

A glimpse into the sexual biology of the “zygiellid” spider genus *Leviellus*

Simona Kralj-Fišer¹, Matjaž Gregorič¹, Tjaša Lokovšek¹, Tatjana Čelik¹, and Matjaž Kuntner^{1,2,3}: ¹Jovan Hadži Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Novi trg 2, SI-1001 Ljubljana, Slovenia. E-mail: simonakf@gmail.com; ²Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; ³College of Life Sciences, Hubei University, Wuhan, Hubei, China

Abstract. We investigated the mating biology of the previously unstudied central European spider *Leviellus thorelli* (Ausserer 1871) by staging laboratory mating trials using males and females of varying mating histories. Our aim was to seek common themes in sexual behaviors of the sexually size-monomorphic “zygiellid” spiders with their putatively close relatives, araneids and nephilids, which are relatively well studied with respect to sexual biology. We found *L. thorelli* mating biology to more closely resemble that of sexually size-monomorphic araneids than that of dimorphic nephilids. Unlike in nephilids with sexually conflicted adaptations, we found no evidence for genital damage or plugging in *Leviellus* Wunderlich 2004, although we found rare cases of half-eunuchs. We suggest that the mating system of *L. thorelli* spiders is determined by short female sexual attractiveness, reduced receptivity after mating and/or intensive mate guarding.

Keywords: Mating system, genital plugging, mate guarding, sexual-size dimorphism, eunuchs

Sexual conflict theory concerns the idea that males and females may have different goals in reproduction (Watson 1991; Chapman et al. 2003; Arnqvist & Rowe 2005). As a consequence of intersexual conflict, various morphological, physiological and behavioral adaptations have evolved, such as complex genitalia, multiple sperm storage organs, toxicity of seminal fluids, sexual cannibalism, and mate guarding (Parker 1984; Austad 1984; Chapman et al. 1995; Kuntner et al. 2009a; Uhl et al. 2010). These adaptations along with other demographic and ecological factors shape the mating system of a species.

Among invertebrates, spiders represent an especially suitable clade for sexual selection research (Eberhard 2004). In spiders, the prevailing mating strategy may largely be determined by two morphological constraints: genital morphology and delayed female maturation. First, spiders are classified into entelegyne and haplogyne species (Austad 1984; Uhl 2000; Uhl et al. 2010; Kuntner et al. 2009a). Haplogyne species possess a single insemination duct connected to spermathecae exhibiting last-male sperm priority (Austad 1984; Uhl 2000; Uhl et al. 2010). Alternatively, the entelegyne spiders have separate insemination and fertilization ducts connected to spermathecae and overwhelmingly exhibit first-male sperm priority (Austad 1984; Uhl 2000; Uhl et al. 2010). As a consequence, males of many entelegyne species have evolved mechanisms to avoid or reduce sperm competition with rival males by pre- or post-copulatory mate guarding and by the production of mating plugs (reviewed in Uhl et al. 2010). Although these plugs are thought to largely prevent or delay subsequent mating, they are not universally effective even in closely related species, as studies on nephilid spiders have shown [contrast e.g., *Nephila pilipes* (Fabricius 1793), *Nephilengys malabarensis* (Walckenaer 1841) and *Herennia multipuncta* (Doleschall 1859)]: Fromhage et al. 2007; Schneider et al. 2008; Kuntner et al. 2009b; Kralj-Fišer et al. 2011). Besides mechanical plugging of stored sperm and mate guarding, males employ other mechanisms to reduce sperm competition. Such an example is chemical manipulation, where products of male genitalia that are transferred during copulation may induce female resistance for further matings or earlier oviposition (Eberhard 1997).

Further behavioral and physiological adaptations also shape the mating system of a given species. For example, in highly dimorphic species that produce mating plugs, the small males are often cannibalized after copulation (Nessler et al. 2009), either due to intersexual conflict (Arnqvist & Rowe 2005; Fromhage & Schneider 2005) or male sacrifice, which may have a selective advantage in increasing paternity (Elgar & Nash 1988; Andrade 1996; Elgar et al. 2000; Schneider et al. 2000) leading to monogynous mating systems. In some species, males are physiologically limited to one mating (Downes 1978; Michalik et al. 2010) or females are receptive to only one mate (Alcock & Buchmann 1985).

