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**TESTING MALE PREFERENCES IN FEMALE SIZE AND CHOOSINESS ALONG THE MATING SEASON IN THE TARANTULA SPIDER *EUPALAESTRUS WEIJENBERGHI***

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**ABSTRACT**

Adult males of *Eupalaestrus weijenberghi* (Thorell 1894) live only for few months, while females live many years and continue molting and growing. Hence, there is a strong female-biased operational sex-ratio. While males move massively during the brief mating season, females are sedentary and remain inside their burrows, which are sometimes very close to each other. We tested if males are able to select females according to their body size and if selectivity of both sexes varies along the mating season. We exposed 5 males to 5 pairs of females of different sizes that occupied a burrow in each of two joined terraria. We performed two series of experiments: one at the beginning (March) and other at the end (May) of the mating season. Threesome combinations varied without repetitions, totalizing 25 trials on each series. We did not find differences in male behaviors: initial orientation, body vibration, palpal drumming and mating attempts regarding female size. We did not find differences in female behaviors as call or rejection. Courtship intensity was lower in May. This decrease could reflect male decadence and/or lower female receptivity. Unexpectedly, females preferred to lose their annual reproductive chance than mate with a poor quality male.

**Keywords:** male preference, *Eupalaestrus weijenberghi*, low female receptivity, tarantulas.

**RESUMEN**

**Poniendo a prueba preferencia masculina por el tamaño de las hembras y selectividad a lo largo de la estación reproductiva en la tarántula *Eupalaestrus weijenberghi*.** Los machos adultos de *Eupalaestrus weijenberghi* viven pocos meses, mientras que las hembras viven varios años y continúan mudando y creciendo. Por ello, existe una fuerte proporción sexual sesgada hacia las hembras. Los machos son móviles durante la breve temporada reproductiva, mientras que las hembras son sedentarias y permanecen en sus cuevas, que pueden estar muy cerca entre sí. Pusimos a prueba si los machos son capaces de seleccionar hembras de acuerdo a su tamaño corporal, y si la selectividad de ambos varía a lo largo de la temporada reproductiva. Expusimos 5 machos y 5 pares de hembras de diferente tamaño, ocupando cuevas en cada uno de dos terrarios enfrentados. Realizamos dos series de experimentos: uno al comienzo (marzo) y otro al final (mayo) de la temporada reproductiva. La combinación de los tríos varió sin repeticiones, totalizando 25 experimentos en cada serie. No encontramos diferencias en los comportamientos de los machos:

orientación inicial, vibraciones corporales, tamborileo palpar e intentos de monta con respecto al tamaño femenino. La intensidad del cortejo fue menor en mayo. Esta disminución podría reflejar la decadencia de los machos y/o una menor receptividad de las hembras. Inesperadamente, las hembras prefieren perder su oportunidad anual para reproducirse que copular con un macho de baja calidad.

**Palabras clave:** preferencia masculina, *Eupalaestrus weijenberghi*, baja receptividad femenina, tarántulas.

## INTRODUCTION

*Eupalaestrus weijenberghi* (Thorell 1874) is a medium-sized tarantula (Fig. 1), very common in Southern meadows of Uruguay (Costa & Pérez-Miles, 2002). According to Pérez-Miles *et al.* (2005) females and juveniles live in burrows, while males, after reaching adulthood, become the walking sex, meaning they actively search for females. The brief mating season goes from late February to early May: late-summer and middle-autumn in the Southern hemisphere (Costa & Pérez-Miles, 2002). At the end of this period, adult males found at the field show bad body condition and die soon after. Females, after reaching maturity, live 8-10 years. Conversely, adult males live for only 3 months. Therefore, a pronounced female-male biased operational sex ratio is expected (Costa & Pérez-Miles, 2002). Considering that adult females are receptive every two years, the actual operational sex ratio could be 4-5 females per male (Postiglioni *et al.*, 2005; Pérez-Miles *et al.*, 2007). Females copulate only once during each reproductive period (sequentially monandrous) and usually early in the mating season (Pérez-Miles *et al.*, 2007). After reaching adulthood, females molt every two years, as occurs with other theraphosids (Costa & Pérez-Miles, 2002). Consequently, females show a high variation in body size, being old females larger than young females.



