

Female and juvenile burrow digging in *Allocosa brasiliensis*, a South American sand-dwelling wolf spider

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Summary

Allocosa brasiliensis is a sand-dwelling wolf spider that constructs burrows along the coasts of rivers, lakes, and the Atlantic Ocean in Argentina, Brazil, and Uruguay. This species shows a reversal in typical sex roles in spiders: females wander searching for males and initiate courtship, while both females and males are selective when taking mating decisions. Females prefer to mate with males that show long burrows. As burrow digging in the sand seems to be an energetically expensive activity, we would expect differences in burrow characteristics according to developmental stage and selection pressures. Our aim was to describe female and juvenile digging behaviour in *A. brasiliensis* and report burrow dimensions, comparing the results with data available for males of this species. We placed each individual ($n = 30$ of each category) in a glass cage with sand as substrate and recorded burrow construction under laboratory conditions. Only five females and nine juveniles constructed burrows after 72 hours. Burrow dimensions did not show significant differences between females or juveniles, but burrow length was markedly lower than reports for males of this species. Burrow digging was stereotyped both in females and juveniles, following a sequence of behavioural units repeated in a cycle. Digging behaviour would be highly conserved in *A. brasiliensis*. However, variations in burrow digging behaviour and final burrow dimensions would reflect differences in strategies according to sex and stage.

Introduction

Animal constructions depend on the application of different behaviours to the materials which are available on each habitat. Constructions can involve the assembly of elements, or the removal of substrate and creation of a stable structure, as is the case for burrow digging (Hansell 2005). Many arthropods are well adapted for digging, and spend part of, or their entire, lives inside their self-constructed

refuges (Gherardi, Russo & Anyona 1999; Hansell 2005; Suter, Stratton & Miller 2011). Arachnid constructions in sandy habitats have received much attention to try to answer questions related to how abiotic and biotic pressures define and mould behaviour, and how animals adapt themselves to inhabit harsh environments (Henschel & Lubin 1992; Henschel, Ward & Lubin 1992; Aisenberg & Peretti 2011a; Jakob, Skow & Long 2011; Suter, Stratton & Miller 2011).

Allocosa brasiliensis (Petrunkevitch, 1910) is a nocturnal wolf spider that inhabits South American sandy coasts of the Atlantic Ocean and of internal lakes and rivers in Argentina, Brazil, and Uruguay (Capocasale 1990). This species shows a reversal in typical spider sex roles and sexual dimorphism (Aisenberg, Viera & Costa 2007; Aisenberg 2014). Males are larger than females and construct deep burrows, while females construct shallow silk refuges where they stay during the day and in the coldest months (Aisenberg, Viera & Costa 2007; Aisenberg & Peretti 2011a). During summer nights, females search for males and initiate courtship (Aisenberg, Viera & Costa 2007; Aisenberg 2014). Mating occurs inside male burrows and, after the final dismount, both sexes cooperate in closing the burrow entrance: males from outside the burrow and females from inside. Males then exit while females stay inside the male burrows, oviposit there, and leave when it is time for spiderling dispersal (Aisenberg, Viera & Costa 2007; Aisenberg 2014).

During courtship, females of *A. brasiliensis* follow males to the base of their burrows and they prefer to mate with males with the longest burrows (Aisenberg, Viera & Costa 2007). This suggests a significant selective pressure on males for efficient digging because their burrows will be the target of female choice and the breeding nest for their future progeny (Aisenberg 2014). Previous field studies showed a heterogeneous distribution of burrows of this species in sand dunes according to sex and reproductive stage (Aisenberg, Costa & Gonzalez 2011). Burrow digging in sand has been described as an energetically expensive activity for spiders, not only for the digging behaviour per se but also due to silk production and deposition: essential behaviours to maintain a firm burrow in sand (Aisenberg & Peretti 2011b; Suter, Stratton & Miller 2011). Furthermore, because both juveniles and females are more mobile than males, and frequently leave their refuges and construct new burrows, we would expect them to invest less in burrow digging compared to adult males. Finally, burrow construction in the base or in the slope of the dune by *A. brasiliensis* individuals could imply differences in burrow dimensions and in the associated energetic costs.

