggs, but the development of these eggs ceases immediately because of deleterious mutations (e.g. Nessler et al. 2009). However, analyses of our data indicated that non-irradiated males had an overall advantage (GLM results, Table 2). As equal numbers of N-males and R-males (randomly chosen from respective subsets) were used in each size class and treatment, this effect is evenly distributed across trials; hence irradiation is unlikely to bias analyses of paternity. Paternity shares of both males in each trial could be estimated by assigning undeveloped eggs to the irradiated male and normally developed offspring to the non-irradiated male, respectively. We used control groups where females mated with either two normal (NN) or two sterile males (RR) to estimate the proportion of eggs that do not develop after non-manipulated matings and to confirm sterility of R-males. In control trials, each male performed a single copulation and was removed from the web afterwards. All 64 females used in experimental treatments as well as the 13 females in control groups built viable egg sacs which were stored separately in air-vented plastic containers at 23-27 °C and preserved in 70 % alcohol after 5-6 weeks. Each egg sac was opened and the content counted under a stereo-microscope. For paternity assignment of sterile males, the proportion of undeveloped eggs in NN-control clutches serves as a correction factor. The hatching success in egg sacs of the NN-control-group was 94.3 % ($N = 8$). No eggs

developed in egg sacs of the RR-control group ($N = 5$). We used 154 males and 77 females in this study, including control treatments.

Statistical analysis

For analyses of paternity, we randomly determined one of both size-mismatched males in each trial to be the focal male, thereby precluding repeated measurements of individual males. Analyses of agonistic male-male interactions, cannibalism, cohabitation duration, and male mating performance included both males in each trial. We analysed potential determinants of paternity conducting a generalized linear model (GLM) performed in R (Version 2.15.2, R Development Core Team 2012) with quasi-binomial error structure (due to overdispersion) and probit-link function. The model included trials of all three treatments. We used the paternity success of the focal male as the response variable by treating the number of eggs fertilized by each male as 'successes' and 'failures' with respect to the focal male's success (using the `cbind`-function in R). The model was simplified by stepwise removal of non-significant effects (beginning with interaction terms) based on ANOVA-model comparisons with F -tests ($\alpha = 0.05$) to confirm order of deletion. Non-significant P -values correspond to the time a variable was removed from the model. Significant P -values denote variables that remained in the minimal adequate model. Interaction terms that were significant in the GLM were further evaluated with post-hoc interaction tests (R package `phia`) to calculate pairwise between-factor level contrasts with ANOVA-based F -tests. Additional statistical tests were performed in JMP IN 7.0 (SAS Institute Inc., Carey, NC, USA). Descriptive statistics are given as mean \pm standard error. Sample sizes may differ within experiments due to missing data. In treatment S versus M, one small male escaped from the mating arena. This male

cohabited with the female over a period of four days. We excluded this male from analyses of cannibalism and cohabitation duration, but included the trial in all other statistical analyses.

Ethical note

Cannibalism is a common behaviour in many spiders (reviewed in Elgar & Schneider 2004). In *Nephila*, mature males spend most of their adult life-span in female webs; cohabiting with females as kleptoparasites (adult males are unable to catch prey on their own). In several *Nephila* species, males' are frequently cannibalized by the much larger females. Males grasped by the female die immediately. In our study, we used the minimum numbers of study animals necessary for meaningful statistical analyses. All study animals were reared specifically for study purposes, using offspring of a minimum number of wild-collected females.

Results

Agonistic male-male interactions

We recorded multiple agonistic interactions of individual male pairs, hence we merely report the percentages of encounters won or lost. In treatment S versus M, we recorded 73 agonistic encounters of which 67 were won by the medium-sized male (91.8 %). In detail, 12 of these encounters were contact fights (11 won by the medium-sized male) and 61 were classified as male-male chases (56 won by the medium-sized male). In treatment S versus L, large males won 6 of 7 contact fights and 45 of 52 male-male chases; thus the large male prevailed in 51 of 59 agonistic interactions (86.4 %). Similarly, large males dominated their relatively smaller competitors in treatment M versus L and succeeded in 16 of 17 contact fights as well as 58 of 66 male-male chases. Collectively, 74 of

83 agonistic encounters in this treatment were won by the large male (89.2 %). Across treatments, the relatively larger male succeeded in 89.3 % of all agonistic encounters observed.

Cannibalism

The majority of all males were cannibalized by the females in the course of the study (79.5 %). Across treatments, small males were cannibalized significantly more often (42 of 44) than medium-sized (33 of 44) and large males (26 of 39) (G -test: $\chi^2 = 13.343$, $N = 127$, $P = 0.001$).

Male-female cohabitation duration

Across treatments, small males survived for considerably shorter periods than medium-sized males and large males, but the difference was not significant (small male

cohabitation = 7.18 ± 1.06 days; medium-sized male cohabitation = 11.02 ± 1.24 days; large male cohabitation = 10.69 ± 1.42 days, Kruskal-Wallis-test: $\chi^2 = 5.852$, $N = 127$, $P = 0.054$).

Male mating performance

In treatment S versus M, we recorded similar numbers of copulations in small males and medium-sized males. Maximum individual copulation durations and total copulation durations in small males exceeded those of medium-sized males, though not statistically significant, and small males achieved first copulations as often as medium-sized males.

In treatment S versus L, we observed twice as many copulations in large males competing with small males. However, small males achieved first copulations as often as large males, and the maximum single

Table 1. Comparison of male mating performance between size classes in individual treatments (S versus M, S versus L, and M versus L).

		Total no. of observed copulations	Maximum copulation duration [s]	Total copulation duration [s]	First copulation achieved (y/n)
S versus M	S-males	1.68 ± 0.27	319.76 ± 62.99	349.44 ± 75.94	12/13
	M-males	1.72 ± 0.23	206.14 ± 32.4	273.38 ± 53.32	13/12
	χ^2	0.132	1.535	2.203	
	P	0.717	0.215	0.652	1
	N	50	43	49	25
S versus L	S-males	1.15 ± 0.22	254.64 ± 49.65	217.2 ± 52.44	10/8
	L-males	2.3 ± 0.35	147.12 ± 37.33	237.05 ± 62.5	8/10
	χ^2	6.425	3.863	0.156	
	P	0.011	0.049	0.693	0.815
	N	40	31	39	18
M versus L	M-males	1.42 ± 0.35	157.15 ± 38.32	154.05 ± 46.26	3/14
	L-males	1.9 ± 0.33	120.88 ± 18.11	152.22 ± 24.05	14/3
	χ^2	1.603	0.012	1.016	
	P	0.206	0.913	0.313	0.013
	N	38	29	37	17

S: small; M: medium-sized; L: large. Parameters measured are the total number of copulations observed, the maximum duration of individual copulations, the total copulation duration observed (Wilcoxon-two-sample-tests) and the numbers of males to copulate first in the initial observation period of each trial (Two-sided binomial test). Significant P -values are shown in bold.

copulation duration in small males significantly exceeded that of their large competitors. Small and large males did not differ in total copulation durations observed.

In treatment L versus M, medium-sized and large males neither differed in total numbers of copulations observed, nor in maximum individual copulation duration or total copulation duration observed. In this treatment, however, large males were the first to copulate with the female much more frequently than medium-sized males (see Table 1 for comparative test results).

Proportions of paternity

Consistent with the hypothesis that differently-sized males achieve similar reproductive success in contest competition, there were no significant differences between average proportions of paternity of focal male size classes. Small males achieved a paternity of 0.7 ± 0.08 ; medium-sized males' paternity was 0.5 ± 0.09 , and large males gained a paternity of 0.58 ± 0.08 (Kruskal-Wallis-test: $\chi^2 = 2.528$, $N = 64$, $P = 0.283$).

Regarding the outcome of competition in specific treatments, we asked if focal males' paternity success varied with the competitor's size class, but again we found no significant differences. Small males achieved a paternity of 0.65 ± 0.12 in competition with medium-sized males and 0.75 ± 0.11 in competition with large males (Wilcoxon-test: $\chi^2 = 0.174$, $N = 23$, $P = 0.677$). Medium-sized males' gained a paternity of 0.5 ± 0.11 when competing with small rivals and 0.49 ± 0.16 in competition with large rivals (Wilcoxon-test: $\chi^2 = 0.335$, $N = 21$, $P = 0.563$). Large males' paternity in competition with small males was 0.62 ± 0.12 and they achieved a paternity share of 0.55 ± 0.12 with medium-sized males (Wilcoxon-test: $\chi^2 = 0.146$, $N = 20$, $P = 0.703$).

In addition to relative paternity success, we also compared absolute numbers of fertilized female eggs between focal male size classes. A small male fertilized 508.7 ± 64.27 eggs, on average; medium-sized males fertilized 300.24 ± 60.53 eggs, and large males fertilized 373.7 ± 65.86 eggs. Although small males fertilized considerably more eggs than their competitors, the numbers did not differ significantly between the three size classes (Kruskal-Wallis-test: $\chi^2 = 4.847$, $N = 64$, $P = 0.089$).

Overall, there was a high variation in proportions of paternity that ranged from 0-1 for any size class in any treatment. In 19 of all 64 trials (29.7 %), the focal male achieved exclusive paternity. The probability of exclusive paternity did not differ between focal male size classes (9 of 23 small males, 5 of 21 medium-sized males, and 5 of 20 large males; G-test: $\chi^2 = 1.514$, $N = 64$, $P = 0.469$).

Determinants of paternity

We analysed potential mechanisms determining paternity across treatments in a generalized linear model (GLM). The paternity success of the focal male in relation to the competitor's success was used as the dependent variable. In order to reveal complex interrelations between competing size classes, we based our model on comparative results obtained from behavioural observations (see 'Male mating performance' and Table 1). Consequently, we included as explanatory variables the number of observed copulations (which markedly differed between small and large males), and whether the focal male or the rival achieved the first copulation (in which medium-sized and large males differed significantly). The number of observed copulations was entered into the model as the proportion of the focal male's number of copulations relative to the rival male's number of copulations, a factor we termed 'focal male copulation ratio'. In

Table 2. GLM results analysing determinants of male paternity success.

Explanatory variable	<i>df</i>	<i>F</i>	<i>P</i>
Treatment	2	0.5265	0.5944
Focal male size class	2	1.8070	0.1756
Focal male copulation ratio	1	0.0046	0.9464
Rival male first to copulate	1	0.2187	0.6423
Focal male irradiation state	1	5.3349	0.0254
Female age	1	0.8731	0.355
Female size	1	0.0014	0.9701
Focal male size class * focal male copulation ratio	2	5.9866	0.0049
Focal male size class * rival male first to copulate	2	1.9917	0.1495
Focal male size class * female age	2	2.6989	0.0817
Focal male size class * female size	2	7.2713	0.0018
Rival male first to copulate * focal male copulation ratio	1	0.3042	0.5843
Rival male first to copulate * female age	1	12.082	0.0011
Rival male first to copulate * female size	1	0.9194	0.343

Significant *P*-values are shown in bold.

order to include zero-copulation observations, the value 0.1 was added to absolute copulation numbers for calculating this factor. We also included the focal male size class (S, M, L), treatment, focal male irradiation state (R/N), female adult age, and female size.

As we expected that mating performances of different size classes and female traits mutually influence one another, we used the following interaction terms: focal male size class × competitor first to copulate, × focal male copulation ratio, × female adult age, and × female size; competitor first to

copulate × focal male copulation ratio, × female adult age, and × female size (see Table 2 for an overview of GLM results).

The model revealed three significant interactions. First, the focal male's size class significantly predicted his paternity success in an interaction with female size. Female size had divergent effects on medium-sized and large males' paternity, in that medium-sized males achieved higher paternity shares with relatively small females, whereas large males benefitted from large females. The paternity success of the small male size class, however, was unrelated to female size (Fig. 1, Table 3).

Table 3. Pairwise between-factor-level contrasts of interactions that significantly influence male paternity (see GLM results, Table 2).

Interaction term	Factor level pairs	Coefficient	<i>df</i>	<i>F</i>	<i>P</i>
Focal male size class * female size	Size class (L – M)	1.5183	1	12.3686	0.0001
	Size class (L – S)	0.6434	1	2.0561	0.1584
	Size class (M – S)	-0.8749	1	3.1588	0.0821
Focal male size class * focal male copulation ratio	Size class (L – M)	-0.0025	1	0.0035	0.9532
	Size class (L – S)	0.1192	1	7.0032	0.0111
	Size class (M – S)	0.1217	1	6.8151	0.0122
Rival male first to copulate * female age	Rival copulated first (no – yes)	-0.2078	1	10.171	0.0026

S: small; M: medium-sized; L: large. Values for each interaction term derive from ANOVA tables calculated as post-hoc interaction analysis (see 'Statistical analyses'). The interaction term 'Rival male first to copulate × female age' (containing only a two-level factor) is included to provide complete estimates associated with significant interaction terms. Significant *P*-values are shown in bold.

Second, the model revealed a significant influence of female age in connection with whether the focal male or the competitor copulated first with the female. The focal male achieved a relatively high paternity with young females, given he secured the first copulation with the female. In contrast, if the rival male copulated first, the focal male benefitted from older females (Fig. 2, Table 3).

Third, we found that the focal male's paternity success was significantly determined by the interaction between the focal male's size class and focal male copulation ratio. Medium-sized and large males' paternity success was directly related to the number of copulations achieved relative to the rival; i.e. by outnumbering their rivals' copulations, those males increased their paternity success. However, this was different in small males. Small males did not benefit from particularly high copulation scores, but achieved high proportions of paternity even with lower copulation ratios (Fig. 3, Table 3).

Discussion

In accordance with the hypothesis that differently-sized males achieve similar average reproductive success in contest competition, our long term cohabitation study showed that there is no general paternity advantage for larger competitors, irrespective of unequal physical strength. Inversely, we found no indications of a general small male advantage; neither did we observe strong effects on paternity distributions in particular combinations of competing size classes.

Our analyses indicate that paternity distributions within trials were modulated by size-related but flexible mating strategies,

allowing males of any size the chance to persist in competition when facing a differently-sized rival; a finding which substantially adds to our understanding of extreme male size variation and SSD in *Nephila*. While large males' mating investment varied with competitive context and female value, small males invested indiscriminately in females.

We found female size to be a key factor in moderating paternity, as female size divergently affected paternity success in medium-sized and large males, whereas small males' paternity was unrelated to female size. While large males achieved higher paternity success with large females, medium-sized males benefitted from relatively small females. As our analyses of mating performance showed, large males regularly secured the first copulation in competition with medium-sized males, and mating successfully with a virgin female often marks the beginning of rigid monopolization strategies in spiders (e.g. through mate plugging or mate guarding; Fromhage & Schneider 2006; Snow et al. 2006; reviewed in Uhl et al. 2009). Therefore, the ability of medium-sized males to increase paternity with small females may appear curious at first, as it reflects limited paternity success of large males, despite large males' mating first in most of these trials. However, based on theoretical results in favour of mating strategies that vary with female quality (Rittschof et al. 2012), we did not expect all males in our study to follow a maximal investment strategy with the present female. Instead, large males should use plastic strategies of differential mating investment based on reliable predictors of reproductive success.

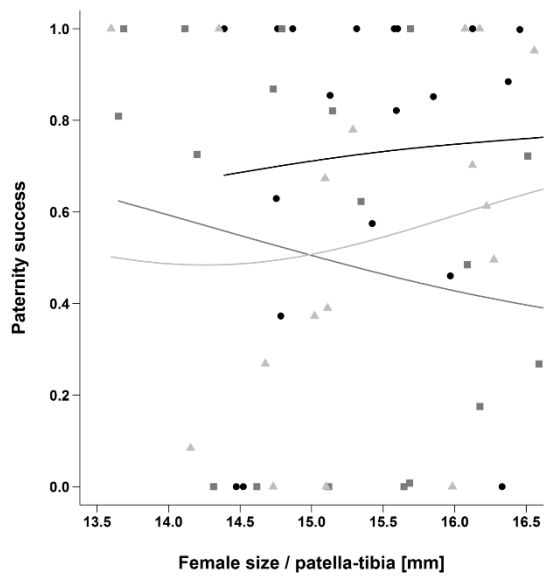


Figure 1. Focal males' paternity in relation to focal male size class and female size. Focal male size classes were small (black circles), medium-sized (dark grey squares) and large (grey triangles). Smoothed lines generated with kernel density estimates based on GLM results were added to visualize the interaction.

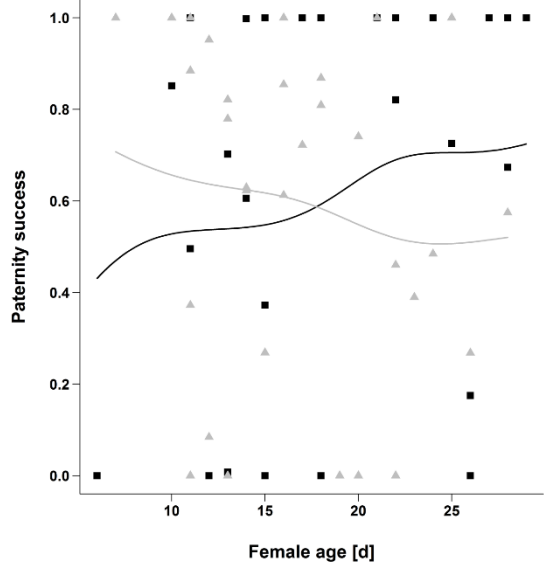


Figure 2. Focal males' paternity in relation to female age and the competitor's ability to copulate first with the female. Black squares: competitors achieving the first copulation; grey triangles: competitors not achieving the first copulation. Smoothed lines illustrating the interaction were generated with kernel density estimates based on GLM results.

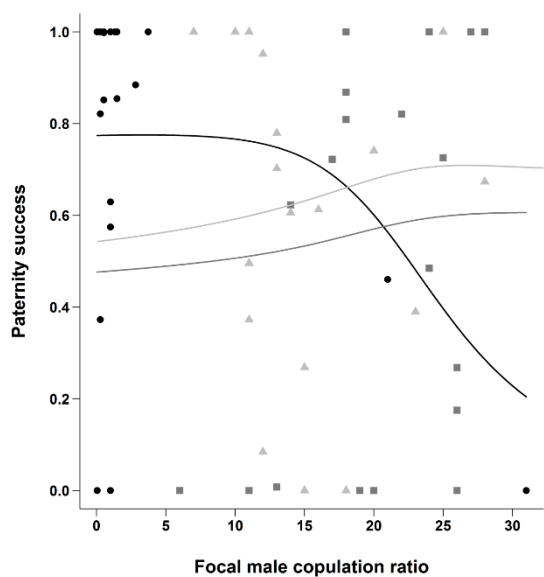


Figure 3. Focal males' paternity success in relation to focal male copulation ratio (the number of copulations relative to the rival) and focal male size class: small males (black circles), medium-sized males (dark grey squares) and large males (grey triangles). Smoothed lines were generated with kernel density estimates based on GLM results to visualize the interaction.

Fecundity, for example, is positively correlated with female size in *Nephila* (Higgins 1992; Miyashita 1986; Rittschof 2011) and the risk of falling prey to a predator is much reduced in large females as well (Chase 1999; Higgins 2002), which considerably increases the chance of successful reproduction. Therefore, large females are especially attractive, which is reflected in increased large male investment. In contrast, large males may have refrained from investing heavily in smaller females as part of a polygynous strategy, although our experimental design precluded mating with another female. In natural populations, however, other females are frequently accessible in the proximity of the first female's web, and large males may leave small females after moderate mating investment to search for another female of potentially higher quality; a strategy of sequential mating investment (Bateman & Fleming 2006; Fromhage & Schneider 2012; Welke & Schneider 2010). Individual copulations in large males were generally shorter than those of smaller rivals, suggesting that large males may have adjusted investment by varying numbers of short copulations.

