

## Funnel-web construction and estimated immune costs in *Aglaoctenus lagotis* (Araneae: Lycosidae)

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**Abstract.** Constructing webs for survival is rare in wolf spiders. However, some species, postulated as basal in the family, live in funnel-webs. *Aglaoctenus lagotis* (Holmberg 1876), a South American lycosid, lives permanently in webs. It is virtually unknown how web construction occurs for this species and the few other lycosid weavers. Also, costs associated with construction have not been studied, although funnels are suggested to be particularly costly webs. This study describes the funnel-web construction behavior of *A. lagotis* (Lycosidae: Sosippinae) and measures its costs in subadult and adult individuals. We recorded web construction, effects of sealing spinnerets in weaving activity, and immune costs of weaving (measuring melanization of an implant) in individuals allowed to weave and prevented from weaving. Construction consisted of three alternating behaviors: deposition of thick threads with a radial orientation and prolonged attachments (mainly involving the anterior spinnerets); deposition of swaths of fine threads without consistent orientation and with short attachments (mainly involving the posterior spinnerets); and motionlessness. No sticky threads are present in the web. The thick threads have a supporting function and the fine threads have a filling function. Subadults and males allowed to weave reduced their immune response compared with those prevented from weaving; no such relationship was observed for females. Males presented the weakest immune response, followed by subadults and females. The web construction process showed greater similarity with agelenid spiders than with the only other lycosid studied, *Sosippus janus* Brady 1972, and appears to be a costly activity, especially for males.

**Keywords:** Sedentary life, wolf spider, Sosippinae, immunity, encapsulation response

Prey-capture web construction is exceptional in lycosid spiders, which are typically characterized by their wandering habit (Foelix 2011). However, the subfamilies Sosippinae (from the Americas) and Venoniinae (from Asia and Australia) present species that construct webs during some or all stages of their lives (Santos & Brescovit 2001; Yoo & Framenau 2006). This web living habit is considered a basal character in the family (Foelix 2011), although is still a controversial hypothesis (Murphy et al. 2006).

Funnel-webs are common in families as Hexathelidae and Dipluridae (mygalomorphs), as well as in Agelenidae and Tengellidae (araneomorphs). This type of web is also constructed by the few species of Lycosidae that weave webs, such as *Aglaoctenus lagotis* (Holmberg 1876). Although variations exist in structures and type of silk threads used in funnel construction (Griswold et al. 2005), their general structure consists of a flat sheet (platform) that connects to a tube (refuge) located at the edge or near the middle of the sheet. Some webs have threads above the sheet that intercept flying insects (intercepting threads), causing them to fall onto the sheet (Foelix 2011). How the funnels are woven is almost unknown (Rojas 2011; Eberhard & Hazzi 2013) because most of the information comes from spiders with orb webs (Eberhard 1990), or with irregular webs (Benjamin & Zschokke 2002). Rojas (2011) has reported one detailed description of the weaving process in an agelenid, but no descriptions are reported in wolf spiders, excepting limited data provided by Prestwich (1977) for *Sosippus janus* Brady 1972. The costs associated with weaving these funnels are not well known.

The measurement of costs associated with any activity of spiders has been little studied in comparison to insects

(Moreno-García et al. 2013). The most common measurement has involved estimating costs from activities directly related to reproduction (Ahtiainen et al. 2006; Aisenberg & Peretti 2011; Cady et al. 2011; Calbacho-Rosa et al. 2012). In terms of costs involved in webs construction, only Prestwich (1977) and Tanaka (1989) have studied two species that converge in weaving funnel-webs, a lycosid (*Sosippus janus*) and an agelenid (*Agelena limbata* Thorell 1897), respectively. Both authors, estimating the costs based on oxygen consumption rate, agreed that these webs are particularly expensive in comparison to orbicular or irregular webs. These high costs may have caused the high fidelity to the web construction site observed in individuals of both species. Other indirect estimators of costs based on fat reserves and immune response (by measuring melanization of a nylon implant) have been used on lycosids in the area of assessing costs of reproduction and burrowing (Ahtiainen et al. 2006; Aisenberg & Peretti 2011).

*Aglaoctenus lagotis* is a lycosid that lives sedentarily in its web, except for adult males who change to a wandering habit and abandon the web completely while searching for females (Bucher 1974; Sordi 1996). This South American wolf spider may be unique in having a web-living habit for all stages of development. Prey capturing, sexual encounters, refuge, protection of the egg sac and spiderlings all occur in the web (Sordi 1996). The species is widely distributed, from Uruguay to Colombia (Piacentini 2011), being present in different environments (Sordi 1996; Santos & Brescovit 2001). The existence of two forms has been reported within the species, differing from each other at least in sexual behavior (González et al. 2013) and phenology (González et al. 2014), suggesting a divergence process (González et al. 2015). One of the forms

(the “similar to southern Uruguay, SU”) constructs its webs exclusively on the herbaceous stratum, and the other (the “similar to central Argentina, CA”) can construct them also on the arbustive and arboreal strata (González et al. 2014).

Considering the importance of web construction in determining phylogenetic relations of spiders (Kuntner et al. 2008), its potentially high costs of weaving, affecting survival and reproduction, and the atypical nature of living in webs for Lycosidae, we here describe the web construction process and assess its immune costs in *A. lagotis*. For these purposes, we observed web construction in untreated spiders and in spiders with pairs of sealed spinnerets, to test their participation in the process. We also measured the melanization of a nylon implant in adult and subadult individuals allowed to weave and prevented from weaving. We expected to find a similar web construction process to those outlined for the other lycosid studied, *S. janus*, assuming that this trait is usually conserved at the family level (Foelix 2011). We also predicted significant construction costs for all developmental stages of the species, as has been noted for other funnel-web spiders (Prestwich 1977; Tanaka 1989). Because adults rarely construct new webs in the field, it suggests that they invest in other life history characters (i.e., reproduction), therefore, we would expect higher costs of weaving for adults than for subadults.

