

No pain, no gain: Male plasticity in burrow digging according to female rejection in a sand-dwelling wolf spider



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ABSTRACT

Behavioral plasticity allows individuals to reversibly respond to short-term variations in their ecological and social environment in order to maximize their fitness. *Allocosa senex* is a burrow-digging spider that inhabits the sandy coasts of South America. This species shows a reversal in typical sex roles expected in spiders: females are wanderers that visit males at their burrows and initiate courtship. They prefer males with long burrows for mating, and males prefer virgin over mated females. We tested whether female sexual rejection induced males to enlarge their burrows and if female reproductive status affected males' responses. We exposed males who had constructed burrows to: a) virgin females or b) mated females, ($n = 16$ for each category). If female rejection occurred, we repeated the trial 48 h later with the same female. As control, we maintained a group of males without female exposure (unexposed group, $n = 32$). Rejected males enlarged their burrows more frequently and burrows were longer compared to unexposed males. However, frequency and length of enlargement did not differ according to female reproductive status. Males of *A. senex* showed plasticity in digging behavior in response to the availability of females, as a way to maximize the possibilities of future mating.

1. Introduction

Animal constructions such as elaborated nests, burrows, pebble mounds or sand hoods, can function not only as refuges but also as signals that transmit reliable information about the condition and motivation of the constructor (Barlow, 2000; Christy et al., 2003; Schaedelin and Taborsky, 2009). The construction of structures can imply costs for the individuals in terms of the energy required for the construction activities per se and, in some cases, in the defense of the resources from intruders. High quality individuals in good physical condition would be expected to produce more elaborate and expensive structures that can be honest signals of their physical and cognitive abilities (Andersson, 1994; Johnstone, 1995). Furthermore, when male constructions are delivered to females prior to or during mating, they can also represent direct benefits to females in terms of avoidance of energetic costs associated to searching and defending a territory, looking for materials and the activity of construction per se, while providing a mating refuge and/or breeding nest (Andersson, 1994). Therefore, during sexual interactions male constructions are well-suited targets of female choice (some examples: Backwell et al., 1995; Kellogg et al., 2000; Madden, 2002; Hansell, 2005; Quader, 2005). However, as

we said before, those constructions imply costs so males will need to scan the environment and adjust their investment according to mating opportunities and the cost of producing and defending the structure.

Phenotypic plasticity – the ability of an individual or genotype to express different phenotypes according to the environment and its variations – is a widespread phenomenon that is an essential component of fitness (West-Eberhard, 2003). Specifically, behavioral plasticity is of particular significance because it allows individuals to reversibly respond to short-term fluctuations in the ecological and social environment in order to maximize their fitness (Bretman et al., 2012, 2014). For example, local sex ratios can vary very quickly during a reproductive season, influencing levels of encounter rates, competition for mate access, mating opportunities and mate choice (Bonduriansky, 2001). Therefore, in those cases in which males invest in the construction of elaborate structures associated with reproduction, they will be selected to respond on time to changes in the environment and allocate energy and resources accordingly (Bretman et al., 2011).

Traditionally, arthropod behavior was considered mainly driven by instinct and inflexible. Nevertheless, during the last decades various studies have challenged those concepts showing that behavioral plasticity and learning occur in this group, suggesting that those

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abilities have been historically ignored or misunderstood (Dukas, 2008; Jakob et al., 2011). Arthropods are able of responding successfully to changes in their environment in foraging, predatory, anti-predatory, social and sexual scenarios (Dukas, 2008; Milner et al., 2010; Allen et al., 2011; Bretman et al., 2011; Jackson and Cross, 2011; Jakob et al., 2011). Studies of behavioral plasticity in spiders in relation to web construction, predatory responses, and sexual behaviors have increased exponentially during the last years (Heiling and Herberstein, 1999; Maupin and Reichert, 2001; Jackson et al., 2002; Wilder and Rypstra, 2008; Blamires, 2010; Gordon and Uetz, 2011; Jakob et al., 2011; Schneider and Andrade, 2011; Hesselberg, 2015). Spiders can show elaborate cognitive behaviors in response to clues in the surrounding ecological and social environment (Hebets and Sullivan-Beckers, 2010; Jackson and Cross, 2011). Regarding sexual behavior in wolf spiders and as some examples, in *Schizocosa ocreata* females exposed during their sub-adult stage to males of certain kind of phenotype (with manipulated sexual traits) were more likely to mate with them than with males of an unfamiliar phenotype (Hebets, 2003). Stoffer and Uetz (2015) also reported that female preference in that same species can vary according to the number of male expositions received under the penultimate stage: penultimate females with more male visits are more selective as adults. Other studies have also shown associative learning capacity in males of the wolf spider *S. ocreata* by means of eavesdropping, in which males can infer the presence of a female in the area by observing nearer males performing courtship (Clark et al., 2015). Therefore, these examples demonstrate that spiders can adjust their behavior according to previous social experiences suggesting that spiders can be much more flexible than it was traditionally assumed.