Medium-sized and large males' divergent investment in small and large females might be based on persistent mate preferences resulting in size-assortative mating investment (e.g. Rowell & Servedio 2009; Servedio & Lande 2006). Assortative mating in general may either arise as a consequence of male-male contests (less competitive males are restricted to mating with lower-quality females, e.g. Johnson 1982), or males with lower competitive abilities may actually seek to mate with less targeted females in order to avoid competition right from the start (prudent mate choice, e.g. Bel-Venner & Venner 2006; Härdling & Kokko 2005; Venner et al. 2010). While male reluctance to mate

with a virgin female altogether has rarely been observed in spiders (but see Zimmer et al. 2012), males may none the less adjust mating investment in particular females. A general preference for small females in medium-sized males would lead them to voluntarily reduce mating investment in large females even in the absence of rivals; a strategy that would only be beneficial if any investment exceeding a threshold value is likely to be erased by subsequent rivals. While specifics of our study system (e.g. male sperm limitation, intense male-male competition, pronounced differences in males' physical strength, and female polyandry) could favour such mating tactics, our data do not permit evaluation of that possibility, and further studies are required. The most intuitive explanation for our findings might be that medium-sized males were unable to gain primary access to large females in contests with large males, and that large males' non-maximal investment in less preferred females may have shifted the chance of winning to the competitor.

Another female trait affecting the outcome of contest competition in our experiment was female age. Depending on the focal male's ability to achieve the first copulation with the female, his paternity success was likely to increase or decrease with female age. Males mating with virgin females gained particularly high paternity shares when the female was young. If, however, the competitor secured the first copulation, thereby altering the female's mating status to non-virgin; the focal male benefitted more from older females. Again, these results reflect interactions between both larger size classes, as only medium-sized and large males competing against each other differed significantly in the probability to achieve the first copulation. In many animal mating systems, virgin females are the most attractive mates, a pattern which is also

observed in spiders (reviewed in Gaskett 2007; Gaskett et al. 2004; reviewed in Huber 2005). Mating with young females before they have laid eggs may yield particularly high fitness payoffs because a male's sperm will then be used to fertilize the first clutch produced, which is typically the largest (e.g. Jones & Elgar 2007), and may also be used in successive clutches (females may produce up to three clutches in nature; pers. observations). Ideally, the female may not remate and the fortunate male will sire all of her offspring. Our results support the assumption that investing substantially in young virgin females is generally the most promising mating strategy, which in our competitive settings especially large males were able to put into practice when facing medium-sized rivals. Interestingly, focal male mating investment shifted to older females when males lost the first mating to the competitor. This finding is consistent with another study on *N. clavipes* in which males were allowed to choose between non-virgin females of different age. Males showed a strong preference for relatively old females close to laying eggs, probably because these females bear a low mortality risk within the short period of time between mating and oviposition. As reduced female mortality entails a higher probability of successful reproduction, males charge this benefit against the costs of non-exclusive paternity (Rittschof 2011).

While female qualities and differences in mating performance of focal males compared to rivals significantly influenced paternity in medium-sized and large males, we found no such relationship in small males. Overall, our results imply that small males' average reproductive success was independent of particular conditions. This became apparent by evaluating the number of focal male copulations relative to the rival, which

affected medium-sized males' and large males' paternity, but not small males' paternity. Males of both larger size classes increased their paternity by performing more copulations than the competitor, whereas small males did not benefit from copulating more often than the rival. We suggest that small males counterbalanced their physical inferiority by adopting an alternative mating strategy most akin to a sneaker strategy, which is known from a range of taxa (e.g. Iwata et al. 2011; Neff 2004; Wikelski et al. 1996). In contrast to large males' differential investment, small males probably seized every chance for investing maximally in the present female. Small males performed significantly longer copulations than large males; a behaviour that has also been reported for *N. edulis* (Schneider & Elgar 2005). Prolonged copulations resulted in similar total copulation durations between small and large males, which probably contributed to balanced reproductive success in our long-term study.

Our results demonstrate that fitness payoffs in male *N. senegalensis* are moderated by context-dependent mating strategies allowing individual males to adjust their mating behaviour in response to different competitive settings and female qualities. In line with current research, our findings emphasize a previously underestimated flexibility of behavioural tactics, particularly in arthropods. For example, Jordan et al. (2014) have shown that males in the Australian *N. plumipes* may alter initial settlement decisions according to changing social conditions, e.g. the intensity of male-male competition. Importantly, adaptive shifts may result in mating with females that would be less preferred in non-competitive situations.

Our study confirms that small, medium-sized, and large males are sufficiently

successful in contest competition to persist in a population. Based on this assessment, however, why a proportion of males grow large at all remains somewhat ambiguous. The abilities of large males to actively vary their investment in females according to female quality evidently exceeded those of smaller rivals, and large males were superior in terms of longevity (see also Uhl & Vollrath 1998). Prolonged survival in female webs may facilitate a polygynous mating strategy that could significantly increase large males' lifetime reproductive success. However, while the ability to fertilize multiple females has been experimentally demonstrated in *N. senegalensis*, males rely on a fixed sperm supply that determines the number of successful matings. Typically, experimental males were able to fertilize two females (Schneider & Michalik 2011). Large males' potential to increase fitness by means of additional mate searching is therefore limited. On the other hand, if a large male succeeded in monopolizing two high quality females, he would probably obtain above average fitness, but this might rarely be possible in a competitive environment.

In nature, *Nephila* males may face distinctly different competitive settings in which mating objectives depend on a range of parameters. While the density of sexually receptive males and females in the population predicts the intensity of competition on a broad scale, fitness prospects of a virgin male arriving at a female's web will be largely determined by local factors, such as female reproductive value, the number of competitors present on the female's web, and each rival's competitive abilities (e.g. signalled by body size). In connection with the male's own potential in contests and seasonal effects influencing the future availability of additional females, these parameters create complex and highly variable settings that

males' best respond to by applying the most promising mating tactic from a set of alternatives.

As large and small males are less common in nature than medium-sized males (Neumann & Schneider, unpublished data), we suggest that their occurrence is mediated by frequency-dependent selection driven by seasonal changes. Field studies in seasonal *Nephila* species show that male and female average body size decreases in the course of the season (e.g. Kasumovic et al. 2009; Miyashita 1994) and these conditions may favour early maturing large males that are able to fertilize large numbers of eggs produced by a large female, or eggs of multiple smaller females. Small males, on the other hand, are more common toward the end of the mating season, probably because unfavourable conditions regarding nutrition and temperature do not allow reaching large size in the time remaining to reproduce. Although contest competition may prevent small males from investing differentially in females and force them into a so-called 'best of a bad job-strategy', our study corroborates theoretical results suggesting that small males' indiscriminate mating investment in fact represents a successful alternative mating tactic.

Mating strategies, where behavioural flexibility elevates average fitness payoffs to a level exceeding what would be obtained by applying static behaviours, have been mainly reported from vertebrate taxa (e.g. Humfeld 2013; Reichard et al. 2004; Rezucha & Reichard 2014; Sato et al. 2004; Schradin & Lindholm 2011), but may be especially relevant when morphological phenotypes in adults are fixed, like in most arthropods. Implications of our findings extend to female-biased SSD, as we show that the physical predominance seen in large males does not reflect general directional selection in favour of large size; yet context- and frequency-

dependent benefits of large size still persist. However, even slight changes in conditions may shift net selection to favour less specialized tactics of smaller males. Such dynamics may generally stimulate the disassociation of body size between the sexes, thereby promoting SSD.

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Chapter 2

Socially cued developmental plasticity in web-building spiders

Rainer Neumann and Jutta M. Schneider

Abstract

Socially cued anticipatory plasticity (SCAP) has been proposed as a widespread mechanism of adaptive life history shifts in semelparous species with extreme male mating investment. Such mating systems evolved several times independently in spiders and male reproductive success should critically depend on timely maturation and rapid location of a receptive and, ideally, virgin female. We experimentally investigated socially cued anticipatory plasticity in two sympatric, closely related *Nephila* species that share many components of their mating systems, but differ in the degree to which male reproductive success depends on mating with virgin females. Juveniles of both species were reared either in the presence or absence of virgin female silk cues. We predicted strong selection on socially cued plasticity in *N. fenestrata* in which males follow a highly specialized terminal investment strategy, but expected a weaker plastic response in *N. senegalensis* in which males lost the ability to monopolize females. Contrary to our predictions, *N. fenestrata* males presented with virgin female silk cues did not mature earlier than siblings reared isolated from such cues. Males in *N. senegalensis*, however, showed a significant response to female cues and matured several days earlier than control males. Plastic adjustment of maturation had no effect on male size. Our results indicate that a strong benefit of mating with virgins due to first male sperm priority does not necessarily promote socially cued anticipatory plasticity. We emphasize the bidirectional mode of developmental responses and suggest that this form of plasticity may not only yield benefits through accelerated maturation, but also by avoiding costs of precipitate maturation in the absence of female cues.

Introduction

In most organisms, genetically identical individuals develop markedly different phenotypes when exposed to different environments (Bradshaw 1965; Liefing et al. 2009; Pigliucci 2009; Schmalhausen 1949; Smith-Gill 1983; Stearns 1982) and such plastic modifications of morphology, physiology, life history or behavior have been frequently shown to be adaptive, yielding increased fitness returns under specific conditions (Stearns 1989; West-Eberhard 2003; Whitman & Agrawal 2009). Juvenile development, maturation, and the period of reproduction in many animal species follow recurrent seasonal gradients (Foster & Kritzman 2009; Helm et al. 2013), thus it is crucial to adjust one's own reproductive period to the opposite sex, particularly in semelparous species experiencing only a single reproductive episode. Often thermal threshold values (Caro et al. 2013) or photoperiod changes are used as indicators of large-scale seasonal progression (Hut et al. 2013b). However, fluctuations of external conditions may alter the density and structure of a population (Angelstam et al. 1985; Owen-Smith 1990; Saether 1997), and sex-specific differences in developmental rates or mortality will further add temporal demographic variation. Such local differences are difficult to predict from large-scale cues and plastic adjustment of life history traits based on local information might be advantageous (Kasumovic et al. 2009).

Recent studies have highlighted the role of social cues in adaptive life history shifts, for example, in response to the density of conspecifics (Bailey et al. 2010; Kasumovic et al. 2011). Since accelerated or delayed juvenile development in response to conspecific cues precedes its fitness-relevant effect at the stage of maturity, these

mechanisms have been termed 'socially cued anticipatory plasticity' (SCAP; Kasumovic & Brooks 2011). Socially cued developmental tactics are hypothesized to be more common than currently appreciated (Kasumovic & Brooks 2011), but plasticity can also involve fitness costs (DeWitt et al. 1998; Relyea 2002) that may constrain the evolution of such traits. Moreover, an established cue from the social environment that benefits plastically responding individuals in a single species may not be similarly relevant in related species, as the value of particular cues will strongly relate to specific features of the mating system under study (Kasumovic & Brooks 2011).

Many spiders show extreme reversed sexual size dimorphism (SSD; Foellmer & Fairbairn 2005a; Kuntner & Elgar 2014) and specialized male strategies to maximize and protect paternity, such as self-sacrifice (Andrade 1996; Foellmer & Fairbairn 2003; Welke & Schneider 2010), genital plugging (Fromhage & Schneider 2006; Nessler et al. 2007; Snow et al. 2006) or remote copulation (Li et al. 2012). In such species, males generally benefit most from locating a virgin female (Fromhage et al. 2005; Gaskett et al. 2004; Huber 2005), which may impose selection on males to mature earlier. The transition to the reproductive stage, however, is critical because maturing males lose the ability to capture prey on their own. Since adult males are restricted to feeding opportunistically on the females' prey (Foelix 1979; Foellmer & Fairbairn 2005b), precipitate maturation may also be unfavorable.

Spider males perceive conspecific females using one or more sensory modalities, but most species lack acute vision and have to rely on mechanical or chemical signals that indicate the presence of receptive females (Pollard et al. 1987; Trabalon & Bagneres

2010). Female sex pheromones, both volatile and incorporated in female silk, have been shown to serve this function in orb-web spiders (Chinta et al. 2010; Nessler et al. 2009; Schulte et al. 2010), but only one study on the Australian red-back spider *Latrodectus hasselti* provides evidence that female pheromones induce adaptive developmental plasticity in males (Kasumovic & Andrade 2006). Like most animals (Stearns 2000), males in this species have to trade-off developmental time against growth, as fast-maturing males stay relatively small, but intense contest competition shifts fitness payoffs to larger males (Kasumovic & Andrade 2009).

Specialized male mating strategies that allow maximizing paternity with a single or very few females and constitute a very high mating effort have evolved at least four times independently in different spider families (Schneider 2014; Schneider & Fromhage 2010), providing ideal model systems to investigate whether such characteristics generally promote socially cued anticipatory plasticity. Differences in specific mating traits may affect selection on such mechanisms and a comparative approach may help to relate the magnitude of plastic responses to the associated adaptive value.

The golden-silk spider genus *Nephila* is an established model system in sexual selection research, which has been used to study, for example, female-biased SSD (Kuntner & Elgar 2014), sexually selected life history traits (Higgins et al. 2011; Rittschof et al. 2012), male-male competition (Christenson & Goist 1979; Fromhage & Schneider 2005b; Rittschof 2010; Schneider et al. 2000), and intrasexual size variation (Schneider & Elgar 2005; Vollrath 1998b). Especially male size varies greatly in some species (Elgar et al. 2003b; Neumann & Schneider 2015). Socially cued developmental plasticity may increase male size variation; even more so as the capacity

for plastic modifications may differ between genotypes (West-Eberhard 2003). *Nephila* females build large orb-webs, whereas males cease web-building after reaching maturity to search for females (Vollrath 1980). Population densities change in the course of the season (Higgins 1992; Miyashita 1986; Miyashita 1993), but in addition, local environmental conditions cause strong between-year variation in some species (Higgins 2000). Socially cued plasticity could serve to adjust male development to female availability and male-male competition, but experimental work is required to determine whether predefined cues induce the expected developmental modifications (Kasumovic & Brooks 2011).

We examined the capacity of males to optimize the timing of maturation in response to female silk cues using two sympatric *Nephila* species that are exposed to almost identical abiotic cues of seasonal changes in their natural habitat, *N. fenestrata* and *N. senegalensis*. Both species are generally similar in their reproductive biology, but differ in certain aspects of male mating strategies. *Nephila fenestrata* males follow a terminal investment strategy aimed at monopolizing a single female by means of mate plugging through copulatory organ breakage (Fromhage & Schneider 2006), whereas in *N. senegalensis*, males do not produce mating plugs and each male is able to fertilize up to four females (Schneider & Michalik 2011). Males in this species adopt flexible mating tactics including male mate choice and polygyny, which reduce the imbalance in reproductive success between males that encounter a virgin or a non-virgin female first (Neumann & Schneider 2015). Hence, although males in both species prefer mating with virgins (Fromhage & Schneider 2005a; Neumann & Schneider 2015; Schneider et al. 2011), lifetime fitness in *N. fenestrata* more strongly depends on locating

an unmated female. These differences affect the value of prospective mates and are expected to generate dissimilar selection on socially cued anticipatory plasticity; an assumption in line with a field study on two other orb-web spider species suggesting anticipatory plastic responses to female densities in the monogynous *N. plumipes*, but not in *Argiope keyserlingi* in which males are usually bigynous (Kasumovic et al. 2009).

We reared juvenile *N. fenestrata* and *N. senegalensis* under standardized conditions in climate-control chambers, presenting spiders in the experimental treatment with virgin female silk. We expected the highly specialized, terminally investing *N. fenestrata* males to accelerate their development in the presence of virgin female silk cues. Males were supposed to mature earlier, but at smaller size, than siblings in the control treatment without virgin female cues. In *N. senegalensis*, males are often polygynous and depend less on locating a virgin female, hence we predicted a weaker developmental response in this species.

Material and methods

Study animals

Spiders used in this study were F2 offspring descending from females that were collected at Mawana Game Reserve, Zululand District, KwaZulu-Natal, South Africa, in 2012 (permit OP 990/2012 from EZEMVELO KZN WILDLIFE PERMITS OFFICE). All families of study animals were derived from mating virgin individuals from different maternal lines. Six family lineages were used in each species, comprising 23.7 ± 5.6 individuals per family in *N. senegalensis* and 25.5 ± 5.4 individuals per family in *N. fenestrata*. We reared hatchlings communally at first, and separated them after approximately two additional molts to maintain them in 200 ml plastic cups, which

were turned upside down. Spiders were kept under standardized conditions in our main laboratory (Schneider et al. 2011) before being transferred to the experimental rooms. The experiments took place at the Zoological Institute, University of Hamburg, between May 23 and August 25, 2013.

Experimental setup and treatments

Spiders were kept in two climate-control chambers, measuring approximately 1.9 m x 4.3 m x 2.4 m each, in order to control temperature, relative humidity, light-dark cycle, and light intensity. Climate-control chambers (Weiss Umwelttechnik GmbH, model type WK 21'/5 – 40) featured identical technical specifications. Both devices were contemporaneously installed, calibrated, and put into operation by the manufacturer's expert staff in 2012.

For each of our study species, we established an experimental treatment in which adult virgin females' silk was introduced to the spiders' rearing cups (referred to as the Female cues treatment). Thereby, we presented the study animals with potential contact pheromones or any properties of silk that may indicate the presence of adult females. In a control treatment, spiders were reared isolated from adult virgin female silk cues (referred to as the No cues treatment). To exclude long distance perception of female silk cues in the control treatment, we arranged the Female cues treatments for both of our study species simultaneously in one climate chamber and used the second chamber for both No cues treatments. Each climate chamber was equipped with six bottom shelves and six top shelves. A tubular fluorescent daylight lamp was mounted above each shelf with a distance of 60 cm. We placed up to twenty-seven rearing cups on each shelf with an equal distance of approximately 15 cm

between cups. Prior to the transfer of study animals to the experimental treatments, we adjusted the climate-control chambers to provide identical conditions of temperature, relative humidity, and light regime. Temperature and humidity were regulated corresponding to periods of artificial daytime and night-time throughout the experiment; i.e. temperature was set to 26 °C during lighting periods and 21 °C during dark periods. We set daytime humidity to 50 % and night-time humidity to 70 %, respectively. These conditions fit well within the range in both species' habitats. In the beginning of the experiment, we used a 14:12 h light-dark cycle and reduced the daily lighting duration by 10 min each week to simulate a decrease in day length, which both of our study species experience during summer and autumn in their habitats of origin.

Transfer of study animals to climate-control chambers

Nephila fenestrata study animals were transferred to the climate chambers on May 23; *N. senegalensis* were transferred on May 26/27. We used a split brood design and allocated equal numbers of randomly chosen individuals from each family lineage to each treatment. After the transfer had been completed, we checked all study animals for presence and condition on the following day and replaced a small number of spiders that had died or vanished from the rearing cups. No study animals were replaced at a later date.

Maintenance and monitoring schedule

The regular monitoring of study animals began on May 29 (defined as the start of the experiment) with the following numbers of study animals: *N. fenestrata*: Female cues treatment: $n = 156$; No cues treatment: $n =$

157; *N. senegalensis*: Female cues treatment: $n = 162$; No cues treatment: $n = 162$. Spiders were fed *Drosophila* flies twice a week on a regular schedule. In the initial stage of the experiment when the spiders were still very small, we used flies that had been killed at -80 °C. When all spiders had reached a minimum body length of approximately 5 mm, we supplemented the diet with live insects. This food supply allowed the spiders *ad libitum* feeding. Water was offered on 6 days per week. At this stage, we checked the animals' condition four times a week and recorded any cases of death, as well as spiders that had vanished from their rearing cups (missing spiders likely dropped from rearing cups during feeding or cleaning of shelves).