While male burrow digging behaviour in *A. brasiliensis* has been described in detail (Aisenberg & Peretti 2011a) there is no such description for female and juvenile burrow digging behaviour in this species. Studying burrow digging behaviour in *A. brasiliensis* and the differences according to developmental stage and sex would help understand the value of male delivery of a long burrow as a nuptial gift in this sex role-reversed species. The aim of the present study was to describe burrow digging behaviour in females and juveniles of *Allocosa brasiliensis*, comparing the results with data available for males of this species, and discussing differences in burrow characteristics and digging behaviours according to the developmental stage, sex, and

	Males	Females	Juveniles	Statistics
Burrow length (cm)	6.71 ± 3.01	6.99 ± 3.39	10.00 ± 5.53	F = 1.62, p = 0.22
Burrow width (cm)	1.06 ± 0.14	1.11 ± 0.14	1.09 ± 0.12	F = 0.20, p = 0.82

Table 1: Burrow dimensions (mean ± standard deviation) in males (Aisenberg & Peretti 2011a), females, and juveniles, and statistical comparisons among them.

sexual strategies of the species. We expected that, due to the potential energetic costs of burrow digging, females (who will occupy male burrows after mating), and juveniles will invest less in burrow digging and show shorter burrows than males.

Methods

Thirty adult females and 30 juveniles (antepenultimate or penultimate sub-adults) of *A. brasiliensis* were collected between January and May 2012, on the bank of the San Antonio river (31°21'03.18"S 64°34'37.26"E), Cuesta

Blanca, Córdoba, Argentina. We housed each spider in a transparent plastic box 5 cm wide, 9 cm long, 2 cm tall, with sand as substrate and damp cotton wool. We fed the individuals twice a week with *Tenebrio molitor* larvae (Coleoptera, Tenebrionidae). Room temperature during breeding was 23.5 ± 1.5°C (mean ± standard deviation, range: 22.5–27.8°C).

One week after their capture at the field, we introduced each spider (n = 30 females and juveniles) in a glass terrarium 15 cm wide, 30 cm long, 40 cm tall, with sand collected from the capture site as substrate (15 cm of dry sand and 5 cm of moistened sand at the base, following Aisenberg

