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SHORT COMMUNICATION

Are multiple copulations harmful? Damage to male pedipalps in the funnel-web wolf spider *Aglaoctenus lagotis* (Araneae: Lycosidae)

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Abstract. Damage to genital structures during copulation has been reported in about twenty spider families, but never in Lycosidae. *Aglaoctenus lagotis* (Holmberg, 1876), a South American wolf spider, is one of the few that live their whole lives in funnel-webs. This work reports on the damage to pedipalpal bulbs observed in males of the "southern Uruguay" form of *A. lagotis* with multiple copulations. Observed damage consists of the irreversible expansion of the hematodocha and even its explosion. A high copulation cost resulting from multiple and long-lasting copulations could be causing this damage, affecting the mechanism of sperm transference in a definitive way. This is the first report of pedipalp damage in lycosids, and also the first report of damage involving the hematodocha in spiders.

Keywords: Copulation cost, expansion, explosion, hematodocha, re-copulation

Damage to genital structures during copulation has been reported in species from about twenty spider families (Uhl et al. 2010), of the 113 families currently known (World Spider Catalog 2017). These structures are almost always from the male (but see Nakata 2016), and consist of pieces of the embolus or the entire pedipalp that the male leaves attached to or inside the female's genitalia, forming a mating plug (Uhl et al. 2014). These plugs are usually associated with a monogynous mating system (Uhl et al. 2010). Among the spiders of the family Lycosidae, genital damage during mating has never been reported (Fernández-Montraveta & Ortega 1990; Norton & Uetz 2005; Jiao et al. 2011; Fernández-Montraveta & Cuadrado 2013). And, although studies reporting the number of copulations that males can achieve (and their associated costs) are scarce, polygamy is likely the most widespread mating system (Huber 2005).

Aglaoctenus lagotis (Holmberg, 1876) is one of the few lycosid spiders that, instead of having the characteristic wandering habit of the family, lives its whole life in funnel-webs (Santos & Brescovit 2001). Only males leave their webs, in order to search for mates (Sordi 1996). The geographic distribution of the species is Neotropical, from Uruguay to Venezuela, although there is a historical taxonomic controversy that questions whether this is a single species (Santos & Brescovit 2001; González et al. 2015). In fact, two "forms" of *A. lagotis,* differing in sexual behaviour, body coloration patterns, and phenology, are currently reported and have been suggested as different species (González et al. 2015). One difference between the two forms is copulation duration: long copulations (averaging 60 minutes) in the "southern Uruguay" form (SU form) and short copulations (averaging 8 minutes) in the "central Argentina" form (CA form) (González et al. 2013). Other studies related to the reproductive biology of the species are scarce (Stefani et al. 2011; González et al. 2015), and there are no reports regarding mating systems or the functioning of sexual structures during mating.

During observations performed to describe the sexual behavior of the species, I had occasionally observed a male of the SU form with a lax, white membrane, the hematodocha, hanging from one of his pedipalpal bulbs (henceforth referred to as "bulb") after copulation. Therefore, I decided to quantify the occurrence of this pedipalp damage in males of this form of *A. lagotis* under laboratory conditions. As I have not found previous references regarding damage in spiders involving the hematodocha, or data about genital damage in the lycosids, this would be the first report about them.

Forty-eight subadult individuals of *A. lagotis* were collected in Piedras de Afilar, Canelones, Uruguay (34°43′44″ S, 55°30′46″ W) during March and April 2016. Spiders were individually maintained in Petri dishes (9.5 cm diam. x 1.5 cm height), with cotton moistened with water. Individuals were fed two times a week with mealworms (*Tenebrio molitor*, Coleoptera, Tenebrionidae) and fruit flies (*Drosophila melanogaster,* Diptera, Drosophilidae), until reaching adulthood. Room temperature during the breeding and trials period averaged 21.6 ± 3.6 °C (mean ± SD).

I exposed seven virgin males to consecutive randomly assigned virgin females and checked male palpal bulbs after each sexual encounter. Each male was exposed to a new female every three days. Copulations and their characteristics were recorded with a Sony DCR-SR85 digital video camera. Bulb observations were performed under an Olympus Stereoscopic microscope with a recessed digital camera. Males were exposed to females until they did not court for two consecutive trials. Experimental trials were carried out in glass cages (length 30 cm x width 16 cm x height 20 cm) following previous work with the species (e.g., González et al. 2013). I placed a layer of 2 cm of sand and 2 cm of wood-chips as substrate, simulating leaf litter, and Y-shaped small plant branches were added as refuge and for web support. Encounters were promoted on female's webs, so I placed each virgin female in the arena five days before the trial to allow funnel-web construction. Males were carefully introduced into the margin of each web and removed after 30 min if there was no courtship, after 60 min if males courted but did not copulate, or after the end of copulation. Experimental males were used between 10–15 days after reaching adulthood; females were at least 10 days of adult age. Females were not reused. Cephalothorax width, a common measure of body size in spiders (Eberhard et al. 1998), and body weight of individuals were measured. Voucher specimens were deposited in the arachnological collection of Sección Entomología, Facultad de Ciencias, Montevideo, Uruguay.

In three of the seven males evaluated (43%), damage appeared in bulbs during successive sexual exposures (Table 1); irreversible expansions of the hematodocha were observed in two cases (males A and D) and an explosion of the hematodocha (male F) from the left bulb in another case (Fig. 1). The three cases of irreversible damage were observed after the second, fourth and fifth copulation, respectively (Table 1). The explosion of the hematodocha was accompanied by the spill of transparent drops (coming from the same bulb), probably hemolymph (see video S1, Supplemental

Table 1.—Copulations characteristics and body measurements of the experimental males.