Allocosa senex (Mello-Leitão, 1945, synonym of *Allocosa brasiliensis* Petrunkevitch 1910 according to Simó et al., 2017) is a nocturnal wolf spider that inhabits the sandy coasts of South America (Capocasale, 1990). They dig burrows in the sand where they stay during the day and in the coldest months, becoming active in summer nights (Costa, 1995; Costa et al., 2006). This species shows a reversal in expected sex-roles and in traditional sexual size-dimorphism in spiders (Aisenberg et al., 2007; Aisenberg, 2014). Males are larger than females, and females are the roving sex that looks for potential mates and initiates courtship (Aisenberg et al., 2007). Females inspect male burrows before accepting mating and they prefer those males that show long burrows (Aisenberg et al., 2007). On the other hand, males are selective too: they prefer virgin females in good body condition and non-preferred females can be rejected for mating and cannibalized (Aisenberg et al., 2011). Copulation occurs inside male burrows and after it ends, the male exits and covers the burrow entrance before leaving (Aisenberg et al., 2007). The female stays inside the male burrow and oviposits there, leaving when it is time for spiderling dispersal (Aisenberg, 2014).

Burrow digging in the sand is expected to be an energetically expensive activity in spiders (Henschel and Lubin, 1992; Craig, 2003; Suter et al., 2011). After mating, males of *A. senex* need to construct another burrow to have access to new mating opportunities and this burrow should be long enough to be accepted by a female (Aisenberg, 2014). Males would be expected to invest in burrow digging according to the possibilities of female visits and mating. The aim of the present study was to test whether males show plasticity in burrow digging behavior and respond to female rejection by enlarging their burrows. We also assessed the influence of female reproductive status on male posterior digging response. We predicted that males would enlarge their burrows more often after female visits and, in agreement with their preference pattern (virgin over mated females and females in good body condition; Aisenberg et al., 2011).

2. Materials and methods

2.1. Collecting and housing

We collected sub-adults and adults of *A. senex* in the coastal sand

beaches of San José de Carrasco (34°50'44.38"S, 55°59'17.64"W), Canelones, Uruguay, from November to April 2012, 2013 and 2014. We captured the spiders during the night using head-lamps to locate them while they are walking or leaning out from the burrow entrances. This spider has been recently named priority species for conservation due to its occurrence only in coastal sandy habitat, environments that are considered endangered and in drastic reduction in Uruguay (Ghione et al., 2017). We decided to work with moderate sample sizes to avoid the extraction of large numbers of individuals from the wild.

We housed the spiders individually in Petri dishes (diameter 9.5 cm, height 1.5 cm), with sand as substrate and cotton wool embedded in water. Individuals were fed twice a week with mealworm larvae *Tenebrio* sp. and domestic flies (*Musca domestica*). We monitored daily the spiders and recorded molting occurrence in sub-adults to determine the exact date of reaching adulthood. Individuals were maintained at an average temperature of 24.4 ± 1.1 °C (range: 21.5–27.0 °C).

2.2. Experimental set-up

The observations took place from December to May in agreement with the reproductive period reported for this species (Costa, 1995). At the field males construct vertical burrows that average 12.94 ± 5.65 cm of length (Albín et al., 2016). So, for the trials we used glass cages of 30 cm length, 16 cm width, and 20 cm height, with a layer of 15 cm of sand as substrate and water supply, following previous studies on this species (Aisenberg et al., 2007). The sand was brought from the capture site of the individuals. Each male was placed in the arena 48 h before the trial to allow burrow construction. Individuals usually construct their burrows against the glass walls, allowing the observation of their behaviors when they are inside the burrows (Aisenberg et al., 2007).

