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Scientific note

Can't you find me? Female sexual response in an Argentinean tarantula (Araneae, Theraphosidae)

¿Puedes encontrarme? Respuesta sexual en una tarántula de Argentina (Araneae, Therphosidae)

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Abstract: We described and analyzed the female sexual response in the tarantula *Grammostola vachoni* Schiapelli and Gerschman 1961 under three series of 10 experiments each with different situations for the male-female encounters: male free in terrarium but with no access to female; male confined into a glass cup at 30 cm of female burrow and; male confined into a glass cup over a heavy stone closing the female burrow. We observed leg palpal tapping and body movements of female in response to male courtship. Leg and palpal tapping could indicate a female receptive condition and her attractiveness. Also, this behavior may serve to orient male towards her location. These females responded with leg and palpal tapping only to males which present long courtships together with active searching (walking) for female location. The female body movements, originated by legs III, are interpreted here as a rejection considering that male stopped courting and tried to escape.

Key words: Mygalomorphae. *Grammostola*. Argentina. Behavior. Sexual display.

Resumen: Se describe y analiza la respuesta sexual en hembras de la tarántula *Grammostola vachoni* en tres series de 10 experimentos cada uno bajo diferentes situaciones de encuentros entre machos y hembras: machos libres en el terrario pero sin acceso a la hembra; machos confinados dentro de un recipiente de vidrio a 30 cm de la cueva de la hembra y machos confinados en el recipiente de vidrio sobre una piedra cerrando la entrada de la cueva de la hembra. Se observaron golpes con patas y palpos y movimientos corporales de las hembras en respuesta al cortejo de los machos. Los golpes con patas y palpos podrían indicar la receptividad de las hembras y su atracción. También, este comportamiento podría orientar al macho hacia su posición. Estas hembras contestaron con golpes de patas y palpos sólo a los machos que realizaron cortejos largos junto a una búsqueda activa de la cueva de la hembra. Los movimientos corporales de la hembra, originados con patas III, se interpretan como un rechazo considerando que el macho dejó de cortejar e intentó escapar.

Palabras clave: Mygalomorphae. *Grammostola*. Argentina. Comportamiento. Repertorio sexual.

Introduction

Spiders use different channels of communication such as chemical, tactile, visual and acoustic/vibratory during courtship (Uetz and Stratton 1983). Acoustic and seismic vibratory sexual signals are frequently used in spider courtship, especially in tarantulas (Uetz and Stratton 1982; Prentice 1992; Quirici and Costa 2005, 2007). Long distance communication is particularly important in male spider coursthip to avoid non sexual behavior of females such as their cannibalistic behaviors (Uetz and Stratton 1983). Seismic signals can reach long distances without loss of effectiveness (Narins 1990; Endler 2000). In spiders, male signals can reach 1 m in ctenids (Barth *et al*. 1988) and at least 1.30 m in theraphosids (Quirici and Costa 2007). The usual indicator of effectiveness of male signals are the changes in female behavior being leg waving, leg tapping or body vibrations the more usual receptive responses (Prentice 1992; Quirici and Costa 2005, 2007; Ferretti and Ferrero 2008).

Theraphosids are widely distributed in tropical and subtropical regions of the world (Platnick 2012), but their biology is poorly known (Costa and Pérez-Miles 2002, Ferretti *et al*. 2011). Studies of mating behavior are limited to a few species (Minch 1979; Costa and Pérez-Miles 1992, 2002; Shillington and Verrell 1997; Yáñez *et al*. 1999; Bertani *et al*. 2008; Ferretti and Ferrero 2008). *Grammostola vachoni* Schiapelli and Gerschman, 1961 is an Argentinean tarantula that lives in burrows under stones in rocky hills (Ferretti and Pérez-Miles 2011; Ferretti *et al*. 2011). Females of *G. vachoni* are the first known of the genus that show sexual response to courting males, but the context and possible function of this response has not been elucidated (Ferretti and Ferrero 2008). Other studied *Grammostola* species such *G. mollicoma* (Ausserer, 1875) and *G. iheringi* (Keyserling, 1891) do not respond to courting males (Pérez-Miles and Costa 1992; Costa and Pérez-Miles 2002; Postiglioni and Costa 2006). Bertani *et al*. (2008) observed in captivity that females perform sexual responses if they were occupying a retreat location as in natural conditions, but when females were inhabit open arenas no responses were observed. The main objective of this work is to elucidate under what context females occupying retreats as in their natural habitat respond to male courtships and to discuss the possible function of these behaviors.

Materials and Methods

Males and females were collected in Sierra de la Ventana 38°07'63"S 61°47'30"W, Buenos Aires, Argentina, during

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October – January 2008 – 2009. Spiders were housed in glass jars of 13 cm diameter and 15 cm height, with 5 cm of soil as substrate and water provision. We fed spiders with *Zophobas* sp. (Coleoptera, Tenebrionidae) larvae weekly. We used a 12 h light/dark cycle. For all experiments, we used females with known reproductive history, which involved females previously mated and molted, so they had no storage sperm. We used a total of 10 males and 10 females. We considered active males those who showed intense courtship during more than 30 min. For the encounters we used glass cages (30 x 35 and 30 cm) containing a 10 cm thick layer of soil as substrate. The room temperature during experiments was 26.7° ± 1.52°C and the mating arenas were illuminated with fluorescent light. In each terrarium, we constructed a burrow attached to the glass wall, allowing visual observations of female behavior inside burrow. We placed each terrarium in different tables in order to minimize ground vibrations and to prevent seismic signals between terraria.

