**SHORT COMMUNICATION**

**Female attack is not necessary for male copulatory organ breakage in the sexually cannibalistic spider *Argiope argentata* (Araneae: Araneidae)**

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**Abstract.** In sexually cannibalistic spiders, males usually only copulate with one female. This selects for male strategies to improve paternity success in their single mate. Male mating strategies can include genital damage during female attack in some cannibalistic orb-weaving spiders, where males are dwarf and females polyandrous. We explored whether sexual cannibalism is necessary for male genital damage in the silver spider *Argiope argentata* (Fabricius 1775) by performing mating trials with recently killed virgin females. We found that males can break off their copulatory organs without female intervention and spontaneously die during copulation. Results suggest that genital damage evolved in response to sperm competition in this species.

**Keywords:** Genital damage, sperm competition, mating plug

Sexual cannibalism, defined as instances where females kill and consume conspecific males before, during, or after copulation, has been considered an extreme case of conflict of interest between the sexes (Elgar 1992). Males benefit by fertilizing more eggs, while females can benefit by remating (Simmons 2005), leading to sexual antagonistic coevolution (Arnqvist & Rowe 2005). Sexual cannibalism has been reported for a variety of invertebrates, including crustaceans, insects, and arachnids, and is particularly frequent among spiders (Elgar 1992). If males transfer sperm successfully, then sexual cannibalism may be part of a male mating strategy (Elgar & Schneider 2004). In these cases, males maximize their paternity in an act of single mating, becoming monogamous. Such terminal investment without parental care has been shown to evolve under a male-biased effective sex ratio with high risk of sperm competition (Fromhage et al. 2005).

In a framework of high sperm competition, males will develop offensive and defensive strategies to protect paternity, including the use of mating plugs. Mating plugs can be substances that become hard while occluding genital ducts (Baer et al. 2001; Polak et al. 2001; Aisenberg & Eberhard 2009), or parts of the entire male copulatory organ, a process known as genital damage (Eberhard 1985; Kamimura 2003; Uhl et al. 2010). Genital damage is widespread among sexually cannibalistic spiders, where males usually break off parts of the entire copulatory organ during copulation (Andrade 1996; Andrade & Banta 2002; Elgar & Schneider 2004; Foellmer & Fairbairn 2004; Miller 2007; Nessler et al. 2008). Male spiders’ copulatory organs are paired (palp bulbs) and the intromittent features, the emboli, are introduced into the paired female genital openings during mating, usually one at a time. In spiders, all known cases of genital damage occur in entelegyne spiders in which the genitalia are sclerotized and the ovipository duct is independent from the copulatory ducts, and therefore not occluded by mating plugs (Uhl et al. 2010).

In spiders, paternity success is usually linked to copulation duration and sperm transfer (Elgar 1995). Schneider et al. (2006) and Nessler et al. (2006) suggested the occurrence of a sexual conflict over copulation duration in the orb-web spider *Argiope bruennichi* (Scopoli 1772), where female attack occurs precisely when the male dislodges the used copulatory organ and tries to insert the other one (Schneider et al. 2006). In the orb-web spider *Nephila plumipes* (Latreille 1804), palp organ breakage increases male survivorship, allowing a second insertion and increased copulation duration, whereas males that do not break their organs are cannibalized (Schneider et al. 2001). In *Argiope lobata* (Pallas 1772), cannibalized males break off their genital copulatory organs more frequently than surviving males, suggesting that sexual cannibalism facilitates genital damage (Nessler et al. 2008).

The silver spider *Argiope argentata* (Fabricius 1775) is an araneid spider with Pan-American distribution (Levi 1968). In the field, Robinson & Robinson (1980) observed that males arrive on females’ webs, court from the periphery, and afterwards move to the hub of the web where mating usually occurs, and sexual cannibalism always occurs during or after copulation. In the laboratory, virgin females are usually receptive to courting males, but they attack them during the first insertion, forcibly dislodging males from their genitalia with their third pair of legs, resulting in 70% of the males dying (Ghione 2008). Surviving males reinitiate courtship and perform a second insertion; after that they are consumed by the females. Males always break the apical region of the embolus, including a singular sclerite or spur of unknown function (Levi 1968), that remains stuck inside the female ducts (Ghione et al. 2006; Ghione 2008).

In the present study, we used freshly killed females to explore experimentally if males can break off their copulatory organs by themselves, or if female cannibalistic attack determines male genital damage. We hypothesize that the female’s attack has a direct impact on the copulatory outcome, both in the removal of the male and the breaking off of the inserted copulatory organ.

We collected nine subadult males, 11 subadult females, and 18 adult males of *A. argentata* between September to March (spring and summer of 2005–2006 and 2006–2007), in meadows at Piedras de Atilar, Canelones, Uruguay (34°45′42.5″S and 55°33′10.8″W), a temperate region. We housed spiders individually in 500-ml glass containers, providing water daily, and *Tenebrio sp.* larvae (Tenebrionidae) twice per week.

