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Proc. R. Soc. B published online 29 April 2009
doi: 10.1098/rspb.2009.0104

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The spider *Harpactea sadistica*: co-evolution of traumatic insemination and complex female genital morphology in spiders

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The males of invertebrates from a few phyla, including arthropods, have been reported to practise traumatic insemination (TI; i.e. injecting sperm by using the copulatory organ to penetrate the female's body wall). As all previously reported arthropod examples have been insects, there is considerable interest in whether TI might have evolved independently in other arthropods. The research reported here demonstrates the first case of TI in the arthropod subphylum Chelicerata, in particular how the genital morphology and mating behaviour of *Harpactea sadistica* (Řezáč 2008), a spider from Israel, has become adapted specifically for reproduction based on TI. Males have needle-like intromittent organs and females have atrophied spermathecae. In other spiders, eggs are fertilized simultaneously with oviposition, but the eggs of *H. sadistica* are fertilized in the ovaries (internal fertilization) and develop as embryos before being laid. Sperm-storage organs of phylogenetically basal groups to *H. sadistica* provide males with last male sperm priority and allow removal of sperm by males that mate later, suggesting that TI might have evolved as an adaptive strategy to circumvent an unfavourable structure of the sperm-storage organs, allowing the first male to mate with paternity advantage. Understanding the functional significance of TI gives us insight into factors underlying the evolution of the genital and sperm-storage morphology in spiders.

Keywords: hemocoelic insemination; hypodermic insemination; mating behaviour; sexual coercion; sperm competition; sexual selection

1. INTRODUCTION

Traumatic (also called hypodermic or hemocoelic) insemination (TI), an anomalous mode of copulation in which the male penetrates the female's body wall with his copulatory organ to inject sperm into her body cavity (Carayon 1966), may be of two types. Intra-genitalic TI is cryptic because, although the male inserts his copulatory organ into the female's genitalia, he then injects sperm by puncturing the inner wall of the genitalia while extra-genitalic TI is more transparent because the male penetrates the female's body wall at some location away from her genitalia. TI has been documented especially in several groups of aquatic invertebrates (Eberhard 1985). However, it is also found among terrestrial animals, namely in a few insect orders. A recent first report of intra-genitalic TI in *Drosophila* (Kamimura 2007), one of the most well-studied of all the insects, suggests that intra-genitalic TI might be more prevalent in insects than currently appreciated. Examples of extra-genitalic TI come from only the orders Strepsiptera (Beani *et al.* 2005), Lepidoptera and Heteroptera. All heteropteran examples are from the infraorder Cimicomorpha (Tatarnic *et al.* 2006), perhaps the best known example being the bed bug, *Cimex lectularius* (Stutt & Siva-Jothy 2001). The lepidopteran examples are the adults of tent-caterpillar species in the genus *Malacosoma*, these being insects practising extra-genitalic TI facultatively (Bieman & Witter 1982).

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsob.2009.0104> or via <http://rsob.royalsocietypublishing.org>.

There is a striking absence of documented examples of TI from the Chelicerata, this being the other major arthropod subphylum. Chelicerates include, among others, scorpions, spiders and mites and, in the vast majority of chelicerates, spermatophores are used by males to inseminate females (Alexander & Ewer 1957). However, a few of the chelicerate orders have evolved copulatory organs with which the male injects the sperm directly into the female's genitalia. The best known example is the spiders. Male spiders have two secondary copulatory organs ('bulbi'), one on the distal end of each pedipalp. Before mating, males prepare for sperm transfer by first extruding a drop of semen from the primary genital opening onto a small web and then filling the bulbi from the drop (Foelix 1982). Numerous bizarre adaptations are known in spiders for males to maximize fitness, thus spiders provide an interesting taxon to examine the evolution of extreme mating tactics. These range from common practices such as mate guarding and the use of sperm plugs, to self-sacrifice during copulation (Andrade 1996) and spontaneous death after copulation (Foellmer & Fairbairn 2003). During copulation, the male inserts his embolus (the intromittent part of the bulb) into the female's genital organ (vulva), which is on the ventral side of her anterior abdomen, and injects sperm from there into spermathecae inside the female's abdomen (Foelix 1982).

