Characterisation of burrow architecture under natural conditions in the sand-dwelling wolf spider Allocosa brasiliensis

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**ABSTRACT**

Allocosa brasiliensis (Petrunkevitch, 1910) is a wolf spider that constructs silk-lined burrows along the coastal sand dunes of Argentina, Brazil and Uruguay. This species shows a reversal in typical sex roles and sexual size dimorphism expected for spiders. Females are the smaller and mobile sex, which initiates courtship at the male burrow entrance. Mating occurs in the male burrow, and when it ends, the male leaves and the female stays inside. Females prefer to mate with those males showing longest burrows, so burrow dimensions would be under strong sexual selection pressures. Previous studies in the laboratory indicated that male burrows are longer than those of virgin females, which were described as simple silk capsules. Preliminary observations suggested that juvenile burrows presented differences from those of adults; however, detailed observations of A. brasiliensis burrow characteristics at the field were lacking. The aim of this study was to characterise the burrows of adults and juveniles of A. brasiliensis under natural conditions. We recorded the dimensions of burrows inhabited by males, females and juveniles (n = 30 for each category) and created beeswax moulds that reflected burrow shape. Juveniles inhabited tubular burrows with two branches and two openings; on the contrary, adults were found in tubular burrows with a single entry. Males and females inhabited burrows of similar length and width, but those of juveniles were shorter and narrower. We discuss the results and their possible functional explanations according to the selective pressures expected for each developmental stage and sex.

**Introduction**

Animals inhabiting open sandy areas need to develop morphological and behavioural strategies that allow them to deal with extreme temperatures, water loss, lack of shelters and strong winds (Cloudsley-Thompson 1982; Henschel and Lubin 1992). Many arthropods build their shelters where they find refuge from climatic conditions, thermoregulate, moult, protect themselves from predators, forage, reproduce and/or
provide parental care (Cloudsley-Thompson 1982; Aisenberg and Peretti 2011). Burrow construction by arthropods has been reported for crustaceans, insects, scorpions and spiders (McLachlan 1991; Henschel and Lubin 1992; Shivashankar 1994; Fallaci et al. 2002; Dolejš et al. 2008; Halfen and Hasiotis 2010; Aisenberg and Peretti 2011).

Many spider species excavate burrows below the surface of the ground as a way to obtain adequate and stable temperature and humidity conditions for their own survival and, on some occasions, for brood care (Henschel and Lubin 1997; Suter et al. 2011). Spider burrows can be temporal or permanent according to the sex and/or species (Suter et al. 2011). Burrowing spiders inhabiting sandy areas need to deposit several silk layers on the walls of the burrows to ensure the stability of the construction (Gwynne and Watkiss 1975; Henschel 1990). This type of silk-lined burrows constructed in the sand has been described for Lycosidae (Gwynne and Watkiss 1975; Shook 1978; Aisenberg and Peretti 2011), Zodariidae (Jocqué 1991, 1993; Ramírez 1995), Eresidae (Scott Turner et al. 1993; Henschel 1995), Sparassidae (Henschel 1990) and Nemesidae (Montes de Oca and Pérez-Miles 2013).

Allocosa brasiliensis (Petrunkevitch 1910) is a sand-dwelling wolf spider that constructs burrows along edges of rivers and lakes and the coast of the Atlantic Ocean in Argentina, Brazil and Uruguay (Capocasale 1990). Individuals of this species show heterogeneous spatial distribution, associated positively with the presence of sand dunes and native vegetation (Costa 1995; Aisenberg et al. 2011). During daylight, individuals stay inside their burrows, and they turn active during the night, when they forage and reproduce (Aisenberg 2014). Females reach maturity after 9.44 ± 1.01 moults and males after 9.83 ± 1.11 moults (Aisenberg and Costa 2008). Females and males mature in November–December and survive for two reproductive periods (1 year; Aisenberg and Costa 2008). This species shows a reversal in typical sex roles and expected sexual size dimorphism described for spiders (Aisenberg et al. 2007). Males are larger than females and they dig deep tubular burrows, whereas virgin females construct shallow silk capsules (Aisenberg et al. 2007). Studies performed under laboratory conditions indicate that male burrows average 10 cm in length and 0.9 cm in width, while female burrows average 3.0 cm in length and 0.8 cm in width (Aisenberg et al. 2007). According to observations performed in captivity by Capocasale (1990), small juveniles of this species construct branched burrows; however, this has not been confirmed under natural conditions.