Introduction of female silk cues

As a consequence of female-biased SSD, *Nephila* females take longer to mature than males, so that early maturing males become adults in populations devoid of adult females (protandry, Miyashita 1993; Vollrath 1980). As our goal in this study was to simulate the beginning of the mating season, we presented males with adult virgin female cues not from the start, but after a period of development in the absence of such cues. In the Female cues treatment, we introduced the first set of silk cues to the rearing cups on days 22/23 from the start of the experiment for *N. fenestrata*, and days 22–24 for *N. senegalensis* (all subsequent sets of silk cues were introduced within one day). We used plastic expansion bolts to present silk samples to the study animals. For this purpose, the expansions of each piece were spread, resulting in a Y-shaped object, which we put up in vertical position using a base of potting clay. These silk fixtures measured 5.5 cm in height. For acquiring silk cues, we used female webs the spiders had built into 40 cm x 40 cm-sized Perspex frames. Webs had

usually been newly built in the previous night, but were at most two days old. Females were removed from their webs and the frames were taken to the female cues chamber. We then twisted a few silk threads from the web's moistened catching spiral around the upper expansions of each silk fixture and used fine scissors to dissect the threads from the web. One silk fixture was placed under each rearing cup, so that the spider inside could easily access the silk threads, especially with its pedipalps and forelegs, bearing the most important sensory organs to perceive physical and chemical cues (Foelix 1970; Foelix & Chu-Wang 1973). Fresh silk cues were introduced on a weekly schedule (on days 29, 36, 43, 50, 57, 64, 71, 78, and 85 from the start of the experiment). On the previous day, we removed all silk fixtures from the rearing cups and cleaned the shelves in the experimental rooms. Each object was cleaned of silk with alcohol and air-dried prior to reuse. In order to standardize experimental conditions, we placed identical objects free of silk under the rearing cups in the No cues treatments. Silk cues were acquired from twenty-four adult virgin female *N. fenestrata* (up to four per turn) and thirty-three *N. senegalensis* (up to six per turn). Females originated from eleven family lineages in *N. fenestrata* and twelve family lineages in *N. senegalensis*. Average female adult age (days passed from the date of maturity) at the time of web production was 13 days (range: 2-30 days) in *N. fenestrata* and 11.5 days (range: 2-29 days) in *N. senegalensis*. How many times a male received fresh silk cues depended on individual developmental durations. Those males in the Female cues treatments that were used in our analysis received fresh cues 5.4 ± 0.1 times in *N. fenestrata* and 6.4 ± 0.1 times in *N. senegalensis* (range in both species: 3-8 times). Individual silk cues were

obtained from a female unrelated to the cues-receiving male (48 % of cues in *N. fenestrata* and 45 % of cues in *N. senegalensis*) or from a female that had one parental lineage in common with the cues-receiving male (52 % of cues in *N. fenestrata* and 53 % of cues in *N. senegalensis*). In < 1 % of cues in *N. fenestrata* and 2 % of cues in *N. senegalensis*, we could not avoid using silk from females that had both parental lineages in common with the cues-receiving male. No male received cues from related females only. With the first implementation of female silk cues, we adjusted the monitoring of study animals and checked the individual state of development on six days per week. For each male, we recorded the duration of development from the start of the experiment to maturity and the duration of the subadult instar (i.e. the last developmental stage; subadult males can easily be detected by the swollen palp tarsi indicating the ongoing transformation into copulatory organs). Juvenile females were immediately removed from the study when they were clearly discernible (body length \geq approximately 12 mm, pedipalps unmodified).

Statistical analysis

We defined the start of the experiment as the first monitoring of study animals after being transferred to the climate-control chambers (May 29). In *N. fenestrata*, some males matured before the first introduction of female silk cues had been completed (June 21). These males were excluded from the analyses (predefined female cues chamber: $n = 5$; no cues chamber: $n = 9$). In each of our study species, we analyzed effects of our experimental treatment (Female cues/No female cues) on male development with separate linear mixed models performed in R 3.2.4 (R Development Core Team 2016).

Dependent variables were (1) Duration of development from the start of the experiment, (2) Duration of subadult stage, (3) Adult size, and (4) Adult weight. The study animals' family lineage was included as a random effect. We tested for statistical significance of Treatment using ANOVA model comparisons with χ^2 tests between the full model and a model that had the variable removed. Using the same dependent variables, we conducted generalized linear models in JMP IN 7.0 (SAS Institute Inc., Carey, NC, USA) to test for an interaction between Treatment and Family lineage. Models were fitted with normal error structure and identity-link function. We removed the interaction term if it was non-significant ($\alpha = 0.05$) while retaining both main effects in the final models. Developmental durations were log-transformed to improve model fit. Descriptive statistics are given as mean \pm standard error. Within experiments, sample sizes may differ due to missing data.

Results

We performed linear mixed models to test effects of our experimental treatment on male development and growth. The models clearly revealed a significant influence of our treatment on the duration of development in *N. senegalensis*. Males in the Female cues treatment matured two to five days earlier, on average, than males in the No cues

treatment, and the mean duration of the subadult stage alone differed by one and a half to two days (ANOVA model comparisons: Duration of development from the start of the experiment: $\chi^2 = 10.563$, $p = 0.001$; Duration of subadult stage: $\chi^2 = 29.724$, $p < 0.001$; Table 1). However, shortened development did not translate into different male size or body mass (ANOVA model comparisons: Adult size: $\chi^2 = 1.134$, $p = 0.287$; Adult weight: $\chi^2 = 2.586$, $p = 0.108$; Table 1). Contrary to our predictions, in *N. fenestrata*, there were no significant differences in various life history parameters between males presented with virgin female silk cues and those reared in the absence of such cues (ANOVA model comparisons: Duration of development from the start of the experiment: $\chi^2 = 0.006$, $p = 0.939$; Duration of subadult stage: $\chi^2 = 1.632$, $p = 0.202$, Adult size: $\chi^2 = 0.528$, $p = 0.467$; Adult weight: $\chi^2 = 1.629$, $p = 0.202$; Table 1).

We ran additional generalized linear models to analyze potential family-specific variation of developmental plasticity. In *N. senegalensis*, the response toward a shortened development was present in all family lineages (Fig. 1). The interaction between Family lineage and Treatment, however, was always found to be non-significant at the 5 % level; although developmental responses varied considerably between families (Fig. 1, Table 2). Corroborating mixed model results, the GLMs showed that in *N. fenestrata*, only Family

Table 1. Developmental parameters of male *Nephila fenestrata* and *N. senegalensis* reared in different experimental treatments.

	<i>Nephila fenestrata</i>			<i>Nephila senegalensis</i>		
	No female cues	Female cues	<i>n</i>	No female cues	Female cues	<i>n</i>
Duration of development (start to maturity) [d]	57.36 \pm 0.78	57.18 \pm 0.86	153	68.83 \pm 0.86	65.36 \pm 0.63	142
Duration of subadult stage [d]	18.51 \pm 0.18	18.26 \pm 0.15	153	21.12 \pm 0.25	19.38 \pm 0.21	142
Adult size/patella-tibia [mm]	5.69 \pm 0.09	5.65 \pm 0.09	150	4.74 \pm 0.09	4.62 \pm 0.1	132
Adult weight [mg]	18 \pm 0.58	18.22 \pm 0.56	153	22.46 \pm 0.66	21.06 \pm 0.7	141

lineage predicted developmental durations, size, and weight, while Treatment had no effect (Table 2). In contrast, both Family

lineage and Treatment significantly determined developmental durations in *N. senegalensis* (Table 2).

Table 2. Effects of family lineage and treatment on developmental parameters in *Nephila fenestrata* and *N. senegalensis*.

<i>Nephila fenestrata</i>												
Explanatory variable	Duration of development (start to maturity)			Duration of subadult stage			Adult size			Adult weight		
	χ^2	<i>p</i>	<i>df</i>	χ^2	<i>p</i>	<i>df</i>	χ^2	<i>p</i>	<i>df</i>	χ^2	<i>p</i>	<i>df</i>
Family lineage	50.65	<.0001	5	14.6	0.012	5	67.07	<.0001	5	61.86	<.0001	5
Treatment	0.002	0.961	1	2.08	0.15	1	0.73	0.391	1	2	0.158	1
Family lineage *Treatment	2.101	0.835	5	2.332	0.807	5	1.588	0.903	5	1.704	0.888	5
<i>Nephila senegalensis</i>												
	χ^2	<i>p</i>	<i>df</i>	χ^2	<i>p</i>	<i>df</i>	χ^2	<i>p</i>	<i>df</i>	χ^2	<i>p</i>	<i>df</i>
Family lineage	21.89	0.0006	5	22.23	0.0005	5	37.56	<.0001	5	40.77	<.0001	5
Treatment	11.34	0.0008	1	31.01	<.0001	1	1.16	0.281	1	2.621	0.105	1
Family lineage *Treatment	10.126	0.072	5	7.924	0.161	5	10.58	0.06	5	9.757	0.082	5

Likelihood-ratio tests and corresponding *p*-values derive from generalized linear models performed in JMP IN 7.0 (SAS Institute Inc., Carey, NC, USA). Non-significant interaction terms were removed from the final models. Developmental durations were log-transformed. Significant *p*-values are shown in bold.

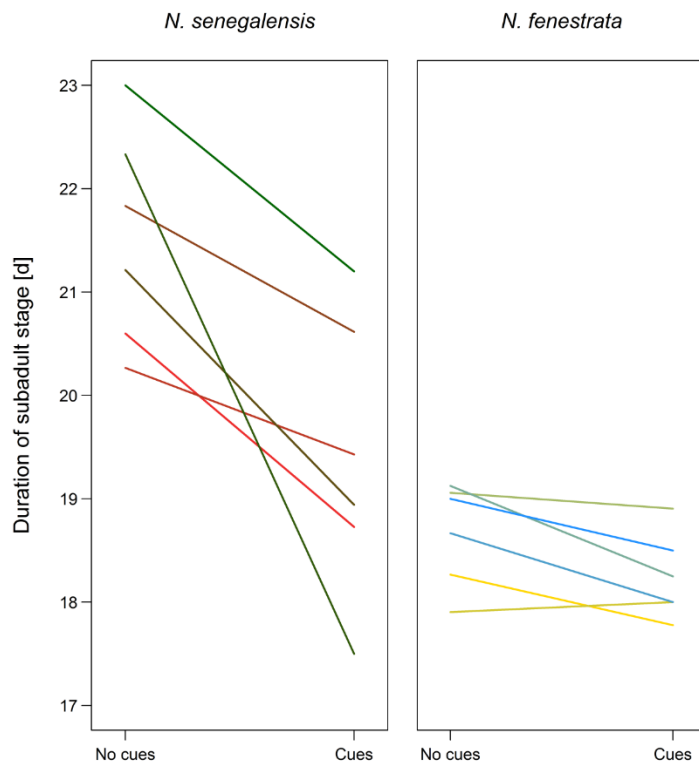


Figure 1. Duration of the subadult instar (i.e. the last developmental stage preceding maturity) in the presence or absence of virgin female silk cues compared between male *Nephila senegalensis* and *N. fenestrata*. Graphs illustrate means of developmental responses according to family lineages.

Discussion

Males in one of our study species, *Nephila senegalensis*, plastically adjusted development and matured significantly earlier in response to female silk cues than those reared isolated from such cues. However, we found no developmental response in *N. fenestrata*. While the plastic adjustment of maturation in *N. senegalensis* is in accordance with our predictions, we expected an even more distinct modification of development in the monogynous *N. fenestrata* males whose fitness strongly depends on locating a virgin female (Fromhage & Schneider 2006). The absence of a plastic response in this species indicates that socially cued anticipatory plasticity is not a universal feature in species with strong developmental differences between the sexes and a high male mating effort.

The plastic adjustment of maturation in *N. senegalensis* is best described as flexibility in the duration of the subadult instar and did not affect male adult size or mass. Males in this species are able to fertilize multiple females (Schneider et al. 2011; Schneider & Michalik 2011) and differential mating investment has been identified as an integral part of a flexible mating strategy in this species (Neumann & Schneider 2015). In nature, individual males visit up to four females (Neumann & Schneider, unpublished observations); hence adjusted maturation in response to the perception of female cues may increase a male's chance to locate a virgin female first, and to mate with further females in a period of low or moderate competitive conditions.

Animals in general have to trade-off developmental duration against growth (Stearns 2000). Increased food intake and

delayed maturation will usually result in larger adult body size, which often is an important determinant of male reproductive success in mating systems involving contest competition (Lindenfors et al. 2007). This relationship also exists in some web-building spiders (Kasumovic & Andrade 2009) and we expected males perceiving the presence of virgin females to mature at smaller size as a consequence of accelerated development. In contrast to a previous study on Australian red-back spiders (Kasumovic & Andrade 2006), however, adjustment of maturation in *N. senegalensis* was not achieved by substantially abbreviating development. Rather, the timing of maturation was modified by differences in the duration of the subadult instar and there was no trade-off between adjustment of development and adult body size. Hence, we found no support for socially cued plasticity to contribute to the extreme male size variation observed in many *Nephila* species (Higgins et al. 2011; Neumann & Schneider 2015; Rittschof et al. 2012; Schneider et al. 2000). While males adjusted the timing of maturation in the same direction across family lineages, we also observed considerable variation between lineages regarding the magnitude of plastic responses. Genotype-specific degrees of plasticity in response to an environmental trigger could contribute to phenotypic variation, but our study found little evidence for such interrelations in our model species.

It is important to realize the bidirectional mode of a plastic response; hence not only the expression of a specific modification appropriate to requirements should be beneficial, but also the non-expression of the same modification in the absence of the corresponding trigger. What is to be gained from staying subadult for a male *N. senegalensis* in the absence of adult females?

With sexual maturation, web-spider males undergo drastic changes in terms of morphology, physiology, and life-style, solely targeted on reproduction (Foelix 1979). Adult male spiders lose weight during mate search (Ceballos et al. 2015), but are no longer able to build capture webs (Christenson & Goist 1979; Vollrath 1980). In order to maintain a sound physical condition, they depend on stealing prey from female webs (Higgins et al. 2011; Higgins & Goodnight 2011; Vollrath 1980). Males maturing without the perspective of locating a female in a short time risk declining physical strength, whereas subadult males residing in their own webs stay relatively safe from predation and may continue feeding on self-captured prey.

Another potential benefit of a delayed maturation may relate to sperm-limitation, which is a universal trait in nephilid spider males (Michalik & Rittschof 2011a). Male *N. senegalensis* produce their lifetime sperm supply in their subadult instar and spermatogenesis is terminated prior to adulthood (Schneider & Michalik 2011). Total sperm numbers vary considerably among males (Ceballos et al. 2015) and a prolonged subadult instar may allow males to increase sperm quantity to prevail in sperm-competition. Taken together, these arguments support the assumption that *N. senegalensis* males significantly benefit from shifting maturation until mating is about to take place, and not to mature when the probability of finding a female is low.

However, most of these arguments apply to *N. fenestrata* as well, and the absence of a plastic response to virgin female cues in this species is puzzling. Owing to mate plugging and copulatory organ breakage, male mating tactics are less flexible than in *N. senegalensis*, and male reproductive success critically depends on the ability of monopolizing a single female (Fromhage et

al. 2005; Fromhage & Schneider 2006). To explain our findings, we might consider between-species differences regarding the value of developmental responses from both male and female perspective. Such differences might be linked to our study species' ecology, as habitat requirements differ slightly between both species, which could affect the predictability of receptive females. Habitats in *N. senegalensis* range from humid areas to bush savannahs and habitat heterogeneity is reflected in varying population densities (Neumann & Schneider, unpublished observations). Predicting female presence is therefore challenging and selection may favor male ability to fine-tune maturation on a local scale. In contrast, *N. fenestrata* occurs in forested areas (Robinson & Robinson 1980; sub *N. pilipes*) providing relatively constant temperature and humidity, and females typically form dense aggregations in preferred sites (Penney, unpublished observations). Given the rather narrow range of tolerated conditions, female presence may directly be indicated by abiotic large-scale cues and habitat quality, making socially cued anticipatory plasticity less needed in *N. fenestrata*.

Finally, the presence and absence of socially cued plastic responses in the respective species could be explained from the female perspective. We cannot unambiguously relate the developmental response in male *N. senegalensis* to silk-borne pheromones, as the physical properties of adult females' silk alone could indicate their presence, but females in various web-building spiders use specific chemical signals to attract males and secure a timely mating (Gaskett 2007; Schulz & Toft 1993; Uhl & Elias 2011). *Nephila senegalensis* females are polyandrous (Schneider & Michalik 2011) and may use pheromone signals to repeatedly attract males. In *N. fenestrata*, however, there may be little need for females to

advertise their presence, as males may easily locate them in their spatially limited habitats; and also because female mating rates are much lower compared to *N. senegalensis*. Pheromone production itself may be costly (Byers 2005; Delisle & Vincent 2002; Harari et al. 2011) and attracting unwanted males could even decrease female fitness, if there are no significant benefits to be gained from multiple matings (Arnqvist 1998; Boulton & Shuker 2015; Umbers et al. 2015). Additional research should investigate whether female *N. fenestrata* produce sex pheromones strategically; e.g. only under a high risk of remaining unmated (Cory & Schneider 2016).

Conclusions

Our results suggest that a strong benefit of mating with virgins due to first male sperm priority does not necessarily promote socially cued anticipatory plasticity. Benefits and costs of using and providing information may differ between the sexes. Even if males, in principle, would benefit from plastic life history shifts, they may sensorially rely on information provided by females. In such cases, the evolution of plasticity may depend on whether females benefit from providing cues, and future studies should take the female perspective into account. In addition, we suggest that the adaptive value of socially cued anticipatory plasticity might not be limited to males that adaptively accelerate development to mature in time, but males that delay maturation in the absence of female cues might also benefit by avoiding potential costs of precipitate maturation.

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Chapter 3

Fitness implications of sex-specific catch-up growth in *Nephila senegalensis*, a spider with extreme reversed SSD

Rainer Neumann, Nicole Ruppel and Jutta M. Schneider

Abstract

Animal growth is often constrained by unfavourable conditions and divergences from optimal body size can be detrimental to an individual's fitness, particularly in species with determinate growth and a narrow time-frame for lifetime reproduction. Growth restriction in early juvenile stages can later be compensated by means of plastic developmental responses, such as adaptive catch-up growth (the compensation of growth deficits through delayed development). Although sex differences regarding the mode and degree of growth compensation have been coherently predicted from sex-specific fitness payoffs, inconsistent results imply a need for further research. We used the African *Nephila senegalensis*, representing an extreme case of female-biased SSD, to study fitness implications of sex-specific growth compensation. We predicted effective catch-up growth in early food-restricted females to result in full compensation of growth deficits and lifetime fecundity (LTF) equivalent to unrestricted females. Based on a stronger trade-off between size-related benefits and costs of a delayed maturation, we expected less effective catch-up growth in males. We tracked the development of over one thousand spiders in different feeding treatments, e.g. comprising a fixed period of early low feeding conditions followed by unrestricted feeding conditions, permanent unrestricted feeding conditions, or permanent low feeding conditions as a control. In a second experimental section, we assessed female fitness by measuring LTF in a subset of females. In addition, we tested whether compensatory development affected the reproductive lifespan in both sexes and analysed genotype-by-treatment interactions as a potential cause of variation in life history traits. Both sexes delayed maturation to counteract early growth restriction, but only females achieved full compensation of adult body size. Female catch-up growth resulted in equivalent LTF compared to unrestricted females. We found significant interactions between experimental treatments and sex as well as between treatments and family lineage, suggesting that family-specific responses contribute to the unusually large variation of life history traits in *Nephila* spiders. The different feeding treatments had no effect on the reproductive lifespan in either sex. Our findings are in line with predictions of life history theory and corroborate strong fecundity selection to result in full female growth compensation. Males showed incomplete growth compensation despite a delayed development, indicating relaxed selection on large size and a stronger trade-off between late maturation and size-related benefits. We suggest that moderate catch-up growth in males is still adaptive as a 'bet-hedging' strategy to disperse unavoidable costs between life history traits affected by early growth restriction (the duration of development and adult size).