Finally, female maturation in extremely sexually size dimorphic species is usually considerably delayed (Higgins 2000; Kuntner & Coddington 2009; Kuntner et al. 2009b). Along with ecological factors such as the duration of the reproductive season, the operational sex ratio, the female or male distribution and/or the travel costs to the mate (Riechert 1974, 1981; Fromhage et al. 2007, 2008), unsynchronized male and female maturation may substantially constrain an individual's copulation frequency.

Clade-wide comparisons in mating behavior are essential for revealing macroevolutionary patterns of mating strategies; however, some groups remain largely understudied. Here, we investigate a spider clade informally named “Zygiellidae”, which contains temperate and subtropical representatives of several genera exhibiting a moderate sexual-size dimorphism, but diverse entelegyne genital morphologies (M. Gregorič unpublished data). Our ongoing phylogenetic work suggests a close association of the “Zygiellidae” group with the families Nephilidae and Araneidae. Within the former, sexual biology has been well studied in many genera. Many exhibit extreme sexual-size dimorphism and sexual cannibalism, where large females devour tiny males (Kralj-Fišer et al. 2011). In addition, males often engage in genital plugging, genital damage and mate guarding (Schneider et al. 2008; Kuntner et al. 2009a,b). In Araneidae, the sexual biology of most genera remains unstudied, but with some notable exceptions, e.g., *Argiope* (Audouin 1826) with similar sexual phenomena as

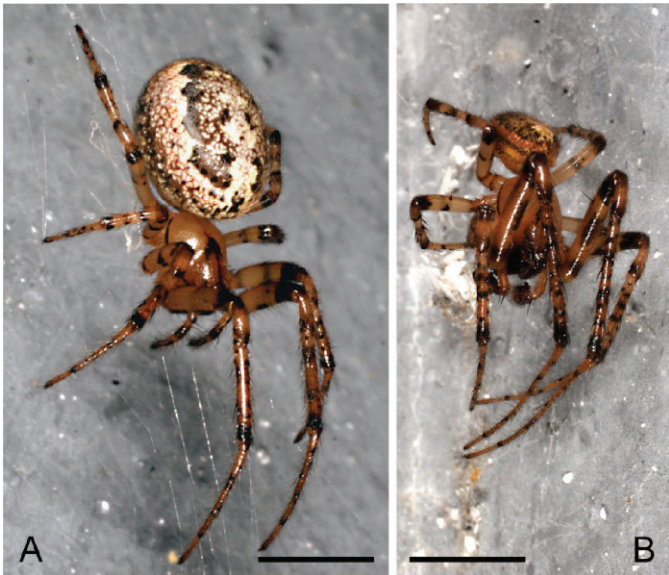


Figure 1.—Female (A) and male (B) of the monomorphic *Leviellus thorelli*. Scale bar = 5 mm.

found in Nephilidae (Fromhage et al. 2003; Foellmer & Fairbairn 2004; Zimmer et al. 2012).

To investigate differences and similarities among the three groups, we studied the sexual biology of a previously unstudied “zygiellid” *Leviellus thorelli* (Ausserer 1871) (Fig. 1). To determine whether the *L. thorelli* mating system is monogamous or polygamous, we collected female and male *L. thorelli* and tested them in staged mating experiments. We measured spider body size to estimate the levels of sexual size dimorphism (SSD), observed male-male competition and determined the occurrence of plugging, genital damage and sexual cannibalism.

METHODS

Study animals.—*Leviellus thorelli* spiders were collected in September and October 2009 on houses near Lukovica, central Slovenia (46°09'43"N, 14°41'30"E). We collected 64 adult spiders (33 females and 31 males) and kept them in the laboratory for behavioral trials. We placed the collected females into glass frames to allow them to build webs, whereas males were kept in foam-covered plastic vials. We watered and fed the spiders twice a week with *Drosophila* flies and mealworms and maintained a seasonal light-dark cycle (16:8).