## METHODS

Eighty-nine subadults of *A. lagotis* were collected from Fray Bentos, Rio Negro (Uruguay; 33°06'46"S, 58°17'11"W) during February and from August to December 2012. We chose this locality because both forms of the species, reported by González et al. (2014) (“similar to SU” form and “similar to CA” form), are present. Collecting at different periods of the year was done to ensure finding subadults of both forms because they present different phenologies (González et al. 2014). Individuals were captured from their webs during daylight, by manually blocking the entrance of the silk tube. Measurements and photographs from 20 webs were taken in the field for later comparisons with webs constructed in captivity. To standardize conditions before starting measurements, spiders were kept in the laboratory for a week, individually housed in Petri dishes (9.5 × 1.5 cm), with moist cotton as a water supply. We fed them with a mixed diet of mealworm larvae (*Tenebrio* sp.; Coleoptera, Tenebrionidae) and small crickets (*Acheta domestica*; Orthoptera; Gryllidae). We analyzed the results using the statistical packages PAST v.1.18 (Hammer et al. 2003) and WINPEPI v.1.6 (Abramson 2011). We checked data for normal distribution with the Shapiro–Wilk test and homogeneity of variances with the Levene test.

**Description of web construction.**—For observations of web construction, we relied on the method described by Rojas (2011). Each subadult individual was placed in a plastic box (10 × 10 × 5 cm) with corrugated cardboard covering the base to facilitate the visualization of the silk threads. The cardboard was pierced by tacks every 2 cm, forming a grid and functioning as support for the threads, and providing reference points for use when analyzing the videos. Also a piece of rolled cardboard (2.5 × 2.0 cm) was stuck in a corner of the box as an artificial tube as refuge for the spider, taking advantage of the high fidelity that funnel-weavers have

to web sites (Tanaka 1989). Observations took place during the morning (beginning between 0730–0930 h) with the boxes placed at the same position with respect to the window (light source). The average temperature was  $24.81 \pm 2.25$  °C. Individuals were measured (cephalothorax and abdomen widths) and weighed. Choosing subadults for the description of the web construction was based on our observations that subadults are those who weave all elements of the webs and are faster in constructing the web (M. González pers. obs.). Additionally, spiders achieving adulthood are already living in the webs they previously wove.

We observed web construction by ten spiders, five of each form (“similar to SU” and “similar to CA”). We chose the largest spiders to facilitate their tracking and the visualization of their spinnerets. The trial started when the spider began to move in the box. We recorded the activity of individuals for a duration of 3 h with a digital video camera Sony DCR-SR85 HD mounted on a tripod positioned in such a way as to have a complete view of the observational boxes (“macro observations”). The videos were analyzed using Ardesia 1.0 (Ardesia Team 2012) for reconstructing the route taken by each spider during weaving, because threads were not visible in the recording. Five additional individuals were videotaped at a shorter distance (“focal observations”) and analyzed in slow motion for visualizing the movements of the spinnerets and threads released during weaving. The software JWATCHEE (Blumstein et al. 2000) was used to determine the behaviors involved in the construction process, as well as their frequencies and durations. Spiders were left in the boxes three additional days, and we photographed them daily for possible modifications in the constructions.

We sealed the spinnerets of another ten subadult spiders (without discriminating between forms) using heated liquid paraffin; the anterior spinnerets (AS) of five of them and the posterior spinnerets (PS) of the other five. Individuals were anesthetized with CO<sub>2</sub> during sealing. Each spider was then placed in a plastic box, similar to the one described above, for weaving. We recorded the webs they had constructed 24 h later and photographed them. Preliminary trials sealing all the spinnerets of some individuals showed absence of silk deposition. In some cases, the individuals molted and we could observe the recovery of silk deposition. The photographs were taken under a stereoscopic magnifier (Nikon SMZ 1500) or a microscope (Nikon Eclipse 50i). Data were analyzed using the Mann–Whitney U-test for non-parametric data.

**Immune costs of web construction.**—For assessing the immune costs of weaving a funnel-web, we measured the generic immune response, in this case melanic encapsulation, that an individual mounts against a foreign agent introduced into the spider’s body. The foreign agent we used was a single sterile nylon filament (1 mm long and 0.08 mm in diameter), previously rubbed with sandpaper and sterilized with 80% ethanol. Once dried, the implant was introduced into the hemocoel of the spider, following the technique described by Ahtiainen et al. (2006). The same measure of melanization of an implant has been applied for another lycosid species by Aisenberg & Peretti (2011) showing that the insertion of a foreign element stimulates the formation of a cover of hemocytes and a melanin matrix (encapsulation). This cover is reduced in size and melanin deposition when the animals are

under specific energetically stressed (i.e., by web construction in our study).

We used 20 adult females, 20 adult males and 20 subadults of *A. lagotis*. Here, we do not discriminate between forms because we did not find differences between them in web construction (see Results below). We inserted an implant in ten individuals of each group immediately after they had been weaving for 48 h (allowed to weave: experimental group). The other ten spiders received the implant after being confined for 48 h in tubes (1.5 × 5.0 cm), preventing web construction (prevented from weaving: control group). None of the individuals were fed during the experimental period. The silk of the constructed webs was collected with a metal clip and weighted with a precision scale (Ohaus PA 114, 0.0001 g).

For the insertion of the implant, each individual was anesthetized with CO<sub>2</sub> and immobilized by taping its legs laterally onto a glass slide with paper tape. We inserted the nylon filament through an incision made on the ventral cuticle of the abdomen, beneath the epigastric furrow. The implant remained inserted with the individual immobilized in the dark during 12 h, similar to Aisenberg & Peretti (2011). After this period, the implant was removed and allowed to dry. All spiders survived the implantation process. After removal, each implant was photographed with a digital camera attached to a stereoscopic microscope (Nikon SMZ 1500). The images were examined using the software UTHSCSA Image Tool, version 2.0 (Wilcox et al. 2002). We measured the percentage of the encapsulated part of the implant (implant cover percentage) and the pigmentation (darkness) of the encapsulation, as it has also been considered an estimator of generic immune response in insects (Rantala & Roff 2007; Bascuñán-García et al. 2010). We categorized encapsulation darkness by dividing the array of possible gray values into thirds. If the percentage value of gray was under or equal to 33%, it was considered light; if it was between 33% and 66%, it was taken as medium, and if it was over 66%, it was considered dark.