The present study provides the first example of a spider that departs from this basic style of mating. *Harpactea sadistica* Řezáč, 2008 (Araneae: Dysderidae) is a spider that practises extra-genitalic TI. Its genital morphology

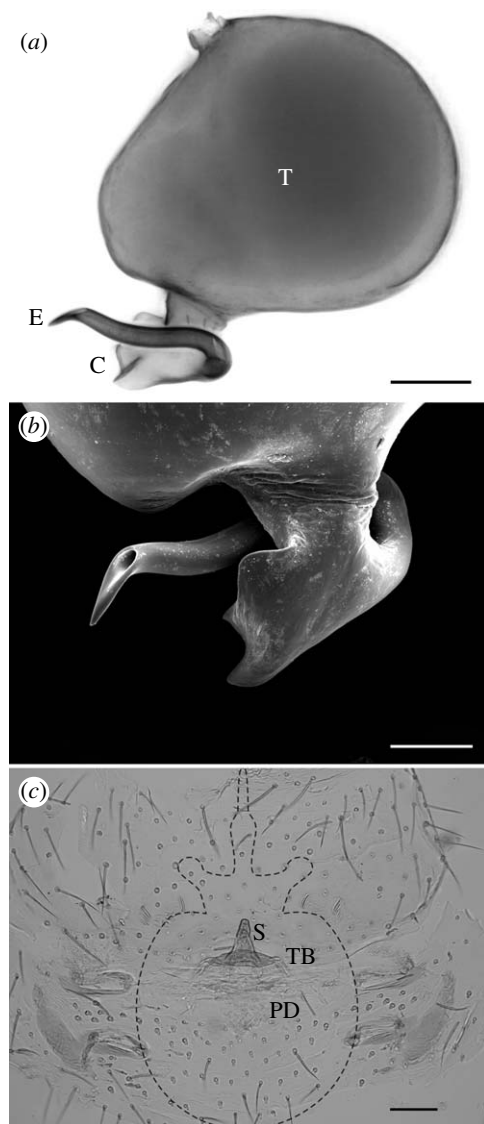


Figure 1. Copulatory organs of *H. sadistica*. (a) Right male copulatory organ. C, conductor (hooking part); E, embolus (intromittent part); T, tegulum (proximal part with reservoir for sperm). Scale bar, 0.1 mm. (b) Detail of the left embolus. Scale bar, 0.05 mm. (c) Atrophied female copulatory organ. PD, posterior diverticulum; S, spermatheca TB, transverse band. Dashed line shows the relative size of functional sperm-storage organs in a morphologically similar species *Harpactea herodias* (Brignoli 1978). Scale bar, 0.1 mm.

and its mating behaviour both show evidence of specialization in the context of TI, and these findings suggest implications for a more general understanding of the evolution of spider genital morphology.

2. MATERIAL AND METHODS

The specimens used in this study were collected in April 2004 and March 2007 from the Adulam Nature Reserve near Jerusalem in Israel (Řezáč 2008). Each specimen was put singly inside a closed vial (diameter 15 mm, length 90 mm) with a perforated plastic plug on the top and, inside, a leaf for substrate and moistened cotton wool for humidity. The vials were kept at room temperature and the spiders were fed vinegar flies (*Drosophila melanogaster*). After 2 weeks, the spiders were paired in small glass tubes (diameter 4 mm, length 45 mm, closed with cotton wool). Subsequent mating behaviour (14 mating sequences performed by different pairs)

