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SHORT COMMUNICATION

Loading the male pedipalps: sperm induction in a subsocial spider

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Abstract. Detailed descriptions of sperm induction are rare in the literature. *Anelosimus vierae* Agnarsson, 2012 (Theridiidae) is a Uruguayan subsocial spider that lives in communal nests. This paper describes the sperm induction of this species under laboratory conditions. We collected spiders in Montevideo, Uruguay, from different trees and placed them in thirty male and female pairs in Petri dishes to observe the occurrence of sperm induction. We witnessed twenty-two males performing sperm induction after copulation. Sperm web characteristics and description of the male´s behavior during sperm induction are described.

Keywords: *Anelosimus vierae*, sperm web building

Spiders have a variety of reproductive strategies with particular characteristics making the m an excellent model for the study of sexual selection (Eberhard 2004). Disconnection between the male pedipalps (copulatory organ) and the sexual glands is a feature unique among spiders. As a consequence, the male must build a sperm web and load his pedipalps with sperm before encountering the female. The sperm web varies in size and shape depending on the family of spider (Foelix 2011). The male deposits a droplet of semen on the web and fills his pedipalps (Foelix 2011). In most Araneomorph spiders the sperm web is a small structure. It may be reduced to a single thread (e.g., that of *Scytodes* Latreille, 1804). Alternatively, it could have a triangular or trapezoidal shape (e.g., *Tetragnatha* Latreille, 1804 and *Theridion* Walckenaer, 1805, respectively), with the induction process taking little time. In contrast, theraphosid spiders and the Araneomorph spider *Kukulcania hibernalis* (Hentz, 1842) build relatively large sperm webs and the entire induction process may take several hours, making it easier to observe (Costa & Pérez-Miles 2002; Barrantes & Ramírez 2013).

Although sperm induction is common, reports in the literature are relatively scarce, from descriptions of sperm webs (Domínguez & Jiménez 2005) and partial observations of the induction process (Fraser 1987), to single observations of the entire process (Levi 1967; Jackson & Macnab 1991). Detailed and rigorous descriptions of the induction process are needed to compare among different species so we can increase our knowledge of this important process.

Anelosimus vierae Agnarsson, 2012 (Theridiidae) is a Uruguayan subsocial spider. Individuals live in communal nests in evergreen trees. The mother cares for young, and the juveniles show a lack of conspecific aggression. Males mature earlier and consistently disperse, resulting in inbreeding avoidance, while females mature asynchronously and may or may not disperse from the natal nest (Viera et al. 2007). Males court females using vibrations induced by plucking silk threads and by touching the female until she adopts the copulation posture with the ventral side upwards, body axis approximately inclined 45° and facing down, and copulation occurs (Albo et al. 2007). Copulation lasts one hour on average (Viera & Albo 2008). During mating trials, we observed several incidents of males performing sperm induction after copulation. Here we describe the sperm induction process of *A. vierae*, including sperm web construction, sperm emergence and sperm induction itself, under laboratory conditions.

Juveniles of *A. vierae* were collected from several nests of different trees in Montevideo, Uruguay (34°54′48″S, 56°10′2″W) during June 2014. In the laboratory, individuals were bred, isolated in plastic Petri dishes (5.5 cm diameter x 1.5 cm height) and maintained with moistened cotton wool. All spiders were fed *ad libitum* with *Drosophila* spp. (Diptera). Individuals were checked daily under a stereo microscope (Nikkon type 102; 10 x ocular) and the recently molted females were housed in smaller Petri dishes (3.3 cm diameter x 1.0 cm height), where the subsequent observations took place.

After 48 h of molting, each virgin adult female was exposed to a male from a different nest. Thirty trials were followed using a video camera (Sony DCRA-C151, 2.7″ Wide Hybrid LCD, Handycam Station Supplied, 12 x optical zoom, 800 x digital zoom) during 2 h. When an individual did not engage in sexual behavior during the first 15 min or mating did not occur during the first 30 min, the trial was excluded. Each individual was used only once. We described the units of male sperm web construction, sperm emergence and sperm induction, and recorded durations and frequency. The sperm web was photographed and the area of it was measured using an image processing and analysis program (ImageJ). Voucher specimens were deposited in the Entomology Collection of the Faculty de Sciences, Montevideo, Uruguay.