Data were analyzed with the Kruskal-Wallis ANOVA test and the Mann-Whitney U-test for non-parametric data. The pigmentation of the encapsulated part of the “allowed to weave” and “prevented from weaving” individuals was compared with the Chi-square test for independent variables and with the Fisher-exact test. With multiple linear regressions, we evaluated the relationships between the immune response and body characteristics of the individuals.

## RESULTS

**Description of web construction.**—All *A. lagotis* webs observed in the field had a platform (sheet area) and a tube, whereas intercepting threads were only present in areas with closed vegetation. The sheet area averaged  $242.07 \pm 94.18$  cm<sup>2</sup> and the tube diameter was  $1.2 \pm 0.3$  cm (mean ± SD) (Fig. 1a), showing no differences between the two forms (“similar to SU” and “similar to CA”) of the species (sheet area:  $U = 17.5$ ,  $P = 0.153$ , tube diameter:  $U = 8.5$ ,  $P = 0.139$ ). Thus for the other aspects studied, we considered individuals of both forms as a whole.

In the laboratory, funnel-webs observed during the first hours of construction had an area averaging  $65.60 \pm 10.45$  cm<sup>2</sup>, but all of them occupied the whole available area (100 cm<sup>2</sup>) (mean ± SD) during the following three days (Fig. 1b). The tube diameter was  $1.6 \pm 0.3$  cm. All webs had a platform

and a tube, but intercepting threads were never seen. The artificial tube was used by 47% of the individuals, whereas 53% constructed a new one. We did not observe a relationship between the location of the tubes and light source (either in the laboratory or in the field). The weaver individuals had a cephalothorax width of  $3.15 \pm 0.28$  mm, abdominal width of  $3.28 \pm 0.55$  mm, and mass of  $0.09 \pm 0.02$  g.

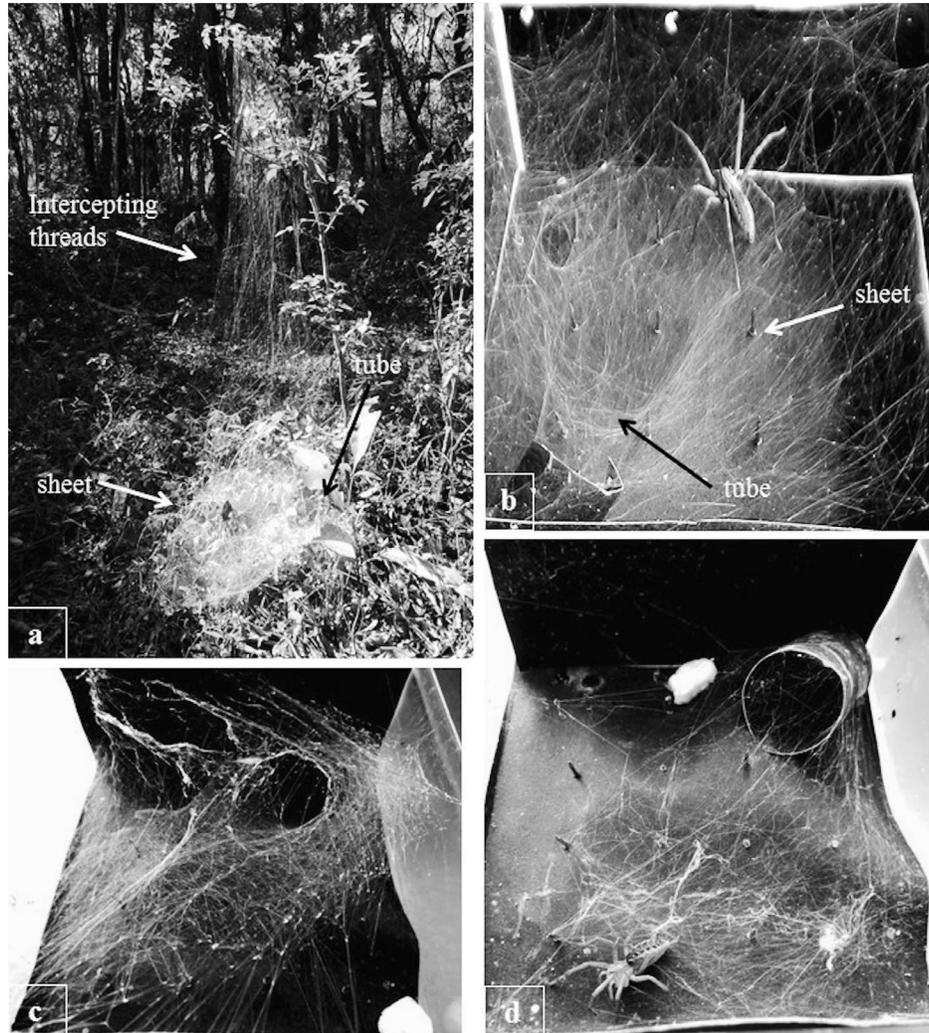
**Untreated spiders.**—The funnel-web construction involved three behaviors under laboratory conditions: deposition of thick threads (DTT), deposition of fine threads (DFT), and motionlessness (M) (Figs. 2, 3).

**DTT behavior:** during this behavior, the spider deposited thick and long threads, generally walking slowly and in a straight line, without bending or tilting the abdomen. The threads (apparently two) seemed to emerge from the AS during this behavior (Fig. 2a). However, a scissor movement of the posterior lateral spinnerets (PLS) (both spinnerets, at the same time, going towards the axis of the body and then separate, quickly and repeatedly) was observed during the displacement of the individual (see Fig. 2c). The spider walked radially from the position where the tube would be constructed towards the edges of the box or the tacks, attaching threads generally at high points. For the attachment, the spider stopped (approximately 1.10 sec—“prolonged attachment”—Fig. 4c) and supported the abdomen and the spinnerets (apparently the AS) on the substrate, although the posterior ones (at least the lateral ones) remained elevated (Fig. 2d) (Supplemental Video 1, online at <http://www.bioone.org/doi/suppl/10.1636/M14-65>). We observed the deposition of a whitish substance during the adhesion. Then the spider returned to the place of tube construction almost by the same route. This behavior tended to be predominant at the beginning of the web construction (Fig. 3).