Females of A. brasiliensis prefer to mate with those males showing the longest burrows (Aisenberg et al. 2007). While courting, females inspect male constructions by following them to the bottom of their burrows. Then, they can accept mount, or turn away and leave (Aisenberg et al. 2007). Copulations occur inside male burrows, and after copulating, males exit and females stay inside. Males cover the burrow entrances completely before leaving, and females cooperate by laying silk from the inside while the male is covering the entrance from outside (Aisenberg et al. 2007). Male burrows are mating refuges and breeding nests in this spider, because females will oviposit there and emerge when it is time for spiderling dispersal (Aisenberg 2014). Females lay up to four egg sacs from January to May (Aisenberg 2014). Female choice in burrow length suggests high selective pressures acting on male digging behaviour in this species. Males donate to females a refuge with stable conditions for egg-sac care and
development for the spiderlings. Additionally, by closing and camouflage the burrow entrance from the outside, they are protecting females from predators such as Anoplius pompilid wasps (Costa 1995; Stanley et al. 2013).

Individuals of A. brasiliensis are located differentially on the dune according to stage and sex (Aisenberg et al. 2011). However, until now the characteristics of the burrows constructed by juveniles, females and males under natural conditions were unknown. The objectives of this study were to characterise the shape and dimensions of burrows constructed by adults and juveniles of A. brasiliensis in the field, and compare the results with data obtained in captivity. Our predictions were that we would find differences in burrow shape and dimensions according to the stage of development and sex. We discuss the selection pressures that could be affecting burrow construction in this species.

Materials and methods

We located burrows inhabited by individuals of each category (males, females and juveniles, n = 30 for each) of A. brasiliensis from December 2012 to March 2013 and from December 2013 to March 2014, at the coastal area of Brisas del Uruguay (33° 51’0.28” S, 50°24’41.4” W), Colonia, Nueva Palmira, Uruguay. We collected only small juveniles categorised according to their size (range of carapace width: 1.5–3.2 mm). We worked during the night after 10 p.m., using headlamps.

We located open burrow entrances and removed the spider by offering it a Tenebrio molitor (Coleoptera) larva tied to a thread. We waited for the spider to attack the prey and bite it so it could be pulled out with the string, similarly to the technique proposed by Carrel (2003) for Geolycosa xera archiboldi and Geolycosa hubbelli. Once the burrow was empty, we measured the diameter of the entrance with a calliper, and the burrow length with a fine wire with an incorporated metric scale. We recorded the temperature inside the burrow (at the base) using a digital thermocouple Digi-Sense, Cole Parmer. In juvenile burrows it was not possible to record burrow temperature, due to the incompatibility between burrow diameter and thermocouple size.

The spiders that were removed from their burrows were taken to the laboratory, where they were sexed and measured. We used carapace width as representative of body size (see Moya-Laraño et al. 2003). After this, individuals were freed in the wild in the same location where they had been collected. We deposited voucher specimens from the study site in the arachnological collection of Sección Entomología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

For determining A. brasiliensis burrow shape we carried out preliminary tests in which we filled empty burrows with a variety of substances (polyurethane foam, plaster, candle wax, paraffin, dentist plaster, natural beeswax). Beeswax moulds best retained the shape of the burrow constructed in the sand. We filled each empty burrow (n = 30 for each category) with recently melted natural beeswax and waited for 20 minutes for the wax mould to harden. We removed the moulds and took them to the laboratory where we took three images of each one. We analysed burrow dimensions (diameter and length) with the THSCSA Image Tool program and averaged the values from each image for later analyses. In the case of juvenile branched burrows, we considered the length of the main branch (the longest and most vertical one). We calculated burrow volume by
measuring fluid displacement after introducing each mould in a glass graduated cylinder of 500 cm$^3$ (± 5 cm$^3$) with water, similarly to Suter et al. (2011). Volumes were calculated only in adult burrows, and not in juvenile burrows due to limitations caused by their size and shape.