Experimental protocol.—In staged mating experiments in the laboratory, we observed mating behavior and occurrences of remating with the same genital organ. Mating was staged by placing a male in the female web, approximately 10 cm away from her. We observed male and female pre-copulatory behavior (courtship), which palp (left/right/both) the male inserted, how long and how many times the male inserted each palp, which female copulatory opening (CO; left/right/both) he inserted into, whether the spiders were aggressive and how they behaved after copulation (e.g., mate guarding). Each observation lasted for two hours. After a trial, we gave a spider 1–12 days of rest before testing for remating.

To make inferences about the mating system, we conducted four types of experimental trials, depending on female and

male mating history in the laboratory. We never staged a mating trial between a male and a female that had been previously tested together. In these trials we mated 1) both sexes with unknown mating history [$n = 45$ trials, $n = 64$ spiders (28 individuals that did not mate in their first trial were reused)], 2) previously copulated female and male with unknown mating history (female remating, $n = 10$ trials), 3) female with unknown mating history and a previously copulated male (male remating, $n = 8$ trials), and 4) both male and female previously copulated [female and male remating, $n = 8$ trials (2 males used in Experiment 3 were reused)]. When pairing already mated individuals, we devised pairs in such a way that the male could insert his virgin palp only into the female's used CO (insertions were always ipsilateral). For example, we paired a male with a virgin left palp and a used right palp with a female with a used left CO and a virgin right CO; hence, the virgin palp could be inserted only in the used CO and vice versa. If remating did not occur in two subsequent trials, we concluded that remating with the used genital organ was not possible.

In three trials we placed two males on a female's web to document male-male antagonistic behavior. At the end of all trials, the spiders were euthanized, fixed in 70% ethanol and examined morphologically. Voucher specimens are available from the authors.

Morphological examination.—We examined all specimens from mating trials for genital damage ($n = 64$) and measured their first tibia+patella lengths, carapace width and carapace length ($n = 50$) under a Leica MZ16 stereomicroscope. Following Kuntner & Coddington (2009), sexual-size dimorphism (SSD) is measured as the ratio of female to male body length (or any other size measure).

We macerated all palps in concentrated KOH overnight in order to make them transparent and expandable in distilled water. We excised and examined all epigyna externally, then macerated each epigynal preparation in concentrated KOH overnight, and carefully cleaned it with needles in distilled water (e.g., Kuntner et al. 2009b). This technique exposes the dorsal epigynal anatomy and renders spermathecae translucent, which allows any embolic leftovers lodged inside spermathecae to be seen under a stereomicroscope.

Statistical analyses.—We examined the difference in body size measures between the sexes using the Mann-Whitney U Test. Correlations between size measures were analyzed using the Pearson correlation. We used a Generalized Linear Mixed Model (GLMM) to test the effect of two fixed factors, male and female mating history in the laboratory (previously unmated in the laboratory, previously mated in the laboratory) and carapace length; and a random factor (individual code) on occurrence of copulation (yes, no). We sequentially deleted fixed terms in order of decreasing significance; only terms with $P \leq 0.1$ remained in the final model. We re-entered the excluded terms one by one into the final model to confirm that they did not explain a significant part of the variation. We ran all analyses in PASW Statistics 18 (Field 2005).

RESULTS

SSD.—Patella + tibia I, carapace width and carapace length were significantly correlated (patella + tibia I, carapace width: $r = 0.63$, $n = 50$, $P < 0.001$; patella + tibia I, carapace

length: $r = 0.62$, $n = 50$, $P < 0.001$; carapace width, carapace length $r = 0.71$, $n = 50$, $P < 0.001$). The sexes differed significantly in patella + tibia I length (Mann-Whitney $U = 91$, $P < 0.001$, $n = 50$) but not in carapace length and width (length: Mann-Whitney $U = 254.5$, $P = 0.264$, $n = 50$; width: Mann-Whitney $U = 231.5$, $P = 0.118$, $n = 50$). Using carapace length, SSD in *L. thorelli* was 1.29, which translates to a sexually-size monomorphic species (Kuntner & Coddington 2009).