Twenty-eight of the thirty trials resulted in mating. In 22 of these trials, males performed sperm induction after copulation. The latency post-mating was 22.33 ± 7.10 min. No male performed sperm induction just before mating.

The males, which were suspended with the ventral side up in the females' webs, built a scaffolding of the sperm web consisting of two nearly parallel threads located at each lateral side of the abdomen and connected to the female's web. After that, males placed their legs II and IV on the parallel threads and positioned legs III where the bridge between parallel threads would be built. Legs I remained on the female web, and males began to lay a swath of threads between the parallel threads using the following movements: (1) laid a line with the end of the abdomen (spinnerets) on one of the parallel threads near the prosoma end; (2) lifted the abdomen and first put it on the center area of the same thread, then on the most distal end in relation to the prosoma; (3) repeated the same abdominal movements on the other parallel thread; (4) once again lifted the abdomen, laying a line on the first parallel thread near the first foothold, and repeating this abdominal sequence a total of 19.64 ± 3.00 times (Fig. 1). The end result was a horizontal hourglass-shaped web (Fig. 2). The process of

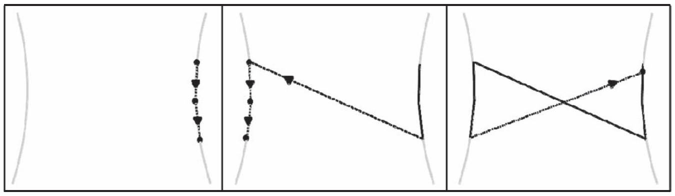

Figure 1.—Sperm web construction sequence. The threads (dotted and solid black lines) are laid between the scaffolding (gray lines). This sequence is repeated several times. The black points are the places where the silk threads are attached.

sperm web building lasted on average 36.64 ± 9.18 s. The mean area of the sperm web was 0.28 ± 0.11 mm^2.

Once the sperm web was finished, males deposited sperm. They made small legs flexions at the femur-patella articulation, with higher intensity of leg IV, and moved their abdomens up and down. They then continued the up-and-down abdominal movements, increasing the frequency and diminishing the amplitude without bending the legs, until a drop of sperm emerged and was later deposited on the thin middle portion of the sperm web. This process lasted 35.32 ± 11.09 s on average.

After sperm deposition, males moved backwards, holding the sperm web with their legs in the same position so the pedipalps could reach the sperm drop. The drop was deposited on the upper side of the web and was absorbed through to the same side with fast vibratory movements alternating between one pedipalp and then the other. This was done 10.45 ± 3.42 times on average (Fig. 2). The time they spent with each pedipalp on the sperm drop increased as the behavior progressed. Most males withdrew most of the sperm after approximately 3.28 ± 1.04 min, except in two cases where males left part of the sperm in their sperm web. The entire process of sperm induction (sperm web building + sperm drop + filling up the pedipalps) took an average of 4.47 ± 1.38 min. When induction was completed some males placed their pedipalps into their mouthparts and shook their abdomens once or twice but they did not consume the sperm web. (To view video of sperm web construction, sperm drop deposition, and loading the palps, see http://dx.doi.org/10.1636/J15-57.s1)

Because sperm induction is essential for successful copulation, it is surprising that there are few studies describing it in detail. In this species, this behavior always took place shortly after copulation.