Virgin females were obtained by individually raising the sub-adults until reaching adulthood. We used virgin females of at least 10 days of adult age, time when they are receptive to matings (Aisenberg et al., 2007). For the trials of mated females we used adult females captured walking during the night. The reproductive status of those females captured at the wild (which could be either virgin or mated females) was confirmed after the end of each trial in the cases that courtship occurred but mating did not occur. If cannibalism occurred we removed the female from the male to determine the reproductive status (in case it was a female captured as adult at the wild). We dissected their spermathecae, embedded them in clove oil and observed sperm receptacles under an Olympus SZ61 dissecting microscope. In each case we checked for the presence of full spermathecae, comparing them with spermathecae under the same treatment of two females of known reproductive history (one virgin female and one female previously mated in the laboratory with full spermathecae). In the cases that females showed empty spermathecae they were discarded from the experimental group corresponding to mated females and they were not considered in the study (we were not sure if they were virgin or mated females they had just ran out of sperm). Mated females and adult males from the wild were used at least 7 days after their capture. Virgin males were used 7 days after their final molt. Males were randomly assigned to each experimental trial. We used males with unknown reproductive history (captured as adults) or virgin males (molted under laboratory conditions) indistinctly because they do not show differences in courtship or mating behavior (Aisenberg et al., 2011). We did not find statistical differences in burrow length between constructions of virgin males and males of unknown reproductive history ($U = 479.0$, $p = 0.74$).

In all the cases we recorded burrow length at the beginning of the trial. Previous studies (Aisenberg et al., 2007; Aisenberg et al., 2011) have shown that individuals of this species tend to construct their burrows against the walls of the terrarium, allowing us to measure burrow dimensions, observe and record male and female behaviors when they are inside their burrows, through the glass walls. We

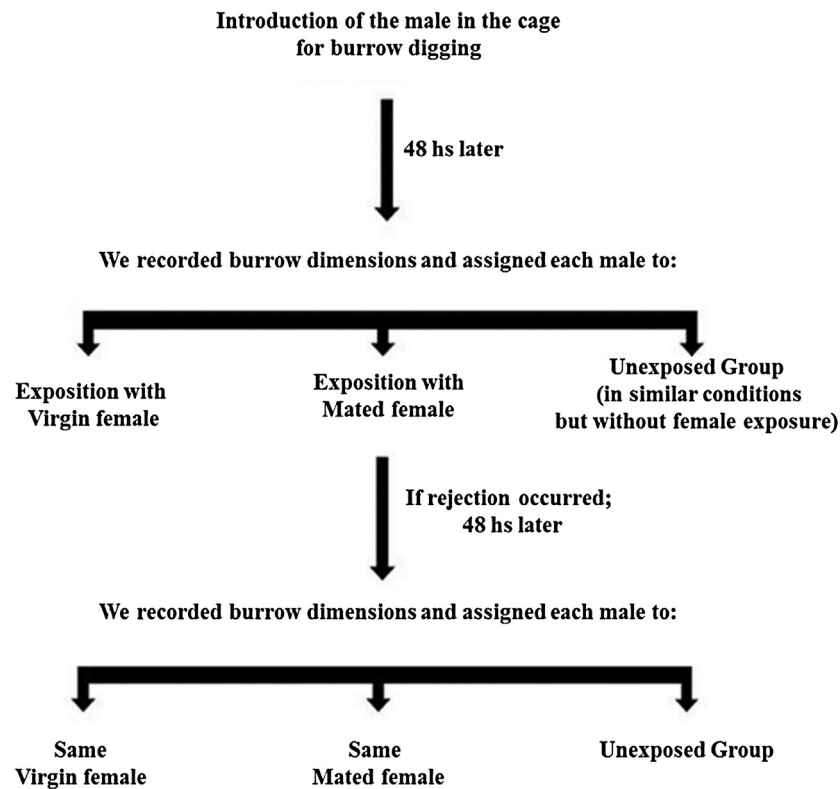


Fig. 1. Schematic representation of the experimental set-up.

exposed each male who had constructed a burrow to: a) one virgin female ($n = 19$), or b) one mated female ($n = 23$) (Fig. 1). If female rejection occurred, we repeated the trial 48 h later with the same male and female in the same glass cage. Each trial had a control group with a male in a glass cage during the same time-period and conditions, but without female exposure ($n = 42$; unexposed group, Fig. 1). Each observation started when we placed the female in the experimental arena. We only considered trials in which the female detected the male's burrow within one hour. We monitored the occurrence of female and male courtship, mating and male attacks on females that resulted on injuries, leg loss and/or cannibalism. We considered female courtship as the bursts of foreleg waving performed while she leans into male's burrow and/or inside the burrow (Aisenberg et al., 2007). Male courtship consists of rhythmic bouts of body shakes (Aisenberg et al., 2007; Garcia Diaz et al., 2015). We only considered trials in which female or male courtship occurred in 30 min after placing the female in the arena. If courtship did not take place, the trial ended 30 min after the female detected the male's burrow. If courtship occurred but mating did not occur, the trial ended 1 h after placing the female in the arena.

