The lives (and loves) of widow spiders

Behaviour and reproductive morphology of a sexually cannibalistic spider

Projektbericht

In his treatise "The Descent of Man and Selection in Relation to Sex" (1871) Charles Darwin postulated that some traits of animals and plants were favoured owing to their contribution to mating, rather than survival. Nowadays, sexual selection is understood to be a powerful force in shaping behaviour and morphology of organisms. Sexual selection can occur via competition within a sex for access to mates or by one sex choosing a mate based on its displays or other characteristics indicative of its quality. Commonly, males compete by various means, while females are choosy (Andersson, 1994). In species lacking extensive parental care of offspring, males are able to increase their share of paternity mainly by searching for, and mating with multiple females. Therefore, male monogamy in species lacking parental care is unusual and requires explanation. When males search actively for sedentary females, they are subject to higher mortality risks than females; in such cases, they may be restricted to mating with a few, or even only a single female. When searching costs are high, males may benefit from investing more resources in mating with a single female than in attempting to find additional females, thus becoming monogamous. In our research on widow spider (Araneae, *Latrodectus*) mating behaviour, we have encountered an extreme case of male monogamy. In the present study, we investigated the behaviour and several other characteristics of monogamous male widow spiders that help to explain the evolution of male monogamy in this group.

All species of widow spiders have a lengthy and elaborate courtship, sometimes lasting several hours. Courtship involves web vibrations and silk deposition by the male, and is energetically demanding (Anava & Lubin, 1993; Segoli et al., 2006; Harari et al., 2009). Males of *L. pallidus*, for example, lose about 5% of their body mass while courting the female. Spider males transfer sperm with the help of transformed body appendages, the pedipalps, that consist of several segments (sclerites). During copulation, males frequently break off part of the terminal sclerite (the embolus) of the pedipalp, through which sperm is released into the female copulatory duct. The tip of the embolus becomes lodged inside the female's spermatheca, which is where the sperm are stored until used to fertilize the eggs. It is suggested that this embolus tip acts as a mating plug that prevents insemination of the female by a second male. However, females of several widow spider species have been found with more than one broken tip in the spermatheca or in the insemination duct (Berendonck & Greven, 2002; Snow et

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Kurzvita

Yael Lubin is Professor emerita of Ecology at Ben-Gurion University of the Negev in Israel. She has spent over 30 years at the Blaustein Institute for Desert Research, on the Sede Boqer Campus of the university, investigating ecology and behaviour of spiders. Her research on spiders include adaptations to deserts, the ecology and evolution of group-living, and the natural history and evolution of sexual behaviour. She also does applied research on the use of spiders in biological control of crop pests. Her Ph.D. in Zoology was from the University of Florida (USA), and she previously conducted research in many tropical countries including Panama, Ecuador and Papua New Guinea.

Fellow-Projekt

» Reproductive Morphology and Physiology of a Sexually Cannibalistic Spider

Sexual selection, first postulated by Charles Darwin in 1871, is a powerful force shaping the behaviour and morphology of organisms. Sexual selection can occur via competition within a sex for access to mates or by one sex choosing a mate based on its displays or other traits indicative of its quality. Commonly, males compete by various means, while females are choosy. In Brown Widow spiders, males are much smaller than females and are frequently cannibalized during mating. As they only mate once, males should be very selective. The long courtship display of male Brown Widow spiders may involve mate choice by both partners. An alternative male tactic, recently discovered in Brown Widow spiders, involves mating with immature females. These females are never cannibalistic and thus the males may go on to mate with additional females. This is expected to be the preferred tactic for the male, but perhaps not for the female. I investigated courtship and mating behaviour, and the morphology of reproductive organs in Brown Widow spiders in the lab of Professor Dr. Gabriele Uhl, Department of General and Systematic Zoology, University of Greifswald.