Introduction

Body size and the duration of development are among the most fitness-relevant life history traits and have been extensively studied in various animal taxa (reviewed in Blanckenhorn 2005; Nylin & Gotthard 1998; Roff 2002). Generally constrained by a trade-off between a favourable size at the onset of reproduction and the time necessary to reach it (Blanckenhorn 2000; Roff 1992), animal growth depends on both inherited growth trajectories and plastic modifications of them (Chase 1999; Dmitriew 2011). Extrinsic factors that may influence the mode of development include parasite infestations and other pathogens (Paez et al. 2015; Vergauwen et al. 2011), cues of present or future environmental conditions (Kasumovic & Brooks 2011), ambient temperature (Kingsolver et al. 2004), and food supply (Stearns 1992; Wilson & Osbourn 1960).

Growth strategies and optimal body size may differ markedly between the sexes, which is particularly evident in sexually size-dimorphic species (Blanckenhorn 2005). As large males tend to succeed in male-male competition across animal taxa, body size in males is often subject to sexual selection, resulting in male-biased sexual size dimorphism (SSD) (Kingsolver & Pfennig 2004). Still enigmatic conditions are found in species with female-biased SSD, which mainly occurs in oviparous animals, like fishes (Barreto et al. 2003), amphibians (Hector et al. 2012; Nali et al. 2014), and many invertebrates (Honek 1993; Smith & Brockmann 2014) including spiders (Cheng & Kuntner 2015; Foellmer & Moya-Laraño 2007; Higgins et al. 2011; Schneider & Andrade 2011). As females are strongly selected to produce large numbers of eggs, increased female size through fecundity selection is generally well supported in these species

(Blanckenhorn 2005; Nylin & Gotthard 1998). Males, however, apparently are selected to stay small, which has been related to improved mobility and agility during mate search (Moya-Laraño et al. 2002; Moya-Laraño et al. 2009), a decreased risk of predation and female sexual cannibalism (Foellmer & Fairbairn 2004), benefits of protandry through rapid maturation (Blanckenhorn et al. 2007), and reduced energy expenditures (Blanckenhorn 2000; Blanckenhorn 2005).

Optimization of development and growth may be difficult in non-constant environments. Basic strategies that permit fitness maximization under invariant conditions may need to be refined in response to environmental changes (Foster & Kritzman 2009). While alterations of environmental conditions often appear as recurring sequences, irregular fluctuations of environmental parameters are also common in a range of habitats. Such unpredictable conditions pose a threat to an individual's fitness, particularly in species in which reproductive success depends on a single reproductive period (Abrams et al. 1996). For example, unfavourable temperatures or food restriction may result in a delay of development, which can increase juvenile predation risk, but also lower reproductive prospects in individuals that reach maturity too late (end-of-season penalty; e.g. De Block et al. 2008; Higgins 2000).

Phenotypic plasticity (the capacity of a genotype to express different phenotypes in different environments; West-Eberhard 2003) provides the potential to counteract a period of unfavourable growth conditions in juvenile stages by means of adaptive developmental responses, e.g. compensatory growth and catch-up growth (Dmitriew 2011; Krause & Caspers 2016; Metcalfe & Monaghan 2001; Walzer et al. 2015).

Compensatory growth refers to elevated growth under improved conditions, whereby a delay of sexual maturation is minimized. In contrast, adaptive catch-up growth is defined as a strategy to reach a favourable adult size at the expense of delaying maturation (Hector & Nakagawa 2012; Livingston et al. 2014). Compensatory developmental mechanisms enable animals to either limit or entirely prevent fitness costs that would follow from a period of adverse growth conditions without the capacity for such flexible responses. However, early food restriction and subsequent growth compensation may entail intrinsic costs that can even lead to a reduction in lifespan (English & Uller 2016; Hornick et al. 2000; Reichert et al. 2015).

As benefits and costs of developmental compensation may differ considerably between males and females, integrating pre-estimated divergent selection on body size may substantiate experimental work on such strategies. Plastic modifications of life history traits have been related to experimental feeding regimes in a range of studies (Bauerfeind & Fischer 2005; Bonneaud et al. 2016; Dahl et al. 2012; Davidowitz et al. 2003; Fernandez-Montraveta & Moya-Larano 2007; Kleinteich & Schneider 2011; Krause & Caspers 2016), but relatively few of these have addressed sex-specific differences regarding compensatory development (Arnold et al. 2007; Chin et al. 2013; Tawes & Kelly 2017).

In general, predictions concerning such differences are based on (1) proposed sex-specific net benefits of growth compensation (i.e. the sex whose fitness depends stronger on large body size is expected to show a higher degree of growth compensation), (2) on the possibility to increase size after sexual maturation (i.e. determinate versus indeterminate growth; with determinate growth generating stronger selection

pressure to compensate growth deficits), and (3) on potential long-term costs of compensatory development (Livingston et al. 2014). Previous studies, however, are inconsistent as to whether predictions were met or not (Barreto et al. 2003; Livingston et al. 2014; Stillwell & Davidowitz 2010; Tawes & Kelly 2017), thus indicating that possible trade-offs between growth compensation, taxon-dependent life history, and environmental conditions that determine the adaptive value of compensation require further research.

Species showing strong SSD are particularly suitable model systems to investigate sex-specific compensatory mechanisms, because especially pronounced sex-differences concerning size selection can be comparatively studied in a single species. Golden-silk spiders (genus *Nephila*, family Araneidae) show some of the most extreme cases of female-biased SSD (Kuntner et al. 2013). Male and female size in these spiders has been suggested to have evolved independently, with steady fecundity-driven selection on increased female size, whereas phylogenetic analyses did not reveal a consistent evolutionary trend towards male size-reduction (Higgins et al. 2011; Kuntner & Elgar 2014). In addition, several studies have reported large male advantages in the context of mating (Christenson & Goist 1979; Elgar et al. 2003a; Elgar & Fahey 1996; Rittschof 2010). Causes of small male size remain thus ambiguous, which also applies to the remarkable within-sex size variation in many species (Elgar & Fahey 1996; Higgins et al. 2011; Schneider & Elgar 2005). *Nephila* spiders are short-lived animals with determinate growth (Fromhage et al. 2007; Miyashita 2005; Rittschof 2011) and individuals of both sexes mature and reproduce within a limited time frame and within their own cohort (Higgins 2000; Higgins et al. 2011).

Natural populations exposed to different environmental conditions have been studied in the American *N. clavipes* (Higgins 1993; Higgins 1992). This species is bivoltine in some populations, where first-generation females mature at larger average size than second-generation females. However, first-generation females pass through early development in the dry season, experiencing low feeding success and hence unfavourable juvenile growth conditions. These females may have adaptively delayed maturation, later taking advantage of improving feeding conditions to eventually mature at large size (Higgins 1992). While these observations hint towards catch-up growth in females, experimental work is needed to test predictions following from this mechanism; specifically in comparison with permanently food-restricted and permanently well-fed individuals. Furthermore, the inclusion of males is essential to access sex-specific differences in an integral procedure to understand selection in this system.

Here, we consider the above-mentioned observations in the light of current research focussing on plastic compensatory mechanisms in sexually size-dimorphic species (Chin et al. 2013; Kahn et al. 2012; Livingston et al. 2014). Using the African *N. senegalensis*, we implemented a comprehensive approach consisting of two successive experimental sections. In the first section, we manipulated the study animals' feeding conditions, rearing split broods under constant low or high food supply, or in treatments in which the food supply was reciprocally reversed at a fixed point in time. Based on strong fecundity-selection for large female size (Higgins & Goodnight 2011; Kuntner et al. 2012b), we predicted effective catch-up growth after treatment reversal in initially food-restricted females to compensate the preceding deficits. As

Nephila males generally benefit from protandry (Danielson-Francois et al. 2012; Kasumovic et al. 2009), selection should act against an exceedingly delayed development in males. Furthermore, flexible mating strategies have been found to balance reproductive success between differently-sized competitors (Neumann & Schneider 2015). Therefore, we assumed weaker selection on large male size and predicted less effective catch-up growth in males.

Following the rearing treatments, we used a subset of adult females to measure lifetime fecundity (LTF), thereby providing a direct test concerning the adaptive significance of growth compensation, which is often omitted in empirical studies (Dmitriew 2011; Hector & Nakagawa 2012). We predicted growth compensation to result in equivalent numbers of offspring in initially food-restricted females compared to constantly well-fed females.

As another measure of fitness, we tested whether growth compensation affected the post-maturation lifespan and hence the potential time-frame of reproduction in both sexes. Finally, we report treatment-related mortality and analyse genotype-by-treatment interactions as a potential cause of the remarkable variation of life history traits in our model system.

Material and methods

Developmental duration, body size and weight, and pre-maturation mortality

We collected eight gravid females near Cradock, Eastern Cape, South Africa, in March, 2008. Field-collected females were transferred to the laboratory and housed individually in 60 x 60 cm-sized Perspex frames. We maintained females under standardized conditions and all of them built

viable egg sacs that were incubated in air-vented plastic containers until the offspring hatched (see Schneider et al. 2011 for our standard methods concerning housing, feeding, and watering of spiders as well as temperature conditions). The hatchlings were separated at very small body size (2-4 mm) before they had reached the third instar (the first two moults in *Nephila* spiders occur inside the egg sac). Separated spiders were housed in small plastic cups but were transferred to larger cups as they increased in body size. We haphazardly allocated the study animals to the following feeding treatments: (1) High-High, (2) High-Low, (3) Low-High, and (4), Low-Low. Equal numbers of spiders from individual maternal lineages were used in each treatment. Spiders in the High-High treatment were provided with *ad libitum* food over the entire duration of development to maturity, whereas spiders in treatment Low-Low were kept at low-food conditions throughout the experiment. Study animals in treatment High-Low received *ad libitum* food during a fixed period of four weeks (defined as early experimental conditions) but were kept under low-food conditions in the period following the first four weeks (defined as late experimental conditions). The inversed pattern was adopted in treatment Low-High. The spiders were fed *Drosophila* and *Calliphora* flies. Low-food conditions conform to 4 *Drosophila* flies per week during early experimental conditions and 6-10 *Drosophila* flies (depending on the spider's size) per week during late experimental conditions, respectively. Low-food spiders large enough to eat *Calliphora* received 2 flies per week. *Drosophila* flies were raised on Carolina Biological Supply instant *Drosophila* medium Formula 4-24, which was enriched with additional nutrients, especially protein and vitamins. For this purpose, we mixed the medium with commercial high quality dog

food according to a study by Mayntz & Toft (2001), which demonstrated positive effects on growth and survival in a wolf spider fed with flies cultivated on this specific mixture. *Calliphora* flies were obtained by incubating fully grown larvae purchased from a commercial supplier. All study animals were reared in a daylight lab and hence were exposed to slight photoperiod changes. We checked the spiders on five days per week and tracked the development of each individual by recording the following data: sex, duration of development from the start of the experiment to maturation, weight at completion of the early experimental period, adult weight, and adult body size (given as patella-tibia length).

As morphology-based sex-determination is impossible in small juvenile spiders, individuals could be sexed only at larger developmental stages in the late experimental period, but 85 spiders died unsexed. Immature males were identified by their swollen pedipalps indicating the ongoing transformation into copulatory organs; the lack of this trait in juveniles with a body length \geq approximately 12 mm indicated female sex.

Post-maturation longevity

In addition to developmental modifications, we analysed treatment-related effects on adult longevity. For this purpose, we chose 137 males and 251 females across treatments upon reaching maturity. The spiders were maintained on our regular laboratory feeding schedule irrespective of the developmental feeding treatment experienced before. Spiders were chosen randomly; paying attention, however, to exclude animals to be used in the mating experiments (see 'Lifetime fecundity and hatching success') or in our general breeding schedule. The remaining study animals were killed by hypothermia after reaching maturity and preserved at -80

°C. In total, 1280 spiders were used in this study, of which 30 disappeared and another three were accidentally killed during daily routine at early juvenile stages.

Lifetime fecundity and hatching success

We randomly chose 38 adult females originating from the treatments High-High ($N = 14$), Low-High ($N = 11$), and Low-Low ($N = 13$) to investigate whether compensatory growth enables females to overcome a period of poor feeding conditions during juvenile development and achieve a reproductive outcome equivalent to constantly well-fed females. We did not include High-Low females in this experiment (females in treatments High-Low and Low-Low did not differ significantly in developmental duration and size; see results; Table 2). Females were maintained on our regular feeding schedule. We randomly chose adult males from the High-High feeding treatment to arrange mating trials. Prior to mating, each female was transferred to a Perspex frame (measuring $60 \times 60 \times 12$ cm) and given at least one day to build an orb-web, which is necessary for courtship and mating to take place. We positioned an unrelated male on the upper frame threads of the web. Each virgin couple was allowed to copulate once in a predefined period of 3 hours. If copulation did not occur within the given time, we excluded the male from the study and arranged a second mating trial with a different male at a later date (each male was used only once). Six females remained unmated after the second trial and were excluded from the study.

At the beginning of each trial, the female received one *Calliphora* fly (males prefer mating with feeding females; Schneider et al. 2011). We measured the duration of copulation and removed the spiders from the web afterwards. Females were maintained in

Perspex frames to build egg sacs until they died of age. Four females did not build egg sacs at all, despite apparently normal copulations. The egg sacs produced were incubated in air-vented plastic containers and preserved in alcohol after approximately five weeks. We carefully opened each egg sac and assessed the number of normally developed spiderlings, undeveloped eggs, and total clutch size. All experiments were carried out at the Zoological Institute, University of Hamburg.

Statistical analysis

The study animals originated from eight maternal lineages from which we allocated equal numbers of individuals to each rearing-treatment. As a premise for further analysis, we tested if family lineages were evenly distributed among individuals that had passed through the feeding treatments and finally matured. The test confirmed no significant differences in the numbers of individuals originating from different family lineages between our feeding treatments (G-tests: males: $\chi^2 = 15.16$, $P = 0.82$; $N = 362$; females: $\chi^2 = 19.24$, $P = 0.57$; $N = 559$). We tested predictions with respect to adult body size, body mass, developmental durations, lifetime fecundity (LTF), and post-maturation longevity using t-tests for normal data with equal variances and non-parametric Wilcoxon or Kruskal-Wallis tests if data diverged from these assumptions (indicated by Shapiro- and Bartlett's tests). Results are given as means \pm SE, providing medians and interquartile ranges (IQRs) for non-parametric tests. Complementary post-hoc analyses were performed using pairwise Steel-Dwass tests that correct for Type I error inflation in multiple comparisons. We performed three linear mixed models to analyse sex-specific and family-related effects of our feeding treatments on development and growth. We

tested effects of the variables Early treatment (High or Low), Family lineage, and Sex on body mass after completion of the early experimental period with a model containing all three variables and the interactions between Early Treatment and Family lineage as well as between Early treatment and Sex. Models on total developmental duration (beginning with the day on which hatchlings were separated and allocated to individual treatments) and adult body size were equally specified with Treatment (early and late conditions), Family, and Sex, as well as the respective interaction terms. The variable Start date (the day of allocation to experimental treatments) was entered into the models as a random effect to account for a potential influence of photoperiod on development (Start date had nine levels ranging from June 11 to July 25, 2008). Body mass and developmental duration were log-transformed to improve the fit of the models. We analysed variation in LTF with a standard least-square model containing the interaction between Treatment and the number of clutches produced. All analyses in this study were conducted in JMP Pro 13 (SAS Institute Inc., Carey, NC, USA). Effect tests for individual variables in JMP are based on ANOVA-model comparisons between the full model and a reduced model lacking the respective variable. Additional statistical tests are denoted in the results section. Sample sizes within experiments may differ due to missing data.

Results

Implications of early experimental conditions

Mortality rates during the first four weeks of the experiment did not differ significantly between early treatments; 59 of 626 spiders died under high feeding conditions (9.4 %)

and 74 of 630 spiders died under low feeding conditions (11.8 %) (G-test: $\chi^2 = 1.79$, $P = 0.18$; $N = 1256$). (Sex-determination is impossible in small juvenile spiders; hence mortality rates were analysed independent of sex.)

Table 1. Effects of interactions between the variables Early treatment, Family lineage, and Sex on body mass at completion of the early experiment.

Explanatory variable	Body mass at completion of the early experiment	
	<i>F</i>	<i>P</i>
Early treatment	1822.1	<0.0001
Family lineage	6.5	<0.0001
Sex	427.6	<0.0001
Early treatment * Family lineage	8.6	<0.0001
Early treatment * Sex	45.5	<0.0001

Results derive from a linear mixed model including the variable Start date (the time of allocating hatchlings to individual treatments) as a random effect to account for a potential influence of photoperiod on development. Body mass was log-transformed. Significant *P*-values are shown in bold.

In both sexes, body mass after the first four weeks differed considerably between early low and high feeding conditions. Males weighed 2.6 ± 0.11 mg at the end of the early low food period (median = 2.35 mg, IQR = 1.65); significantly less than those reared under early high feeding conditions, weighing 9.56 ± 0.43 mg (median = 8.26 mg, IQR = 6.46) (Wilcoxon test: $Z = 13.96$, $P < 0.0001$; $N = 363$). In females, early low feeding conditions resulted in a body mass of 4.08 ± 0.12 mg (median = 3.84 mg, IQR = 2.15), whereas early high-feeding females weighed 23.95 ± 0.68 mg (median = 22.25 mg, IQR = 17) (Wilcoxon test: $Z = 21.26$, $P < 0.0001$; $N = 665$). We conducted a linear mixed model to test for sex-specificity of juvenile growth as well as potential family-relatedness of growth responses. The model revealed highly

significant effects of the interactions between Early feeding treatment and Family lineage as well as between Early treatment and Sex on the spiders' body mass at completion of the early experiment (Table 1).

Implications of full experimental conditions

Pre-maturation mortality

We analysed pre-maturation mortality in spiders that survived to experience both early and late experimental conditions and found distinctly different mortality rates (G-test: $\chi^2 = 29.1$, $P < 0.0001$; $N = 1114$; Table 2).

As mortality rates during the first four weeks did not differ between early high and low feeding conditions (see above), mortality differences concerning full experimental conditions may have solely been caused by late conditions. Pairwise between-treatment comparisons corroborated this assumption; irrespective of early feeding conditions, pre-

maturation mortality rates differed significantly in treatment pairs exhibiting different late feeding conditions, but not in treatment pairs in which late feeding conditions were identical (Table 2).

Adaptive catch-up growth

In accordance with our predictions, late developmental durations in Low-High females significantly exceeded those of females in the High-High treatment (Late female developmental duration L-H: mean = 57.39 ± 1.04 days, median = 54 days, IQR = 8); H-H: mean = 46.72 ± 0.59 days, median = 46 days, IQR = 8.75; Wilcoxon test: $Z = 9.81$, $P < 0.0001$; $N = 292$). This delay of development resulted in full compensation of adverse early feeding conditions (Fig. 1). Females matured at similar size and weight in both treatments (Female adult size L-H: mean = 12.24 ± 0.13 mm, median = 12.22 mm, IQR = 1.9; H-H: mean = 12.45 ± 0.59 mm, median = 12.32 mm,

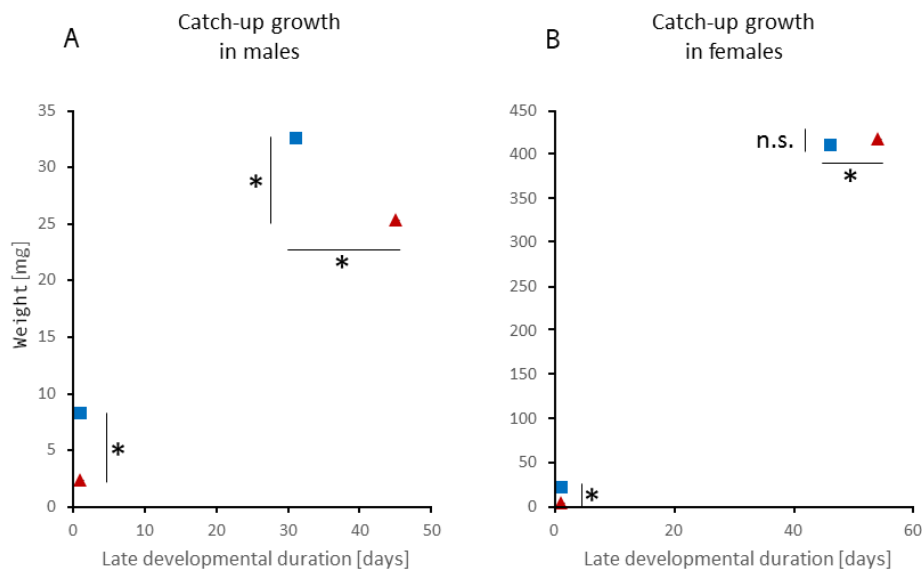


Figure 1. Adaptive catch-up growth in (A) male and (B) female *Nephila senegalensis*. Symbols indicate median values for body mass at the beginning of the late-experiment development and body mass at sexual maturation in treatments High-High (blue squares) and Low-High (red triangles). Late-experiment development in the High-High treatment followed four weeks of early high feeding conditions. Late-experiment development in the Low-High treatment followed four weeks of early low feeding conditions. Body mass is given as a proxy of body size (*, indicates significant differences).