**DFT behavior:** during this behavior, the spider moved quickly and deposited fine threads, emerging numerously from each spinneret (Fig. 2b). The spider attached these threads every short distance, bending and tilting its abdomen towards each side (a zigzag movement). The spinnerets that were principally involved appeared to be the PLS. The spider walked radially from the site chosen for the construction of the tube (back and forth), but also more randomly, attaching threads mostly over other threads (thick threads from the DTT). For the attachment (Fig. 2e), the individual stopped walking briefly (0.65 sec; “short attachment”), bent the abdomen towards the substrate, the PS (at least the lateral ones) separated from each other remaining almost perpendicular to the body axis, and usually one of them supported on the substrate (Supplemental Video 2, online at <http://www.bioone.org/doi/suppl/10.1636/M14-65>). The more independent movement of the spinnerets for spinning and attaching threads gave the funnel-web the appearance of a mesh (Fig. 4a). This behavior tended to be the predominant one after the first 20 min of weaving and was the one that filled the web (Fig. 3).

**M behavior:** during this behavior, the spider did not walk and stayed generally inside the tube (refuge). The time spent motionless increased as the construction of the web progressed (Fig. 3).

All ten individuals performed the three behaviors described above, but differed somewhat in sequence and duration. The spiders spent an average of 79.34% of the experimental time weaving (mean ± SD:  $142.81 \pm 15.72$  min,  $n = 5$ ) and the



**Figure 1.**—a) Funnel-web of *A. lagotis* in the field. Note the component parts: tube (refuge), sheet (platform) and intercepting threads; b) Funnel-web observed in the laboratory; c) web constructed by an individual with their posterior spinnerets sealed; d) web constructed by an individual with the anterior spinnerets sealed.

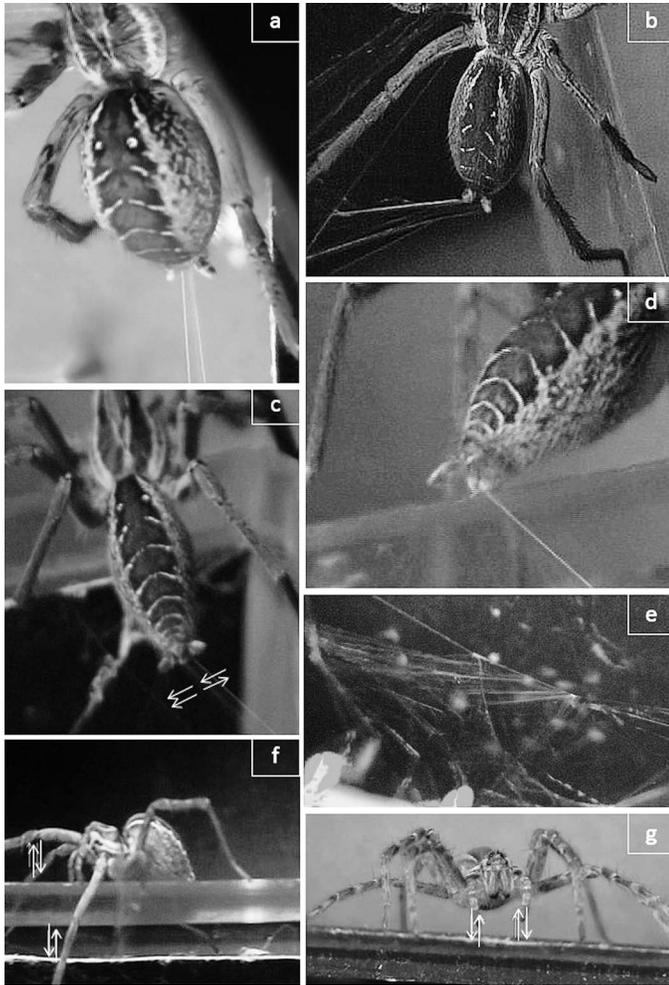
remaining time motionless. Within the time spent weaving, 38.52% ( $55.0 \pm 19.15$  min,  $n = 5$ ) was spent in DTT and 61.48% ( $88.06 \pm 28.09$  min,  $n = 5$ ) in DFT. In some cases, the spiders changed from DTT to DFT, and vice versa, without returning to the tube. The number of attachments was lower at the beginning, but the time expended in this behavior was longer (according to the predominance of the DTT) (Fig. 5). As construction time progressed, the number of threads deposited was higher, and the time expended on attachment was shorter (consistent with the predominance of DFT). We did not observe manipulation of threads with the legs or the deposition of sticky drops. As the days passed, the spider continued adding layers of threads, giving the web a more dense and whitish aspect.

**Other behaviors of untreated spiders.**—Prior to the beginning of construction, individuals usually walked through the different parts of the experimental container, including the edges of the box (exploration), and frequently waved their forelegs (8 of the 10 individuals) (Fig. 2f). Four individuals came out from the box and tried to attach threads in higher

and more distant points, returning posteriorly to the box. Four individuals cut threads with the chelicerae, mostly during the tube construction. Also palpal drumming was seen in the five individuals (Fig. 2g), on box walls or at the tube entrance. In one opportunity, we observed forelegs rubbing.

**Spinneret-sealed spiders.**—Photographs taken under the microscope showed at least the presence of thick and fine threads (Fig. 4b,d) that would correspond with the behaviors described above for the individuals. Individuals with sealed PS tended to construct webs with taut, thick threads, but a more open network (Fig. 1c) than untreated ones (Fig. 1a,b). In contrast, when the AS were sealed, the webs appeared flaccid and were composed of many fine threads (Fig. 1d). Sealing the anterior spinnerets seemed to have a stronger effect on the web structure than sealing the posterior ones.

**Immune costs of web construction.**—Subadults that constructed webs (weavers) showed less pigmented (less dark) encapsulations than those prevented from weaving (non-weavers) ( $\chi^2 = 8.00$ ,  $P = 0.005$ ), as was also the case with males ( $\chi^2 = 6.35$ ,  $P = 0.01$ ) (Fig. 6a,c). However, females



**Figure 2.**—Funnel-web construction behaviors in *A. lagotis*: a) threads observed during the deposition of thick threads (DTT); b) threads observed during the deposition of fine threads (DFT); c) scissor movement of the posterior lateral spinnerets observed during DTT; d) adhesion of thick threads; e) adhesion of fine threads; f) forelegs shaking; g) palpal drumming.

allowed to weave (weaver females) and those prevented from weaving (non-weaver females) showed no differences in the encapsulation pigmentation ( $\chi^2 = 0.18$ ,  $P = 0.91$ ) (Fig. 6e). Web construction did not decrease the implant cover percentage either in subadults ( $U = 49$ ;  $P = 0.97$ ) (Fig. 6b) or in males ( $U = 29$ ;  $P = 0.07$ ) (Fig. 6d). However, females allowed to weave showed higher implant cover percentages than those prevented from weaving ( $U = 26$ ;  $P = 0.01$ ) (Fig. 6f).