**Fig. 1.** Adult female (A) and male (B) of *Eupalaestrus weijenberghi*. The bar reference indicates 10 mm length.

Burrow density of *E. weijenberghi* is very high, reaching 0.65 individuals per square meter with a minimal distance between entrances of 33 cm (Pérez-Miles *et al.*, 2005). Consequently, from the male perspective, a high availability of receptive females is expected. Male courtship includes body vibrations, which consist of spasmodic movements of the third leg pair (Baruffaldi *et al.*, 2008) and palpal drumming. Quirici & Costa (2005) found that the main signals used by males during courtship of *E. weijenberghi* are seismic and these signals would be generated by body vibrations. These authors also reported that these seismic signals elicited sexual responses of females in burrows 136 cm away from the male (Quirici & Costa, 2007). Considering the proximity of burrows found in the field, the distance at which females can respond and the possibility of males as a scarce resource, this is a propitious scenario for female competition and male mate choice (Avilés *et al.*, 2000; Huber, 2005).

Male mate choosiness is widespread among insects and other animals (Gwynne, 1991). Most common male preferences are associated to maximize their fertilization success on each mating. Male preferences could favor female phenotypes related with high fecundity (Gwynne, 1991; Bonduriansky, 2001). In spiders, male mate choice has received little attention and mainly restricted to sexual cannibalistic species. In these cases males do not remate and/or the density and the variation in the quality of females is high (Huber, 2005). A clear case of male choice was observed in *Allocosa brasiliensis* (Petrunkevitch 1910) and *A. alticeps* (Mello-Leitão 1944), two sand-dwelling wolf spiders, where males copulate more frequently with virgin females (Aisenberg *et al.*, 2011; Aisenberg & González, 2011). In *A. brasiliensis*, males show an extreme case of choosiness, being able of cannibalize females, preferentially those non-virgin or with a low body condition (Aisenberg *et al.*, 2011).

Choosy males are also expected in spiders when females perform courtship (Huber, 2005). Costa & Pérez-Miles (2002) reported this kind of behavior in *E. weijenberghi*, where female courtship includes an attracting response: foreleg tapping against the substrate, a behavior that was named female call. Females can also reject males performing forward and backward body movements, a display called piston behavior. When females finally accept mating with the courting male, they partially emerge from the burrow. Then, the males clasp female cheliceral fangs with their tibial spurs and raise females, allowing male palpal insertions on the female genital openings (Pérez-Miles *et al.*, 2007).

Since females mate only once per mating season (every two years), the number of available females will decay as soon as the season advances. This fact will reduce the initial female-biased operational sex-ratio, becoming females a more limited resource (Pérez-Miles *et al.*, 2007). At the beginning of the mating season, when female-biased sex-ratio is achieved, females are highly choosy (Pérez-Miles *et al.*, 2007). As predicted, if females are too choosy, they increase the risk of losing their reproductive opportunities and a whole reproductive period. In this scenario, females could become less restrictive as the sexual period advances and decrease choosiness.

Many aspects of the biology of this type of spiders, referring in particular to most of the tarantulas, still remain unknown. In the same way, sexual selection and other studies of the reproductive biology of these animals are very scarce (Costa & Pérez-Miles, 2002; Pérez-Miles *et al.*, 2007).

In this work we tested if body size affects female attractiveness by evaluating the orientation of the male to a female burrow and the occurrence and intensity of courtship displays. We additionally tested if female attractiveness or receptivity varied from the beginning to the end of the sexual period. The underlying hypotheses were: a) large females would be preferred instead of smaller ones because larger size in spiders in general implies more eggs (Beck & Connor, 1992) and consequently males could increase their reproductive success, and b) virgin females would be more receptive at the end of the reproductive period to avoid the risk of losing mating opportunities during the reproductive period.

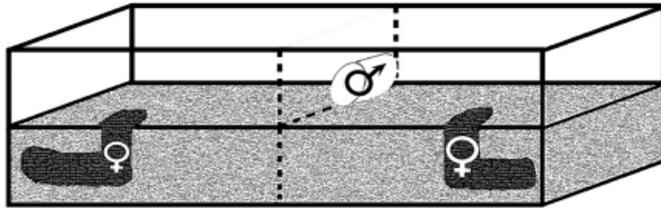
## MATERIALS AND METHODS

Five adult males of *E. weijenberghi* were captured in February and early March 2007, walking on the roads of Northern Salinas, Canelones, Uruguay (34°44'11.44"S, 55°51'40.57"W). Ten adult females were selected considering their body size: five large females (14.9 ± 0.49 mm carapace width; range 14.05-15.45 mm) and five small females (12.78 ± 0.76 mm, range 12.05-13.60 mm). All females were maintained at least for two years before experiments under laboratory conditions, allowing us to know at least their more recent reproductive history. Females were collected in burrows at Southern Uruguay, in the Provinces of Canelones, Colonia, Durazno, Flores and Montevideo. All individuals were maintained in glass jars (9 cm diameter, 14 cm height) with a layer of soil and water provision. Spiders were fed *ad libitum* with cockroaches (*Blaptica dubia*, Blattaria, Blaberidae). All the females were considered sexually receptive, as they molted between December 2006 and February 2007 and they did not copulate after that (Pérez-Miles *et al.*, 2007).