Mating results.—In all staged mating experiments ($n = 71$), a male signaled a female by pulling or drumming on her web. Typically, he initially remained at the edge of the female's web where he attached silk, created a mating thread, plucked the threads of the female's web with his front legs and rubbed his palps. Eventually he walked on the mating thread toward the female resting in her retreat and sometimes touched her legs with his front legs. Then he retreated and rhythmically plucked and beat the mating thread with his front legs. The male repeated this sequence until the female emerged from her retreat, if receptive. During courtship, the female usually moved her first legs and palps and sometimes her abdomen, and turned toward the male. When (if) the female joined the male, they touched with legs in venter to venter position, then suddenly grasped each other with legs to form a ball-shaped outline (S1.—available online at <http://www.bioone.org/doi/suppl/10.1636/Hi13-08>). The male inserted one of his palps ipsilaterally. After approximately 7 min (mean \pm SE, 6.82 ± 1.35 min, $n = 17$) the female and the male abruptly separated, the male usually hanging on the mating thread, and the female retreating (S1). Then, the female typically rubbed her copulatory openings with the third and fourth legs, whereas the male positioned himself approximately 3–5 cm away from the female, plucked the threads, and rubbed and cleaned his palps. A male always continued to court after copulation, but in no case did the pair copulate again. In most trials, the female was not highly aggressive toward the male during or after copulation, and sexual cannibalism was only observed after one mating (5.9%). In some cases, however, the female and the male were aggressive to each other before the copulation, shaking the web and approaching each other with open chelicerae. In such cases, mating never ensued.

If two males were introduced into the same female web, they assumed an aggressive pose toward each other with front legs extended, shook the web, fought vigorously and chased and bit each other. In all three cases the larger male chased off the smaller one (S2.—available online at <http://www.bioone.org/doi/suppl/10.1636/Hi13-08>).

Of 71 mating trials (Fig. 2), copulation occurred in only 17 cases (23.9%). The occurrence of mating depended on male and female mating history ($F_{95,7,1} = 41.81$; $P < 0.001$). The male and the female copulated in 37.8% ($n = 45$) of the trials when both of them had not previously copulated in our experiments; however, we never observed spiders to copulate in experiments 2, 3 and 4. That is, spiders never remated and reused the genital organ they had previously used ($n = 26$ trials). The random effect was not significant.

Genital damage.—Two mated males ($n = 17$) emasculated one palp to become half-eunuchs (Kuntner et al. 2009b) after separating from the females they had copulated with. We found the damaged palps in the males' vials, implying that

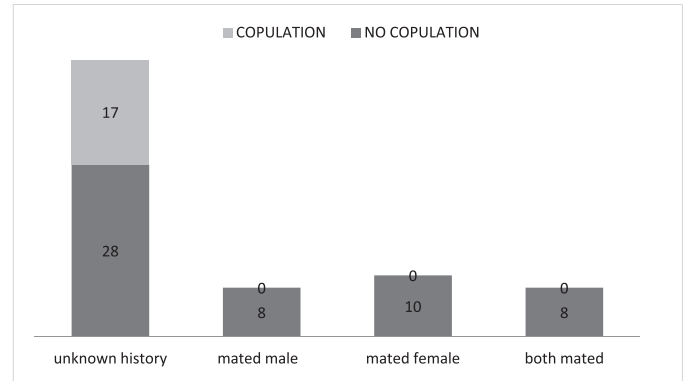


Figure 2.—Copulation success in four different combinations of female and male mating history in the laboratory. Unknown mating history = previously not mated in the lab.

they were not stuck in the female genitalia during copulation but were rather self-removed after mating. Our morphological examination revealed no further damage to male pedipalps ($n = 31$) or any plug formation in female copulatory openings, ducts or spermathecae ($n = 33$).

