Long-term persistence and water resistance of female sex cues in the tarantula *Eupalaestrus weijenberghi* (Araneae: Theraphosidae)

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Summary

The presence of pheromones has been reported for several spider species but their persistence in field conditions has scarcely been studied. We test the activity and persistence of female sex cues outdoor in the tarantula *Eupalaestrus weijenberghi* (Thorell, 1894), using male courtship as an indicator of cue attraction. We studied female silk fields in two conditions: protected and exposed and in both we found a long persistence of sex signals, up to 55 and 46 days, respectively. We discuss the nature and adaptive significance of these cues.

Introduction

Contact pheromones are widespread in the sexual communication of spiders (Schultz 2004, 2013; Gaskett 2007). These chemical signals are usually associated with silk threads. Conversely, as far as we know, the persistence of sex pheromone in natural conditions has only been reported in one previous work (Baruffaldi *et al*. 2010). Chemical sexual communication in theraphosid tarantulas was suspected by Baerg (1958) and observed by Minch (1979), Prentice (1997), Shillington & Verrell (1999), and Costa & Pérez-Miles (2002). Recent studies suggest that the presence of sex pheromones is the rule among theraphosids (Pérez-Miles *et al*. 2005, 2007; Copperi *et al*. 2012; Almeida-Silva *et al*. 2008; Ferretti & Ferrero 2008; Ferretti *et al*. 2013). However, nothing is known about the persistence of sex pheromone in Theraphosidae. Information about the persistence of chemical signals under natural conditions provides fundamental data for understanding how males respond to such cues over time in the field, and for deducing the sexual strategies involved in mate location for males and females. Climatic factors such as sun, wind, dew and rain are expected to wash or degrade the semiochemicals, as suggested by Dondale & Hegdeckar (1973), Wilder *et al*. (2005), Gaskett (2007), and Baruffaldi *et al*. (2010). Fukushima, Bertani & Da Silva (2003) found that the sex pheromone of the theraphosid *Acanthoscurria gomesiana* can be extracted with polar solvents, in agreement with the wash action of rain and dew. However, Dor *et al*. (2008) found chemically mediated recognition in the burrow entrance of *Brachypelma vagans*; these substances could be extracted with apolar solvents. In the present study, we test the persistence of sex cues of female *Eupalaestrus weijenberghi* (Thorell, 1894) in natural conditions using male courtship as indirect evidence.

Eupalaestrus weijenberghi is a medium-sized tarantula common in the meadows of Uruguay which inhabits burrows in the soil (Pérez-Miles *et al*. 2005). The reproductive season is generally in March and April (end of summer and beginning of autumn in the Southern Hemisphere), when males actively search for females and initiate courtship after detection of female silk at the burrow entrance (Costa & Pérez-Miles 2002; Pérez-Miles *et al*. 2005, 2007). Walking males are commonly observed on stormy and rainy days, even during daylight. Male courtship includes palpal drumming and body vibrations generated by the third pair of legs (Baruffaldi *et al*. 2013) which allow seismic communication (Quirici & Costa 2005, 2007). Males live only two months as adults and die after the sexual period (Pérez-Miles *et al*. 2005).

Considering the restricted sexual period and the possible deactivating effect of rain and dew on sex pheromones, we predict its short persistence.

Methods

We used 18 males and 15 females collected at Canelones and Paysandú, Uruguay. All females were housed in the laboratory until they moulted and were presumed receptive (Pérez-Miles *et al*. 2007). Experiments took place on the campus of the IIBCE, in a grassland field. Nine females were placed in a grassy outdoor area, covered by two concentric inverted pails. These pails were plastic and translucent; the inner one (containing the female) was 13 cm in diameter, the external one being 21 cm in diameter. Both pails had a heavy weight on them to prevent escape. Females were able to walk inside the pail. After 48 hours, females were removed and the cues remaining marked with a small paper flag for observations. To test the persistence of the pheromones, males were deposited on cue fields for 5 min duration and the occurrence and frequency of courtship movements (body vibrations) were monitored. In all observations, males were confined by an inverted cylindrical transparent glass container (19 cm diameter). Males were always deposited carefully in the centre of the arena to allow cue detection. Initially, nine field arenas were tested: six exposed to weather conditions and three protected by an inverted 21 cm diameter pail, both exposed and protected arenas were tested concurrently. The level of cue attractiveness was tested at variable intervals (Fig. 1): brief when most males vibrated and longer when few males vibrated. Trials were performed for 55 days, between April 4 and May 29 of 2014. We tested each arena using a male in each trial. We re-used males randomly to avoid repeating the male on the same female cue. Males were re-used after a minimum interval of 24 hours.