Males showed lower encapsulation pigmentation than both subadults and females (Fig. 7a). Females, in turn, presented higher values than subadults. Implant cover percentage showed a similar order (Fig. 7b): males had the lowest, followed by subadults and females (males vs. subadults:  $U = 132$ ,  $P = 0.041$ ; males vs. females:  $U = 148$ ,  $P = 0.033$ ; females vs. subadult:  $U = 50$ ,  $P = 0.53$ ). The weight of silk from the webs woven in each group varied: subadults  $0.5 \pm 0.4$  mg, males  $0.4 \pm 0.3$  mg, and females  $0.9 \pm 0.3$  mg ( $H = 6.488$ ,  $P = 0.037$ ). When compared in pairs, the silk from the webs constructed by the males was significantly lighter in weight than those of the females

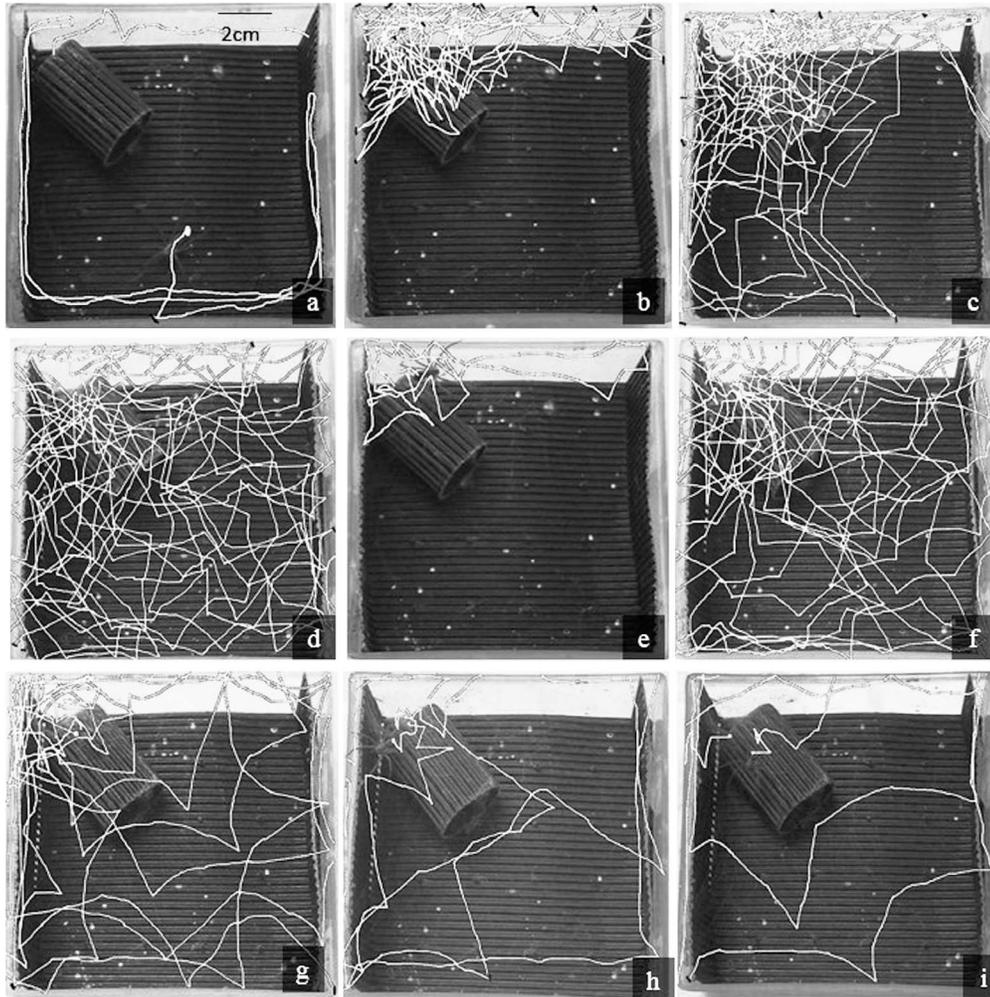
( $U = 6.0$ ,  $P = 0.019$ ). No significant differences occurred between the silk of males and subadults ( $U = 17.0$ ,  $P = 0.473$ ) or between the silk of the females and subadults ( $U = 22.5$ ,  $P = 0.670$ ). When we conducted a retrospective analysis of adults that were previously used as subadults, we found that weaving subadult females showed darker encapsulations in their implants than weaving subadult males (Fisher-exact test:  $P = 0.001$ ). However, we did not find such differences between the non-weaving individuals (Fisher-exact test:  $P = 0.562$ ). We also found no differences between subadult males and subadult females in the implant cover percentage (weavers:  $U = 7.0$ ,  $P = 0.701$ ; non-weavers:  $U = 7.0$ ,  $P = 0.901$ ).

The body condition index differed between stages/sexes ( $H = 38.82$ ,  $P < 0.0001$ ), being lower in males, followed by subadult and then females (subadults vs. females:  $U = 137$ ,  $P = 0.023$ ; females vs. males:  $U = 5.0$ ,  $P = 0.0001$ ; subadults vs. males:  $U = 28.0$ ,  $P = 0.0001$ ). We found no correlation between body condition and immune reaction, in both measurements taken. This occurred for weavers (males:  $R^2 = 0.015$ ,  $df = 1$ ,  $F = 0.031$ ,  $P = 0.735$ ; females:  $R^2 = 0.090$ ,  $df = 1$ ,  $F = 1.411$ ,  $P = 0.336$ ; subadults:  $R^2 = 0.045$ ,  $df = 1$ ,  $F = 0.311$ ,  $P = 0.548$ ) and non-weavers (males:  $R^2 = 0.0034$ ,  $df = 2$ ,  $F = 0.031$ ,  $P = 0.864$ ; females:  $R^2 = 0.124$ ,  $df = 2$ ,  $F = 1.411$ ,  $P = 0.262$ ; subadults:  $R^2 = 0.037$ ,  $df = 2$ ,  $F = 0.311$ ,  $P = 0.592$ ).