The experiments were performed in two different series: a first series in March (early mating season) and a second series in May (late mating season). Along the first series room temperature was 25.98 ± 0.34 °C, and in the second series it was 21.06 ± 0.71 °C. Both series showed statistical differences in temperature (Mann-Whitney U-test: U = 0, p < 0.0001).

During the experiments, females were kept in individual terraria of 30 x 14 cm base and 19 cm height, with a layer of soil of approximately 7 cm height. Males were maintained in their respective glass jars. To facilitate the observation of behaviors, an artificial burrow was constructed against the glass wall of each terrarium and their entrances were placed at the middle of the terraria. The initial section of the burrow was vertical and turned to horizontal before reaching the terrarium floor (Fig 2), emulating burrows in natural conditions: approximately cylindrical, 25 mm of diameter and 200 mm length (Pérez-Miles *et al.*, 2005).

In order to test male preferences between two different sizes of females we joined two female terraria, by removing one of the lateral glass walls of each one. This removal allowed the contact of the two soil layers (ensuring the connection of seismic media) and permitted the free transit of the male throughout the surface. The burrow of the right terrarium was directed towards the right and the left terrarium towards the left (Fig. 2), keeping females as far as possible from each other. Since burrows were designed to allow the observation



**Fig.2.** Experimental arena consisting in two joined terraria after removal of lateral glass walls. Each terrarium had one female burrow, left one had the small female and right burrow had the large one. The male confined into the plastic glass was deposited on the joint of both soils allowing the emergence and giving the chance of taking one of two ways.

of female behaviors, both females were necessarily placed in front of the observer. Given that also burrows had to maximize distances between females, which implied the removal of only one glass wall, and no mixing of different females sizes and terraria was allowed, the small female occupied the left terraria and the large female the right one (Fig. 2).

The location of the terraria in the laboratory during the observations varied randomly to avoid effects of light, noises or other environmental factors that could affect spider behavior. Females were placed in their terraria at least a week before the beginning of the first series of experiments and in all the cases they occupied and modified their burrows. In all trials, males were carefully placed on the joint of the two terraria, inside of a tumbled plastic glass (Fig. 2), until they emerged by themselves. The trial began when male contacted the substrate. This procedure allowed males to choose any of the two sides. The trials ended after 30 min or when the couple clasped the female cheliceral fangs with the male tibial spurs. Then, males were immediately removed from the terraria, avoiding mating. Male and female activities were followed by direct observation. We registered male first choice (right or left), locomotion, body vibration, palpal drumming, contact with burrow entrance, entering to the burrow and female clasping (for more details see Pérez-Miles *et al.*, 2007). In females we registered: locomotion, female call, emergence from the burrow, piston behavior and other rejecting behaviors.

We performed five trials by day during five days, totalizing 25 trials on each of the two series. Females were combined in different pairs without repetitions. Males were used only once with each female pair in each series.

For comparisons, we indexed the number of bouts per minute of male behaviors, as the trial duration was extremely variable (see Pérez-Miles *et al.*, 2007). We considered bouts of body vibration and palpal drumming performed until first female response. We compared frequencies using McNemar (for dependent samples) and Chi-square test with Yate's correction (for two independent samples). We tested normality (Shapiro-Wilk test) and homogeneity of variances (Levene test). We also used paired tests for comparisons of means: Student t-test for paired samples (parametric test) and Wilcoxon T test (non-parametric test). We used the Past Statistical package (Paleontological Statistics, version 1.18; Hammer *et al.*, 2004). Once the specimens die, voucher specimens will be deposited at the Arachnological collection of the Sección Entomología, Facultad de Ciencias, Montevideo, Uruguay (FCE-MY).

## RESULTS

### Male behavior

For comparisons we only used the data of body vibrations. We could not compare differences in palpal drumming owing to the low number of paired data ( $n=2$  in March,  $n=1$  in May). In the first experimental series, males did not show significant differences in initial orientation to female arenas according to female size ( $\chi^2 = 0.02$ ,  $P = 0.89$ ): 12 went towards large females and 12 towards small females; one remained immobile in the center during the whole trial. When comparing the body vibration (bouts/min) regarding female size, we did not find significant differences ( $t = -1.3$ ,  $n = 4$ ,  $p = 0.28$ ).