We also tested the potential influence of learning (conditioning) and/or spontaneous activity by depositing six males on a neighbouring grassy outdoor area free of tarantulas (clean substrate) six days after the beginning of the trials (April 10). To test male ability to court after two months, we exposed them to recently deposited pheromone cues

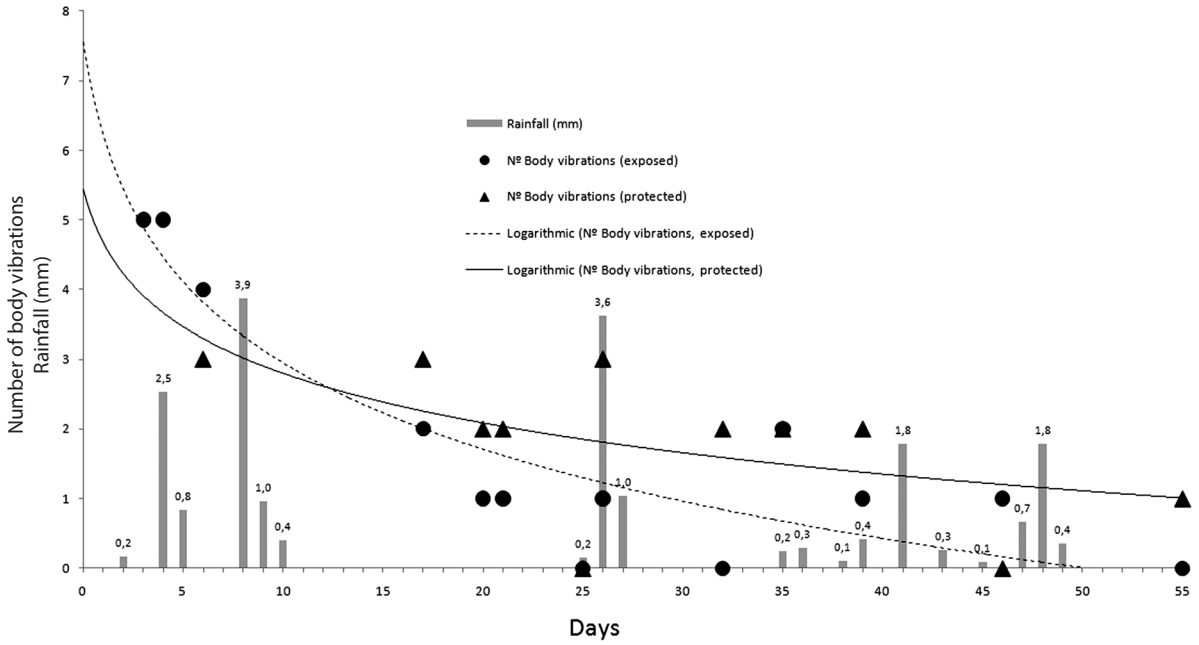

Fig. 1: Changes of the sexual responses of males confronted to exposed and protected female cues during a 55 day period. Rainfall is represented by grey bars (cumulative water during a day, in millimetres).

in the field and in the laboratory. In the field, six females left the cues during 48 hours (May 7–8), and males were tested immediately after female removal and four days after. Finally, in the laboratory, 15 males were placed in a terrarium (50 × 14 cm base with an 8 cm soil layer) with a female in her burrow and male courtship was observed; we used five females exposed to three males each. Comparisons were done using the χ^2 test and the Fisher exact probability test for two independent samples. Means were compared with the unpaired Student t-test.