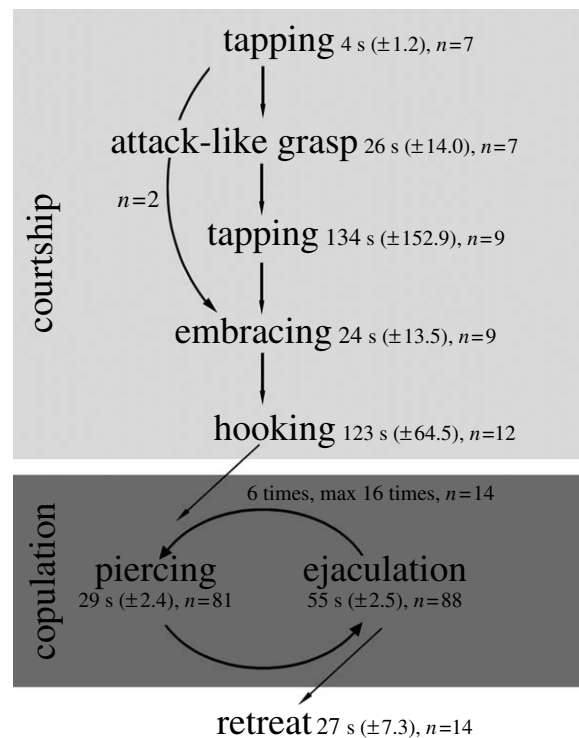


Figure 2. The sequence of mating events of *H. sadistica*. Means of duration of particular events and standard errors are indicated. In a few cases the courtship was missed; therefore, the number of observations (n) of courtship is lower than that for copulation. The total length of the mating sequence took on average 878 s ($n=14$), with 306 s ($n=10$) spent in courtship and 629 s ($n=14$) in copulation.

was video-recorded using a charge-coupled device camera attached to a stereomicroscope. Each mated female produced egg sacs.

For morphological detail, male copulatory organs were separated, dried at room temperature, mounted on a stub, sputter-coated with gold and examined using a scanning electron microscope. Female genitalia of alcohol preserved specimens were dissected, cleaned using 5 per cent KOH and observed under a light microscope.

3. RESULTS

(a) Genital morphology

The bulbus of *H. sadistica* (figure 1a) is a single sclerite. The ejaculate is stored in the proximal part of the bulbus (tegulum). The distal part, which is used for sperm transfer, consists of a needle-shaped intromittent organ (embolus, figure 1b) and a hook-shaped lobe (conductor).

It is characteristic of the genus *Harpactea* for females to have two types of ‘cul-de-sac’ sperm-storage organs (a sclerotized rod-shaped spermatheca and a spherical membranous posterior diverticulum). In *H. sadistica* females (figure 1c), however, both sperm-storage organs are very small and apparently degenerate (spermatheca only slightly sclerotized, no cavity for sperm storage present; posterior diverticulum is hardly visible).

(b) Mating behaviour

Mating sequences (figure 2) began by the male tapping and embracing the female with his forelegs and then suddenly grasping the female’s abdomen and inserting his cheliceral fangs into her integument (video S1 in the