DISCUSSION

One of our goals was to look for common themes in sexual behaviors of the sexual-size monomorphic “zygiellids” with their close relatives, araneids and nephilids. Copulation behavior of *Leviellus thorelli* resembles that of typical araneid species; males construct and court on a mating thread, with responsive females emerging out of the retreat and copulating with the male in a “hug posture” on the mating thread (Robinson 1982). Similar to other spiders with low levels of SSD, male *L. thorelli* apparently do not damage their genitals obligatorily and do not produce mating plugs, and females exhibit low levels of sexual cannibalism. We found little resemblance to nephilids, where extremely sexual-size dimorphic spiders engage in many ritualistic, sexually conflicted behaviors and strategies (Kuntner 2005, 2006, 2007; Schneider et al. 2005, 2008; Fromhage et al. 2007; Kuntner et al. 2009a, b; Zhang et al. 2011). However, laboratory and field observations of *L. thorelli* indicate intense mate guarding probably due to first-male sperm priority, where males should have reduced fitness benefits when mating with a previously mated female (Austad 1984). Yet, the question of the mating system in *L. thorelli*—and hence questions about macroevolutionary patterns in mating strategies among the three clades—remains open.

Among our aims was to determine the mating system in *L. thorelli*. A male or a female that had previously copulated in the laboratory was never observed remating, which could suggest that both sexes in *L. thorelli* might be either monogamous or at most bigamous. However, we acknowledge here a serious limitation of our study, precluding such definitive conclusion; we collected adult spiders from their natural setting with unknown mating histories, whereas to be conclusive, a study would better rear subadults to ensure virginity. Despite these limitations, the fact is that we never observed polygamy in *L. thorelli*, even though each individual was tested at least twice, with two different potential mates.

Hence, (extreme) polygamy seems unlikely in the system studied.

It is important to note that 60% of pairs failed to mate in the staged experiments. We presume that those spiders had mated before capture. If so, the females that received and stored enough sperm might bias their energy investment in egg production and fertilization, and hence might be sexually unreceptive. It is also likely that females were only receptive during molting and a short period thereafter (e.g., Alcock & Buchmann 1985; Gaskett 2007). Mated or older spider females can be aggressive and exhibit decreased receptivity to subsequent courting males (Elgar 1998), e.g., *Pholcus phalangioides* (Fuesslin 1775) (Schäfer & Uhl 2002), *Argiope keyserlingi* Karsch 1878 (Herberstein et al. 2002) and *Tegenaria atrica* C.L. Koch 1843 (Trabalon et al. 1997).

The alternative/additional explanation for the absence of remating is that males do not find the mated females sexually attractive, as is the case in *Tegenaria atrica* (Trabalon et al. 1997) and *Agelenopsis aperta* (Gertsch 1934) (Papke et al. 2001), both monogamous species that do not produce mating plugs. Male spiders in general prefer virgin over mated females, when females mate only once in several spiders; e.g., *Agelenopsis aperta* (Riechert & Singer 1995). It may vary among species whether a mated male or a female itself reduces female attractiveness. One or more such mechanisms might exist in *L. thorelli*, but this remains to be tested.

Based on our data, we cannot clarify why males did not remate (with the used palp) with a newly introduced female. Research on the closely related *Zygiella x-notata* indicates male choosiness for mates (Bel-Venner et al. 2008; Venner et al. 2010), where only 3% of guarding males switched to another female (Bel-Venner & Venner 2006). Although prolonged tandems during the reproductive season are known to reduce sperm competition and to lower sexual harassment of a mated female (Greenfield & Coffelt 1983; Schöfl & Taborsky 2002), it would be worth studying if and what mechanisms cause *L. thorelli* pairs to persist together in nature, or even to remain monogamous after separation. A phenomenon of prolonged tandems may relate to why no *L. thorelli* males use both palps during mating. In the field and laboratory, we observed that the male persists with the female for a long period with recurrent courting phases. Hence, it is possible that males use both palps with the same female, but over a longer episode than the observed two-hour trial in the laboratory.