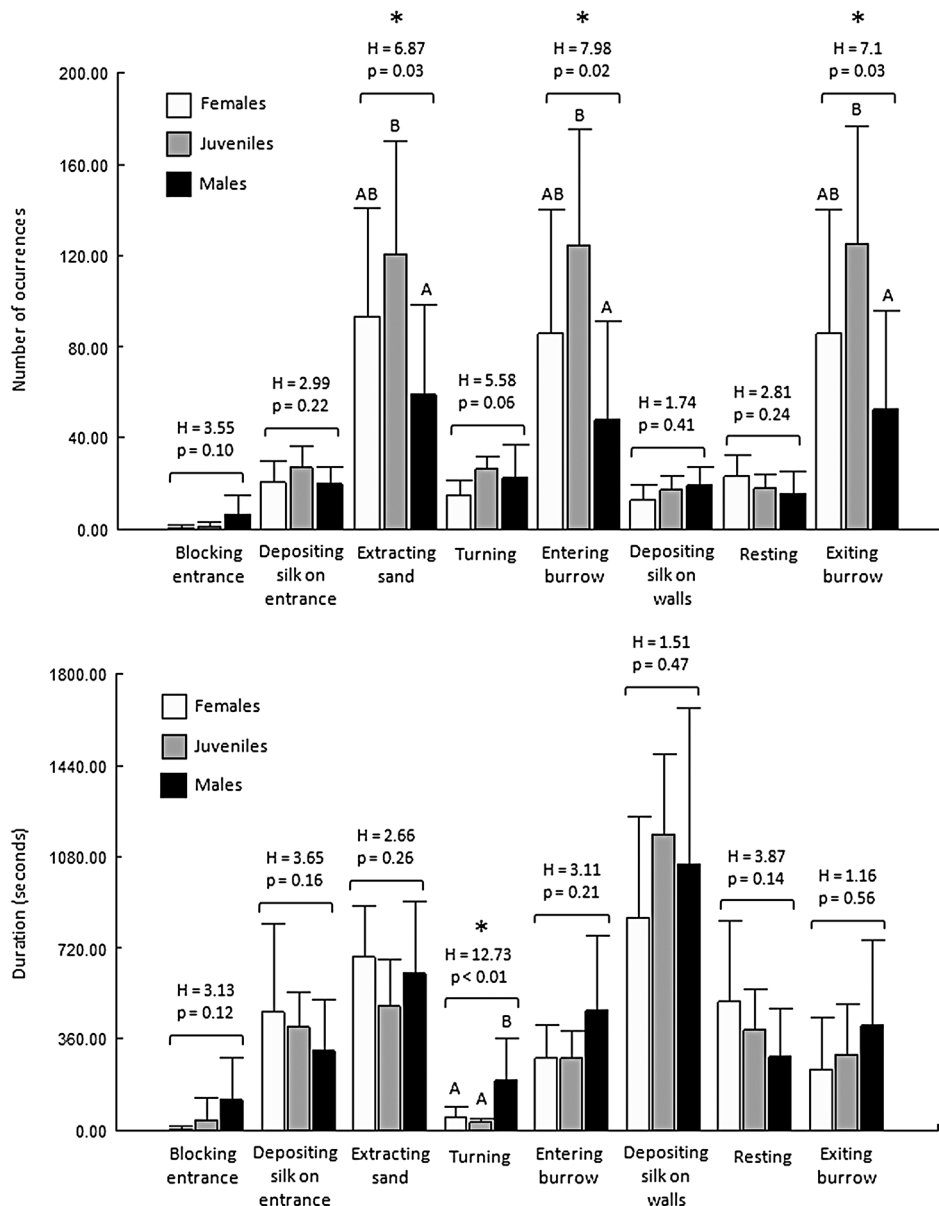


Fig. 1: Mean values and standard deviations of the number of occurrences of behavioural units associated with digging (upper graph) and their corresponding durations (lower graph), distinguishing between behaviours performed by males, females, and juveniles of *A. brasiliensis*.

Females

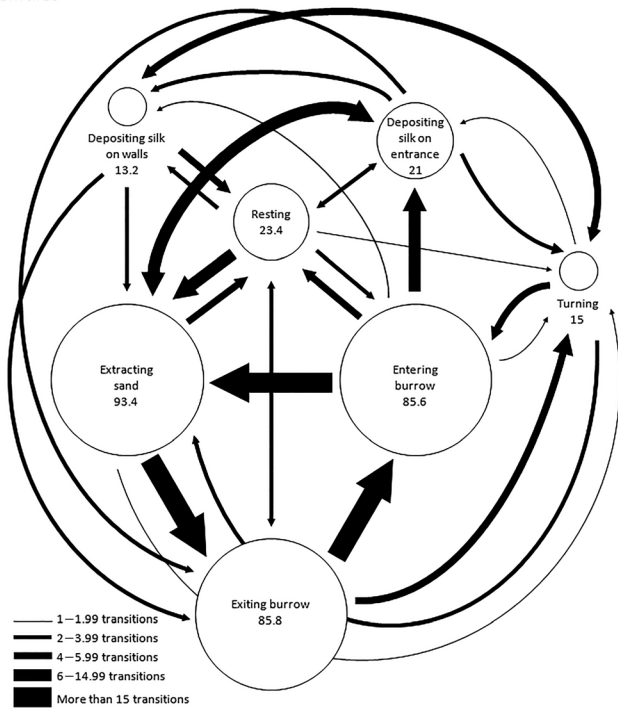


Fig. 2: Flow diagram showing the mean number of transitions (arrow length) for each digging behavioural unit performed by females of *A. brasiliensis*. The circle diameters represent absolute frequencies of occurrence for each behaviour (corresponding to mean values of each individual, $n = 5$).