To minimize observer bias, blinded methods were used when all the behavioral data were recorded and/or analyzed. All the trials were recorded in darkness, with a Sony DCR-SR85 digital video-camera equipped with night-shot. The video recordings were analyzed with JWatcher software (Blumstein et al., 2000). Courtship in this species includes burst of female leg weaving when facing the male, male body shakes and forelegs contacts between the two sexes (Aisenberg et al., 2007; Aisenberg, 2014). We analyzed courtship durations of each sex, frequencies of bursts of female leg waving, and frequencies of bursts of male body shaking in each experimental group. Female courtship duration was considered since she first leaned inside male burrow and performed the first burst of leg waving and until the last burst of leg waving; male courtship duration was considered from the first and until the last burst of body shake. We measured carapace width of the individuals – a measurement considered representative of body size in spiders (Eberhard et al., 1998)–, abdominal width, and weight of each

individual measured immediately before they were placed in the experimental cage. The index abdominal width/carapace width was considered as representative of body condition, as described by Moya-Laraño et al. (2003) for *Lycosa tarantula*. Voucher specimens were deposited in the arachnological collection of Sección Entomología, Facultad de Ciencias, Montevideo, Uruguay.

2.3. Statistical analysis

We analyzed the results using Past Palaeontological Statistics version 1.18 (Hammer et al., 2003), WINPEPI version 1.6 (Abramson, 2004) and RStudio (2009–2016). We performed comparisons of the occurrence and length of burrow enlargement between males exposed to: virgin females, mated females, and altogether (without considering female reproductive status), with the corresponding unexposed groups. As we wanted to test males responses to female rejection, for those comparisons we used only those cases in which the males were rejected by females in their first exposure (we did not considered cases of mating or cannibalism in the first exposure). We also compared courtship characteristics between expositions with virgin and mated females (non-paired tests), and between first and second expositions in each group (paired tests). Variables were checked for normal distribution (Shapiro–Wilk test) and homogeneity of variances (Levene test). Variables neither following a normal distribution nor having homogeneous variances were analyzed with non-parametric Mann-Whitney *U* test. We used the parametric Student paired *t* or non-parametric Wilcoxon paired test to compare courtship behavior and burrow length in first and second expositions. We used Chi-square tests to compare frequencies of mating and cannibalism across the experimental groups.

We analyzed a regression model controlled by male body weight and body index, and evaluated whether the interaction between treatment (female exposition) and female status (virgin or mated) was associated with the frequency (logistic multiple regression) or the length (multiple regression) of enlargement of male burrow. We repeated this analysis only for the males that were exposed to females

(not considering the controls). In this case, the characteristics of females (weight, body condition index) were added as predictors. Finally, we used a logistic regression considering the mating acceptance of the female in the second exposure as a response variable and the frequency of enlargement of the burrow and male body characteristics (weight, body condition index) as predictors. Variables that did not follow a normal distribution were adjusted in a lognormal model.

3. Results

During the first expositions with virgins we obtained one mating, two cases of sexual cannibalism (one with previous female courtship), and 16 cases with courtship but with no mating (6 only with female courtship, 3 only with male courtship and 7 with courtship by both sexes). In their second expositions in three cases we obtained matings, in one case sexual cannibalism occurred (with absence of female courtship) and in 12 cases courtship occurred but mating did not take place (3 only with female courtship, 3 only with male courtship and 6 with courtship by both sexes). In first expositions with adult females captured at the wild we obtained 2 matings, 5 cases of sexual cannibalism (2 with previous female courtship), and 16 cases with courtship but with no mating (6 only with female courtship, 1 only with male courtship and 9 with courtship by both sexes). Finally, in their second expositions, in three cases we obtained matings, in two cases sexual cannibalism occurred (both with previous female courtship) and in 11 cases courtship occurred but mating did not take place (4 only with female courtship, 2 only with male courtship and 5 with courtship by both sexes). Nineteen males enlarged their burrows in the exposed treatment and nine in the unexposed treatment. We obtained five matings in the exposed treatment; all the matings occurred with males which had enlarged their burrows in comparison with their first exposition.