We compared the behaviour of adult males when given a choice between immature and adult virgin females. The aim was to determine if males distinguished these females from a distance by airborne chemicals released by the females, and if their behaviour differed when encountering web-silk of immature and adult females, based on silk-borne chemicals. Contrary to expectation, males were more attracted to adult than to immature females. Once web-silk was encountered, males courted adult females, but immediately attempted to mate with juveniles. The genitalic (reproductive) structures are fully formed in immature females before their final moult. However, the external openings, through which copulation occurs, are covered by cuticle, which the male tears during mating. Precocious genital development in immature females is previously unknown in spiders. The consequences of immature mating for females remain an open question.

al., 2006). Interestingly, the broken embolus does not prevent males from re-mating and transferring sperm (Anava et al., 1993; Segoli et al. 2009).

Contrary to popular ideas, sexual cannibalism is uncommon in most widow spider species. In *L. pallidus* and *L. revivensis*, two species studied in Israel, only 10-13% of males were cannibalized during or after mating (Segev et al., 2003; Segoli et al., 2006). However, males of the Australian redback, *L. hasselti*, somersault into the female's mouthparts during mating (Andrade, 1996), and similar behaviour occurs in the brown widow spider, *L. geometricus* (Segoli et al., 2008). In both species, male-assisted cannibalism by females occurred at high frequencies (> 60% of matings). Consequently, in these two species, males are nearly always monogamous. A benefit to males of this suicidal behaviour may be a larger share of offspring sired by the cannibalizing female (Snow & Andrade 2004). Surprisingly, however, males of both species were found to engage in an alternative strategy that enables them to escape from strict monogamy, namely, mating with immature females (Biaggio et al. 2016). In some spider species males cohabit with immature females and mate with them just after the final moult to adult stage or even while the female is moulting (Uhl et al. 2015). Immature females lack exposed genital openings and therefore were assumed to be unable to mate. Biaggio et al. (2016) showed that males are sometimes able to tear the female's cuticle to expose the genital openings. Thus, mating with an immature female may represent an extreme alternative mating tactic.

In our research, we focused on the brown widow spider, *L. geometricus,* a cosmopolitan species that is common in urban areas. Brown widow females that mate as immatures store the sperm in their sperm-storage organs (spermathecae), and produce viable egg sacs after moulting to adult (Biaggio et al. 2016). Males invest less energy and time when mating with immature females than when courting adult females. When mating with immatures, males do not perform the typical somersault into the mouthparts of the female while copulating, and they are not cannibalized. These males are then able to mate with an additional female, thereby circumventing monogamy.

We suggested that for males, mating with an immature female should be a preferred mating tactic. For females, however, it remains unclear if mating as an immature carries a cost, and if so, whether females have any counter-tactic to prevent mating as an immature. Preliminary data suggested that immature females do not produce a male-attractant pheromone, leading to the possibility of a conflict between male and female mating tactics. Immature and adult females may differ in the state of development of internal genitalic structures, such as the spermathecae or the glands associated with sperm transfer and fertilization. Thus, aside from the damage to the cuticle caused by the males, we asked whether females suffer additional costs to mating as immatures.

The research was conducted in collaboration with Professor Dr. Gabriele Uhl (General and Systematic Zoology, University of Greifswald) and a post-doctoral fellow, Dr. Lenka Sentenska (from the partner Masaryk University, Brno, Czech Republic). One bachelor student (Lucie Hoffschlaeger) and one Master project student (Aileen Neumann) participated in the study. The study built on the complementary fields of expertise, behavioural ecology and morphology, of Professors Lubin and Uhl.

The research comprised two parts: behavioural experiments and morphological analyses.

Behavioural experiments

We conducted behavioural experiments to compare the attraction and behaviour of adult males when presented with a choice between

Fig. 1: Male mating with an adult female in the choice test. Note the size difference between the small male and large female. The male is standing on the ventral side of the female and inserting one pedipalp into her genital opening. (Photo: L. Hoffschlaegger)

immature and adult virgin females and when presented with each alone (no-choice). The aim was to determine if males distinguished these females from a distance (based on airborne pheromones) and if the male's behaviour differed when encountering the female's web-silk.

Methods: Prior to the experiments virgin females of different stages (see below) were placed separately in clean boxes and left within for five days to build a web. The females were fed a day before being placed in the experimental boxes. Once inside they were offered no food to prevent odours other than female-produced chemicals to be present in the boxes.