We placed females in terraria and trials were carried out at least five days later to allow accommodation. To test for the occurrence of female sexual response, a series of three consecutive experiments were carried out using the same ten pairs of male/female individuals. Each experiment was performed in different situations for the encounters male-female. In the experiment "A", we placed free male in terrarium but with no access to female due to the heavy stone covering the burrow. In the experiment "B", we placed males far off from the female burrow (30 cm) and confined into a glass cup. In the experiment "C", we placed males over the heavy stone that closed the burrow of female and confined into a glass cup (Fig. 1). We elicited the courtship of males by adding abundant silk threads of females inside the glass cup. We recorded encounters with a Handycam Panasonic SDR-S7 and analyzed the video records with a PC program (Sony Vegas 9.0) in order to accurately describe behavioral patterns, using slow motion and single frame advances modes.

Results

We observed two types of responses of females to male courtships: leg and palpal tapping and body movements. In video analyses, females that performed leg and palpal tapping made palpal and leg flexing, lifting and lowering against the substrate at high frequency. The mean number of leg movements during leg and palpal tapping was 16.44 ± 15.26 SD, n = 51 and the mean number of bouts of leg and palpal tapping per interaction was 12.75 ± 11.58 SD, n = 51. The mean duration of leg tapping was 1.63 s ± 0.99 SD, n = 51 and the mean duration between bouts of female leg tapping was 48.20 s ± 37.49 SD, n = 8. The second response observed consist in body movements. Females displayed a high frequency downward and upward movement of the entire body. By using slow motion in video analyses, we observed a contraction of the third pair of legs during these movements.

In experiments A and C females made their sexual display by tapping vigorously with palps and first pair of legs or only with palps, while in series B we observed no responses from females. Four of 10 females responded to male courtship, three females responded in experiment A and one female responded in experiment C. Three of four responses were after the palpal drumming of males and long courtships (approximately 1 hour), and one response was before the male initiates courtship. All females that responded with palpal and leg tapping scraped vigorously the stone with first pair of legs. After female tapping and in experiment A, males frequently oriented to the female burrow and stayed courting in the vicinity.

Three females responded with body movements in experiment C. These responses were observed after male palpal drumming and the number of the movements was highly variable, ranging from 2 to 61 (16.6 ± 24.96 SD, n = 72). One female that performed body movements made three abrupt emergences, which consisted in a fast walking to the burrow entrance.

Discussion

Our data suggest that seismic communication by substrate occur during courtship in *G. vachoni* in agreement with findings of Quirici and Costa (2005) in *Acanthoscurria suina* Pocock, 1903 and *Eupalaestrus weijenberghi* (Thorell, 1894). Female leg and palpal tapping as response to some male courtship seems to indicate her receptive condition and her attractiveness, as was found for *A. suina* and *E. weijenberghi* (Quirici and Costa 2005; Pérez-Miles *et al*. 2007). However, female leg and palpal tapping response was more frequent in experiment A, which males freely walked in the terrarium and also after extremely long courtships (from 30 min to 1 hour approximately). We additionally proposed that this female response could orient male towards her location at long distance. Also insistence of males could be a positive honest signal of males with good condition to mate, and consequently selected by females. Moreover, females responded predominantly with leg and palpal tapping to males that showed long courtships together with active searching (walking) for female location (experiment A). Although these confined males courted, only one received female leg and palpal tapping response. Though we cannot discard an airborne acoustic communication (e.g. stridulation) of males, one male that courted inside the glass cup obtained a female response. Additionally, it has been demonstrated that airborne acoustic communication it is not

Figure 1. Schematic representation modified from Quirici and Costa (2005) showing the experimental series used for *Grammostola vachoni*. Male always on right and female always on left.

important in some theraphosids such as *A. suina* and *E. weijenberghi*, though seismic signals are sufficient to elicit a complete female response during courtship (Quirici and Costa 2005). In addition, the sexual role of the stridulatory setae of *Acanthoscurria suina* has no relevance in a sexual context; it may be involved in a defensive function (Pérez-Miles *et al*. 2005). The female body movements are interpreted here as a rejection considering male stopped courting and tried to escape after this female behavior during experiments C. Body movements seem to be different from the rejection piston behavior of female observed for *E. weijenberghi* (Pérez-Miles *et al*. 2007). In *G. vachoni*, this behavior consisted in downward and upward body movements, has higher frequency, and is probably originated by third pair of legs. This threatening behaviour occurs inside the burrow and probably generates air currents or air vibrations through the burrow entrance, and could additionally be generating seismic signals as was observed in *E. weijenberghi* (Pérez-Miles *et al*. 2007). Piston behavior was proposed as a ritualized rejection response, considering that after that, males immediately escaped (Pérez-Miles *et al*. 2007). Female rejection could help the male to save time and energy spent in courtship and also to reduce the risk of attracting predators. From the female perspective, this rejection of male could be contributing to avoiding her distraction from other biological activities as was found in *E. weijenberghi* (Pérez-Miles *et al*. 2007). We observed body movements of females predominated in experiments C, where males were confined in the nearest possible location to female burrow.

In conclusion, females of *G. vachoni* showed two sexual responses to courting males: one positive maybe through seismic signals in the substrate that when male perceived it, he was able to successful location of the female burrow and oriented towards it. Moreover, this sexual display by female could be indicating not only their position in the field, but also a good signal of her receptiveness. Additionally, a negative response from female to courting male that involved body movements could be acting as a rejecting mechanism to male on early instances of courtship. The special acceptance or rejecting signals seem to be very complex and effective during sexual communication, reducing the time that males spend outside the female burrow. Moreover, courting males could represent a cost for females because they could attract predators or compete with other important biological female activities (Pérez-Miles *et al*. 2007). Finally, this complex communication system would allow not only a mutual assessment between both sexes during courtship, but also an estimation of the female condition and reluctance without taking main risks.

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