In the 16 cases for each category (virgins, mated females) in which neither mating nor cannibalism occurred in the first exposition, we proceeded with a second exposition with the same female and male. Female weight was the only significant predictor of enlargement frequency ($p = 0.01$), but not female status (virgin or mated) ($p = 0.48$, Fig. 2). However, we did not find a significant relation

between the frequency of female leg weaving (bursts/second) and female body weight ($R = 0.013$, $F = 0.004$, $p = 0.95$). Burrow length increased after 48 h after female rejection in the exposed treatment ($W = 348$, $p = 0.0009$) but not in the unexposed treatment ($W = 120$, $p = 0.88$). We summarize the results on how often (percentages) and how much males enlarged their burrows after rejections, in treated and untreated groups in Fig. 3. Males exposed to females (considering altogether virgins and mated females) that were rejected during their first exposition enlarged their burrows more frequently than in unexposed males ($p = 0.01$) (Fig. 3). When we considered the length of burrow enlargement (difference between the length of the burrow in the second exposure and the first one), the exposition to females was the only significant predictor ($p = 0.0009$) (Fig. 2). When we analyzed the enlargement length, we did not find a significant relation with female status ($p = 0.58$), female weight ($p = 0.57$), female body condition index ($p = 0.47$), male weight ($p = 0.46$), or male body condition index ($p = 0.60$) (Table 1). Finally, we found that both the frequency of burrow enlargement ($p = 0.01$) and male body condition index ($p = 0.0009$) were positive significant predictors of female mating acceptance during second exposures.

In general we did not find statistical differences in female or male courtship behavior in trials with virgins compared to mated females when we compared the first or second expositions (Table 2), with the only exception of the frequencies of female leg waving in the first exposition that were higher in mated females compared to virgins. When we compared courtship behavior in the first and second exposition we found that virgin females intensified their leg waving courtship behavior during their second exposition, while mated females that had not mated in their first exposition reduced this behavior in the second instance (Table 3). On the other hand, males showed longer courtship duration in their second expositions with mated females (Table 3).

4. Discussion

According to our results males of *A. senex* can assess the immediate social environment and, consequently, modify the size of their burrows after being rejected by females in order to increase the possibilities of mating success. We observed this tendency in a higher frequency of