## DISCUSSION

The process of funnel-web construction in *A. lagotis* includes the deposition of thick and thin threads, separated by variable periods of motionlessness, without addition of sticky silk or the use of legs for manipulating silk threads. The location of the tube appears to be planned from the beginning; the behaviors involved in its construction did not differ from those used for weaving the sheet and both anterior and posterior spinnerets are involved. Interestingly, the web construction seems to match more with the description reported for agelenids by Rojas (2011) than with the few data available for the funnel-web wolf spider *Sosippus janus* (Prestwich 1977). Immune costs associated with web construction would be significant for *A. lagotis* individuals, as has been reported for other funnel-web spiders (Prestwich 1977; Tanaka 1989), particularly for males.

*Aglaoctenus lagotis* shares with the mygalomorph spiders Dipluridae and Hexathelidae (also funnel-web weavers) (Foelix 2011) the asymmetric use of the spinnerets during weaving, although they differ in the glands involved in threads adhesion (Eberhard & Hazzi 2013). Moreover, at least in the diplurids, the behaviors performed during the construction of the sheet are different from those performed during the construction of the tube, unlike *A. lagotis* that performs DTT and DFT during the construction of all parts of the web (sheet, tube). Among the araneomorph spiders with funnel-webs, tengelids add cribellate silk (Eberhard et al. 1993), something also not observed in *A. lagotis* or the other araneomorph family with funnel-webs, Agelenidae (excepting a single genus of New Zealand, according Griswold et al. 2005). *Sosippus janus*, the only other wolf spider for which we have some previous information about web construction (Prestwich 1977), shares with *A. lagotis* the foreleg shakings during the initial stages of construction. Additionally, both



**Figure 3.**—Route made by an individual of *A. lagotis* during the first 180 min of construction of the funnel-web. Each frame shows the movements that occurred during 20 min. In the first frame (a) the starting point of the route is indicated with a white circle. The black points are attachments classified as “prolonged attachments” during the DTT).

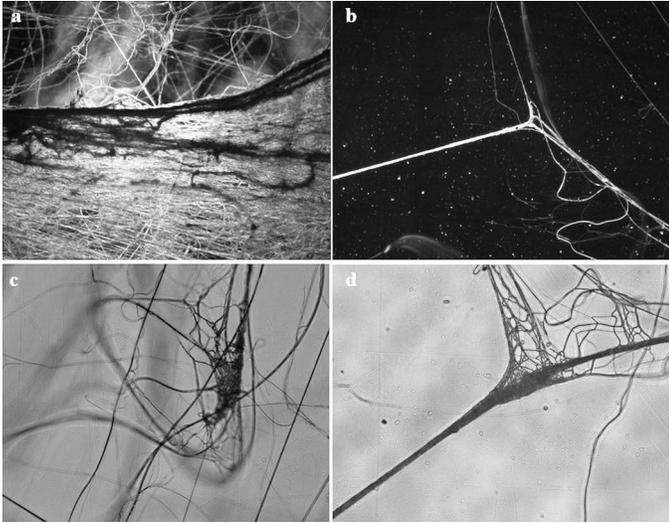
species intersperse periods of rest (motionlessness) in the area serving as a refuge. However, in *A. lagotis*, it is not usual to construct the tube from a hole generated on the sheet, and the sheet is not woven from an area previously delimited by silk threads as occurs in *S. janus*.

The web construction process reported for the agelenid *Melpomene* sp. (Rojas 2011) appears most similar to that described here for *A. lagotis*, both in the behaviors involved and the spinnerets used. The deposition of thick threads and the deposition of thin threads in *A. lagotis* appear equivalent to the “laying support threads” and “filling of the sheet” respectively, reported for the agelenid (Rojas 2011). The proportion of time spent in the deposition of supporting and filling threads (Rojas 2011) also seems to be similar to the deposition of thick and thin threads in our lycosid. The differences detected between the two families include scroll speed during the deposition of thick threads (faster in *Melpomene* sp.) and the total time spent in construction (shorter in the agelenid) (Sordi 1996; Rojas 2011).

Given the similarities noted above, it is feasible that the glands involved in each construction behavior are similar

between *A. lagotis* and agelenids, despite the fact that these tend to vary at higher taxonomic levels (Eberhard 1990). Rojas (2011) suggests the ampullate glands as mainly responsible for supporting thread production (which would be the thick threads in *A. lagotis*), and the piriform glands as the ones for the attachments, both connected to the anterior spinnerets. The filling threads (and their equivalent fine threads in our lycosid) would involve principally the aciniform glands that are connected to the posterior spinnerets. The use of anterior spinnerets has been featured in typical lycosids and the other wandering spiders for their role in the deposition of draglines and attachment disks (Moon 1998). However, the posterior spinnerets have been thought to be involved in molting and the construction of the sperm web (Richter 1970), but have not been reported for web construction in wolf spiders.