IQR = 1.39; Wilcoxon test: $Z = 0.81$, $P = 0.416$; $N = 283$; Female adult weight L-H: mean = 433.84 ± 14.07 mg, median = 416.7 mg, IQR = 174.73; H-H: mean = 441.7 ± 12.6 mg, median = 410.83 mg, IQR = 159.67; Wilcoxon test: $Z = 0.22$, $P = 0.826$; $N = 282$).

Males in the Low-High treatment also delayed development compared to High-High males (Late male developmental duration L-H: mean = 45.46 ± 1.16 days, median = 45 days, IQR = 15; H-H: mean = 33.11 ± 1.05 days, median = 31 days, IQR = 11; Wilcoxon test: $Z = 7.56$, $P < 0.0001$; $N = 190$). In contrast to females, however, prolonged development did not fully compensate differences in male adult size and body mass (Male adult size L-H: mean = 4.95 ± 0.11 mm; H-H: mean = 5.62 ± 0.09 mm; t-test: $t = 4.86$, $P < 0.0001$; $N = 184$; Male adult weight L-H: mean = 26.59 ± 1.09 mg, median = 25.25 mg, IQR = 13.81; H-H: mean = 34.23 ± 1.03 mg, median = 32.57 mg, IQR = 14.05; Wilcoxon test: $Z = 5.06$, $P < 0.0001$; $N = 191$) (Fig. 1).

Developmental durations and adult size in the remaining treatments

Complementary post-hoc analyses of the remaining treatment-pairs confirmed the overall limiting effects of low experimental nutrition on development and growth (Table 3). Females in the Low-Low and High-Low treatments took much longer to mature and were still very much smaller than females in the two other treatments (Fig. 2, Table 3).

Males responded differently from females, as they took longer to reach adulthood and matured at smaller size in the Low-Low treatment compared to High-Low males (Fig. 2, Table 3). Further different from females, males showed intermediate developmental durations and adult size in the High-Low and Low-High treatments relative to Low-Low and High-High males (Fig. 2), indicating less adverse effects of late low feeding conditions in males. In both sexes, High-High conditions resulted in the shortest developmental durations as well as the largest adult size (Fig. 2).

Effects of Treatment, Sex, and Family lineage on developmental duration and adult size

We used linear mixed models to test whether phenotypic variation regarding developmental duration and adult body size can be attributed, in part, to family-specific plastic responses and to ascertain overall sex differences with respect to development and growth. The models were specified with Treatment, Sex, and Family lineage as well as the Treatment-by-Sex and Treatment-by-Family lineage interactions as explanatory variables (Start date was included as a random effect to account for slight photoperiod changes; see methods). Highly significant effects of both interactions confirmed sex-specific development and family-specific plasticity (Table 4).

Table 2. Pre-maturation mortality compared between feeding treatments.

Treatment pair (% mortality)	χ^2	P	N
High-Low (21 %) – Low-Low (24.7 %)	1.044	0.307	569
High-Low (21 %) – High-High (9.3 %)	15.393	<0.0001	564
High-High (9.3 %) – Low-High (13.9 %)	2.816	0.093	545
High-High (9.3 %) – Low-Low (24.7 %)	24.156	<0.0001	563
Low-High (13.9 %) – Low-Low (24.7 %)	10.264	0.001	550
High-Low (21 %) – Low-High (13.9 %)	4.886	0.027	551

Treatment pairs were compared using G-tests. Significant P -values are shown in bold.

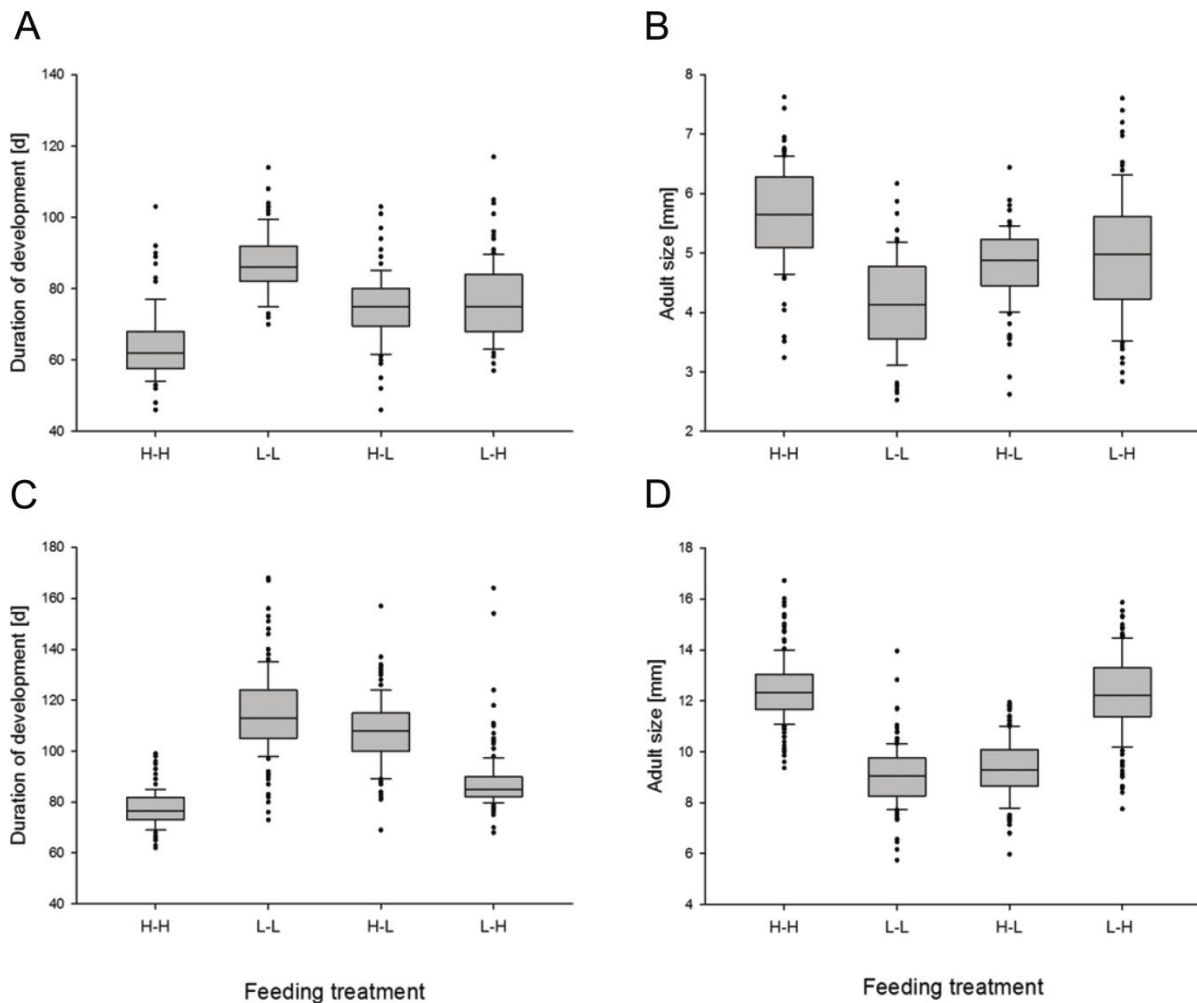


Figure 2. Effects of feeding treatments on the duration of development and adult size in *Nephila senegalensis*. Top row (A, B): Males. Bottom row (C, D): Females. Treatments were High-High (H-H), Low-Low (L-L), High-Low (H-L), and Low-High (L-H).

Post-maturation implications

Lifetime fecundity and hatching success

Females originating from the Low-High treatment delayed maturation, but achieved a similar adult body size and weight as High-High females through catch-up growth (see above). We predicted equivalent fecundity in Low-High females compared to High-High females and tested differences between feeding treatments High-High, Low-High, and Low-Low (we did not include High-Low females; High-Low and Low-Low females did not differ significantly in developmental

duration and size; see Table 2). Fecundity was measured as the total number of eggs (comprising hatched and undeveloped eggs) produced by each female during her entire reproductive lifespan (lifetime fecundity, LTF). The mean number of clutches was 2.82 ± 0.25 (range 1-6) and did not differ significantly between treatments (pairwise Tukey-Kramer HSD tests: $P > 0.1$; $N = 28$). Females originating from the Low-High treatment achieved the highest LTF of all treatments (LTF L-H = 2832.8 ± 448.02 ; $N = 10$), producing more eggs than the High-High females (LTF H-H = 2071.64 ± 320.12 , $N = 11$) and about twice as many as females originating from the Low-Low treatment (LTF

Table 3. Total developmental durations and adult size compared between feeding treatments.

Treatment pair	Males				Females			
	Total duration of development		Adult body size		Total duration of development		Adult body size	
	Z	P (N)	Z	P (N)	χ^2	P (N)	Z	P (N)
High-High – Low-Low	10.42	<0.0001 (184)	9.06	<0.0001 (181)	284.84	<0.0001 (283)	13.55	<0.0001 (275)
High-High – High-Low	7.17	<0.0001 (182)	6.86	<0.0001 (179)	226.58	<0.0001 (296)	13.74	<0.0001 (285)
Low-Low – High-Low	7.24	<0.0001 (172)	5.32	0.0008 (168)	4.67	0.2 (267)	2.11	0.151 (262)
Low-Low – Low-High	6.47	<0.0001 (180)	4.92	<0.0001 (173)	108.55	<0.0001 (263)	12.26	<0.0001 (260)
High-Low – Low-High	0.15	0.999 (178)	0.95	0.776 (171)	71.9	<0.0001 (276)	12.14	<0.0001 (270)

Treatments were compared with Steel-Dwass pairwise tests (excluding treatment pair High-High – Low-High subject to predefined analysis of catch-up growth; see results). Significant *P*-values are shown in bold.

L-L = 1343.86±262.65; *N* = 7). A linear model showed that the interaction between Treatment and the number of clutches explained a large proportion of variation in LTF ($F = 4.154$, $P = 0.03$, adjusted $R^2 = 0.83$). The model suggested that LTF increased over a series of clutches similarly in Low-High and High-High females, whereas Low-Low females were unable to achieve an equivalent increase of fecundity (Fig. 3). A Tukey-Kramer HSD test performed on model least square means showed significant differences in LTF between the Low-Low treatment and both other treatments (pairwise comparisons: L-H – H-H: $P = 0.264$, $N = 21$; L-H – L-L: $P = 0.0002$, $N = 17$; H-H – L-L: $P = 0.005$, $N = 18$).

Treatment effects on LTF did not correspond to absolute hatching success in our study. Although the total number of hatchlings produced differed considerably between treatments, variation was high and the differences were not significant (Number of hatchlings H-H: mean = 962.45±290.47, median = 701, IQR = 2287; L-L: mean = 421.57±133.05, median = 528, IQR = 738; L-H: mean = 1726.2±364.68, median = 2138, IQR = 2070.75; Kruskal-Wallis test: $\chi^2 = 4.95$, $P = 0.084$; $N = 28$). We also asked if feeding

regimes affected relative hatching success (i.e. the proportions of normally developed hatchlings and undeveloped eggs), but again there were no significant differences between treatments (Proportions hatched H-H: mean = 46.9±9.86, median = 57.58, IQR = 71.81; L-L: mean = 38.01±12.76, median = 35.31, IQR = 53.1; L-H: mean = 55.41±10.41, median = 56.56, IQR = 49.19; Kruskal-Wallis test: $\chi^2 = 1.5$, $P = 0.47$; $N = 28$).

Table 4. Effects of interactions between Treatment, Family lineage, and Sex on total duration of development and adult size.

Explanatory variable	Total duration of development		Adult body size	
	F	P	F	P
Treatment	339.96	<0.0001	343.43	0.007
Family lineage	10.3	<0.0001	2.31	0.033
Sex	821.48	<0.0001	7219.27	<0.0001
Treatment * Family lineage	3.14	<0.0001	1.94	0.007
Treatment * Sex	27.54	<0.0001	115.98	<0.0001

Results derive from linear mixed models including the variable Start date (the time of allocation of hatchlings to individual treatments) as a random effect to account for a potential influence of photoperiod on development. Developmental durations were log-transformed. Significant *P*-values are shown in bold.

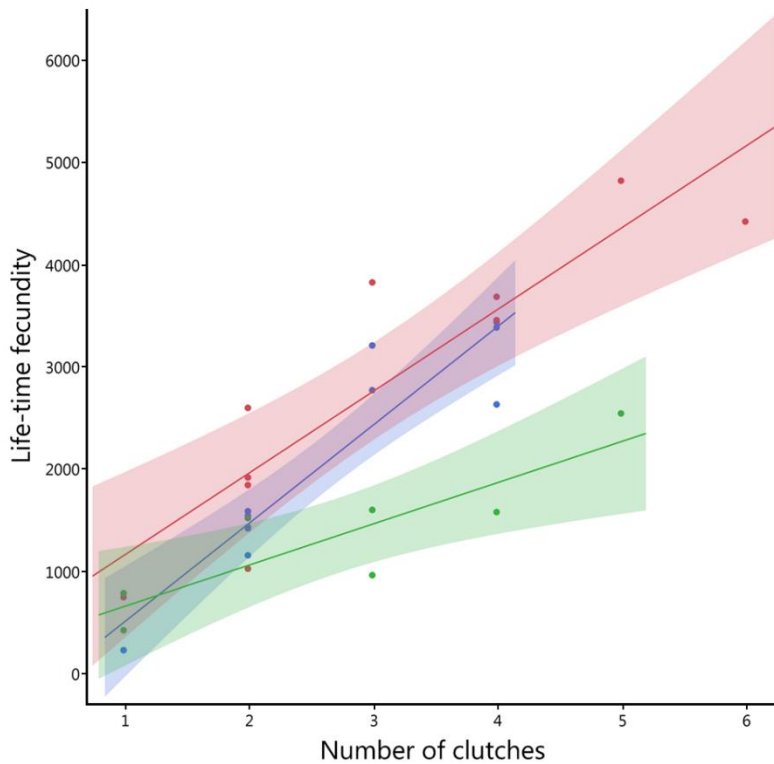


Figure 3. Effects of feeding treatments on lifetime fecundity (LTF) in *Nephila senegalensis*. The interaction between Treatment and the number of clutches produced explained lifetime fecundity in a linear model (adjusted $R^2 = 0.83$). Treatments were High-High (blue), Low-High (red), and Low-Low (green). Shaded areas indicate 95% confidence intervals.

A post-hoc test revealed a positive correlation between copulation duration and the total number of hatchlings ($F = 18.97$, $P < 0.001$, $N = 28$, $R^2 = 0.42$). In nature, female *N. senegalensis* are polyandrous and females have been shown to mate repeatedly in other studies (Neumann & Schneider 2015; Schneider & Michalik 2011). In this study, single copulations were probably insufficient to fertilize all eggs produced by a female.

Post-maturation longevity

We tested whether a period of juvenile food restriction and subsequent catch-up growth reduced the study animals' post-maturation lifespan, but found no significant effects in both sexes (Male adult lifespan H-H: mean = 148.08 ± 7.85 days, median = 144 days, IQR = 46; L-L: mean = 169.91 ± 9.98 days, median = 178 days, IQR = 75; L-H: mean = 150.98 ± 5.77 days, median = 147 days, IQR = 34; H-L: mean = 147.31 ± 8.53 days, median = 150.5 days, IQR = 38; Kruskal-Wallis test: $\chi^2 = 4.83$, $P = 0.185$; $N = 137$; Female adult lifespan H-H: mean =

61.55 ± 4.09 days, median = 60 days, IQR = 55; L-L: mean = 72.1 ± 4.61 days, median = 69.5 days, IQR = 40; L-H: mean = 57.75 ± 3.9 days, median = 54 days, IQR = 34.5; H-L: mean = 63.08 ± 5.46 days, median = 61 days, IQR = 38; Kruskal-Wallis test: $\chi^2 = 4.67$, $P = 0.198$; $N = 251$).

Discussion

Female *Nephila senegalensis* reared under food restriction in early development (Low-High females) used catch-up growth to counteract the restriction period and attain an adult body size and mass similar to those reared under constant food abundance (High-High females). As expected, Low-High females delayed maturation to fully compensate previous growth deficits. Size compensation had to be charged against an average developmental delay of 9-12 days; extending development by approximately 23 % compared to High-High females. This additional investment in time and growth

enabled Low-High females to achieve a lifetime fecundity (LTF) equivalent to High-High females; substantially exceeding LTF in constantly food-restricted females (Low-Low females). These findings further corroborate strong fecundity selection on large female size in *Nephila*. Consistent with our predictions, males did not implement catch-up growth as efficiently, showing incomplete compensation of body size. Although Low-High males delayed their development by 10-14 days, extending development by approximately 37 % compared to High-High males, they matured significantly smaller than the latter. The divergence between the sexes likely reflects generally weaker selection on large male size and hence compensatory ability, but may also indicate a stronger trade-off between timely maturation and growth in males. Juvenile food restriction and compensatory development did not affect the post-maturation lifespan in either sex.

The benefits of catch-up growth have to be offset against costs of a delayed development, which certainly affect fitness under natural conditions. Environmental parameters, such as temperature, food abundance and weather conditions, may generally fall off in quality with the season approaching its end (Hut et al. 2013a). This constitutes an 'end-of-season penalty' for late maturing individuals in semelparous species (De Block et al. 2008; Higgins 2000). Determinate growth and annual life cycles eliminate the opportunity to optimize body size after sexual maturation and to increase fitness in future reproduction, forcing animals into a narrow time frame in which to grow and to reproduce.

In *N. senegalensis*, females produce long-lasting egg sacs which overwinter and hatch in the following spring. The spiders generally mature in late summer and early autumn to experience a relatively short reproductive

period that declines with increasingly adverse weather conditions in late autumn (R. Neumann, 2011, 2012; J. Schneider, 2003, 2008, 2017, pers. obs.). Such strong seasonality generates several trade-offs to cope with. For instance, a prolonged pre-maturation development entails a higher predation-risk (Blanckenhorn 2000), but at the same time, large *Nephila* females outgrow the risk of being preyed upon by various invertebrate predators and parasitoids (Chase 1999; Higgins 2002). However, they may become more attractive to larger vertebrate predators. To make use of large body size in terms of fecundity also requires an increased amount of food and more time to produce the large numbers of eggs that can potentially be laid in multiple clutches (Higgins 2000; Neumann & Schneider 2015). Therefore, the adaptive significance of catch-up growth may vary between environments, for example, depending on predation pressure and the level of physical disturbance caused by extreme weather events (Higgins 2000). Females are hence expected to integrate environmentally-cued information into implementing catch-up growth.