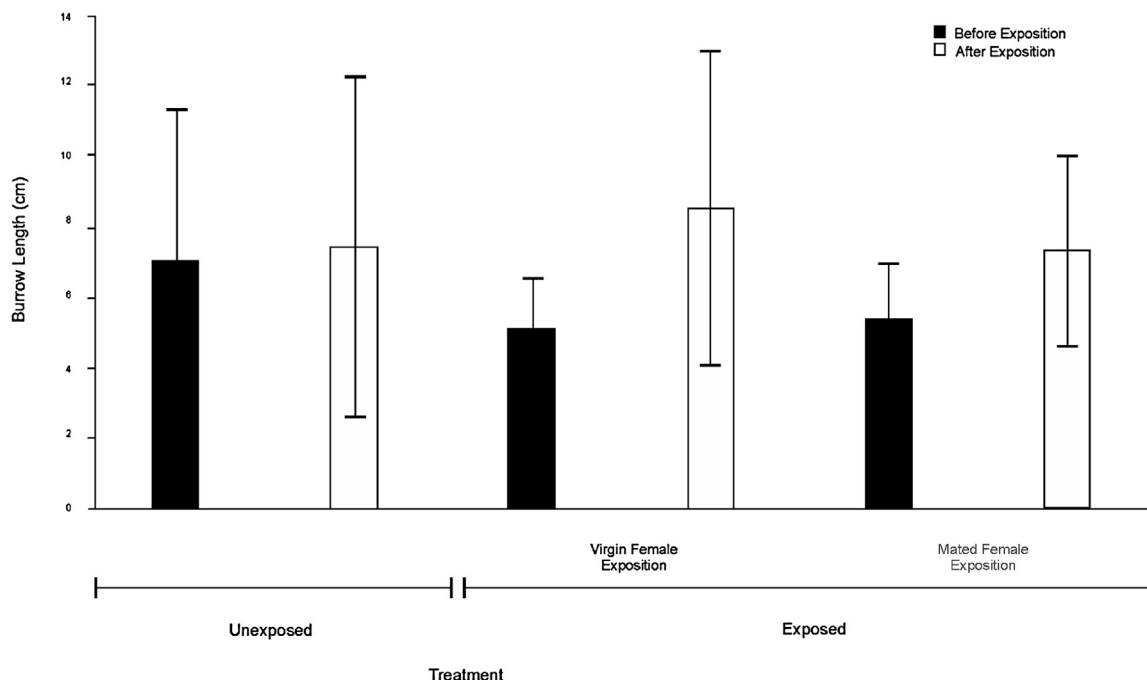


Fig. 2. Comparison of length of enlargement (mean ± SD) between the unexposed and exposed treatments. In the exposed treatment we distinguish between expositions with virgin and mated females.

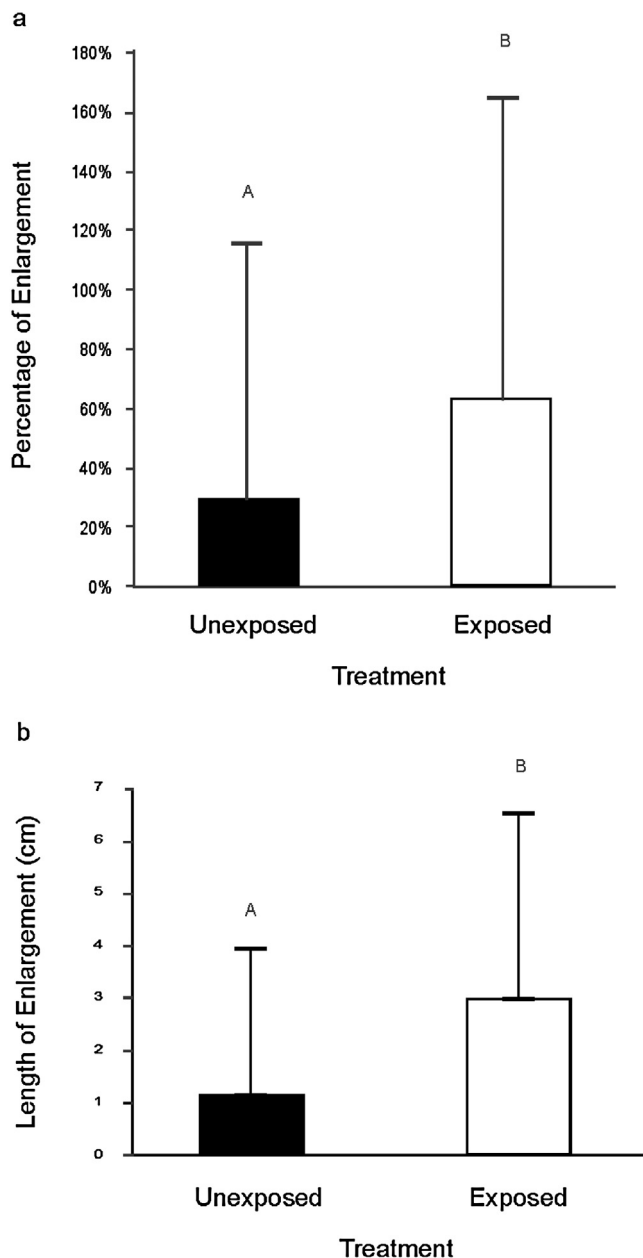


Fig. 3. Comparison of percentages of enlargement (above) and length of enlargement in mm (below) between the unexposed and exposed (virgins and mated females altogether) treatments 48 h after the first measurements. Different letters (A, B) stand for statistical significant differences between the groups.

Table 1

Body characteristics (mean \pm standard deviation) of individuals used in each experimental group.

	Carapace width (mm)	Body condition index
Virgin females	4.90 \pm 0.60	1.25 \pm 0.15
Mated females	5.00 \pm 0.50	1.15 \pm 0.15
Males exposed to virgin females	6.20 \pm 0.70	1.00 \pm 0.10
Males exposed to mated females	6.10 \pm 0.70	1.00 \pm 0.10
Unexposed males	6.11 \pm 0.80	0.99 \pm 0.14

burrow enlargement and longer-sized burrows in the group exposed to females compared with the unexposed group. All these results agree with a modulation of male digging behavior in response to female visits and mating rejection.