We compared burrow dimensions in the three categories. For that purpose, we checked variables for homogeneity of variances and normality. Variables with normal distribution (Shapiro–Wilk test) and homogeneity of variances (Levene test) were analysed with global analysis of variance (ANOVA) and post-hoc Student t-tests for independent samples. Variables neither following a normal distribution nor having homogeneous variances were analysed with non-parametric Kruskal–Wallis tests and Mann–Whitney U-tests. We performed simple linear regressions with carapace width as independent variable and burrow length as dependent variable, and with burrow length as independent variable and temperature as dependent variable. The data were analysed using PAST (Paleontological Statistics) version 1.18 (Hammer et al. 2003) and NCSS 2001 (Hintze 2000).

**Results**

Burrows inhabited by adults were tubular with a single entry, and all of them showed a terminal chamber (Figure 1A and B). On the other hand, burrows inhabited by juveniles presented tubular branching burrows with two entrances but without a terminal chamber (Figure 1C).

![Figure 1. Beeswax moulds showing the shape of burrows inhabited by (A) females, (B) males and (C) juveniles. The black arrow indicates the location of the terminal chamber.](image-url)
When we compared the dimensions of the burrows in the three categories we found statistical differences among them (Table 1): burrows inhabited by juveniles were shorter and narrower compared to burrows inhabited by males and females, but we did not find differences in burrow dimensions between burrows inhabited by males or females. Burrow length did not show a correlation with carapace width either in males ($R = 0.19$, $F_{1,28} = 1.11$, $p = 0.30$) or females ($R = 0.25$, $F_{1,28} = 1.91$, $p = 0.18$), but showed a positive relation in juveniles ($R = 0.40$, $F_{1,28} = 5.36$, $p = 0.03$, beta = 0.40). Burrow width did not show a correlation with carapace width in males ($R = 0.33$, $F_{1,28} = 3.52$, $p = 0.07$), females ($R = 0.08$, $F_{1,28} = 0.21$, $p = 0.64$) or juveniles ($R = 0.02$, $F_{1,28} = 0.05$, $p = 0.82$). We did not find differences in internal temperature between burrows inhabited by adults (Table 1), and when we looked for a correlation between burrow length and temperature we did not find a significant relation in males ($R = 0.34$, $F_{1,28} = 3.56$, $p = 0.06$), or females ($R = 0.33$, $F_{1,28} = 3.56$, $p = 0.07$). We did not find differences in volume between burrows inhabited by males or females (Table 1).

**Discussion**

Our results confirm that burrows of *A. brasiliensis* at the field show differences in length, width and shape, according to the inhabitant. Burrows inhabited by adults were longer and wider compared to those corresponding to juveniles. Furthermore, while burrows inhabited by adults were tubular with only one entrance, those inhabited by juveniles showed a ramification and two entrances. These differences in shape and dimensions could be associated with divergences in the intensity and direction of natural and sexual selection forces driving burrow-digging behaviour. Also, the clustered distribution of burrows according to developmental stage already reported for this species (Aisenberg et al. 2011) could have consequences for burrow dimensions and shape due to the characteristics of the substrate, and vegetation, and other factors such as wind, humidity and solar radiation, and the consequent needs of the individuals according to the location of their burrows.

Unexpectedly, though the length of burrows inhabited by males was similar to data obtained in captivity, burrows inhabited by females were markedly larger: about four times the size reported for this species under laboratory conditions (Aisenberg et al. 2007). Furthermore, we did not find differences in burrow width, burrow volume and internal temperature between burrows inhabited by males or females.

**Table 1.** Burrow characteristics (mean ± standard deviation) in *Allocosa brasiliensis* for each category, with the corresponding statistical comparisons among them.