Juveniles

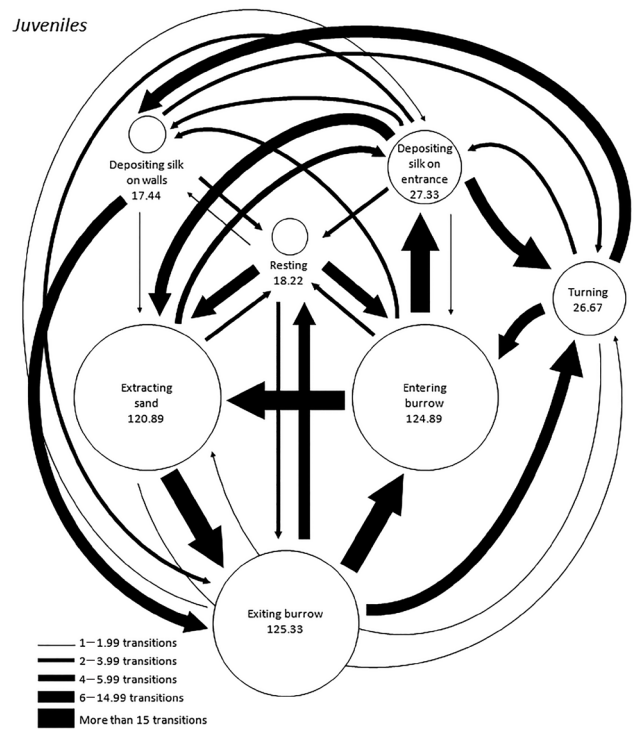


Fig. 3: Flow diagram showing the mean number of transitions (arrow length) for each digging behavioural unit performed by juveniles of *A. brasiliensis*. The circle diameters represent absolute frequencies of occurrence for each behaviour (corresponding to mean values of each individual, $n = 9$).

& Peretti (2011a), and a plastic top with damp cotton wool as water provision. We performed all observations between 12.00 pm and 17.00 pm, as did Aisenberg & Peretti (2011a), as a way to motivate fast burrow construction when individuals of this species are exposed to daylight. We recorded the behaviour of the individuals for one hour after we detected the occurrence of the first sand extraction behaviour. For detailed analysis, we recorded all the observations with a HD Sony Handycam HDR-XR150 video digital camera with 1 \times , 2 \times and 4 \times lenses. If burrow digging behaviour did not occur for 30 minutes, we concluded the observation and it was recorded as a negative digging attempt. After 72 hours, we measured the burrow dimensions (length and diameter of the burrow entrance).

The results were analysed using NCSS 2001 (©2000, Jerry Hintze). We compared burrow digging results obtained in the present study with females and juveniles with that obtained for males by Aisenberg & Peretti (2011a) under similar conditions. We checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene test) of data corresponding to burrow dimensions. Thus, we compared burrow dimensions among females, juveniles, and males with the ANOVA test. We also compared digging occurrence between females, juveniles, and males with the χ^2 test. We analysed the number of occurrences, duration, and sequences of the behaviours using JWatcher software (Blumstein, Daniel & Evans 2000). We followed the nomenclature of digging behavioural units of Aisenberg & Peretti (2011a), and elaborated descriptive flux diagrams (Lehner 1996) taking as a diagram model the one described by Benton (1993).

Voucher specimens are deposited at the Colección de Referencia de Cátedra de Diversidad Animal I, F.C.E.F.N., Universidad Nacional de Córdoba, Argentina, and at the Colección Entomológica de Facultad de Ciencias, Universidad de la República, Uruguay.