After five days, two boxes, each with one female, were placed in diagonally-opposing corners of a square testing arena. Each box was randomly assigned to a corner. The lid of each box was removed exposing female's web and allowing the male to walk in. A virgin male was released into one of the corners unoccupied by a box, equidistant from each box containing a female. We observed the male's behaviour for 30 minutes, recording (1) which female was approached first, and (2) the latency to first contact with female's web; (3) adding silk to the female's web; (4) cutting and removing silk from female's web; (5) abdomen vibration and (6) contact with female body.

After 30 minutes, courting males were left to continue courtship and to mate with the female (Figure 1). As a control, the not-chosen female was paired with another male in order to determine if she was receptive to mating. The choice experiment was included in the analyses only if the female mated. After 24 hours the mating couples were separated and it was noted whether cannibalism occurred. Males that survived were sacrificed and their copulatory organs were checked to determine whether copulation occurred and the genital area of the immature females was checked to determine if they had mated.

Virgin males were exposed simultaneously to (1) one adult female (AF) and one immature female (IF, up to 4 days before the final moult to the adult stage); (2) one young immature female (YIF, less than 8 days after the moult into the last immature stage) and one immature female (within a 4 day period before the final moult to adult); (3) one young

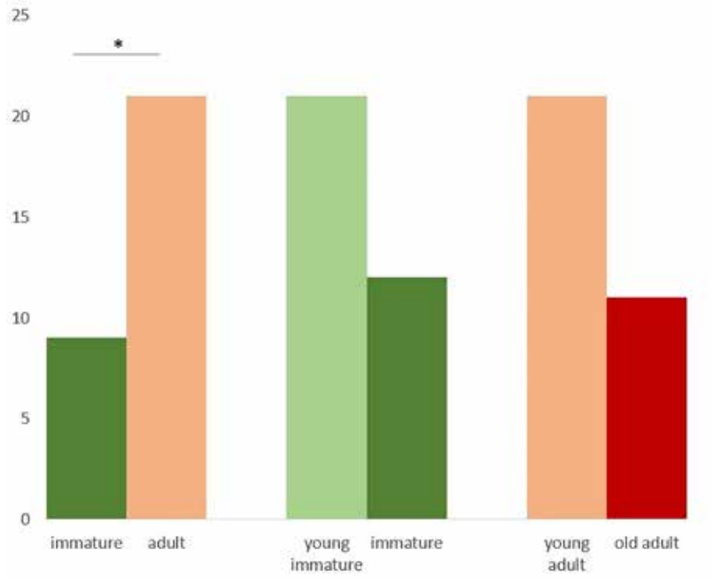

Fig. 2: Comparison of the frequencies of choice in three types of binary choice tests: Immature females within four days of the final moult to adult vs. virgin adult females (N=30); young (recently moulted) immature females vs. immatures within four days of final moult (N=33); and young vs. old adult females (N=32). Adult females were chosen significantly more often than immatures (*, $p<0.05$). All other comparisons were not statistically significant.

adult (YA, 10 to 14 days after the moult to adulthood) and one old adult female (OA, min. 2 months after the moult to adulthood). The readiness to mate in immature females was verified by inspection of female genital area, which changes colour at this stage (Biaggio et al. 2016).

Results: Given a choice between an adult (AF) and immature female (IF), 70% of the males went to adult females (see Figure 2), suggesting the presence of a pheromonal attraction (volatile cue?) either in the adult webs or on the adult females themselves, but lacking in immature females. There was no significant difference in choice of the other combinations: old vs. young adult and young immature (YIF) vs. immature (IF).

Males encountering immature females did not add silk or cut silk, but they vibrated more than with adult females (see Figure 3). By contrast, males that chose adult females engaged in web cutting and removal and laying of silk, both of which are components of courtship behavior. This suggests that males encountering immature females need to test for female aggressiveness by vibrating (distance courtship), and if not attacked by the female, they proceed straight to mating without further courtship. Adult females are choosy and males encountering them engage in lengthy courtship (cutting, laying silk) before being accepted (or rejected) by the female.