Our results show no evidence for genital plugging, but we recorded two cases of male *L. thorelli* becoming eunuchs by severing their palps subsequent to mating. This resembles the eunuch behavior of *Herennia Thorell* 1877 (Kuntner 2005; Kuntner et al. 2009b), but not that of other nephilids where males leave a palp in the female genital tract (Kuntner et al. 2009c; Kralj-Fišer et al. 2011; Li et al. 2012), nor that of *Tidarren* Chamberlin & Ivie 1934 where the single-palped male spontaneously dies while copulating and thus functions as a whole-body mating plug (Knoflach & van Harten 2001). Although the eunuch's behavior in *Leviellus* is clearly not obligate, it may nevertheless be suggestive of some level of post-mating sterility in males.

In conclusion, *L. thorelli* sexual biology resembles that of araneids with low SSD and not that of nephilids, which exhibit

pronounced SSD. Although our data require further corroboration with lab-reared spiders, they suggest that the mating system of *L. thorelli* spiders is shaped by a short period of female sexual attractiveness and/or reduced receptivity after mating and intensive mate guarding.

ACKNOWLEDGMENTS

We thank Eva and Irena Kuntner and Cene Fišer for logistic help, Jutta Schneider for comments on the early manuscript version, and Martin Marzidovšek for making available the video on male antagonism. This work was funded by the Slovenian Research Agency (grant J12063 to M. Kuntner).

LITERATURE CITED

- Alcock, J. & S.L. Buchmann. 1985. The significance of post-insemination display by male *Centris pallida* (Hymenoptera, Anthophoridae). *Zeitschrift für Tierpsychologie—Journal of Comparative Ethology* 68:231–243.
- Andrade, M.C.B. 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science* 271:70–72.
- Arnqvist, G. & L. Rowe. 2005. *Sexual Conflict*. Princeton University Press, Princeton, New Jersey.
- Austad, S.N. 1984. Evolution of sperm priority patterns in spiders. Pp. 223–251. *In* *Sperm Competition and the Evolution of Animal Mating Systems*. (R.L. Smith, ed.). Academic Press, Orlando, Florida.
- Bel-Venner, M.C., S. Dray, D. Allaine, F. Menu & S. Venner. 2008. Unexpected male choosiness for mates in a spider. *Proceedings of the Royal Society B—Biological Sciences* 275:77–82.
- Bel-Venner, M.C. & S. Venner. 2006. Mate-guarding strategies and male competitive ability in an orb webspider: results from a field study. *Animal Behaviour* 71:1315–1322.
- Chapman, T., G. Arnqvist, J. Bangham & L. Rowe. 2003. Sexual conflict. *Trends in Ecology & Evolution* 18:41–47.
- Chapman, T., L.F. Liddle, J.M. Kalb, M.F. Wolfner & L. Partridge. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* 373:241–244.
- Downes, J.A. 1978. Feeding and mating in insectivorous Ceratopogoninae (Diptera). *Memoirs of the Entomological Society of Canada*. Entomological Society of Canada, Ottawa, Ontario.
- Eberhard, W.G. 1997. Sexual selection by cryptic female choice in insects and arachnids. Pp. 32–57. *In* *The Evolution of Mating Systems in Insects and Arachnids*. (J.C. Choe & B.J. Crespi, eds.). Cambridge University Press, Cambridge, UK.
- Eberhard, W.G. 2004. Why study spider sex: Special traits of spiders facilitate studies of sperm competition and cryptic female choice. *Journal of Arachnology* 32:545–556.
- Elgar, M.A. 1998. Sperm competition and sexual selection in spiders and other arachnids. Pp. 307–339. *In* *Sperm Competition and Sexual Selection*. (T.R. Birkhead & A.P. Möller, eds.). Academic Press, San Diego, California.
- Elgar, M.A. & D.R. Nash. 1988. Sexual cannibalism in the garden spider *Araneus diadematus*. *Animal Behaviour* 36:1511–1517.
- Elgar, M.A., J.M. Schneider & M.E. Herberstein. 2000. Female control of paternity in the sexually cannibalistic spider *Argiope keyserlingi*. *Proceedings of the Royal Society of London Series B—Biological Sciences* 267:2439–2443.
- Field, A. (ed.). 2005. *Discovering statistics using SPSS*. Second edition. Sage Publications, London.
- Foellmer, M.W. & D.J. Fairbairn. 2004. Males under attack: sexual cannibalism and its consequences for male morphology and behavior in an orb webspider. *Evolutionary Ecology Research* 6:163–181.