Results

Initially, four or five males on exposed arenas and three in protected ones performed body vibration (Fig. 1). Occurrence and frequency of body vibration decreased along an extended period: up to 46 days in exposed cues and 55 days in protected cues. After the rain, the attraction of exposed and protected cues persisted and even seemed to increased: for example, cues 26 days old did not elicit any male response but the next day after the rain we observed male body vibrations in one of six males in exposed cues and three males on protected ones (Fig 1).

When we compared the cumulative occurrences of male body vibration in exposed cues with protected ones (16 occurrences in 66 exposed and 20 in 33 protected, corresponding to 11 observations during 49 days), we found significantly higher frequencies in protected cues than in exposed ones (χ^2 = 16.57, P = 0.0001). Also, male body vibration frequency was higher in protected cues (mean 3.72 ± 2.82 SD) than exposed cues (mean 2.03 ± 1.07 SD), but we found no significant differences (t = 1.59, P = 0.13, using log-transformed data).

None of the six males performed body vibration on clean ground six days at the beginning of the observations. Males performed body vibration on fields where a second series of females were used for cue donation and then removed (reloaded cues). Three of six males performed body vibration immediately after female removal, and four of six after four days. An additional test to confirm the good condition of males was performed at the end of the observation period. Eight of 15 males performed body vibration in terraria inhabited by females at their burrows (fresh cues). Body vibration occurrence of these males was compared with courtship performed on old cues five days before (when only one vibrated in nine trials on exposed cues). Significant differences were found in the Fisher test (P = 0.048).

Discussion

The most remarkable finding was the long-term persistence of female cues, eliciting male courtship up to at least 46 days in exposed cues and 55 in protected ones. This persistence is unexpectedly high, considering the persistence of pheromone of *Schizocosa malitiosa* outdoor is about a week (Baruffaldi *et al*. 2010); this is the only previous report we found of pheromone persistence in spiders.

Male courtship responses declined slowly over time, and could reflect two non-exclusive factors. First, the attractiveness of female cues may have declined over time and second, male body condition may have declined with age, considering they live for a short period as adults. This second factor seems not to be the main reason for the declining male response, considering that old males continued responding to recently released female cues. The attractiveness decreasing of female cues could be explained by the declination of a sex-contact water-resistant pheromone. This water resistance agrees, and seems to be adaptive with, the fact that males intensively search for females during stormy and rainy days (Pérez-Miles *et al*. 2005). Conversely, the only pheromone reported for a theraphosid is a polar substance soluble in acetonitrile (Fukushima, Bertani & Da Silva 2003). As males did not vibrate on clean fields, the occurrence of associative learning (conditioning)

seems improbable, considering we used the same males more than once.

The higher persistence and attractiveness of protected cues could be explained by the absence of mechanical damage of silk threads by rain drops. Although protected cues suffered higher constant humidity than exposed cues, direct effect of rain and sun was minimized. Our results suggest, again, the importance of silk threads as the physical support of the cues. Furthermore, silk threads could constitute the cues themselves. The absence of sex pheromones would be strange among spiders where their presence seems to be the rule. The only exception known is the thomisid *Misumena vatia* in which males follow female draglines without chemicals (Leonard & Morse 2006). However, no courtship was reported for this species. Further research using several solvent types could help to elucidate the presence and nature of putative pheromones.

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References

ALMEIDA-SILVA, L. M., CAMACHO, A., BRESCOVIT, A. D., LUCAS, S. M. & BRAZIL T. K. 2008: Redescription and notes on the natural history of the arboreal tarantula *Iridopelma seladonium* (Araneae: Theraphosidae: Avicullariinae). *Revista Brasileira de Zoologia* **25**: 728–736.

BAERG, W. J. 1958: *The tarantula*. Lawrence: University of Kansas Press.

BARUFFALDI, L., COSTA, F. G., RODRÍGUEZ, A. & GONZÁLEZ, A. 2010: Chemical communication in *Schizocosa malitiosa*: evidence of a female contact sex pheromone and persistence in the field. *Journal of Chemical Ecology* **36**: 759–767.

BARUFFALDI, L., PERAFÁN, C., PERDOMO, C., MONTES DE OCA, L., PÉREZ-MILES, F. & COSTA, F. G. 2013: Third pair of legs is a key feature for eliciting female receptivity in the road tarantula spider *Eupalaestrus weijenberghi* (Araneae: Theraphosidae). *Journal of Arachnology* **41**: 213–214.