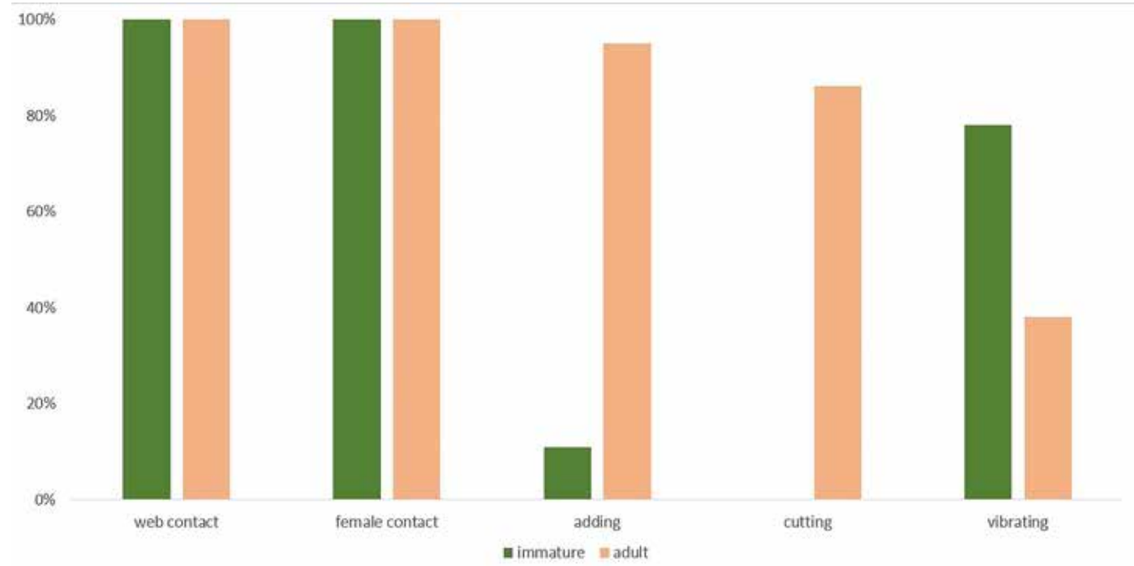

Fig. 3: An example of the percent of occurrences of different male behaviours in 30 trials comparing male response to adult virgin vs. immature females.

The results support the view that immature females (IF) do not advertise their presence, but also do not actively deter males from attempting to mate. By contrast, young immatures (YIF) actively reject the male's approach. Thus, it may be that mating with an immature female is purely opportunistic, contingent on the possibly low probability of a male encountering an IF in the short interval of four days before the final moult. From the female's perspective, however, there remains the unanswered question of benefit vs. cost of mating as an immature. Immature females are not choosy, and males do not court them. In theory, male courtship allows a female to choose the best male, thus females should prefer to mate as adults. We addressed this question by examining whether there are some differences between immature and adult females in sperm storage and in the ability of males to plug the female's genitalia and prevent re-mating.

Morphological analyses

Methods: This part of the study is still in progress. A post-doctoral fellow (Dr. Lenka Sentenska) and a M.Sc. student at the University of Greifswald (Aileen Neumann) are presently engaged in completing the research.

For morphological analyses, female opisthosomata were fixed in Dubosq-Brasil fixation medium. Some of the opisthosomata were prepared for an X-ray microscopy scan (Xradia XCT-200, Carl Zeiss Microscopy GmbH) in

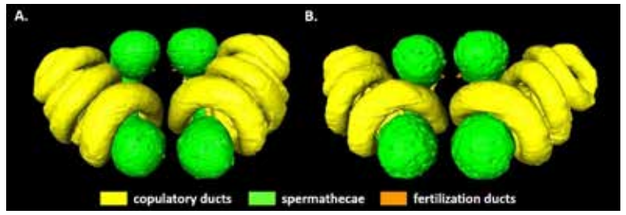

Fig. 4: 3D reconstruction of female genitalia of an (A) immature and (B) adult female of *Latrodectus geometricus* based on 3D X-ray microscopy (ventral view). Note the ‚dumbbell-shaped' spermathecae in green and the copulatory ducts that are very long and coiled around the spermathecae. By contrast, the fertilization ducts, through which the sperm passes to fertilize the eggs in the oviduct, are very short.