The prospects of fitness optimization through catch-up growth seem to be more limited in males, which showed less growth compensation despite a significant developmental delay.

In *Nephila*, body size has often been shown to play a role in male-male competition (Kuntner & Elgar 2014), but the relationship between physical dominance and increased reproductive success has been oversimplified in the past. Indeed, large males may successfully execute their physical strength in specific competitive settings, e.g. in mating contests involving multiple males (Rittschof 2010). On the other hand, there is evidence from both experimental work (Neumann & Schneider 2015; Schneider & Elgar 2005) and

theoretical modelling (Rittschof et al. 2012) that small and medium-sized males adopt alternative mating strategies that balance overall paternity in competition with large rivals. Furthermore, the modelling approach suggested a decrease of average male reproductive success over the course of the season (Rittschof et al. 2012). This may be due to the fact that unmated females become increasingly rare and mated males may guard their females against successive competitors (Cohn et al. 1988; Schneider et al. 2008). In addition, late maturing females are generally smaller and hence less fecund than early females (Higgins 2000; Miyashita 1986). These factors may favour protandry and amplify the trade-off between developmental time and adult size in males. The importance of a timely maturation was further supported in an experimental study, showing that male *N. senegalensis* are able to adjust the duration of their subadult instar (i.e. the last developmental stage preceding maturity) to the presence of receptive females by shifting maturation in the order of several days (Neumann & Schneider 2016). Immature males use female silk (or probably silk-borne pheromones) as a cue to perceive females. Such plastic fine-tuning of life history may increase males' chances to locate receptive females in time and avoid male-male competition, thus further relaxing selection on large male size. However, since males at least showed incomplete catch-up growth, such compensation to the minor extant should nevertheless be adaptive. Males may use moderate catch-up growth to disperse unavoidable fitness costs between both traits affected by early food restriction (the duration of development and adult body size) rather than to mend only one of them.

Sex-specific differences with respect to adaptive developmental modifications addressed in this study probably result from

an evolutionary history of divergent size selection, giving way to sexual size dimorphism (SSD). Extreme reversed SSD in *Nephila* is likely facilitated by the genetic uncoupling of body size between the sexes (Kuntner & Elgar 2014). The task of explaining the evolution and maintenance of extreme SSD requires identification of sex-related selection pressures. In our experiment, individuals of both sexes developed more slowly and matured smaller in the Low-Low treatment than in the High-High treatment, but apart from that, we observed considerable differences between the sexes. Males in the High-Low treatment showed similar life history responses as Low-High males; both treatments resulting in intermediate average developmental duration and adult size relative to High-High and Low-Low males.

In females, however, the respective treatments had markedly different effects. In High-Low females, the late decrease of food supply resulted in severe limitations, as these females neither matured significantly faster nor achieved a larger size than Low-Low females. Thus, in contrast to males, females in the High-Low treatment significantly fell behind Low-High females in terms of developmental compensation, probably bearing high fitness costs. The fact that development and adult size did not differ between High-Low and Low-High males indicates less adverse effects of late-development food stress in males. A previous study addressing sex differences in *Nephila* with respect to food quantity showed that females demand an increased food supply and especially require more food than males to reach sexual maturation (Higgins & Goodnight 2010). The reduction of energetic requirements associated with small male size may therefore help to avoid fitness costs under food stress (Blanckenhorn et al. 1995);

potentially representing an important evolutionary driver to promote the uncoupling of body size between the sexes in spiders.

Although females in the Low-Low and High-Low treatments faced significant limitations regarding pace of development and adult body size, it is important to note that a large proportion of those females were still able to reach sexual maturity. Moreover, Low-Low females included in our mating trials and analyses of fecundity proved to be able to reproduce; albeit at a lowered level. Such small females that are also observed in natural populations may be able to escape reproductive failure by making ‘the best of a bad job’ (Higgins et al. 2011). These findings indicate that the general trade-off between the time invested in growth and the resulting adult size can be enforced substantially by periods of food limitation. Favourable conditions, on the other hand, may alleviate this trade-off, as males and females in the High-High treatment took the shortest average developmental duration to reach the largest average body size.

Irrespective of sex and treatment, variation of developmental duration and adult size was high. Our analyses revealed that these life history traits were modulated by an interaction between experimental treatments (i.e. the spiders’ environment) and family lineage, representing inherited genetic variation and/or maternal effects. Hence, part of the variation in these traits was caused by family-specific responses to feeding conditions, which has also been observed in other web-building spiders (e.g. Kleinteich & Schneider 2010).

The ability to survive under different feeding conditions is a basic requirement to implement adaptive developmental plasticity against impending costs of food restriction. Our results suggest that juvenile *N. senegalensis* are well able to survive a period

of poor feeding conditions in early developmental stages. Although early high- or low-food conditions significantly influenced the study animals’ growth, these differences did not affect the chance of survival. Very low metabolic rates in general enable spiders to subsist on low quantities of food (Foelix 2011; Mayntz et al. 2003). It is possible that phenotypic plasticity is also used to adjust metabolic rates to present conditions in order to survive food stress (Collatz & Mommsen 1975). Larger juvenile stages, however, develop higher nutritional requirements to maintain all vital physiological functions, making fluctuations in food supply more dangerous (Higgins & Goodnight 2010). Accordingly, we recorded a significant increase of mortality rates in spiders experiencing low-food conditions during late development.

Adaptive catch-up growth clearly bears costs of a delayed sexual maturity, but may also involve intrinsic long-term costs arising from developmental compensation, e.g. through partial elevated growth (Hector & Nakagawa 2012; Metcalfe & Monaghan 2001). Physiological stress can even reduce an organism’s longevity (English & Uller 2016; Hornick et al. 2000), but our feeding treatments had no effect on the spiders’ adult lifespan. In contrast, permanent juvenile food restriction reduced adult longevity in another araneid, the Bridge spider, *Larinioides sclopetarius* (Kleinteich et al. 2015). As in *Nephila*, female Bridge spiders delayed development and grew as large as control females; hence there was no apparent elevated growth and it remains unclear whether adverse effects on longevity resulted from dietary restrictions or the compensatory mechanism itself. Although female Bridge spiders reared under food restriction fully compensated adult size, their fecundity lagged behind control females, because they produced smaller clutches

(Kleinteich et al. 2015). Such findings point to limitations of fitness approximations based on size measurements. It is thus important that our study could not only confirm the proposed size-increase through delayed maturation (Higgins 1992), but also evaluated LTF as a direct consequence of experimentally induced developmental responses.

While our results are in accordance with our predictions, findings in other studies addressing compensatory development in size-dimorphic species diverged from predictions in whole or in part. For example, similar to our model system, fecundity-selected females in the mosquitofish, *Gambusia holbrooki*, were expected to show pronounced catch-up growth after juvenile food restriction, whereas minor catch-up growth was expected in the much smaller males whose fitness was proposed to depend less on large size. Different from predictions, however, both sexes delayed maturation and grew as large as control fish (Livingston et al. 2014). Male mosquitofish exhibit large size variation in nature and the authors suggest that size-related fitness consequences may depend on variable external conditions, including the social environment. Male developmental strategies may thus be influenced by population density and the intensity of male-male competition (or cues of such conditions) (Livingston et al. 2014). It is important, in general, to note that other environmental variables may often interact with food supply to induce specific responses, which is challenging to incorporate into experimental work (Davidowitz et al. 2004; Stillwell & Davidowitz 2010).

In the pholcid spider *Pholcus phalangioides*, presenting a rare case of male-biased sexual size-dimorphism in spiders (Uhl 1994), males benefit from both timely maturation and large body size by

avoiding male-male competition, or by succeeding in it (Schaefer & Uhl 2003). Food-restricted males were predicted to use developmental plasticity to increase body size either by delaying development or through accelerated growth. However, although males took longer to mature than control siblings, they could not catch up in terms of adult size (Uhl et al. 2004). Males were apparently unable to resolve the trade-off between benefits of protandry and advantages of large size under dietary restrictions (Uhl et al. 2004). Similarly to our findings, the observed developmental response may reflect a way of dispersing disadvantages with respect to developmental duration and adult size across both traits. It would be worthwhile, in general, to evaluate whether this kind of 'bet-hedging' through intermediate life history traits in moderate catch-up growth is truly adaptive and how external conditions may influence developmental responses. Future studies addressing these aspects should integrate field-based knowledge regarding existing phenotypes, the social environment, and time regime, and preferably include potential interactions between multiple environmental factors that might be involved in the expression of plastic traits.

Conclusions

Male and female *Nephila senegalensis* performed significantly different with respect to catch-up growth. Our study indicates strong fecundity selection on females, resulting in efficient growth compensation and hence prevention of fecundity-related fitness costs. Matching our predictions, catch-up growth in males did not evolve to the same capacity as in females. Relaxed selection on large male size and a stronger trade-off between costs of a delayed

maturation and size-related benefits were reflected in incomplete growth compensation. Nonetheless, the moderate degree of catch-up growth in males is likely adaptive in dispersing unavoidable costs of food restriction across affected traits. The adaptive value of moderate compensatory development and the potential adjustment of such mechanisms to environmentally or socially cued conditions should be addressed in future studies.

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Chapter 4

Males in the sexually cannibalistic spider *Nephila fenestrata* sacrifice their legs to pacify aggressive females

Rainer Neumann, Julia Becker and Jutta M. Schneider

Abstract

Sexual selection favours male adaptations to optimize the transfer of sperm and possible accessory substances to the female genital tract. Males in a range of invertebrates donate nutritious secretions or parts of their own body to the female during mating, which also serves to overcome female resistance in some cases. In spiders, monogynous male mating strategies have repeatedly evolved along with female-biased SSD and extreme male mating investment. However, female spiders often aggressively counteract male attempts to monopolize paternity. Male African golden-silk spiders, *Nephila fenestrata*, are regularly attacked by females during copulation, and such attacks can escalate into sexual cannibalism. Attacked males show a unique behaviour; they actively cast off their front legs and copulation continues while the female is feeding on these legs. Since the loss of legs is costly in reducing males' ability of postcopulatory mate guarding, it should yield significant reproductive advantages in return. We investigated the behavioural mechanism of copulatory male leg ejection and proposed that males sacrifice their legs to prevent female aggression and to invest particularly in high-quality mates. Conducting two mating experiments, we first evaluated whether male leg sacrifice is related to female quality, which was varied by manipulating male-female relatedness. Second, we offered male legs to females and asked whether simulating male leg ejection reduces the risk of copulatory female attacks and sexual cannibalism. In addition, we tested the attractiveness of male legs as food items for females. Our results confirm a significantly reduced probability of attacks in females that had been offered a male leg, but we found no relationship between simulated leg ejection and male survival. Male leg sacrifice did not depend on male-female relatedness and there also was no evidence for special properties of male legs. Female attacks were associated with increased leg loss in males, but also with prolonged copulations. While copulation duration is under male control, we suggest that male *N. fenestrata* may be forced to sacrifice their legs and hence their mate guarding ability to the female in prolonged copulations.

Introduction

Sperm competition and sexual conflict have caused a variety of male traits and strategies to increase the duration of copulation and to promote sperm transfer. Apart from morphological adaptations that facilitate prolonged genital contact, e.g. by means of hooking or coupling mechanisms or copulatory embrace (Jormalainen 1998; Khila et al. 2012; Kwan et al. 2013), males in many animal species donate nutritious gifts to the female either before or during copulation. Such gifts often consist of a species' regular food, but males in a number of invertebrates produce special secretions or offer nutritious body parts to females (Vahed 1998).

In the latter cases, the amount of nutrients to be gained for the female is often positively correlated with the duration of copulation and the number of sperm transferred. Well-known examples among insects include the fleshy hindwings in Sagebrush crickets (Sakaluk et al. 2004) and the salivary droplets produced by male scorpionflies (Engqvist & Sauer 2001). In predatory arthropods, such as spiders, nuptial gifts are less well documented (Vahed 1998). Among the few notable exceptions is the Nursery web spider *Pisaura mirabilis*. Males in this species offer wrapped prey to the female to increase their chance of mating as well as copulation duration and sperm storage (Albo et al. 2013; Stalhandske 2001). Males in the dwarf spider *Oedothorax retusus* possess a special cephalic hump inside of which a nutritional liquid is produced that the female ingests during copulation (Kunz et al. 2012).

In a range of spider species, monogynous mating systems have evolved along with extreme reversed sexual size dimorphism (SSD). The tiny males mate with only one or very few females and evolved special traits to monopolize paternity with a single female

(Schneider & Fromhage 2010). As an extreme form of terminal mating investment, males may even actively sacrifice their body to the female during or after copulation (Andrade 1996; Andrade et al. 2005; Schwartz et al. 2014). Such special cases of sexual cannibalism evolved several times independently in spiders (Miller 2007; Schneider 2014).

The question why females kill their mates and why some males do not attempt to escape cannibalism has been debated since Darwin (Darwin 1871; Elgar 1992). Current knowledge suggests female mate choice and prevention of paternity monopolization as the most likely explanations in species with monogynous mating systems (Elgar & Schneider 2004; Prenter et al. 2006; Schneider & Andrade 2011). Male and female interests over the event and timing of male death often diverge, so that the timing of cannibalism can be considered a trait under sexually antagonistic selection. Accordingly, sexual cannibalism represents an extreme example of sexual conflict and is expected to spark antagonistic co-evolution with strong selection on counter-adaptations in the male victim (Schneider 2014).

Male counter-adaptations to sexual cannibalism are manifold and comprise reducing feeding harm (Andrade et al. 2005), feigning death (Bilde et al. 2006), mating with subadult females (Biaggio et al. 2016), remote copulation (Li et al. 2012), and mating with a moulting and hence defenceless (Uhl et al. 2015), or a feeding and hence occupied and distracted female (Fromhage & Schneider 2005a). Mating while the female is feeding has been experimentally shown to reduce sexual cannibalism in the African golden-silk spider *Nephila fenestrata*, resulting in significantly higher paternity (Fromhage & Schneider 2005a). In another spider species, *Argiope bruennichi*, mating with a moulting

female has been shown to allow males successful monopolization of paternity by inseminating and plugging both copulatory tracts with parts of their genitalia (Uhl et al. 2015). Most of these males die after their second copulation without any active part on the female side. Since these males have achieved their maximum mating rate and their mating plugs efficiently protect paternity, self-sacrifice is adaptive in *A. bruennichi* (Nessler et al. 2007). In two other spider species, males even die during their first copulation without female influence (Knoflach & van Harten 2001; Schwartz et al. 2013).

This is different in *N. fenestrata*, representing an independently evolved mating system in which males also possess one-shot genitalia that break off during copulation to produce mating plugs (Fromhage & Schneider 2006). However, these plugs are not as efficient as in *A. bruennichi* and successive rivals pose a significant threat to a male's paternity. It is therefore important for males to survive mating and to defend the female against rivals as a measure of paternity protection (Fromhage & Schneider 2005b).

In *N. fenestrata*, copulating males regularly lose their front legs, which females then feed on. Males actively cast off these legs in reaction to female attacks that may serve to counteract male monopolization (Fromhage & Schneider 2006). Autotomy of appendages is a common anti-predation behaviour in spiders (Foelix 2011), but copulatory male leg ejection in *N. fenestrata*, as yet, is the only known example of autotomy of extremities during ongoing copulations (Fromhage & Schneider 2006).

However, sacrificing one or more legs is a costly behaviour, as it may generally reduce male locomotion and manoeuvrability (Gerald et al. 2017; Wrinn & Uetz 2008). Previous experiments have shown that male

competitive ability was affected by the number of legs lost, as males with fewer legs were less successful in male-male contests and postcopulatory mate guarding (Fromhage & Schneider 2005b). Based on the idea that copulatory male leg ejection (and female occupation with feeding on the legs) serves to reduce the risk of sexual cannibalism, another study compared the frequency of cannibalism between males that had their front legs removed before copulation and control groups in which males either had two other legs removed or no legs removed at all (Fromhage & Schneider 2006). Although males missing legs were cannibalized more than twice as often as intact males, the differences were not significant. However, a potential relationship between leg ejection and cannibalism may have been obscured by a high degree of variation and limited sample sizes.

Enhancing our hypothesis regarding the function of copulatory male leg ejection, we propose that males sacrifice their legs as an investment in paternity success with a given female. We performed a series of experiments to test the following predictions: (1) conspecific male legs should be particularly attractive food items for females (e.g. by providing a nutritional coating); (2) feeding on a male leg should reduce female aggression and resistance behaviour to the benefit of the male, and (3) males should preferentially invest in high quality mates. We first compared female handling and feeding duration between male legs and normal insect prey items, as well as between sanitized and untreated male legs, to test for special properties of the latter. Second, we staged mating trials with sibling and non-sibling pairings to vary relative mate quality and genetic compatibility. We predicted males to invest more in unrelated, higher-quality females and hence to eject more legs in non-sibling trials. Third, we experimentally

simulated copulatory male leg ejection by offering male legs to females during mating and expected the probability of female attacks and cannibalism to be reduced significantly compared to a control group.

Material and methods

Study animals

The study animals used in experiment 2 were F1-offspring reared from egg sacs produced by ten gravid females collected at Mawana Game Reserve, Zululand District, KwaZulu-Natal, South Africa in 2012 (permit OP 990/2012 from EZEMVELO KZN WILDLIFE PERMITS OFFICE). F2-offspring derived from pairings of unrelated F1-couples were used for experiment 1 and 3. The spiderlings were separated after approximately two additional moults and housed individually in air-vented plastic cups. We maintained the spiders on a diet of *Drosophila* and *Calliphora* flies (see Schneider et al. 2011 for a detailed description of housing and rearing). After the experiments, the study animals were killed at -80 °C and preserved in ethanol. The experiments were carried out between November 2012 and October 2013 at the Institute of Zoology, University of Hamburg.

Experimental procedure and data recording

Experiment 1: handling experiments with male legs and insect prey items: male leg properties and female preferences

We designed this experiment to investigate whether conspecific male legs possess special properties, e.g. a coating of the cuticular surface containing nutritional substances, which could make them especially attractive for females. Females were either offered a leg that had been sanitized (i.e. washed with 95 % ethanol to remove potential substances

from the cuticle) or an untreated leg. We measured female handling/feeding duration and expected that females would be longer occupied with untreated male legs than washed legs. In addition, we carried out tests in which females were offered *Carabus* beetle legs or *Gryllus* cricket body parts in order to compare female handling/feeding duration between conspecific male legs and normal insect prey of web-building spiders.

We randomly allocated 42 females to one of two treatments; (1) the male leg handling treatment in which females were offered an untreated leg on one day and a washed leg of the same male on a different day, or (2) the insect prey handling treatment in which females were offered a *Carabus* beetle leg and a body part of a *Gryllodes sigillatus* cricket in the same manner. Insect prey items were cut to resemble the average mass of a male leg (accepting a 0.5 mg tolerance). Objects were offered in random order in both treatments.

To test females' handling/feeding behaviour with respect to conspecific male legs, it was necessary to obtain fresh legs in a controlled procedure. Spiders, in general, are able to autotomize any leg at the basal leg joint (coxa-trochanter joint) with little impairment aside from the loss of the extremity itself. This behaviour is often used as a measure of last resort to escape from a predator (Foelix 2011). We seized the front legs of each male with forceps and the male ejected his legs immediately (see Fromhage & Schneider 2006). Male legs were obtained from males unrelated to the female within 15 min before starting the trial.

The trials were conducted with females normally positioned at the hub of their webs. To be able to reach the female's mouthparts, we cut a hole of approximately 2 cm in diameter into the female's web prior to each trial using fine scissors (females showed little

or no signs of being disturbed by web-manipulation, but were given a 10 min resting period before a trial was started). We used fine forceps to hand the food items to the female through the hole in the web. Each male leg or insect prey was brought in contact with the female's mouthparts up to three times (with 10 s intervals), so that the female was able to grasp the leg with her chelicerae. Female handling duration was defined as the period of time a female was occupied with the item, irrespective of whether the spider was actually feeding on it or not. Females were not fed between the day of maturation and handling trials.