Results

Out of 30 attempts for each category, only five females and nine juveniles excavated. Burrow dimensions are summarized in Table 1. We found no difference in burrow length ($U = 6$, $p = 0.52$) or width ($U = 4$, $p = 0.23$) between antepenultimate or penultimate juveniles. All the constructions, both of females and juveniles, were tubular with only one entrance. Burrow digging frequencies showed differences between the three categories ($\chi^2 = 23.54$, $p = 0.0001$): males dig their burrows more frequently than females ($\chi^2 = 22.22$, $p = 0.0001$) and juveniles ($\chi^2 = 14.74$, $p = 0.0001$), but females and juveniles did not show differences between them ($\chi^2 = 1.49$, $p = 0.22$). When we compared burrow dimensions among males, females, or juveniles, we found no significant differences between the three categories (Table 1).

Burrow-digging behaviour in females and juveniles was similar and highly stereotyped. We distinguished eight behavioural acts associated with burrow construction: extracting sand, exiting the burrow, entering the burrow, depositing silk on burrow walls, deposition silk at the burrow entrance, turning 180°, blocking the burrow entrance, and resting (Fig. 1).

Burrow digging starts with extracting sand using the palps and chelicerae, incorporating the tibia and metatarsus to help compact and transport the substrate away. Once holes in the sand are formed, individuals start depositing silk lines around the entrances, alternating this behaviour with extracting sand. Sand extraction consists of exiting the hole with the grains of sand, depositing the sand outside the hole, and re-entering the burrow. This pattern is repeated several times, both in females and juveniles, in sequence with depositing silk at the burrow entrance, turning 180° outside the burrow, entering the burrow, and depositing silk on burrow walls (Figs. 2, 3). On three occasions in females, and five in juveniles, turning occurred inside the burrow. Resting occurred frequently throughout the whole digging process (Fig. 1). Blocking the burrow entrance occurred only on three occasions (one in females and two in juveniles), so this behaviour was not considered in the flux diagrams (Figs. 2, 3). When individuals blocked entrances, they did so from inside the burrow, dragging to the opening partially compacted sand with silk, and alternating this behaviour with silk deposition on the burrow entrance from inside until the opening was occluded. The most frequent behavioural acts were extracting sand, exiting the burrow, and entering the burrow, and the act with the highest duration was depositing silk on burrow walls, for both females and juveniles (Fig. 1).

When we compared the frequencies of occurrence of burrow-digging behavioural units between males, juveniles, and females, we found that juveniles showed higher frequencies of extracting sand, entering burrow, and exiting burrow. Males showed a higher duration of turning compared to females and juveniles.

Discussion

In the present study, digging behaviour by adult females and sub-adult juveniles of *A. brasiliensis* was highly stereotyped, following a sequence of few behavioural acts in a repetitive cycle. This is in agreement with the digging behaviour described for males of this species (Aisenberg & Peretti 2011a), which suggests that burrow construction is a conservative character in this species. However, we found some differences in burrow-digging behaviour between juveniles and adults of *A. brasiliensis*. Their higher frequencies in extracting sand, and entering and exiting the burrow compared to adults could be related to their smaller size, which could imply a higher locomotion rate due to their lower capacity for carrying large quantities of sand. Frequencies of burrow digging in females and juveniles of *A. brasiliensis* were very low compared to males of this species (Aisenberg, Viera & Costa 2007; Aisenberg & Peretti 2011a), in spite of the fact that, in both studies, the observations were performed under similar laboratory conditions. A possible explanation is that males are under strong selective pressures to be good diggers, due to the fact that male burrows are under selection by female choice (Aisenberg 2014). Males need to construct long burrows and wait for female visits (Aisenberg, Viera & Costa 2007). After each mating, males donate their burrow to the female, so they need to construct new burrows to find a new refuge

