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SHORT COMMUNICATION

Third pair of legs is a key feature for eliciting female receptivity in the road tarantula spider *Eupalaestrus weijenberghi* (Araneae: Theraphosidae)

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Abstract. Using the road tarantula *Eupalaestrus weijenberghi* (Thorell 1894), we tested the importance of the third pair of legs in male courtship behavior. Our results showed that the third pair of legs is essential for males to elicit female sexual receptivity. Males with their second pair of legs immobilized elicited receptive responses from females, but males with the third legs immobilized did not. The potential role of the third pair of legs in the generation and/or transmission of seismic signals via the substrate is discussed.

Keywords: Courtship, leg vibration, seismic communication

In therapsids, males usually perform body vibration, palpal drumming, and leg tapping during courtship (Costa & Pérez-Miles 2002; Pérez-Miles et al. 2005, 2007; Almeida-Silva et al. 2008; Ferretti & Ferrero 2008). These body vibrations constitute an important communication channel, mainly for burrowing theraphosids (Quirici & Costa 2005). Although Costa & Pérez-Miles (2002) highlighted the use of leg III in body vibrations, the importance of the vibrations generated and/or transmitted by leg III to the substrate and their implications in female receptivity remain obscure. Here we present experimental evidence of the role of the leg III pair in female receptivity in the therapsid species, *Eupalaestrus weijenberghi* (Thorell 1894).

Eupalaestrus weijenberghi is a medium-sized tarantula that inhabits burrows in meadows of the Pampean biogeographic province (see Pérez-Miles et al. 2005). The reproductive season of this species is between February and April (autumn in the Southern Hemisphere). Males search for females using chemical cues and live only two months as adults, while females are sedentary and live up to ten years as adults (Costa & Pérez-Miles 2002; Pérez-Miles et al. 2005). As in other mygalomorphs, females continue molting through their adult stage. During the molt, the lining of the seminal receptacles is shed and the females become “virgin” again, needing new sperm after each molt (Foelix 2011). Therefore, these new virgin, sexually receptive females call males by tapping the substrate with their forelegs. Males can perform multiple matings, whereas females are monandric in each reproductive season, which occurs in alternate years and is associated with the year that they molt again (Pérez-Miles et al. 2007).

For this study, we collected male *E. weijenberghi* from Southern Uruguay, Canelones Salinas Norte (34°44'56"S, 55°52'15"W) and from neighboring areas in February and March 2011. We used female *E. weijenberghi* from our laboratory populations, which were maintained as in Costa & Pérez-Miles (2002) for at least one year before the experiments; they also originated from Southern Uruguay. Only those females that had molted within six months of the experiment were used in the experimental trials in order to guarantee their need for sperm and, therefore, their potential receptivity. Females were individually maintained in containers (50 cm length, 15 cm width, and 20 cm height) containing a layer of soil. We constructed artificial burrows, similar to those found in the field (see Pérez-Miles et al. 2005, 2007), against the glass walls of the containers, to facilitate observation. We maintained the males in cylindrical glass containers of 7.5 cm diameter with soil for at least

one week before the trials. All individuals were fed ad libitum with *Blaptica dubia* (Serville 1839) (Blattaria: Blaberidae). We designed three experimental treatments: in one of them (pair-three-tied group), the third pair of the male legs was tied between them at the joint of the patella and femur, above the carapace, using cotton threads (Fig. 1). In a second treatment, we tied pair two in the same way (pair-two-tied group); in a third treatment (control group), males remained with their legs free but were manipulated to simulate the ligature. Five minutes prior to the experimental trials, we manipulated or tied the legs of the males. After this period, males were slowly introduced into the females' container. Males and females experienced all the treatments in a random sequence over consecutive days; however, couples were never repeated.

In each experimental trial, we recorded the number of male body vibrations, female calling (tapping the first and second pair of legs against the substrate), and female rejection of males (piston behavior, attacks or abrupt emergence). Piston behavior consisted in forward and backward movements of the female, usually in the burrow. For detailed descriptions of courtship behaviors see Costa and Pérez-Miles (2002) and Quirici & Costa (2005). We finished the experimental trials when the female attacked the male, when the female accepted clasping (male clasped female chelicerae with his tibial apophyses before mating), or 30 min after the male was introduced. We interrupted the experimental trials after clasping, avoiding copulation (to retain female virgins and sexual receptivity). We performed all trials in three consecutive days (9–11 March 2011). During the experimental trials, the room temperature varied between 26 and 28°C, with a mean of 26.9°C (\pm 0.6 SD). Statistical analyses were carried out using the Past package (Paleontological Statistics version 2.05, Hammer et al. 2010). Bonferroni-corrected critical values (α = 0.0167) were used in McNemar and Wilcoxon test results for multiple comparisons.