Experiment 2: male mating investment in relation to relative mate quality and genetic compatibility

In line with the mating investment hypothesis, we based this experiment on the assumption that males prefer mating with unrelated females (i.e. higher quality mates in terms of genetic compatibility) over mating with sibling females. We therefore expected increased male mating investment in non-sibling females. Because females may counteract male monopolization attempts by attacking the male, strong male mating investment (i.e. prolonged copulations and/or second copulations) should be reflected in an increased number of legs ejected in a mating trial. In addition, we also assessed a potential female counter-adaptation, namely whether female attacks during first copulations reduce males' chances for a second copulation with the same female.

The mating trials in this experiment were staged as less risky 'opportunistic matings' (Robinson & Robinson 1980) with feeding females; however, with the exception of sexual cannibalism, feeding females as well as males copulating with those females show generally the same behavioural repertoire as

can be observed in matings with non-feeding females.

We arranged 30 trials in which males and females were related (i.e. siblings from the same maternal lineage) and 32 trials in which mates originated from unrelated maternal lineages. In some cases, *Nephila fenestrata* males do not separate from the female and the male pedipalp remains inserted for several hours. Mating trials with overlong insertions (≥ 2.5 h) were excluded from the analyses (2 non-sib trials). Males weighed 24.82 ± 0.9 mg at maturation and female adult weight was 353.33 ± 10.96 mg. The study animals' adult age (the number of days passed from the date of maturation) was 24.87 ± 0.88 days in males and 27.9 ± 1.22 days in females.

Before being used in a mating trial, each female was transferred to a 40 x 40 x 10 cm-sized Perspex frame and given several days to build a normal orb-web inside. At the beginning of each trial, we introduced the male by randomly positioning him either on the left or right upper frame threads of the female's web. The pre-set time for a trial was 3 h and all couples mated within this time (observations and data recording were extended in case of an ongoing copulation). We fed the female one *Calliphora* fly three minutes after starting the trial and a second fly after another five minutes in case no apparent male mating attempts (i.e. the male mounting the female) were observed. If the spiders copulated, the feeding of the second fly was delayed until the copulation had ended. A third fly was fed when the female had finished feeding. The male performed one or two copulations within a trial; observations ended with the end of a second copulation. We recorded the occurrence of copulatory female attacks (i.e. the female bent her body inwards and at the same time kicked, pushed, and pressed the copulating male with her hind legs), copulatory male leg

ejection, the number of copulations, and copulation duration (the time between the inflation of the haematodocha, a balloon-like organ generating haemolymph pressure for sperm transfer, and the collapsing of the organ). Coupling attempts ≤ 1 min were not considered as copulations (such attempts always left the male pedipalp intact and were often used for a regular copulation afterwards).

Experiment 3: experimentally simulated copulatory male leg ejection

We aimed to test the effect of conspecific male front legs offered to females during copulation and manipulated the regular mating procedure by offering a male leg independently of initial female aggression. We predicted this treatment to inhibit female aggression towards the male, thereby reducing the probability of copulatory female attacks and sexual cannibalism.

Fifty males and the same number of females were randomly allocated either to the male leg ejection simulation treatment or the control group, where no food item was offered. The study animals used in each trial were unrelated to each other. All males had their front legs removed before being used in the experiment. Each male was allowed a single copulation. If copulation did not occur within the pre-set time (1.5 h), the trial was repeated on the following day. In case of another failure, the trial was excluded from the study (9 control trials). A proportion of males did not separate from the female after the first copulation and immediately inserted the second pedipalp into the opposite copulatory opening. As we focused on first copulations in this experiment, trials were terminated after the first copulation had ended. Males weighed 20.84 ± 0.86 mg at maturation and female adult weight was 282.2 ± 10.47 mg. The study animals' adult

age was 18.89 ± 0.94 days in males and 18.18 ± 0.76 days in females.

We removed the front legs of males as described for experiment 1. Males were given at least 15 min for recovery afterwards. The legs offered in the mating trials were obtained from males who were unrelated to the focal male and female. Before a trial was initiated, we cut a hole into the female's web as described for experiment 1. Mating trails were started as described for experiment 2. In order to clearly determine the potential effect of male leg ejection, we offered the leg after 14 min of copulation; approximately four minutes before the average time of initial female aggression in preliminary observations (Neumann, pers. observations). The leg was offered up to three times for a period of 10 s (using 10 s intervals) by bringing it in direct contact with the female's mouthparts. In the event of a female attack at an earlier time, the leg was offered immediately. We recorded the occurrence of copulatory female attacks, cannibalism, and copulation duration.

Statistical analysis

All analyses in this study were conducted in JMP Pro 13 (SAS Institute Inc., Carey, NC, USA). We ran generalized linear models (GLMs) to analyse data of experiment 2, using the following dependent variables: the number of male legs ejected, the number of copulations performed (one or two), and total copulation duration. Explanatory variables were male-female relatedness (sib-sib/sib-non-sib mating), male and female adult weight (a proxy of adult size) and age, copulatory female attack (yes/no) in the first copulation (used only with number of copulations as dependent variable), female attack (yes/no) in the first and/or second copulation, and the number of copulations (used only with total copulation duration and

number of male legs ejected as dependent variables). GLMs to analyse data of experiment 3 were specified with the following dependent variables: female copulatory attack (yes/no), sexual cannibalism, and copulation duration. As explanatory variables, we used the leg ejection simulation treatment, male adult weight and age, and female condition (the ratio between the female's weight at the day of the trial and her adult weight to incorporate the increase of female body mass after maturation). We specified models with binomial distribution and logit link function for character variables; Poisson distribution with log link function for count data, and normal distribution with identity link function for continuous numerical data (log-transformed to improve model-fit). Models were simplified by stepwise removal of non-significant effects. Effect tests for individual variables in JMP are based on ANOVA-model comparisons between the full model and a reduced model lacking the respective variable. Non-significant P -values correspond to the time a variable was removed from the model. Significant P -values denote variables

that remained in the final model. Additional statistical tests (denoted in the results section) were performed using t -tests for normal data with equal variances (indicated by Shapiro and Bartlett's tests) and non-parametric Wilcoxon-tests for non-normal data. Sample sizes within experiments may differ owing to missing data.

Results

Experiment 1

We recorded a female handling duration of 1091.10 ± 444.25 s for sanitized male legs and 1245.40 ± 549.59 s for untreated legs. There was no significant difference in handling duration (Wilcoxon-test: $\chi^2 = 0.82$; $P = 0.364$; $N = 20$). In the insect prey handling treatment, females were offered a *Carabus* beetle leg or a body part of a *Grylloides sigillatus* cricket. Female handling duration did not differ between the different food items (beetle leg: 886.20 ± 1199.58 s; cricket: 794.50 ± 500.10 s; Wilcoxon-test: $\chi^2 = 0.3$; $P = 0.584$; $N = 11$). However, the spiders more often consumed the crickets than the beetle

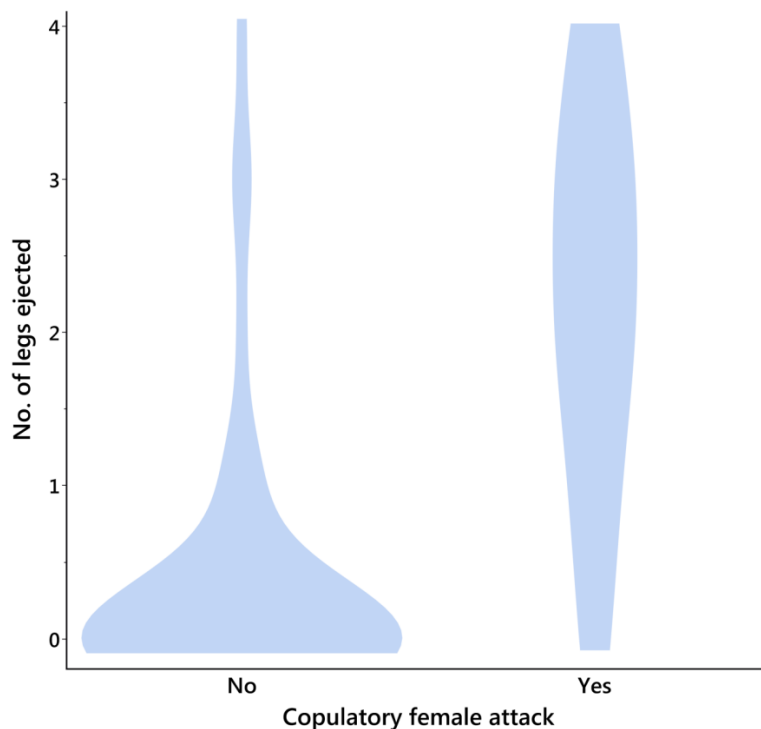


Figure 1. Relationship between male leg loss and the occurrence of copulatory female attacks in mating trials (Experiment 2).

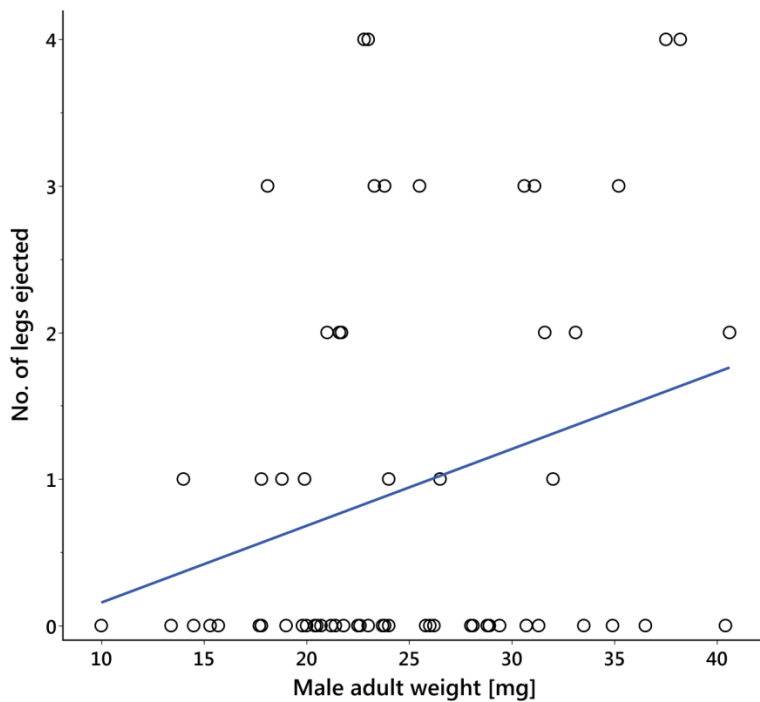


Figure 2. Relationship between male adult weight (a proxy of body size) and the number of male legs ejected in mating trials (Experiment 2).

legs (G-Test: $\chi^2 = 4.27$; $P = 0.039$; $N = 18$). As the absolute handling durations for sanitized and untreated male legs exceeded those for beetle legs and cricket body parts, we pooled the data for male legs and insect food items, but the difference was not significant (male legs: 1092.83 ± 484.95 s; insect prey: 840.35 ± 849.84 s; t -test: $t = 0.83$; $P = 0.476$; $N = 35$).

Experiment 2

Copulatory male leg ejection occurred in 15 of 60 trials (25 %). Contrary to predictions, our models showed that male-female relatedness had no effect on measured parameters of male mating investment. Whether males mated with sibling or non-sibling females neither predicted the number of legs a male ejected in a mating trial (non-sib trials: 0.77 ± 0.23 ; sib trials: 1.1 ± 0.26 , $N = 60$), nor the number of copulations performed (non-sib trials: 1.63 ± 0.09 ; sib trials: 1.63 ± 0.09 , $N = 60$) or total copulation duration (non-sib trials: 2120.18 s \pm 160.71; sib trials: 2716.93 s \pm 361.62 s, $N = 58$) (Table 1).

Of 60 males, 38 copulated twice (63.3 %). Copulatory female attacks occurred in eight

of all first copulations (13.3 %) and ten of all second copulations (26.3 %). Three males were cannibalized (5 %). The models revealed that the number of male legs ejected was related to the occurrence of copulatory female attacks (Fig 1; Table 1). A negative correlation of the number of legs ejected with male adult weight (a proxy of adult body size) indicated that small males lost fewer legs than large males (Fig 2; Table 1). The number of copulations was negatively correlated with both male and female adult weight as well as female adult age, hence two copulations occurred more often in trials with small males and when the female was small and young (Table 1). Furthermore, we found a relationship between female aggression and total copulation duration, as copulation duration in trials where females attacked the male significantly exceeded copulation duration with quiescent females. Copulation duration also was positively correlated with female adult age and the number of copulations in a trial (Table 1).

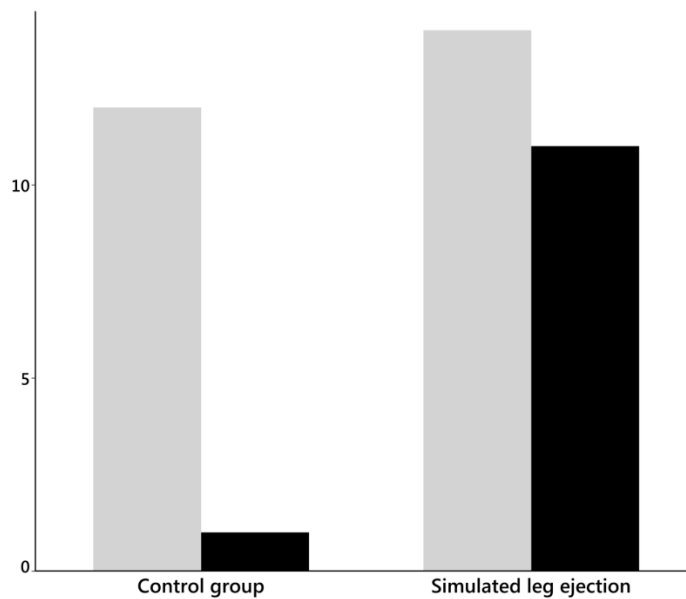


Figure 3. Numbers of females that attacked the male (grey bars) and non-aggressive females (black bars) in the leg ejection simulation treatment and in the control group (Experiment 3).

Experiment 3

Our results confirm that females that had been experimentally offered a male leg during copulation were significantly less likely to attack males than females that had not been offered a leg. Fourteen of 25 females (56 %) in our leg ejection simulation treatment showed copulatory attacks, whereas 12 of 13 females (92.3 %) attacked the copulating male in the control group (Fig 3; Table 2). However, we note that 3 treatment females did not accept the offered

leg and nonetheless remained quiescent during mating. If we exclude these trials from the model for a more conservative test, the treatment effect is still significant ($\chi^2 = 4.01$; $P = 0.045$; $N = 35$).

All males had their front legs removed, but a number of males ejected own second-pair legs (2 in the leg ejection simulation treatment and 5 in the control group). Male second-pair leg autotomy invariably occurred in reaction to female attacks and thus could not prevent initial attacks. Therefore, autotomy did not confound the predicted

Table 1. Summary of generalized linear models to test for effects of male-female relatedness on male mating investment and to analyse correlations between male and female traits and male mating success. Trials were staged as ‘opportunistic matings’ with feeding females.

Explanatory variable	No. of male legs ejected in trial ($N = 60$)			No. of copulations in trial ($N = 60$)			Total copulation duration ($N = 58$)		
	χ^2	P	df	χ^2	P	df	χ^2	P	df
Male-female relatedness	1.16	0.281	1	0.11	0.744	1	0.008	0.928	1
Male adult weight	4.46	0.035	1	9.57	0.002	1	0.31	0.577	1
Male adult age	0.03	0.865	1	<.0001	0.997	1	0.26	0.609	1
Female adult weight	2.06	0.151	1	5.45	0.02	1	1.71	0.191	1
Female adult age	0.23	0.633	1	5.51	0.019	1	5.57	0.018	1
Female copulatory attack in 1 st copulation	-	-	-	0.53	0.465	1	-	-	-
Female copulatory attack in 1 st and/or 2 nd copulation	37.03	<.0001	1	-	-	-	16.03	<.0001	1
No. of copulations in trial	0.13	0.72	1	-	-	-	18.32	<.0001	1

Total copulation duration was log-transformed for the analysis. Significant P -values are shown in bold.

Table 2. Summary of generalized linear models to test predictions related to simulated copulatory male leg ejection.

Explanatory variable	Female copulatory attack (<i>N</i> = 38)			Sexual cannibalism (<i>N</i> = 31)			Copulation duration (<i>N</i> = 37)		
	χ^2	<i>P</i>	<i>df</i>	χ^2	<i>P</i>	<i>df</i>	χ^2	<i>P</i>	<i>df</i>
Leg ejection simulation	6.05	0.014	1	1.18	0.277	1	0.6	0.439	1
Male adult weight	0.75	0.387	1	0.3	0.584	1	0.59	0.442	1
Male adult age	0.32	0.571	1	0.31	0.577	1	2.52	0.112	1
Female condition	0.73	0.392	1	0.63	0.426	1	6.24	0.013	1

Female condition was calculated as female weight at the day of the trial/female adult weight. Copulation duration was log-transformed for the analysis. Significant *P*-values are shown in bold.

effect of our treatment to inhibit female aggression completely in a proportion of mating trials. On the other hand, male second-pair leg ejection could potentially have influenced the probability of sexual cannibalism, and we excluded these trials from the respective model. Relatively few males in this subset did not survive mating. Males were cannibalized by 3 of 23 females in the leg ejection simulation treatment (13 %) and 2 of 8 females in the control treatment (25 %), but the difference was not significant (Table 2). We also analysed potential correlations between our explanatory variables and copulation duration, using the full data set. Mating trials in this experiment were terminated after a single copulation, for which we recorded a duration of 1530.7 ± 82.05 s (*N* = 37). The model indicated that copulation duration was positively correlated with female condition (Table 2).

Discussion

Our results confirm that copulatory male leg ejection enables males to pacify females, as significantly fewer females attacked the copulating male when they had been experimentally offered a male leg during mating. However, our experiments found no support for a protective function with regard to sexual cannibalism. The number of legs a

male ejected did not depend on whether he mated with an unrelated or a sibling female; hence there was no relationship between male leg sacrifice and mate quality. Finally, there was no evidence for special properties of male legs, which could make them especially attractive for females.

A male front leg autotomized during mating is often grasped and fed on by the female, which may prevent the female from further attacks. In various spiders, including *Nephila*, males are more willing to mate with females engaged in prey capture and feeding or directly after the female has moulted to maturity (Foellmer & Fairbairn 2003; Robinson & Robinson 1980). Males in some spider species, e.g. *N. plumipes* and the Autumn spider *Metellina segmentata*, even specialize exclusively in such 'opportunistic matings' where the risk of injury or cannibalism is significantly reduced (Elgar & Fahey 1996; Prenter et al. 1994; Robinson & Robinson 1980). However, such opportunities are generally rare. For example, only few spider females have been observed feeding at a given time in field studies (Higgins & Goodnight 2011; Venner & Casas 2005). In addition, a male-biased operational sex-ratio generates intense competition for receptive females in many species showing reversed SSD. A male should therefore engage in a risky mating with a potentially dangerous female, particularly if the female is unmated

(Fromhage & Schneider 2005b), or of high reproductive value in general. Copulatory male leg ejection may thus serve to distract and pacify especially those females that are not occupied with self-caught prey and become aggressive towards the male.

Sexual cannibalism in *N. fenestrata* is always initiated by copulatory female attacks, but in our study, the majority of attacks did not escalate into cannibalism. Sexual cannibalism occurred in 5 % of all trials in experiment 2, where females were continuously provided with insect prey. However, we also recorded only five cases of cannibalism (16 %) in experiment 3 (the simulation of copulatory leg ejection), and these occurred in females that had been offered a male leg as well as in the control group. Despite the relative infrequency of cannibalism, it should be noted that the arising fitness costs for individual males are often severe, probably reflecting benefits on the female side in the presence of sexual conflict. For example, all but one male victim in our study were cannibalized directly after their first copulation, leaving one of the female's sperm storage organs empty and unplugged. Hence, from the female's perspective, the act of cannibalism eliminated the possibility of another mating attempt and subsequent mate guarding by the first male. Under natural conditions, the female could practice sequential mate choice and mate with a second male of higher quality (Kempnaers et al. 1992; Welke & Schneider 2009).