Male	N° copulations	Cephalothorax width (mm)	Body weight (g)	Pedipalp damage	Attacks (by females)
A	2	4.7	0.203	yes	yes
B	2	4.7	0.205	no	yes
C	2	4.9	0.251	no	yes
D	4	4.7	0.224	yes	no
E	4	4.9	0.256	no	no
F	6	4.6	0.207	yes	yes
G	6	5.1	0.277	no	no

material, online at http://dx.doi.org/10.1636/JoA-S-17-017.s1, and video caption S2, online at http://dx.doi.org/10.1636/JoA-S-17-017.s2). The damage was always more accentuated in one of the male bulbs than in the other, generating asymmetries in the number of ejaculations performed, but males continued inserting both pedipalps until the end of copulation. I had already observed that the males with four and five copulations had brought their pedipalps to the mouth constantly after their previous copulation, and that their hematodochae had remained somewhat expanded even after the copulation was finished, returning to the resting position few minutes later. After the occurrence of two events of irreversible damage, females attacked males at the dismounting. However, attacks (always during dismounting) were also recorded in two other males that did not damage their bulbs (Table 1). Despite the small sample size, it is worth noting that males that achieved several copulations but did not damage their bulbs were larger and heavier to those which incurred damage.

The present study suggests that the occurrence of damage in bulbs of the males of the SU form of *A. lagotis* would occur when they copulate multiple times (lycosids always used both pedipalps during copulation). No male damaged his bulbs during the first copulation. Additionally, damage would be more frequent in smaller, lighter weight males. Therefore, considering all mentioned above, and the fact that copulations last an hour on average (longer than in the other form of the species and other lycosids, González et al. 2013), I suggest that the alteration in the functioning of the bulbs is produced by a high copulation cost, associated with extreme fatigue in pedipalps use (Rovner & Wright 1975), and that individuals with better body condition are better able to cope with this stress.

Reported events of damage in male bulbs are usually related to their functions as mating plugs, and involve parts of the embolus (Fromhage & Schneider 2006), the whole pedipalp (Ramírez & González 1999) or the entire body of the male (Andrade 1996; Foellmer & Fairbairn 2003), but no hematodochae damage, as happens in *A. lagotis*. Furthermore, within the Lycosidae family, reports of plugs are scarce (Kronestedt 1987; Szinetár et al. 2005) and do not involve parts of the body, but substances adhering to the females' epigynes. As the only two reports are based on collection data collection, is difficult to know how the plugs were produced.

I have not found references to the type of pedipalp damage reported here for other spiders. I have also not found reports about the relation between genital damage and polygyny (but see Lynam et al. 2006). Among the few species of lycosids for which there are data regarding male mating systems, *Schizocosa ocreata* (Hentz, 1844) exhibits polygamous males and long copulations (155 min on average) (Norton & Uetz 2005), but pedipalp damage is not observed. Pedipalp damage is also absent in the CA form of *A. lagotis,* although males copulate with several females (a greater number than in the SU form studied here) (Peretti et al. 2016). However, copulations are significantly shorter (8 min) (González et al. 2013), perhaps with less energetic costs per copulation and associated to a different sexual strategy, related to populations density. Finally, the experimental design employed here probably favored males' copulations, even with over enforced bulbs, as males were repeatedly exposed to virgin females, but the same procedure has been followed for the CA form without producing similar damage.

Studying the copulatory mechanism and functional morphology of *A. lagotis* pedipalps during copulation, as well as female receptivity and the amount of sperm in their spermathechae after copulating with damaged and undamaged males, will be of interest to better understand the implications of the present report. Also, expanding research on the sexual history of males to additional families will clarify how widespread this type of genital damage is in spiders.

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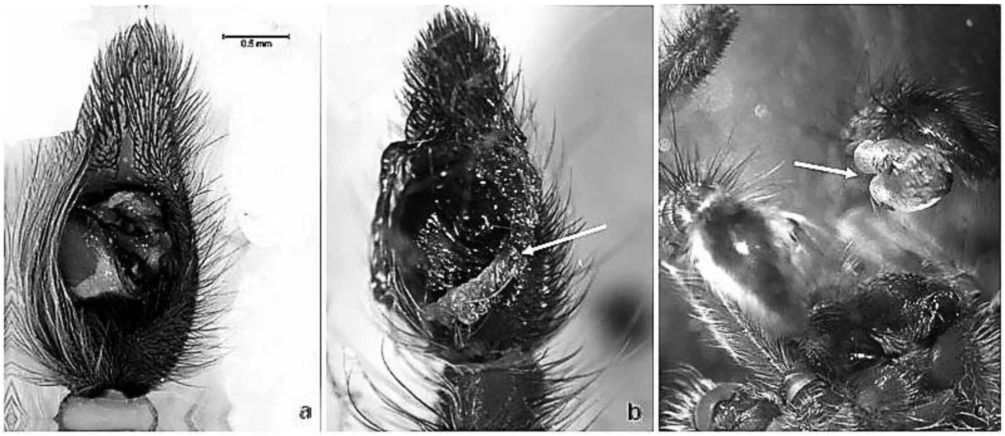

Figure 1.—Bulbs of the SU form of *A. lagotis* with damage found after copulations. (a) Normal bulb in the resting position; (b) Bulb observed with the hematodocha torn and loose ("exploded bulb"); (c) Pedipalps of a male with the left bulb irreversibly expanded.

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