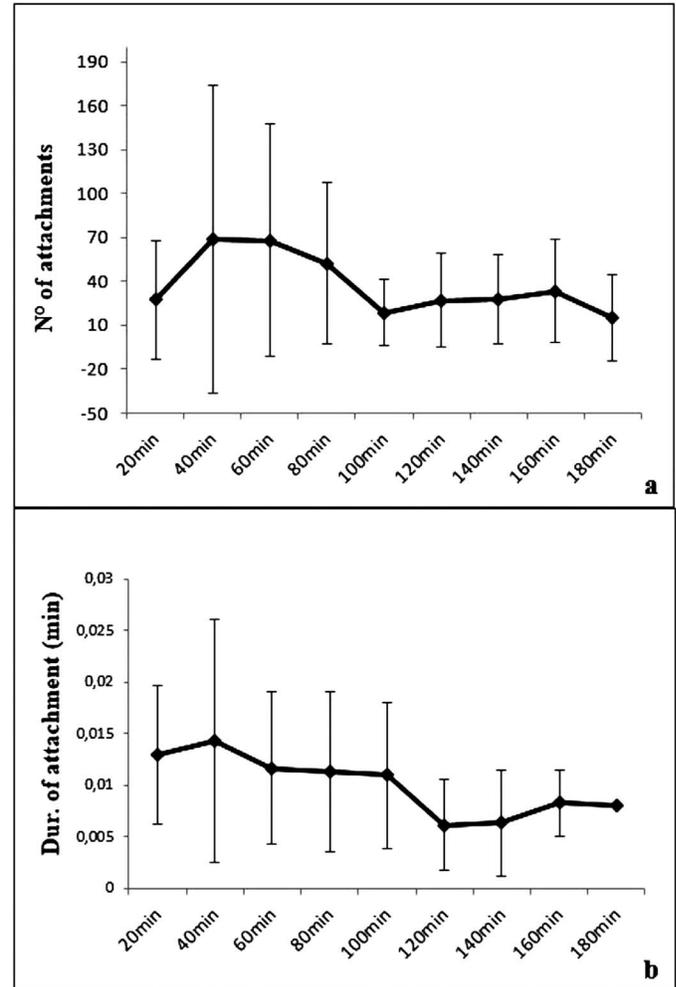
Agelenids usually present long posterior lateral spinnerets (Foelix 2011; Morphbank 2014; J. Coddington: <http://www.morphbank.net/?id=366552>, V. Power.: <http://www.morphbank.net/?id=506814>), which is related to their function during web construction (Griswold et al. 2005). The relation



**Figure 4.**—a) Layers of silk threads from the sheet area of a web observed under a stereomicroscope (1x); b) silk threads observed under the microscope (10x); c) prolonged attachment point; d) short attachment point. Note the thick threads and fine threads that compose the web.

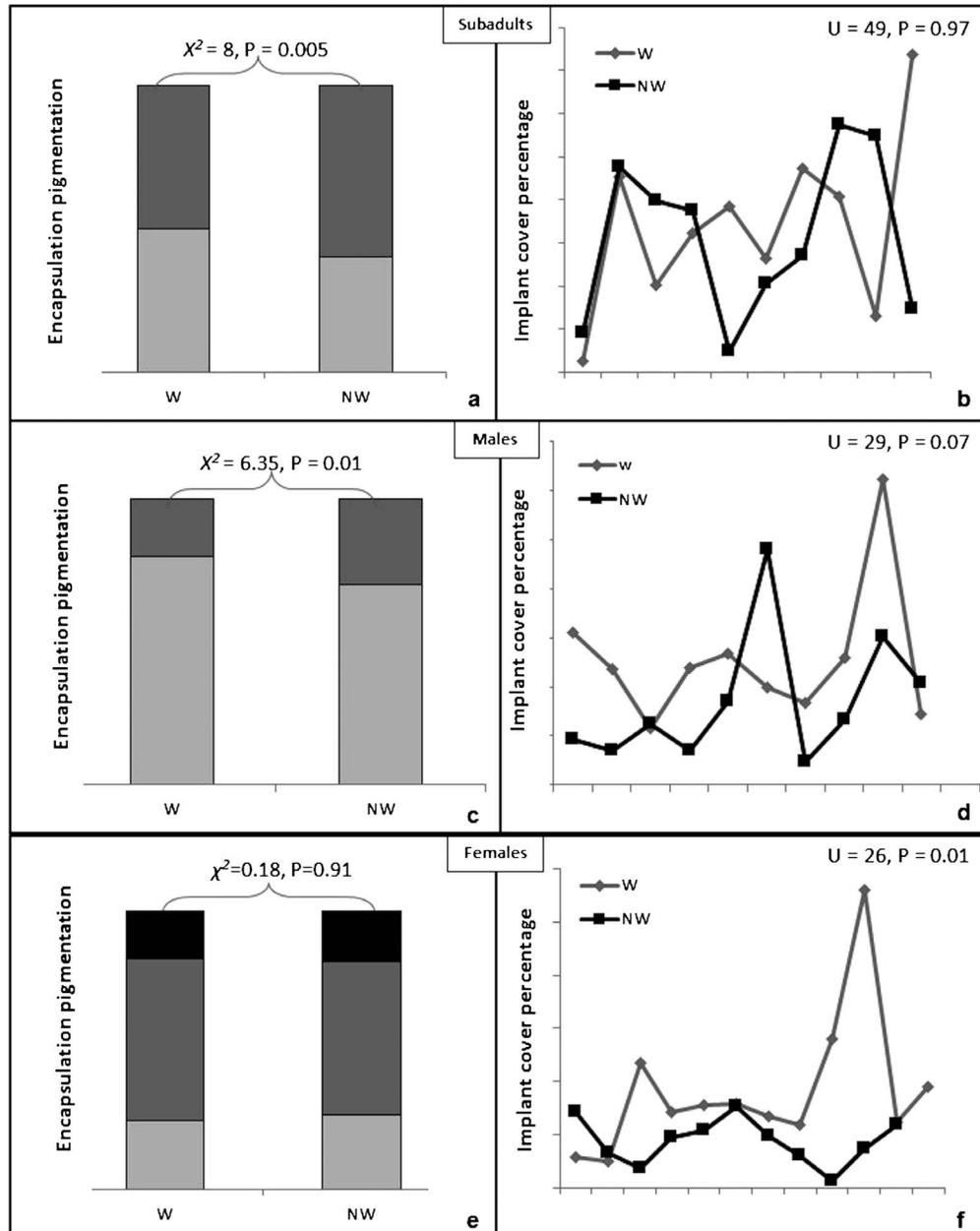
has also been suggested for weaving wolf spiders (Murphy et al. 2006). *Sosippus* Simon 1888 and *Venonia* Thorell 1894, two of the few genera of lycosids that construct funnel-webs, show this pair of spinnerets to be enlarged (Brady 1962; Yoo & Framenau 2006), but this characteristic does not seem to appear in *A. lagotis* (Morphbank 2014; M. Ramírez: <http://www.morphbank.net/?id=476815>). We do not know how this difference could impact the constructing process, but it may be related to the longer time spent by *A. lagotis* to weave compared with *Melpomene* sp. Neither can we point out the role played by the scissor movement of the PLS, the forelegs shaking also reported by Prestwich (1977) for *S. janus*, and the palpal drumming we observed during weaving. As we recorded leg shaking during the beginning of construction and at the edges of the observational boxes, we suggest that the spider uses them for detecting the highest points available for attaching threads. Scissor movements could be useful for bringing silk together in the two threads observed, and palpal drumming may have a sensory function. The relationship between the funnel-web spiders and associated morphological structures needs further study (Murphy et al. 2006).