- Fromhage, L., K. Jacobs & J.M. Schneider. 2007. Monogynous mating behaviour and its ecological basis in the golden orb spider *Nephila fenestrata*. *Ethology* 113:813–820.
- Fromhage, L., J.M. McNamara & A.I. Houston. 2008. A model for the evolutionary maintenance of monogyny in spiders. *Journal of Theoretical Biology* 250:524–531.
- Fromhage, L. & J.M. Schneider. 2005. Safer sex with feeding females: sexual conflict in a cannibalistic spider. *Behavioral Ecology* 16:377–382.
- Fromhage, L., G. Uhl & J.M. Schneider. 2003. Fitness consequences of sexual cannibalism in female *Argiope bruennichi*. *Behavioral Ecology and Sociobiology* 55:60–64.
- Gaskett, A.C. 2007. Spider sex pheromones: emission, reception, structures, and functions. *Biological Reviews* 82:27–48.
- Greenfield, M.D. & J.A. Coffelt. 1983. Reproductive behaviour of the lesser waxmoth, *Achroia grisella* (Pyralidae: Galleriinae): signalling, pair formation, male interactions and mate guarding. *Behaviour* 84:287–315.
- Herberstein, M.E., J.M. Schneider & M.A. Elgar. 2002. Costs of courtship and mating in a sexually cannibalistic orb-web spider: female mating strategies and their consequences for males. *Behavioral Ecology and Sociobiology* 51:440–446.
- Higgins, L. 2000. The interaction of season length and development time alters size at maturity. *Oecologia* 122:51–59.
- Knoflach, B. & A. van Harten. 2001. *Tidarren argo* sp. nov. (Araneae: Theridiidae) and its exceptional copulatory behaviour: emasculation, male palpal organ as a mating plug and sexual cannibalism. *Journal of Zoology* 254:449–459.
- Kralj-Fišer, S., M. Gregorič, S. Zhang & M. Kuntner. 2011. Eunuchs are better fighters. *Animal Behaviour* 81:933–939.
- Kuntner, M. 2005. A revision of *Herennia* (Araneae: Nephilidae: Nephilinae), the Australasian ‘coin spiders’. *Invertebrate Systematics* 19:391–436.
- Kuntner, M. 2006. Phylogenetic systematics of the Gondwanan nephilid spider lineage Clitaetrinae (Araneae, Nephilidae). *Zoologica Scripta* 35:19–62.
- Kuntner, M. 2007. A monograph of *Nephilengys*, the pantropical ‘hermit spiders’ (Araneae, Nephilidae, Nephilinae). *Systematic Entomology* 32:95–135.
- Kuntner, M. & J.A. Coddington. 2009. Discovery of the largest orbweaving spider species: The evolution of gigantism in *Nephila*. *PLoS ONE* 4(10):e7516.
- Kuntner, M., J.A. Coddington & J.M. Schneider. 2009a. Intersexual arms race? Genital coevolution in nephilid spiders (Araneae, Nephilidae). *Evolution* 63:1451–1463.
- Kuntner, M., S. Kralj-Fišer, J.M. Schneider & D. Li. 2009b. Mate plugging via genital mutilation in nephilid spiders: an evolutionary hypothesis. *Journal of Zoology* 277:257–266.
- Kuntner, M., I. Agnarsson & M. Gregorič. 2009c. Nephilid spider eunuch phenomenon induced by female or rival male aggressiveness. *Journal of Arachnology* 37:266–271.
- Li, D., J. Oh, S. Kralj-Fišer & M. Kuntner. 2012. Remote copulation: male adaptation to female cannibalism. *Biology Letters* 8:512–515.
- Michalik, P., B. Knoflach, K. Thaler & G. Alberti. 2010. Live for the moment—Adaptations in the male genital system of a sexually cannibalistic spider (Theridiidae, Araneae). *Tissue & Cell* 42:32–36.
- Nessler, S., G. Uhl & J.M. Schneider. 2009. Sexual cannibalism facilitates genital damage in *Argiope lobata* (Araneae: Araneidae). *Behavioral Ecology and Sociobiology* 63:355–362.