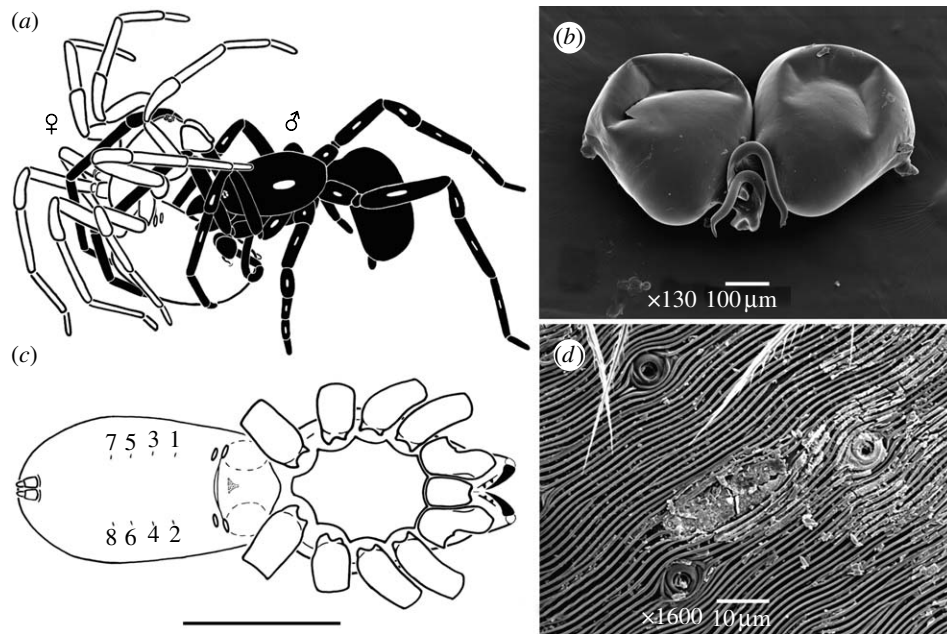


Figure 3. The copulation of *H. sadistica*. (a) Mating position. (b) Arrangement of male copulatory organs during penetration of female body wall. The conductor of the bulbus (to the right in the picture) is hooked to the basal part of the embolus of the left bulbus. In this way the left embolus is stabilized for penetration of female integument. (c) The position of insertions on the ventral side of the female abdomen and their sequence (1–8). Scale bar, 1 mm. (d) The dash-shaped wound after insertion of the embolus.

electronic supplementary material). The female usually became passive and the male moved beneath her prosoma and, in this position, he anchored the tarsi of his first two pair of legs onto the dorsal side of female's abdomen, bringing his pedipalps with copulatory organs closer to the ventral side of the female's abdomen (figure 3a). The male then hooked the conductor of one bulbus on to the basal part of the embolus of the other bulbus ('fixed the embolus'; figure 3b, video S2 in the electronic supplementary material) and next placed the tip of the anchored embolus on the ventro-lateral side of the female's abdomen. Rotating his anchored pedipalp, the male pierced the female's integument and inserted his embolus entirely into her abdomen (video S3 in the electronic supplementary material), after which the male stopped the rotary movements and ejaculated. After ejaculation, the male retracted his embolus and inserted his other embolus into the opposite ventro-lateral side of the female's abdomen. The mean number of insertions per male was 6 with regular alternation between the two copulatory organs. Between insertions, the male moved from the anterior to the posterior of the female abdomen, particularly in the area between the vulva and the spinnerets (figure 3c). No haemolymph was observed leaking from the female's wounds. Males were never seen inserting the embolus into the female genital organ. Males retreated from the female immediately after the last embolus insertion. The total length of the mating sequence was $15 \text{ min} \pm 1.7 \text{ min}$ (mean $\pm$ s.e.), with $5 \pm 1.9 \text{ min}$ spent in courtship and $10 \pm 1.6 \text{ min}$ in TI (figure 2). After mating, round fang wounds and dash-shaped embolus scars (figure 3d) were visible on the female's cuticle and the female, after first passing the legs through her mouthparts, wiped her wounds using the apical segments of her hind legs. Males and females both tended to copulate repeatedly, accepting a second mate as soon as one day later. Up to one month after copulating, the female usually produced an egg sac, but with the eggs

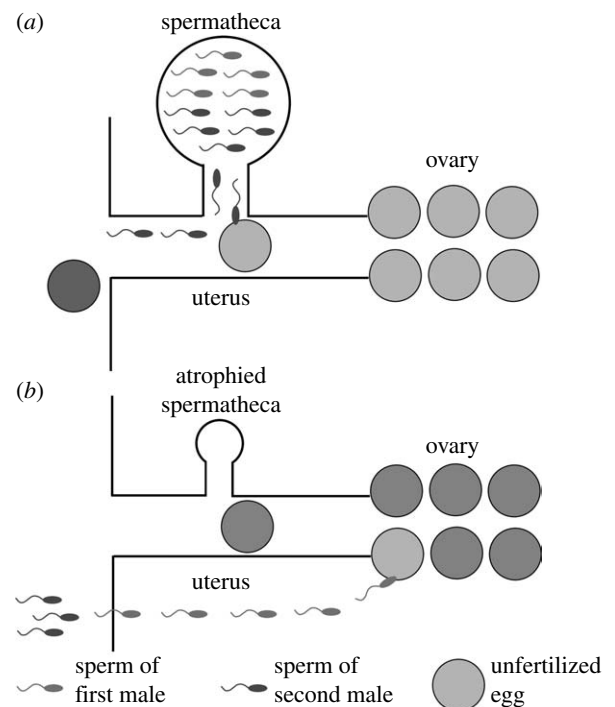


Figure 4. A schematic of two types of female genitalia with hypothetical sperm route. (a) 'Cul-de-sac' type representing all dysderid spiders (including *Harpactea*) with 'normal' insemination. (b) Atrophied type in *H. sadistica*.

already having undergone embryonic development to the morula stage (about 100 cells).