|                | Females       | Males         | Juveniles     | Statistics          |
|----------------|---------------|---------------|---------------|---------------------|
| Length (cm)    | 11.38 ± 3.84  | 12.94 ± 5.65  | 7.24 ± 1.95   | $H_2 = 30.6$, $p = 0.0001$  |
|                |               |               |               | Females vs males: $U = 404.5$, $p = 0.51$ |
|                |               |               |               | Males vs juveniles: $U = 122.5$, $p = 0.001$ |
|                |               |               |               | Females vs juveniles: $U = 134.0$, $p = 0.0001$ |
| Width (cm)     | 1.54 ± 0.37   | 1.67 ± 0.69   | 0.88 ± 0.37   | $H_2 = 40.7$, $p = 0.0001$  |
|                |               |               |               | Females vs males: $U = 4.0$, $p = 0.43$ |
|                |               |               |               | Males vs juveniles: $U = 86.0$, $p = 0.0001$ |
|                |               |               |               | Females vs juveniles: $U = 70.5$, $p = 0.0001$ |
| Internal temp. (°C) | 21.43 ± 2.45 | 24.76 ± 5.19 | --            | $U = 443$, $p = 0.92$ |
| Volume (cm$^3$) | 16.70 ± 12.00 | 11.50 ± 7.20  | --            | $U = 346$, $p = 0.12$ |
A possible explanation could be that female burrow dimensions reported by Aisenberg et al. (2007) were based on data obtained from virgin females in captivity. Due to their lower expectations of being accepted for mating and receiving as nuptial gift a male burrow (Aisenberg 2014), they could allocate more energy in digging their own long burrows that allow them to lay their egg sacs in adequate conditions for the development of progeny. Another possible explanation could be that females found in the field inside the burrows with open entrances could be recently mated females that are occupying male burrows. Additionally, we could expect that shallow silk refuges constructed by females in captivity (see Aisenberg et al. 2007) are abandoned by females after sunset, so they would remain undetected during the field samplings described in the present study, with observers trying to locate open burrows with individuals inside. All these interesting hypotheses require further testing.

Burrows inhabited by adult males and females showed terminal bowl-shaped chambers lateral to the bottom of the burrow, similar to those described for *Eupalaestrus weijenberghi* (Pérez-Miles et al. 2000) and for the wolf spider *Donacosa merlini* (Alderweireldt and Jocqué 1991). In *E. weijenberghi*, the narrow terminal tube functions as a defensive mechanism against pompilid wasps (Costa et al. 2004). Though these terminal chambers had already been described for male burrows of *A. brasiliensis* (Aisenberg and Peretti 2011), once again they had never been observed in female burrows (Aisenberg et al. 2007). The terminal chamber could provide adults with protection from *Anoplius* wasps that are frequent parasitoids of this species (Costa 1995; Stanley et al. 2013). The chamber would function as a trench, making it difficult for the wasp to manipulate the spider and sting it.

Surprisingly, burrow width did not increase with individuals’ size in any category. However, burrow length showed a positive relation with carapace width only in juveniles, and their burrows were shorter compared to adult burrows. Miller and Miller (1984) reported a significant correlation between burrow length and carapace size for *Geolycosa* wolf spiders. Burrow construction in the sand by spiders has been described as an expensive activity not only due to the energetic cost involved in digging behaviours, but also because of the deposition of several layers of silk necessary to provide stability to the structures (Henschel and Lubin 1992). Spiders possibly take their digging decisions based in the equation between the high costs of construction vs the benefits of constructing longer burrows. Due to the fact that male burrow length is subject of female choice and, additionally, male burrows (and perhaps mated female burrows, too) function as nests for the future progeny, they are expected to have more intense selection pressures on building long burrows compared to juveniles. Once again, the clustered and differential location of juvenile burrows at the slope of the dune (Aisenberg et al. 2011) could also explain differences in burrow characteristics between the categories.

Burrows inhabited by juveniles showed one branch and two entrances, similar to the description by Capocasale (1990), and contrary to adult tubular burrows. According to occasional observations (A. Aisenberg, unpublished data) the two entrances would allow juveniles to escape from wasp attacks when they enter their burrows by escaping through a cryptic ‘back door’ entrance. On the other hand, a larger size and larger chelicerae would allow adults of this species to adopt more offensive behaviours during
wasp attacks inside their burrows. Additionally, the terminal chambers located strategically inside male and female burrows would provide them refuge and complicate stinging by the wasp. Plasticity in anti-predatory behaviour has been cited for several spider species (Persons and Rypstra 2001; Jakob et al. 2011).

In summary, individuals of *A. brasiliensis* show variations in burrow characteristics according to their developmental stage. Future studies will focus on testing whether long burrows found occupied by females in the field correspond to virgin and/or mated females or, on the other hand, they are burrows constructed by males and donated to females after mating. It would also be interesting to test whether juveniles vary the characteristics of their burrows according to their instar. This study lays the foundation to study the differences in defensive or evasive strategies to avoid predation by parasitoid wasps according to the developmental stage and its relation to burrow characteristics in *A. brasiliensis*.

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**Disclosure statement**

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