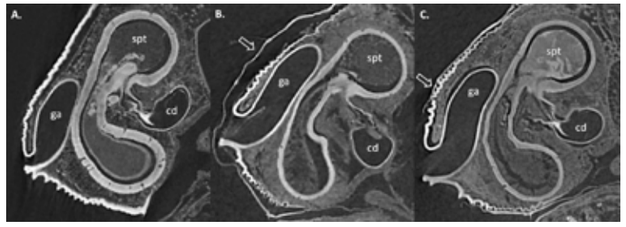

Fig. 5: Virtual longitudinal section through genitalia of an (A) adult, (B) virgin immature and (C) mated immature female of *Latrodectus geometricus*. Note that the cuticle covering the genital opening in the virgin immature female (arrow in B) is ruptured in the immature mated female (arrow in C). cd - copulatory duct; ga - genital atrium; spt - spermatheca.

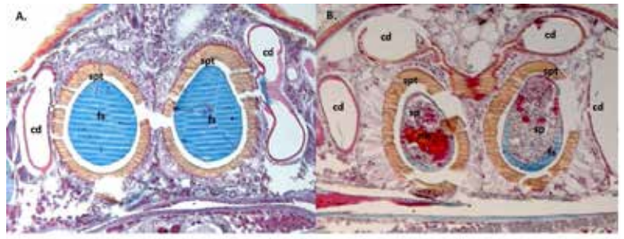

Fig. 6: Histological cross sections through genitalia of a (A) virgin and (B) mated adult female. cd - copulatory duct; fs – female secretion; ms – male secretion; sp – sperm; spt - spermatheca.

the following way: after dehydration, samples were stained overnight using 1% iodine solution (in pure ethanol). After washing in pure ethanol, samples were critical point dried (BalTec 30) and subsequently mounted on insect pins using super glue. Scans were then obtained using the 10x and 4x objective lens. Reconstructed image stacks were created using XMReconstructor software (Carl Zeiss Microscopy GmbH) and the subsequent segmentation (delineation) of the structures of interest in the male and female genitalia was performed with Amira 5.4.5 (Visualization Science Group, FEI). Several opisthosomata were embedded in paraffin and cut into 5µm thick sections and stained with Azan staining.).

Results: There are preliminary results comparing the external and internal genital structures in virgin and mated adult females and immature females. In immature females, the genital structures seem to be fully developed and similar to adults (Figure 4). The only difference from the adult female (Figure 5a) is the presence of cuticle covering the entrance to the genitalia present in the immature female (Figure 5b), but broken in the mated immature female (Figure 5c). Histological sections may reveal other differences.

Histological sections revealed that, apart from their sperm, males transfer a secretion which stains differently (red in Fig. 6B) than the secretion present in female spermathecae before copulation (blue in Figs. 6A and 6B). Sections of genitalia of immature females (IF) before and after mating as an immature will be compared to those of adult virgin and mated females (study in progress).

Conclusions

Precocious genital development in immature females is previously unknown in spiders. While immature females do not advertise their state to attract males, males clearly distinguish them upon contact with their webs. The consequences for the female of mating as an immature remain an open question. Further study of internal genitalic structures of females, and of the fate of sperm stored during the final moult may provide some answers.

Am Kolleg entstandene Veröffentlichungen

Two journal publications are in preparation. Tentative titles are:

1) Immature mating and male choice in a sexually cannibalistic widow spider.
2) Precocious development of genitalia in immature females of the widow spider Latrodectus geometricus.

Two oral presentations were given at scientific conferences:

1) Sentenska et al. Costs and benefits of immature mating in the brown widow spider, Latrodectus geometricus. The 31st European Congress of Arachnology, Vac, Hungary 8-13 July, 2018.
2) Sentenska et al. Immature mating and male mate choice in the cannibalistic brown widow spider, Latrodectus geometricus. 111th Annual Meeting of the German Zoological Society (DZG), Greifswald 10-15 September 2018.