4. DISCUSSION

All dysderid spiders studied adopt the same copulatory posture of *H. sadistica* (e.g. Jackson & Pollard 1982; Gerhardt 1933), but in no other species is it known for males to pierce the female's body with their cheliceral fangs during courtship or to inject sperm by piercing the

female's body wall. Furthermore, the genitalia of both the male and the female of *H. sadistica* are modified in ways that are compatible with TI. The other species of the genus *Harpactea* usually possess emboli which lack a terminal point (e.g. Chatzaki & Arnedo 2006). The needle-like structure of the male's embolus functions in penetrating the female body wall, and the organs that females of other dysderid species would use for sperm acceptance and storage have atrophied in the female of *H. sadistica*. Apparently TI is obligate in this species. Other spiders fertilize eggs in the uterus by moving sperm from spermathecae shortly before oviposition (Foelix 1982), but *H. sadistica* eggs are fertilized in the ovaries (internal fertilization) and the female lays embryos instead of single-cell eggs. The eggs of spiders bulge out from the ovaries into the body cavity (Foelix 1982), and this might facilitate the finding and fertilizing of the eggs by spermatozoa travelling through the female's body cavity.

Females of dysderid spiders have cul-de-sac spermathecae (i.e. a single duct serves as both inlet and outlet for sperm) and the expectation for species with cul-de-sac spermathecae is that the last male to inseminate the female will tend to have his sperm used first to fertilize the eggs (Austad 1984; figure 4a). Moreover, when females have cul-de-sac spermathecae, the sperm from males that mate with the female first are vulnerable to removal by males that mate later (Huber & Eberhard 1997). Among dysderid species, it is common for females to mate readily with different males (M. Řezáč 2007, unpublished data), an inevitable consequence of this being strong competition among males for paternity. By practising TI, *H. sadistica* males seem to have reversed the last-male sperm priority pattern, i.e. for *H. sadistica*, the sperm from the first male to mate via TI should reach the eggs first (figure 4b) and are protected from removal. There are three mutually exclusive hypotheses that might potentially explain the evolution of TI:

Hypothesis 1. Sexual selection, particularly sperm competition (*sensu* Parker 1970), underlies the evolution of TI, which might have evolved as an adaptive strategy by which first males circumvent an unfavourable structure of the female's sperm-storage organs. In other words, the males partly wrest control of fertilization from females. Nevertheless, intersexual conflict of interests should not be considered responsible for the evolution of this male trait as the primary selective force is the competition for paternity, while subduing female interests is its consequence. This hypothesis could be tested by studying the pattern of paternity in *H. sadistica* and in its close relatives. It predicts that in *H. sadistica* first males will be fathers of higher percentage of offspring than in relatives practising the conventional mode of fertilization. The sperm competition hypothesis may also help explain the evolution of TI in other groups of arthropods (Cimicomorpha, Strepsiptera, *Malacosoma* and *Drosophila*), as the out-group of each of these also has cul-de-sac spermathecae (Snodgrass 1935).

Hypothesis 2. TI could have evolved under natural selection. Internal fertilization leading to vivipary could be advantageous if juveniles laid as embryos possess higher survivorship than juveniles laid as eggs. But the female might secure internal fertilization more easily than via TI. This hypothesis could be tested by studying the survivorship of juveniles of *H. sadistica* and juveniles of its close

relatives. It predicts that juveniles of *H. sadistica* will exhibit higher survivorship than juveniles of oviparous relatives.

Hypothesis 3. TI might also have evolved through female selection for good genes. For polyandrous females, it might be advantageous to create an inhospitable environment that allows only the most vigorous sperm to fertilize their eggs. In such a race among females to create the most difficult arena for sperm competition, they could have reduced the genitalia and sperm storage organs. TI could have been the male solution for where to deposit the sperm. The same process could be explained by intersexual conflict over control of mating and fertilization which, however, rests on specific assumptions, that are poorly known. This hypothesis could be tested by comparative study of female genitalia in the genus *Harpactea*. It predicts that there is tendency to reduce female genitalia in this genus.