As we expected, considering the time spent in weaving and the site fidelity reported for other funnel-web spiders (Tanaka 1989), web construction generates significant changes in the immune responses of *A. lagotis* individuals. However, weaving does not appear more costly for adults than for subadults. Web construction would be most expensive for adult males, who wove the smallest webs and showed the lowest pigmentation rates. Perhaps this fact explains why males do not construct webs again after their sexual period starts. Costs would even be increased because, with adulthood, they also stop feeding. Indeed, males *per se* (after weaving or not) showed values that indicate a weaker immune response compared to subadults or females. Adult females deposited the highest silk amount during construction and achieved the darkest encapsulations, whereas subadults showed intermedi-



**Figure 5.**—a) Number of attachments (average  $\pm$  SD) and b) average durations (min) of each attachment every 20 min of construction, based on four individuals of *A. lagotis*.

ate values. This could indicate that weaving a web is not significantly costly for females or that they invest all their energy to ensure the necessary area for mating, hatching, and caring for their offspring (all instances occurring in webs) (Sordi 1996). In this regard, recent observations (M. González pers. obs.) have shown that females with egg sacs that lose their webs are capable of weaving a new one, but it is smaller than their previous webs. Another explanation, which is not exclusionary, could be that the ability to weave a second web in the laboratory is due to the superior body condition of the laboratory females or the space given for web construction, the latter of which is smaller than the area usually occupied by females with a web (females webs in the field usually exceed 100 cm<sup>2</sup>). Meanwhile, we could not explain the unexpected higher implant cover percentage recorded for weaving females compared to those prevented from weaving. Perhaps the dimensions of the tubes selected for preventing weaving are too small for females, which are larger than subadults and males (González et al. 2013), making confinement stressful (and probably indirectly affecting immune cost). Another consideration might be that females who wove coincidentally had better body conditions.

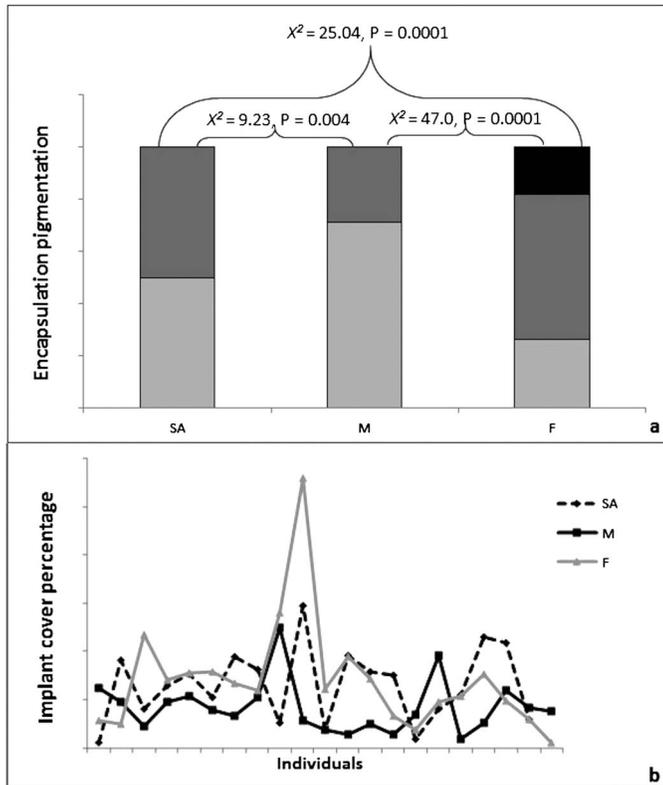


**Figure 6.**—Encapsulation pigmentation (shown with three gray intensities of the bars; light, medium and dark grey) and the implant cover percentage registered in subadult individuals (a, b), males (c, d) and females (e, f) of *A. lagotis*. W: weaver individuals; NW: non-weavers.

The few previous studies (Prestwich 1977; Tanaka 1989) that measured costs of funnel-web construction (both based on oxygen consumption, not on implant encapsulation) found no differences in costs between developmental stages, unlike the present work. However, for other lycosids, it has been shown that the sex that lives longer and/or takes on activities of high immune costs (oviposition, construction of burrows, intrasexual contests) starts with better immunological conditions, which in turn would not decrease rapidly (Ahtiainen et al. 2006; Aisenberg & Peretti 2011). These last results are in agreement with our findings. We cannot be sure that poor body condition by itself determines a low immune response in all stages, sexes, and species. For example, the association between body conditions and immune response was not demonstrated within

adult males. Males with a lower body condition index did not generate less pigmented encapsulations or less implant cover percentage than males in better condition. The fact that immune response was not correlated to body condition in males could indicate that sexual selection is a very strong force compromising an individual's immune ability.

The phylogenetic history of *A. lagotis* and the other few lycosids that construct funnel-webs is controversial (Santos & Brescovit 2001). Whether the web is basal (Foelix 2011) or derived in the wolf spiders is still unclear (Murphy et al. 2006). Regardless of how the webs originated in this species, web construction appears to be an expensive activity for individuals and would explain site fidelity (Sordi 1996). The energetic investment (and associated immune cost) of web construction



**Figure 7.**—Comparisons of (a) the encapsulation pigmentation and (b) implant cover percentage registered in the three groups of *A. lagotis*: subadults (SA), males (M) and females (F).

would affect the energy available for reproduction in adults (because, except for males, all other the stages cannot avoid those costs). Future studies will attempt to quantify the qualitative differences in web structures. These studies could be extended to other lycosids and closely related families. It will be important to see how widespread this type of weaving (and use of the spinnerets) is, and to shed light on the paths that webs have followed phylogenetically.

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