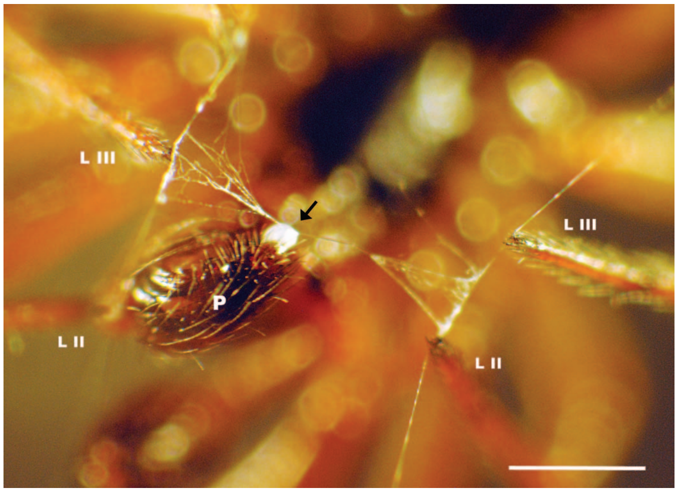

Figure 2.—Male loading one of his pedipalps with sperm from the sperm web. P: pedipalp; L II: leg II; L III: leg III. The arrow points to the sperm drop. Scale = 0.5 mm.

The small size of the sperm web in *A. vierae*, as in most araneomorph spiders (Foelix 2011), represents a low cost in time and effort, in contrast to mygalomorph spiders, which build a large sperm mat involving a significant cost for males (Costa & Pérez-Miles 2002). Also, the short induction duration of *A. vierae* implies low costs relative to most other spider species, in which many minutes, but usually less than half an hour, are required to perform it (Gertsch 1979). The position of the legs prior to sperm web construction is similar to the position of several species of the same family like *Theridion* sp., where the parallel threads are held by legs II and IV, and the bridge is spread by legs III (Knoflach 1998). The horizontal position of the sperm web by the males of *A. vierae* is similar to that commonly observed in other spiders (Foelix 2011). However, its hourglass shape differs from the common triangular or square shape described for other Araneomorph spiders (Gertsch 1979; Foelix 2011) or the trapezoidal shape seen in *Theridion* (Knoflach 1998).

As in many spider families, intensive movements of the abdomen just before ejaculation indicate the activity of the epigastric glands that support the building of the sperm web (Knoflach 1998). These intensive movements were seen in *A. vierae* and could imply the same activity of the epigastric glands. Filling the pedipalps with sperm corresponds to the direct form of sperm induction observed in web-building spiders (Foelix 2011). This process is characterized by a male extending his pedipalp on the same side of the sperm web as the droplet of sperm and contacting the droplet, which absorbs the sperm (Jackson & Macnab 1991). The fact that males performed sperm induction behavior after mating suggests that their pedipalps are loaded with sperm prior to mating. Thus, it may be the case that males load their pedipalps immediately after they molt into adults, as is the case with most spiders (Austad 1984; Michalik & Rittschof 2011). Under laboratory conditions, we observed similar sperm induction characteristics in *A. jabaquara* Levi, 1956, and it also occurred after mating (unpublished results).

Here, males did not copulate again with the same female, but another study reported that males of *A. vierae* made two or three reloads of pedipalps and remated with the same female (Viera et al. 2007). By remating with the same female, males can reduce the probability that she mates with another male, thus reducing the risk of direct sperm competition (Alcock 1994). This makes sense because in this species the first and second males that copulate with a female have similar percentages of paternity (Lorieto et al. 2010). Another option for males is to completely fill the female's spermathecae with sperm. However, the pedipalps may not be able to support all the sperm needed to do so, thus requiring the male to load them more than once. In the future, it would be interesting to evaluate the capacity of female's spermathecae compared with the capacity of the male's pedipalps.

Although the physical cost of sperm induction may be low, there may be other types of associated costs. First, males of *A. vierae* fight for access to females and the loser remains as a satellite male (Albo et al. 2007). When the winner leaves the female to engage in the sperm induction maneuvers, the satellite male could take advantage and copulate with the abandoned female. Females are permissive, tolerant, and accept re-matings with the same male and with others (Viera et al. 2007). Secondly, the brief sexual season of *A. vierae* can represent a pressure on males to load their pedipalps with sperm immediately after mating, because males may seek to copulate with many females over a short period of time (Viera et al. 2007). Future studies are required to investigate how often and under which circumstances (e.g., in the presence of one, two or three females, or other males) the males reload the pedipalps with sperm.

Given that *A. vierae* is a subsocial spider, this study is important for future comparative studies, and also for investigating how sociality may have evolved in spiders via reduced conspecific aggressiveness.

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