The following paragraphs further elaborate the hypothesis 1 based on sperm competition. The evolution of TI may have started in species where sperm occasionally escaped from the female's genital tract and travelled through the haemocoel to her ovaries, as is known to happen in some mite species (Alberti 2002). Further evolutionary steps could be represented by species where the males regularly injure the female genital organ during copulation in order to help sperm to escape and where males directly inject sperm into the coelom through the inner wall of the female's genitalia (intra-genitalic TI), as is known for *Drosophila* (Kamimura 2007). This requires sperm mobility and the female immune system to co-evolve for sperm to reach the eggs by moving through the female's haemocoel. These adaptations might, in turn, have added to the advantage for the male in shortcutting the route and injecting sperm outside the female genitalic region, closer to the ovaries. Hence, in groups which have acquired adaptations related to hemocoelic insemination, the evolution of extra-genitalic TI might be expected. In particular, this hypothesis might explain the multiple independent emergence of extra-genitalic TI in the heteropteran group Cimicomorpha (Tatarnic *et al.* 2006).

Injuries inflicted by males through TI might incur a significant cost to females through physical damage and the risk of infection (see Reinhardt *et al.* 2005). Therefore, one would expect that females would have evolved some defensive adaptations. Such adaptations were not found. Avoiding males that practise TI would be disadvantageous, as sons of females mated with males bearing the TI trait should themselves be more successful in circumventing the structure of the female spermathecae and thus obtaining fertilizations (in accordance with the theory of sexy sons). If the health costs outweighed the profit from good genes, females would probably eliminate this male strategy. Possible protecting adaptations (e.g. integument preventing haemolymph loss) would also be advantageous for the male because they protect his offspring. Females of other arthropods appear to have mitigated TI-derived costs by evolving secondary genitalic structures (Siva-Jothy 2006). In some *Drosophila* species, secondary spermathecae evolved in places where males of related species damage the genitalic wall (Kamimura 2007). In the Cimicidae secondary sperm induction pores and secondary ducts leading to primary genitalia have evolved (Carayon 1966). Similar secondary structures occur in butterflies (ditrysian genitalia; Grimaldi & Engel 2005)

and in spiders (secondary spermathecae or entelegyne genitalia, both of which have evolved independently several times; Brignoli 1978; Forster 1980). The evolution of these features has been heretofore difficult to explain. Yet it is of interest that, in both spiders and butterflies, there appears to be preadaptation of sperm to travel through the female's haemocoel *en route* to fertilizing her eggs. Perhaps the secondary genitalic structures of butterflies and spiders could have originated via TI as well. Instead of being merely a zoological curiosity, TI may be more common among arthropods than previously thought and it may have been an important driving force in evolution of arthropod genitalia.

I thank especially R. R. Jackson for extensive linguistic help with the manuscript, and Y. Lubin, S. Pekár, P. Michalik, P. Cushing, J. Král, T. Bilde and T. Russell-Smith for comments on the manuscript. Specimens were collected by permission of Y. Lubin (25018). This work was supported by grant no. 206/09P521 from the Czech Science Foundation.