In order to determine if the male can break off his copulatory organs by himself or if it is the female that breaks them off when she abruptly removes the male from her genitalia, we carried out experiments with recently killed virgin females. We killed them by means of hypothermia, placing them at a low temperature for 20 minutes. Afterwards, dead females were carefully attached ‘face down’ (typical ‘sit and wait’ and mating posture) to their own web’s radii, fixed by adding melted paraffin onto each of their eight leg tarsi. Once females were fixed to the web in the proper position, we
carefully removed silk with forceps from their spinnerets and added it to the hub or center of the web, as naturally occurs when females are positioned in the web.

For each trial, two adult males were carefully placed equidistantly from the margin of the orb-web of a recently attached killed female. We placed two males to increase the likelihood of copulation. We simulated female behaviors observed in a sexual context (Ghione, pers. obs.) by softly shaking the web, using a pencil to prod the female’s corpse, in response to male courtship. We replaced males when a male did not court during a 15-min period, a male courted from the periphery but did not move to the hub within one hour, or a male initiated courtship but did not proceed to copulation over the course of two hours. This exchange of unsuccessful males continued until either copulation occurred or a total of 5 hours had elapsed, limited by the rapid decay of the dead female in warm experimental conditions. When one male achieved copulation, the other male was immediately (but carefully) removed from the web in order to avoid male-male interferences.

We performed a total of 11 trials and obtained four successful copulations. The occurrence of copulations was highly unpredictable.

Figure 1.—a) Dead male attached to female genitalia in *Argiope argentata*; b) the same male inserting the right palpal organ and showing the left one with the broken embolus tip (arrow).
and difficult. Male genital damage was determined under a dissecting microscope. We deposited voucher specimens in the arachnological collection of Facultad de Ciencias, Montevideo, Uruguay.

In all the 11 trials, males courted the females and responded to the simulations of female sexual behavior. All copulating males died immediately after copulation. Three males performed two palpal insertions. They jumped off the female epigynum a few seconds after their first insertion and escaped from the female web, but immediately returned and courted again. After their second insertion, all three died spontaneously, remaining attached to female genitalia. One male died spontaneously after performing a single palpal insertion. We did not know the exact time of death, but death was confirmed after we proceeded to carefully touch unmoving males (in mating position) with a candy pin after an arbitrary period of 30 minutes of immobility. Each of the three males that performed two insertions broke off the first inserted organ and the embolus tip remained inside the female reproductive tract. The second inserted copulatory organs were not broken off due to males dying and remained connected to female epigynum (Fig. 1).

Results indicate that males spontaneously die in copula, evidenced by the absence of female intervention, as was observed for other Argiope species (A. aurantia [Lucas 1833]; Foellmer & Fairbairn 2003; A. semita [Walckenaer 1841]; Sasaki & Iwahashi 1995; A. keyserlingi Karsch 1878; Herberstein et al. 2005; A. bruennichi: Schneider et al. 2006). In entelegnye spiders, the hematodocha expands during copulation, allowing the penetration of the embolus into the female genital tract (Foelix 1996). In A. argentata, the expansion of the haematodocha probably requires sequestration of a large percentage of hemolymph from body circulation, provoking males’ deaths. The single male which died after his first insertion could possibly have mated previously in the field. However, this male was previously examined under the dissecting microscope, and possessed both intromittant organs intact. Therefore, a previous mating in the wild of this individual is improbable.

Three males were able to disengage and break off their copulatory organs without female intervention, contrary to our prediction. Our results confirm that males alone engage in genital autotomy; female action during cannibalism is not required. This is the first demonstration of voluntary emasculation by males during copulation in Argiope species. Each male of A. argentata did not dislodge himself from the epigynum after the second insertion and died firmly attached to female genitalia, suggesting that the entire male body could act as a whole-body mating plug, as was stated by Foellmer & Fairbairn (2003) for Argiope aurantia. In A. argentata, the expanded copulatory organ could continue ejaculating seminal fluids after the male’s death, as was indicated by Knoflach & van Harten (2001) for theridiid spiders. Interestingly, males would be impeded from remaining attached to the genitalia if the female was alive.

In the present study, we found that males of A. argentata can voluntary break off their genital organs, suggesting that there is no obligate relationship between genital damage and sexual cannibalism in this species. This suggests that sperm competition would be the sexual selection mechanism that underlies this particular behavior of voluntary genital mutilation. Male monogyny has been stated to evolve under a male-biased effective sex ratio (Fromhage et al. 2005), favoring extreme male strategies to ensure paternity. Nevertheless, an increased sample size and experiments with other modifications interfering with sexual cannibalism would help to confirm this hypothesis in this spider.

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