In the second experimental series, we did not find significant differences when we compared the initial orientation of males to female terraria ( $\chi^2 = 0.18$ ,  $P = 0.67$ ): 16 males went towards large females and 9 towards small females. When comparing the frequencies of bouts of body vibration of males faced with females of the two different sizes, we did not find statistical differences ( $t = 0.04$ ,  $n = 7$ ,  $p = 0.97$ ).

In order to compare the performance of male courtship between March and May, we grouped the data of body vibrations of males exposed to both large and small females. The body vibrations were more intense in March ( $1.18 \pm 0.43$  bouts/min) than in May ( $0.61 \pm 0.32$  bouts/min) (paired  $t$ :  $t = 3.19$ ,  $n = 5$ ,  $p = 0.03$ ).

### Female behavior

No differences were found in the occurrence of female call between large and small females, either in the first and the second series. Eight large and four small females called in the first series ( $\chi^2 = 0.89$ ,  $p = 0.34$ ) and three large and one small females in the second series of experiments ( $\chi^2 = 0.27$ ,  $p = 0.60$ ). The same occurred for females that accepted clasping: six large and four small females in the first series ( $\chi^2 = 0.89$ ,  $p = 0.34$ ) and a single clasping of large female in the second series. We did not find significant differences in rejecting behaviors between large and small females (none of the large and two of the small females in the first series; six large and six small in the second series).

We did not find significant differences in the number of females that called (McNemar  $\chi^2 = 0.8$ ,  $p = 0.37$ ), clasped (McNemar  $\chi^2 = 0.5$ ,  $p = 0.48$ ) or rejected males (McNemar  $\chi^2 = 0.25$ ,  $p = 0.62$ ) at least once, when comparing first and second series.

Additional single evidence of decreasing attractiveness was the case of a large female that received body vibrations from the five males and palpal drumming from four males, and called three times and clasped four times in the first series. In the second series the same female elicited only two body vibrations and one palpal drumming and did not perform any sexual behavior. We did not observe any simultaneous call of large and small females in any trial.

## DISCUSSION

Our results did not evidence male preferences related to female size. Males did not show

any preference both after the contact with female silk or after female response. Mate choice by males in the absence of role reversal could be expected when some variation in female quality is observed and when the costs of male mating are such that a small fraction of the available females can be mated by an individual male (Huber, 2005). This unexpected result was not in agreement with the direct reproductive benefit of males, considering that usually large females lay more eggs (Beck & Connor, 1992), and particularly for spiders as stated by Uhl (1998), Danielson-François *et al.* (2002) and Huber (2005). These authors stressed the value of female size in male mate choice. The absence of male choice could be explained by the probably low cost of searching, courtship and copulation. This low cost is presumed considering the high density of females, low predation risks and the presence of only one brief palpal insertion during copulation (Costa *et al.*, 2004; Pérez-Miles *et al.*, 2005, 2007). All these factors could reduce the selective pressure for choosy males. Although we did not find any evidence of a biased initial orientation of the male, we cannot discard an intrinsic tendency of the males to turn to the right or left. Since large and small females were always put in the same position, additional evidence modifying female position is needed to discard this possibility.

On the other hand, females showed no decrease in selectiveness through the reproductive season (Pérez-Miles *et al.*, 2007) and they apparently prefer to lose a mating opportunity and consequently a reproductive period, than to copulate with a poor quality or maybe a late-season male. The decreased male courtship intensity in May regarding March could reflect a decrease in pheromone attractiveness, probably because May is about the end of the reproductive period. Another interpretation is that males are too old and that is why they court with low intensity, considering they live only two months as adults in the wild (Pérez-Miles *et al.*, 2007). Usually, females select intense and costly male signals which reflect a good health condition (Andersson, 1994). Males with honest signals would reflect their poor condition and consequently will be rejected by females. However, male rejection at the end of the reproductive period implies the loss of a reproductive opportunity for an annual cycle (Pérez-Miles *et al.*, 2007). Unexpectedly, females preferred this option than to decrease their attractiveness threshold and mate with a male in a poor body condition, which (on the other hand) could reflect the high cost of accepting a low quality male.

We did not find any evidence of the presumed female-female competition. In fact, in none of the trials the pair of females called together the same male, which could suggest a possible inhibition between the females. In our results, females only called when males were near the burrow. However, Quirici & Costa (2007) reported that females of this species can call males at more than 1.3 m from the burrow entrance. This contradiction is in agreement with our hypothesis of female-female inhibition.

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