Behavioral plasticity shown by males of *A. senex* implies an

interesting discovery in the context of sexual selection and energy investment on sexual traits in this species. Indeed, in this spider the burrow represents a crucial gift that the males donate to females, where females will lay eggs and care for the spiderlings in a safe place until dispersal (Aisenberg, 2014). This gift seems to be the most decisive trait that females evaluate in males to choose or reject them for mating (Aisenberg et al., 2007; Aisenberg, 2014). We show here that males are not passive in front a rejection, being the burrow length a non-fixed variable that can be adjusted depending on previous short-term experience in a sexual interaction.

Not all *A. senex* males that enlarged their burrows mated. However, we found that when males enlarged their burrows they had more chances of mating with the same female that had rejected them during their first encounter. Also, males with higher indexes of body condition were more successful in obtaining matings in their second exposition. Females of *A. senex* are extremely selective and in our study the frequencies of mating occurrence were low. One explanation could be that the degree of burrow enlargement was still not enough to convince the female to mate. Nevertheless, this does not minimize the fact that the male can modulate the burrow construction in order to reach a better quality (variable “burrow length” in this study case) of the trait that the female will choose at the beginning of the sexual interaction.

In other groups where males offer nest or territories to females, male mating success also depends on female inspection and evaluation of the offered resources. This scenario has been observed in fish (e.g. in *Pseudolabrus celidotus*) where males offer territories in deeper waters to reduce egg predation (Andersson, 1994). In male zebra finches previous mating success may influence subsequent nest construction. Males either can use the same elements for nest building if they had got sexual success or avoid those if mating failed (Collias and Collias, 1984; Muth and Healy, 2011). Also, in many birds like *Ploceus cucullatus*, the female chooses its partner in relation to the quality of the nest built by the male (Andersson, 1994). Therefore, flexibility in design and elaboration could be predicted for the male burrow in *A. senex*, in particular because the cost of burrow construction is expected to be high in terms of silk production, deposition and digging activities (Aisenberg and Peretti, 2011).

One interesting aspect of our results is that the frequency of occurrence of burrow enlargement (i.e., yes versus no) was higher after rejection of females compared to unexposed groups but we did not find a significant relation with this behavior and female reproductive status (virgin or mated). This disagrees with our previous expectations based on the preference of males of *A. senex* for virgin over mated females according to Aisenberg et al. (2011). One possible explanation could be that males enlarge their burrows after female visits with two functions: to obtain matings or to cannibalize females. Indeed, male cannibalism on mated females is more frequent on virgin females in this species (Aisenberg et al., 2011) and it occurs after female inspection of the male burrow and courtship. It is important to point out that males do not cannibalize all mated females and in some cases they obtain mating (Aisenberg et al., 2011). This could explain why males also enlarged their burrows also after the rejection by mated females. Furthermore, female body weight – regardless of female status- proved to be a good predictor of male decision to enlarge the burrow. However, we did not find that this characteristic was associated with female courtship intensity. During courtship females and males contact each other with the forelegs (Aisenberg et al., 2007; Aisenberg, 2014), so during this interaction males could be estimating the female body mass. Female weight in spiders is positively associated with higher expected fecundity, reflected by the number of eggs (Wise and Wagner, 1992), what could explain male choice on this trait.

From a perspective of energetic investment, males would have a dilemma: to build or not to build a long burrow. As burrow construction in the sand is expected as an energetically expensive activity (Henschel and Lubin, 1992; Craig, 2003; Suter et al., 2011), one option could be to initially invest less in digging activities and construct a short or

Table 2

Courtship characteristics (mean ± standard deviation) with the corresponding sample size between brackets, for trials with virgin and mated females. Data is shown with the results of statistical comparisons between virgin and mated females for the first and second exposition, for each courtship variable.