during daylight and gain access to new mating partners. The high value of this resource in *A. brasiliensis* males would determine the high digging response in this sex. On the other hand, juveniles and females would not suffer that intense selective pressure. Both juveniles and females are mostly wanderers and frequently abandon their burrows when they leave to forage and/or (in the case of females) when they search for mates (Aisenberg 2014), so we would expect a lower energetic expenditure in burrow construction. Unexpectedly, though previous studies in this species have reported that male burrows in *A. brasiliensis* are longer than those constructed by females (Aisenberg, Viera & Costa 2007; Garcia Diaz, Aisenberg & Peretti 2009), we did not find differences in burrow length or width between burrows constructed by females and juveniles and those reported for males of this species. We can discard the idea that the lack of differences is an artifact of captivity because both the experimental cages and laboratory conditions were very similar to previous studies performed under laboratory conditions (Aisenberg, Viera & Costa 2007; Garcia Diaz, Aisenberg & Peretti 2009). Preliminary observations in the field suggest that juveniles from this population construct long burrows similar to those constructed by males (burrow length, male burrow: 13 cm, juvenile burrow: 9 cm; De Simone, unpublished data); however, sample sizes are small and new observations are required. Regarding females, a possible explanation for the lack of differences in burrow length could be that adult females used in the present study were collected as adults and, consequently, they had unknown reproductive histories. Females used in previous studies in this species (Aisenberg, Viera & Costa 2007; Garcia Diaz, Aisenberg & Peretti 2009), were virgins, captured as sub-adults and bred in isolation until adulthood. According to Aisenberg *et al.* (2010), males are selective on female reproductive status, preferring virgins over mated females. Virgin females have high possibilities of being accepted for mating and, as a result, receiving male burrows as nuptial gifts. However, females of this species can lay up to four egg sacs during a reproductive period (Postiglioni, González & Aisenberg 2008) and, as they leave male burrows for spiderling dispersal, they will need new burrows for laying their next egg sacs. As mated females are frequently rejected and/or cannibalized by males (Aisenberg *et al.* 2010), they could invest more than virgins in burrow construction. Some females used in the present study could be already mated and ready for ovipositing, which could explain why they constructed long burrows. This argument is supported by the fact that the reproductive period of this species runs from November to May (Costa 1995; Costa *et al.* 2006) and, because of the period of collection in the present study, there is a high probability of obtaining mated females. Burrows in females and juveniles were tubular, with only one entrance. However, according to Capocasale (1990), juveniles of *A. brasiliensis* from Uruguayan populations construct branched burrows with two entrances. Branched burrows seem to provide juveniles an additional escape route from parasitoid *Anoplius* wasps (Stanley, Toscano-Gadea & Aisenberg 2014). The fact that juveniles from Argentinian populations construct tubular burrows could have several explanations. There could be differences in predation risk for *A. brasiliensis* between the two sites.

Substrate composition also seems to vary between the two populations, and this could have consequences on burrow dimensions. In fact, the substrate from the collection site of individuals used in the study of Capocasale (1990) in Uruguay contained predominantly fine sand grains, whereas the substrate of the present study had a high percentage of coarse sand (Aisenberg, Costa & Gonzalez 2011; G. De Simone, unpublished data).

Similar to the description for males of *A. brasiliensis* (Aisenberg & Peretti 2011a), burrow digging in females and juveniles was characterized by the occurrence of high frequencies of occurrence of the sand extraction unit, and numerous entering the burrow and exiting the burrow units. All these behaviours were performed very quickly, perhaps as a way to minimize the exposure outside the burrow and predation risk. In agreement with the description for males in this species (Aisenberg & Peretti 2011a), the duration of silk deposition, both at the burrow entrance and on walls, was high, suggesting a potential high cost for burrow digging associated to silk production in this species.

Finally, the present study lays the foundation for future work testing the plasticity in digging behaviour in *A. brasiliensis*, decision making in relation to burrow digging and burrow dimensions, costs associated with this activity, and variations in burrow digging among populations of this South American wolf spider.

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