REFERENCES

- Alberti, G. 2002 Ultrastructural investigations of sperm and genital systems in Gamasida (Acari: Anactinotrichida) current state and perspectives for future research. *Acarologia* **42**, 107–126.
- Alexander, A. J. & Ewer, D. W. 1957 On the origin of mating behavior in spiders. *Am. Nat.* **91**, 311–317. (doi:10.1086/281992)
- Andrade, M. C. B. 1996 Sexual selection for male sacrifice in the Australian redback spider. *Science* **271**, 70–72. (doi:10.1126/science.271.5245.70)
- Austad, S. N. 1984 Evolution of sperm priority patterns in spiders. In *Sperm competition and the evolution of mating systems* (ed. R. L. Smith), pp. 223–249. Cambridge, MA: Harvard University Press.
- Beani, L., Giusti, F., Mereati, D., Lupetti, P., Paccagnini, E., Turrilazi, S. & Dallai, R. 2005 Mating of *Xenos vesparum* (Rossi) (Strepsiptera, Insecta) revisited. *Ž. Morphol.* **265**, 291–303. (doi:10.1002/jmor.10359)
- Bieman, D. N. & Witter, J. A. 1982 Mating wounds in *Malacosoma*: an insight into bed bug mating behavior. *Fla. Entomol.* **63**, 377–378. (doi:10.2307/3494314)
- Brignoli, P. M. 1978 Some remarks on the relationships between the Haplogynae, the Semientelegynae and the Cribellatae (Araneae). *Symp. Zool. Soc. Lond.* **42**, 285–292.
- Carayon, J. 1966 Traumatic insemination and the paragenital system. In *Monograph of Cimicidae* (ed. R. L. Usinger), pp. 81–166. Philadelphia, PA: Entomological Society of America, Thomas Say Foundation.
- Chatzaki, M. & Arnedo, M. A. 2006 Taxonomic revision of the epigean representatives of the spider subfamily Harpactinae (Araneae: Dysderidae) on the island of Crete. *Zootaxa* **1169**, 1–32.
- Eberhard, W. G. 1985 *Sexual selection and animal genitalia*. Cambridge, MA; London, UK: Harvard University Press.
- Foelix, R. F. 1982 *Biology of spiders*. Cambridge, MA: Harvard University Press.
- Foellmer, M. W. & Fairbairn, D. J. 2003 Spontaneous male death during copulation in an orb-weaving spider. *Proc. R. Soc. Lond. B* **270**, 183–185. (doi:10.1098/rsbl.2003.0042)
- Forster, R. R. 1980 Evolution of the tarsal organ, the respiratory system and the female genitalia in spiders. In *Proceedings. 8. Internationaler Arachnologen-Kongress* (ed. J. Gruber), pp. 269–284. Vienna, Austria: H. Egermann.
- Gerhardt, U. 1933 Neue Untersuchungen zur Sexualbiologie der Spinnen. Insbesondere an Arten der Mittelmeerländer und der Tropen [New survey of sexual biology of spiders. Especially the species from the Mediterranean and the tropics]. *Zeitschrift Morphol. Ökol. Tiere* **27**, 11–75. [In German.]
- Grimaldi, D. & Engel, M. S. 2005 *Evolution of the insects*. Cambridge, UK: Cambridge University Press.
- Huber, B. A. & Eberhard, W. G. 1997 Courtship, copulation, and genital mechanics in *Physocylus globosus* (Araneae, Pholcidae). *Can. J. Zool.* **74**, 905–918. (doi:10.1139/z97-109)
- Jackson, R. R. & Pollard, S. D. 1982 The biology of *Dysdera crocata* (Araneae, Dysderidae): intraspecific interactions. *J. Zool. Lond.* **198**, 197–214.
- Kamimura, Y. 2007 Twin intromittent organs of *Drosophila* for traumatic insemination. *Biol. Lett.* **3**, 401–404. (doi:10.1098/rsbl.2007.0192)
- Parker, G. A. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567. (doi:10.1111/j.1469-185X.1970.tb01176.x)
- Reinhardt, K., Naylor, R. A. & Siva-Jothy, M. T. 2005 Potential sexual transmission of environmental microbes in a traumatically inseminating insect. *Ecol. Entomol.* **30**, 607–611. (doi:10.1111/j.0307-6946.2005.00730.x)
- Řezáč, M. 2008 Description of *Harpactea sadistica* n. sp. (Araneae: Dysderidae)—a haplogyne spider with reduced female genitalia. *Zootaxa* **1698**, 65–68.
- Siva-Jothy, M. T. 2006 Trauma, disease and collateral damage: conflict in cimicids. *Phil. Trans. R. Soc. B* **361**, 269–275. (doi:10.1098/rstb.2005.1789)
- Snodgrass, R. E. 1935 *Principles of insect morphology*. New York, NY; London, UK: McGraw-Hill.
- Stutt, A. D. & Siva-Jothy, M. T. 2001 Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*. *Proc. Natl Acad. Sci. USA* **98**, 5683–5687. (doi:10.1073/pnas.101440698)
- Tatarnic, N. J., Cassis, G. & Hochuli, D. F. 2006 Traumatic insemination in the plant bug genus *Coridromius* Signoret (Heteroptera: Miridae). *Biol. Lett.* **2**, 58–61. (doi:10.1098/rsbl.2005.0394)