In many animal species, copulation duration is related to sperm transfer and is therefore an important predictor of male fertilization success (Simmons 2001). Particularly males in highly specialized terminal investing species with one-shot genitalia should evolve mechanisms to optimize copulation duration (Schneider & Elgar 2001; Schneider et al. 2006). Female

aggression towards males may thus be interpreted as a measure to reduce copulation duration in order to counteract male monopolization as well as the general costs of mating (Edvardsson & Canal 2006). For example, female attacks have been shown to reduce copulation duration significantly in the black widow spider *Latrodectus tredecimguttatus* (Neumann & Schneider 2011), but we found that in *N. fenestrata*, female attacks were instead related to prolonged copulatory insertions. As males are able to monopolize a female most efficiently by copulating twice with her, we asked whether female attacks could be adaptive in restricting males to a single copulation. However, whether a male performed a second copulation with a female did not depend on female aggression during his first copulation. Nonetheless, female enforcement of leg ejection through copulatory attacks is associated with the cost of impaired mobility (Wrinn & Uetz 2008) and reduced mate guarding ability in males (Fromhage & Schneider 2005b), which likely serves to prevent male monopolization as well.

We expected males to invest differentially in females, preferring mates of relatively higher reproductive value (Welke & Schneider 2010), and presented males with either an unrelated or a sibling female; the latter potentially bearing a risk of fitness costs through negative effects of inbreeding. However, the relatedness between male and female had no effect on male mating investment and performance. Fitness effects of inbreeding in spiders have been shown, for example, in the Wasp spider *Argiope bruennichi*, where sibling matings resulted in lower hatching rates (Zimmer et al. 2014). Inbreeding also affected fecundity and hatching rates in the dwarf spider *Oedothorax apicatus*, but these findings were reported for inbreeding over three

generations (Bilde et al. 2007). First-generation inbreeding in *N. fenestrata* might not cause significant effects in terms of the offspring's fitness, and hence selection in favour of an avoidance mechanism may be weak. As some females mate with two males, they also may execute postcopulatory choice to bias paternity, which has been shown in *A. lobata* (Welke & Schneider 2009; Welke & Schneider 2010).

Our analyses revealed interrelations between male and female traits and mating performance that may hint towards size-related male mating strategies and trade-offs involved in them. It is important to note, however, that these considerations are derived from analyses of mating trials with feeding females, whereas risky matings with non-feeding females are likely more common in nature, and such different settings are known to have a strong impact on male mating strategies (Fromhage & Schneider 2005a; Fromhage & Schneider 2006). Similar to many other spider species showing extreme reversed SSD (Neumann & Schneider 2015; Schneider et al. 2000; Wilder & Rypstra 2008), male and female body size in *N. fenestrata* has a significant influence on the process of mating, including copulatory male leg ejection. Less risky 'opportunistic matings' with feeding females still involved loss of legs in 25 % of males in our study, and large males ejected significantly more legs than small males. Females may be better able to enforce leg sacrifice in larger males, probably because large males present an easier target for female attacks. Furthermore, we found that two copulations occurred more often in pairings of small males and females; hence large males more often left one of the females' copulatory tracts unplugged. Assuming that this pattern also occurs in nature, successful mate guarding may be particularly important for large males.

Although large males are generally more successful in guarding their female against rivals, the higher number of legs lost compromises this ability. Small males, on the other hand, may often increase their paternity by copulating twice without sacrificing legs. Having achieved their maximum mating rate, they remain functionally sterile and engage in especially vigorous mate guarding (Fromhage & Schneider 2005b). Fitness prospects of this mating strategy, however, may be limited because two copulations typically occurred with young and small females, and small female size in spiders is generally related to a lower fecundity (Higgins 1992; Hirt et al. 2017; Neumann et al. 2017). In addition, young females have not yet developed mature eggs and the relatively long period of time between insemination and the laying of the eggs may involve an increased risk of pre-reproduction mortality in those females (Rittschof 2011).

In summary, our findings suggest that the behavioural mechanism of copulatory male leg ejection in *N. fenestrata* differs in its function from other reported cases of nutritional donations, where males provide females with special substances or parts of their own body during mating (Kunz et al. 2012; Sakaluk et al. 2004; Vahed 1998). In our study species, conspecific male front legs are not particularly attractive for females and likely do not serve as a vector for nutritional substances that females could in turn reward with longer copulation duration or increased sperm storage. In contrast to many spiders (Herberstein et al. 2011; Schneider et al. 2006; Zhang et al. 2011), copulation duration in *N. fenestrata* seems to be largely under male control (Fromhage & Schneider 2005b). This is because the male's pedipalp stays firmly attached to the female's genital opening during copulation and is usually

withdrawn by breaking off a part of the organ, thereby producing a mating plug. The adaptive value of copulatory male leg ejection may be better understood when taking female interests into account. The occurrence of female attacks was associated with a higher number of male legs ejected, but also with prolonged copulation duration. This suggests that males have to pay for copulations exceeding the preferred duration from the female's perspective by sacrificing legs and hence mate guarding ability to the female. While the adaptive significance of prolonged copulations in *N. fenestrata* is unclear (Fromhage & Schneider 2006), copulations lasting longer than required for transferring sperm can increase male reproductive success in other spider species; e.g. by fulfilling an extended mate guarding function (Linn et al. 2007) or biasing paternity in postcopulatory competition (Bukowski & Christenson 1997; Snow & Andrade 2004). The underlying mechanisms in the latter cases remain unresolved, but studies on insects provide evidence for the transfer of accessory substances to the female genital tract that take up space to store other males' sperm, manipulate female receptivity, or induce egg-laying before another mating

takes place (reviewed in Arnqvist & Nilsson 2000). Males in our study copulated longer with females of relatively high body mass, which are generally closer to oviposition than light-weighting females. Single copulations lasted 25 minutes, on average, and often much longer; thus clearly exceeding the time required for sperm transfer. These observations raise the question whether *N. fenestrata* males transfer non-gametic accessory substances in addition to sperm to maximize paternity. The pacifying effect of male leg sacrifice may aid in implementing such a mechanism.

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General discussion

This thesis comprises experimental studies in which I investigated adaptive plasticity of development, body size, and mating behaviour in two species of African golden-silk spiders. These spiders show extreme female-biased SSD as well as extraordinary within-sex size variation, particularly in males. Our understanding of these characteristics has been challenged by a wealth of studies reporting large size advantages in male-male competition, and the modes of selection causing and maintaining size variation and extreme reversed SSD are still not fully understood (Higgins et al. 2011; Kuntner & Elgar 2014; Schneider & Fromhage 2010). I studied contest competition and differential mating investment in size-mismatched males, sex-specific developmental plasticity in the form of adaptive growth compensation, socially cued life history plasticity, and the unique male mating behaviour of copulatory leg sacrifice to overcome female resistance.

Using *Nephila senegalensis*, I conducted a long-term mating experiment in which dyads of size-mismatched males competed for a single female. I investigated whether differently-sized males achieve equivalent average paternity success and how size-related mating strategies potentially balance reproductive success between phenotypes (**chapter 1**).

My analyses revealed high variation regarding paternity success in each size class, but equivalent average paternity could be confirmed. In mating systems involving conditional strategies, there typically is an 'inferior tactic', allowing less competitive males to gain higher reproductive success than they were to expect if adopting the mating tactic of superior rivals (reviewed in Shuster 2010). Similar to many other taxa, the decision of which tactic to use depends

on body size in *Nephila* males. Depending on his own size, a male may invariably apply an unconditional strategy or plastically adjust his mating strategy to present conditions. I could show that large males varied their mating investment according to female reproductive value, whereas small males followed an indiscriminate investment strategy. Intermediate-sized males benefitted from large males' restrained investment in females of lower reproductive value, which shifted chances to increase paternity to the competitor. Both larger size classes adjusted basic strategies to present conditions, integrating their own competitive ability and female quality, as well as the intensity of sperm competition into their mating decisions. My results show that in *N. senegalensis*, conditional mating strategies allow males of distinctly different size to achieve equivalent paternity in contest competition, so that an imbalance in terms of physical strength can be equilibrated under competitive conditions as specified in my study. Competitive settings are much more variable in nature, e.g. involving more variable and often higher numbers of competing males, but also the opportunity to move away and continue searching for another mate (Neumann & Schneider; unpublished data). However, my findings are consistent with a theoretical study in which male mating strategies were modelled in a similar species, *N. clavipes*. In line with my results, the model showed that condition-dependent male mate choice and unconditional indiscriminate mating can coexist as stable alternative strategies, resulting in balanced fitness payoffs between small, intermediate, and large phenotypes (Rittschof et al. 2012). Although small males in these systems are considered to make 'the best of a bad job', associated with relatively low fitness, my results suggest that

supposedly inferior tactics can be equally successful as dominant tactics in specific mating systems. In addition, these findings exemplify the significance of behavioural plasticity in precluding directional large size selection, and thus contribute substantially to understanding female-biased SSD in *Nephila*.

I also expected behavioural plasticity in male *N. fenestrata* in which I studied copulatory male leg ejection within the framework of male mating investment and sexual conflict. Similar to differential mating investment in *N. senegalensis*, male mating decisions in *N. fenestrata* should relate to prospects of reproductive success and hence mate quality. This experiment was based on the assumption that males sacrifice their legs to pacify females, losing increasing numbers of legs with stronger mating investment. I analysed whether males preferentially invest in high-quality females; consequently sacrificing a higher number of legs in the respective mating trials. In order to verify the function of male leg sacrifice, I tested whether experimentally simulated leg ejection reduces the risk of female attacks and sexual cannibalism (**chapter 4**).

Females were less likely to attack males when they had been offered a male front leg during mating. This finding confirms the pacifying function of male leg sacrifice, although this treatment did not affect the probability of cannibalism. While male mating investment in terms of leg sacrifice was unrelated to experimentally manipulated female quality (i.e. male-female relatedness), my analyses indicate that males valued another, probably more fitness-relevant trait. Males copulated longer with females of higher body mass relative to size, which is generally associated with large numbers of eggs and close oviposition (Higgins 1992; Rittschof 2011). As copulatory female attacks were correlated with prolonged copulation duration and a higher number of male legs

ejected, I interpret these findings as reflecting sexual conflict between males that attempt to monopolize fertilization, and females counteracting the restriction to only one male for siring their entire offspring. Aggressive females probably enforce male leg sacrifice to reduce the efficiency of males' postcopulatory mate guarding performance. Since there is remarkable male size variation in *N. fenestrata*, I asked whether my observations indicate a potential significance of body size in male mating strategies of this species as well. I found that male mating success was modulated by benefits and costs of relatively small or large size; further interrelated with age, size, and body mass of the respective female. Small males more often copulated twice than large males and even sacrificed less of their legs. However, this successful mating performance may yield limited fitness returns, because it occurred mainly with small and hence less valuable females. As large males more often left one of the female's copulatory tracts unplugged, successful mate guarding may be particularly important, but compromised by the higher degree of leg sacrifice. Small males, on the other hand, may engage in particularly vigorous mate guarding after having achieved their maximum mating rate of two copulations (Fromhage & Schneider 2005b). These results point towards condition-dependent strategies in male *N. fenestrata*. Since I measured mating success in this study without extending analyses to paternity success, it remains unclear, however, how these differences translate into individual fitness, and specifically, whether they may balance fitness payoffs between differently-sized phenotypes as shown in *N. senegalensis*.

My studies described above provide examples of behavioural plasticity, showing how mature male individuals make use of the ability to adjust their mating strategy to given

conditions, including their own adult size, but also the size of their female mate. However, phenotypic plasticity is also an important factor in the period of time before an individual reaches maturity. It can be useful to study plasticity of growth and size comparatively between both sexes in systems with pronounced sexual size dimorphism and determinate growth, where males and females apparently underlie different selection pressures affecting their adult size. These differences between the sexes should also be reflected in juvenile individuals that are exposed to external conditions restricting optimal development and growth, such as low or variable food supply (Livingston et al. 2014; Tawes & Kelly 2017). Experimental studies can help to identify sex-specific selection pressures that are expected to result in distinctly different modes of adaptive life history plasticity, which also adds to our understanding of SSD.

I studied fitness implications of sex-specific growth compensation in *N. senegalensis* and analysed the study animals' developmental duration and adult size in different feeding treatments. A second experimental section was conducted to assess the adaptive value of growth compensation in terms of lifetime fecundity (LTF). I investigated whether males and females differ as expected in the way how adaptive catch-up growth is implemented in the face of divergent selection pressures. Natural selection should favour optimization of fecundity in food-restricted females, whereas sexually selected benefits of early maturation should generate a stronger trade-off between size-related benefits and costs of a delayed maturation in males (**chapter 3**).

Males and females showed different degrees of catch-up growth. Corroborating strong fecundity selection, efficient compensation in growth-restricted females

resulted in equivalent fecundity compared to unrestricted females. Catch-up growth was less efficient in males and compensation remained incomplete, probably reflecting the expected trade-off between costs and benefits of catch-up growth and relaxed selection on large male size. However, this moderate compensatory development may represent the most adaptive degree of plastic responses in males, as unavoidable costs of early growth restriction are dispersed across affected life history traits. My analyses also revealed differences between maternal lineages with respect to plastic responses in individual treatments, thus indicating interactions between genotype and treatment (i.e. the experimental environment) that are a potential cause of variation in life history traits in general (Stearns 1989).

Developmental plasticity can also act in response to environmental parameters affecting individuals less directly than food regime, such as cues that allow predicting future environmental conditions. For example, animals have been found to modify their juvenile development according to social information regarding the reproductive environment to be anticipated for their own mating period. Socially cued anticipatory plasticity (SCAP) has been proposed as a widespread mechanism, allowing for adaptive life history shifts as part of male mating strategies. Particularly short-lived males with terminal mating investment are proposed to adjust the timing of maturation to cues indicating the availability of receptive females. Such modifications may also affect male adult size and potentially increase size variation in nature (Kasumovic & Brooks 2011; Kasumovic et al. 2009).

Using a comparative approach, I investigated socially cued anticipatory plasticity in male *N. fenestrata* that specialize

in terminal investment and monopolizing single females, and *N. senegalensis* in which males depend less on locating a virgin female. I analysed whether my study species' show sexually selected life history plasticity according to theoretical expectations, i.e. a distinct developmental response in *N. fenestrata*, but a weaker response in *N. senegalensis* (**chapter 2**).

I could show that immature *Nephila* males, in principle, are able to perceive the presence of virgin females only by assessing their silk, which had previously been proposed (Kasumovic et al. 2009), but never demonstrated. The species that did show a distinct plastic response, however, was *N. senegalensis*, whereas female cues had no effect in *N. fenestrata*. Male *N. senegalensis* adjusted the duration of the subadult instar to the simulated presence of virgin females and matured several days earlier than control males. This fine-tuning of maturation, which subsequently has also been demonstrated in another web-building spider, *Argiope bruennichi* (Cory & Schneider 2018), is certainly adaptive. The reasons for male *N. fenestrata* not responding to virgin female cues remain unclear, however. A previous study using the Australian red back spider *Latrodectus hasselti* reported alterations of male developmental duration as well as adult size in response to female cues (Kasumovic & Andrade 2006), and similar degrees of socially cued plasticity have been proposed to be common on the basis of relatively few studies (Kasumovic & Brooks 2011). However, I found that a species possessing several critical traits suggested to favour this ability (Kasumovic et al. 2009) did not show the expected response. In addition, developmental plasticity in *N. senegalensis* did not affect adult size, which was the case in *A. bruennichi* as well (Cory & Schneider 2018). My findings imply that socially cued anticipatory plasticity as part of male mating strategies may not be

as common as proposed. Furthermore, plastic developmental responses shifting the timing of maturation in the order of days may generally not affect adult size; hence this mechanism may not contribute significantly to male size variation in web-building spiders. In systems in which unintentional cues from the social environment are lacking, this form of adaptive plasticity may depend on whether females benefit from providing cues, which they may produce only temporarily (e.g. sex pheromones; Chinta et al. 2010; Cory & Schneider 2018).

In this thesis, I proposed that sex-specific selection pressures act to optimize particularly fitness-relevant traits in my study species. While selection should intrinsically optimize these traits, environmental conditions often prevent individuals from ideal trait expression (e.g. the prevalent food regime may not allow reaching the largest possible adult size in the shortest possible developmental time). Thus, I also expected selection to optimize the capacity to adjust these traits to the environment. I predicted individuals experimentally forced into reproductive environments deviating from ideal conditions to show appropriate plastic responses. The results obtained allowed me to evaluate whether the relevant selection pressures have been correctly identified, and whether the observed plastic response is actually adaptive. I could reveal sex-specific divergent selection, which helps to explain the evolution of extreme reversed SSD (e.g. selection for protandry resulting in incomplete growth compensation in males, and fecundity selection resulting in complete growth compensation in females). Moreover, I could show how adaptive phenotypic plasticity contributes to the extraordinary size variation in both sexes of my study species (e.g. females under low food provisioning, although staying small, can avoid reproductive failure; condition-dependent

mating strategies allow small males to reproduce successfully). My results show how behaviourally plastic mating strategies can balance fitness between differently-sized male phenotypes, thereby precluding directional large size selection. Thus, my work also helps to explain how principal large male size benefits can be dissolved.

In conclusion, I could show that adaptive phenotypic plasticity enables individuals to

modify basal patterns of life history and behavioural strategies, allowing them to elevate their reproductive success to a level that would not be within reach without this ability. These mechanisms promote size variation and extreme female-biased SSD over evolutionary time.

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.

Rainer Neumann

Hamburg, November 2018

Author contributions

Summary, Zusammenfassung

Rainer Neumann (RN) wrote both sections.

General introduction

RN wrote the General introduction.

Chapter 1:

Differential investment and size-related mating strategies facilitate extreme size variation in contesting male spiders

Jutta M. Schneider (JMS) conceived and designed the experiments. JMS and RN developed the hypotheses and predictions. RN collected the study animals in Namibia and reared laboratory populations with support from the department's technical personnel. RN performed the experiments, analysed the data, crafted the tables and figures, and wrote the chapter, with contributions from JMS.

Chapter 2:

Socially cued developmental plasticity in web-building spiders

JMS and RN conceived and designed the experiments and developed the hypotheses and predictions. RN collected the study animals in South Africa and reared laboratory populations with support from the department's technical personnel. RN performed the experiments, analysed the data, crafted the tables and figures, and wrote the chapter, with contributions from JMS.

Chapter 3:

Fitness implications of sex-specific catch-up growth in *Nephila senegalensis*, a spider with extreme reversed SSD

JMS and Nicole Ruppel (NR) conceived and designed the experiments. RN developed the hypotheses and predictions. JMS collected the study animals. NR reared laboratory populations with support from the department's technical personnel and performed the experiments. RN analysed the data, crafted the tables and figures, and wrote the chapter. JMS reviewed and edited the chapter.

Chapter 4:**Males in the sexually cannibalistic spider *Nephila fenestrata* sacrifice their legs to pacify aggressive females**

JMS conceived and designed experiment 1 and 3 and developed the associated hypotheses and predictions. RN and JMS conceived and designed experiment 2 and developed the associated hypotheses and predictions. RN collected the study animals. Julia Becker (JB) and RN reared laboratory populations with support from the department's technical personnel. JB and RN performed the experiments. JB analysed the data for experiment 1. RN analysed the data for experiment 2 and 3 and crafted the tables and figures. RN and JMS wrote the chapter.

General discussion

RN wrote the General discussion.

Prof. Dr. Jutta Schneider

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