We observed that males performed courtship behavior in all 21 trials of the control group, in 19 of the pair-two-tied group, and in 19 of the pair-three-tied group. Females called the males in 16 trials of the control group, in 16 of the pair-two-tied group, and in 3 of the pair-three-tied group. Using the Cochran Q-test for repeated measures, we compared female calling among the three groups and found that it varied significantly with male treatment ($\chi^2_Q = 14.700$, $P = 0.001$). In the pair-wise comparisons for repeated measures (McNemar test), the pair-three-tied group differed from the control group ($P = 0.002$) and from the pair-two-tied group ($P = 0.007$),



Figure 1.—Male *Eupalaestrus weijenberghi* with the third pair of legs tied with a cotton thread.

whereas the control and pair-two-tied groups showed no significant differences between them ($P = 0.72$). We did not find significant differences in the latency of female calling between pair-two-tied and control groups using the non-parametric Wilcoxon test for the pairwise comparisons ($Z = 1.57$, $P = 0.12$). We did not compare the latency of the female call in pair-three-tied group because only three females responded. We also tested whether the female's response to the male's treatment changed over time, but found no statistical differences in the latency of calling behavior over the days of the experiment (Kruskal-Wallis, $H = 0.26$, $P = 0.88$). Four females rejected males (one attack and three piston behaviors) in the leg-three-tied group, while we did not observe any rejection in the other groups.

The latency of male courtship was 5.04 ± 4.46 min in the control group, 5.82 ± 5.20 min in the leg-two-tied group and 9.43 ± 9.67 min in the leg-third-tied group. We did not find significant differences using the Friedman test for repeated measures ($\chi^2 = 1.08$, $P = 0.59$). Male body vibrations occurred at 1.90 ± 1.22 bouts per min in the control group, 2.00 ± 2.20 per min in the leg two-tied-group, and 1.04 ± 0.93 per min in the leg-three-tied group. We found significant differences in rates of body vibration between the leg-three-tied group and the control group using the Wilcoxon test for pairwise comparisons ($Z = 2.43$, $P = 0.015$). However, we did not find significant differences between the leg-two-tied group and the leg-three-tied group ($Z = 1.82$, $P = 0.068$) or the control group ($Z = 0.40$, $P = 0.68$).

We conclude that the third leg is essential for female receptivity. The experimental tying of legs II or legs III did not inhibit male courtship behavior, but affected female sexual response. The absence of differences in the latency of male courtship showed that males will start courtship behavior, regardless of the treatment, when they are in contact with female silk threads. Furthermore, males with the third legs tied did not differ statistically in body vibration regardless of the leg-two-tied groups, suggesting that which legs have been tied does not appear to have an important effect in this stereotyped behavior, and also because females called to males with the second legs tied. In addition, we did not find significant differences in the frequencies of vibratory bouts between the control and the leg-two-tied group. However, when males had their third legs tied, their body vibrations on average were less frequent than with legs two tied and also statistically different from the control groups. These results suggest the importance of the third free leg for increasing body vibrations and also for a complete female response. When third legs were tied, spasmodic movements of the third pair were observed. Most likely, transmission through the remaining free legs was not adequate to elicit female sexual response. We did not observe such spasmodic movements when the second pair was tied. Legs of the third pair,

when free, are involved in the lateral equilibrium of the body, and they can be firmly placed on the substrate on both sides of the body. Legs I and II are placed in front of the body, and legs IV are placed in the back of the body, and these legs are responsible for the transmission of body vibrations from the front to the back and from the back to the front of the male's body. Therefore, legs III are in the best position and are the best candidates for transmitting vibrations from the top to the bottom of the body (F.G. Costa pers. observ.). This relationship allows the spiders to transfer the mechanical energy produced by the leg muscle contractions to the substrate and to vibrate their bodies. A comparable mechanism was proposed for the sparassid *Heteropoda venatoria* (Linnaeus 1767) by Rovner (1980) and by Rovner & Barth (1981) for the ctenid *Cupiennius salei* (Keyserling 1877).

We could not attribute the differences in female behavior to differences in the frequency of male sexual display because we did not find any significant differences in the male sexual behavior between the two groups with tied legs. Consequently, the third legs appear to be key features for eliciting a positive sexual response from the female. As far as we know, this is the first experimental evidence of a mechanism underlying this widespread courtship behavior in tarantulas and other mygalomorph spiders.

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