	First exposition	Second exposition
Female courtship duration (sec)	Virgin females: 1396.34 ± 872.88 (14) Mated females: 1353.06 ± 1217.85 (15) Statistics: U = 101.0; p = 0.88	Virgin females: 1662.95 ± 1482.66 (12) Mated females: 1482.64 ± 1125.84 (12) Statistics: t = 34.0; p = 0.74
Frequency of female leg waving (bursts/sec)	Virgin females: 0.023 ± 0.051(14) Mated females: 0.065 ± 0.12 (15) Statistics: U = 55.0; p = 0.03	Virgin females: 0.07 ± 0.14 (12) Mated females: 0.03 ± 0.08 (12) Statistics: U = 69.0; p = 0.89
Male courtship duration (sec)	Virgin females: 1216.94 ± 1200.39 (10) Mated females: 1355.72 ± 1011.62 (11) Statistics: U = 48.0; p = 0.65	Virgin females: 1975.15 ± 1243.44 (11) Mated females: 1833.19 ± 1299.02 (10) Statistics: U = 49.0; p = 0.70
Frequency of male body shaking (bursts/sec)	Virgin females: 0.23 ± 0.36 (10) Mated females: 0.07 ± 0.09 (11) Statistics: U = 43.0; p = 0.42	Virgin females: 0.08 ± 0.10 (11) Mated females: 0.17 ± 0.22 (10) Statistics: U = 46.0; p = 0.55

Table 3

Results of statistical comparisons of courtship characteristics in first and second expositions of virgin and mated females. For mean values of each characteristic and their standard deviations please see Table 2. We applied paired tests for the comparisons (parametric Student paired t-test or non parametric Wilcoxon paired test).

	Virgin females	Mated females
Female courtship duration (sec)	W = 18.0, p = 1.0	t = -1.1, p = 0.3
Frequency of female leg waving (bursts/sec)	W = 34.0, p = 0.03	W = 40.0, p = 0.04
Male courtship duration (sec)	W = 10.0, p = 0.50	W = 15.0, p = 0.04
Frequency of male body shaking (bursts/sec)	W = 10.0, p = 0.50	W = 9.0, p = 0.69

suboptimal (in terms of length) burrow that will serve as refuge, and invest most of the energy to enlarge the burrow after a female is detected within the area. Though, these species is strictly associated with environments that are reducing in area and increasing their fragmentation, when the species occurs the density of individuals is high (Costa, 1995; Costa et al., 2006). So, waiting for female other visits would be reasonable at certain stages of the reproductive period. If the result of this decision is a definite female rejection (maybe copulating with another male in the proximities) the cost would be high. The alternative decision, to construct directly from the beginning a long burrow would be optimal if a desirable female is within the area but implies unnecessary efforts if the availability of potential mating partners is low. Possibly, communication by airborne sexual pheromones may play an important role for initial decisions. Chemical communication is widespread in spiders and, in species with traditional sex roles, females emit airborne pheromones or deposit them in their web or draglines (Schulz, 2004; Gaskett, 2007). However, in the sex role-reversed spider *A. senex* previous studies have confirmed the emission of male airborne pheromones that would aid females in locating males inside their burrows (Aisenberg et al., 2010). Nevertheless the occurrence of female volatile pheromones remains to be further tested.

The changes observed in female courtship during the first and second expositions are also remarkable. Mated females performed higher frequencies of occurrence of leg waving during the first exposition compared to the second one. In contrast, virgin females showed higher frequencies of this behavior in the second expositions. These patterns could be interpreted from perspectives of mate choice. On one side, in this species mated females suffer higher levels of male cannibalism compared to virgin females (Aisenberg et al., 2011). Thus, mated females may diminish the risk of being cannibalized by the male and being accepted to mating by exhibiting high intense courtship from the beginning of the sexual interactions. Then, these females could diminish their courtship once the male shows predisposition to mate and low demands on mating status. In the case of the virgin females, they are always initially more attractive to males (Aisenberg et al.,

2011), and they do not need to invest in an intense courtship from the beginning. These females could intensify their courtship as a positive response towards male motivation to mate and also as a way to ensure insemination, avoiding the costs of remaining unmated. An intensification of female courtship signals while aging has been reported for insects (Eberhard, 1985) and other spider species (Roberts and Uetz, 2005; Baruffaldi and Costa, 2010).

In conclusion, our study offers new insights on male plasticity on a trait used as nuptial gift in a sex-role reversed spider species. Further studies on *A. senex* will focus on examining changes along the reproductive period in the occurrence of conservative burrows (initial short size) versus highly attractive burrows (initial large size). We will also study male investment in burrow digging according to their body condition, age and degree of advance of the reproductive season. Finally, we will test if male plasticity in digging behavior in this species is an immediate response or is part of a long-term learning process.

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