- Papke, M.D., S.E. Riechert & S. Schulz. 2001. An airborne female pheromone associated with male attraction and courtship in a desert spider. *Animal Behaviour* 61:877–886.
- Parker, G.A. 1984. Sperm competition and the evolution of animal mating strategies. Pp. 1–60. *In* Sperm Competition and the Evolution of Animal Mating Systems. (R.L. Smith, ed.). Academic Press, Orlando, Florida.
- Riechert, S.E. 1974. Pattern of local web distribution in a desert spider—mechanisms and seasonal variation. *Journal of Animal Ecology* 43:733–746.
- Riechert, S.E. 1981. The consequences of being territorial spiders, a case study. *American Naturalist* 117:871–892.
- Riechert, S.E. & F.D. Singer. 1995. Investigation of potential male mate choice in a monogamous spider. *Animal Behaviour* 49:715–723.
- Robinson, M.H. 1982. Courtship and mating behavior in spiders. *Annual Review of Entomology* 27:1–20.
- Schäfer, M.A. & G. Uhl. 2002. Determinants of paternity success in the spider *Pholcus phalangioides* (Pholcidae: Araneae): the role of male and female mating behaviour. *Behavioral Ecology and Sociobiology* 51:368–377.
- Schofl, G. & M. Taborsky. 2002. Prolonged tandem formation in firebugs (*Pyrrhocoris apterus*) serves mate guarding. *Behavioral Ecology and Sociobiology* 54:426–433.
- Schneider, J.M., L. Fromhage & G. Uhl. 2005. Copulation patterns in the golden orb-web spider *Nephila madagascariensis*. *Journal of Ethology* 23:51–55.
- Schneider, J.M., M.E. Herberstein, M.J. Bruce, M.M. Kasumovic, M.L. Thomas & M.A. Elgar. 2008. Male copulation frequency, sperm competition and genital damage in the golden orb-web spider (*Nephila plumipes*). *Australian Journal of Zoology* 56:233–238.
- Schneider, J.M., M.E. Herberstein, F.C. De Crespigny, S. Ramamurthy & M.A. Elgar. 2000. Sperm competition and small size advantage for males of the golden orb-web spider *Nephila edulis*. *Journal of Evolutionary Biology* 13:939–946.
- Trabalon, M., A.G. Bagnères & C. Roland. 1997. Contact sex signals in two sympatric spider species, *Tegenaria domestica* and *Tegenaria pagana*. *Journal of Chemical Ecology* 23:747–758.
- Uhl, G. 2000. Female genital morphology and sperm priority patterns in spiders (Araneae). Pp. 145–156. *In* European Arachnology 2000. (S. Toft & N. Scharff, eds.). Aarhus University Press, Aarhus.
- Uhl, G., S. Nessler & J. Schneider. 2010. Securing paternity in spiders? A review on occurrence and effects of mating plugs and male genital mutilation. *Genetica* 138:75–104.
- Venner, S., C. Bernstein, M. Dray & M.C. Bel-Venner. 2010. Make love not war: When should less competitive males choose low quality but defendable females? *American Naturalist* 175:650–661.
- Watson, P.J. 1991. Multiple paternity and first mate sperm precedence in the Sierra Dome spider, *Linyphia litigiosa* Keyserling (Linyphiidae). *Animal Behaviour* 41:135–148.
- Zhang, S., M. Kuntner & D. Li. 2011. Mate binding: male adaptation to sexual conflict in the golden orb-web spider (Nephilidae: *Nephila pilipes*). *Animal Behaviour* 82:1299–1304.
- Zimmer, S.M., K.W. Welke & J.M. Schneider. 2012. Determinants of natural mating success in the cannibalistic orb-web spider *Argiope bruennichi*. *PLoS One* 7:e31389.