COPPERI, S., FERRETTI, N., POMPOZZI, G. & PÉREZ-MILES, F. 2012: Can't you find me? Female sexual response in an Argentinean tarantula (Araneae, Theraphosidae). *Revista Colombiana de Entomología* **38**: 164–166.

COSTA, F.G. & PÉREZ-MILES, F. 2002: Reproductive biology of Uruguayan theraphosids (Araneae, Theraphosidae). *Journal of Arachnology* **30**: 571–87.

DONDALE, C. D. & HEGDECKAR, B. M. 1973: The contact sex pheromone of *Pardosa lapidicina* Emerton (Araneida: Lycosidae). *Canadian Journal of Zoology* **51**: 400–401.

DOR, A., MACHKOUR-M'RABET, S., LEGAL, L., WILLIAMS, T. & HÉNAUT, Y. 2008: Chemically mediated burrow recognition in the Mexican tarantula *Brachypelma vagans* female. *Naturwissenschaften* **95**: 1189–1193.

FERRETTI, N. & FERRERO, A. 2008: Courtship and mating behavior of *Grammostola schulzei* (Schmidt 1994) a burrowing tarantula from Argentina. *Journal of Arachnology* **36**: 480–483.

FERRETTI, N., POMPOZZI, G., COPPERI, S., GONZÁLEZ, A. & PÉREZ-MILES, F.. 2013: Sexual behaviour of mygalomorph spiders: when simplicity becomes complex, an update of the last 21 years. *Arachnology* **16**: 85–93.

FUKUSHIMA, C. S., BERTANI, R., & DA SILVA JR, P. I. 2003: Feromônio sexual em aranhas migalomorfas? *Actas IV Encontro de Aracnólogos do Cone Sul*, *São Pedro, Brazil*: 201.

GASKETT, A. C. 2007: Spiders sex pheromones: emission, reception, structures, and functions. *Biological Reviews* **82**: 27–48.

LEONARD, A. S. & MORSE, D. H. 2006: Line-following preferences of male crab spiders, *Misumena vatia*. *Animal Behaviour* **71**: 717–724.

MINCH, E. W. 1979: Reproductive behaviour of the tarantula *Aphonopelma chalcodes* (Araneae: Theraphosidae). *Bulletin of the British Arachnological Society* **4**: 416–420.

PÉREZ-MILES, F., COSTA, F. G., TOSCANO-GADEA, C. & MIGNONE, A. 2005: Ecology and behaviour of the "road tarantulas" *Acanthoscurria suina* y *Eupalaestrus weijenberghi* (Araneae, Theraphosidae) from Uruguay. *Journal of Natural History* **39**: 483–498.

PÉREZ-MILES, F., MONTES DE OCA, L., POSTIGLIONI, R., BARUFFALDI, L. & COSTA, F. G. 2007: Mating system in the tarantula spider *Eupalaestrus weijenberghi* (Thorell, 1894): evidences of monandry and polygyny. *Zoology* **110**: 253–260.

PRENTICE, T. R. 1997: Theraphosidae of the Mojave desert west and north of the Colorado river (Araneae, Mygalomorphae, Theraphosidae). *Journal of Arachnology* **25**: 137–176.

QUIRICI, V. & COSTA, F.G. 2005: Seismic communication during courtship in two burrowing tarantula spiders: an experimental study on *Eupalaestrus weijenberghi* and *Acanthoscurria suina*. *Journal of Arachnology* **33**: 159–66.

QUIRICI, V. & COSTA, F. G. 2007: Seismic sexual signals design of two sympatric burrowing tarantula spiders from meadows of Uruguay: *Eupalaestrus weijenberghi* and *Acanthoscurria suina* (Araneae, Theraphosidae). *Journal of Arachnology* **35**: 38–45.

SCHULZ, S. 2004: Semiochemistry of spiders. *In* R. T. Carde & J. G. Miller (eds.), *Advances in insect chemical ecology*. Cambridge: Cambridge University Press: 110–150.

SCHULTZ, S. 2013: Spider pheromones – a structural perspective. *Journal of Chemical Ecology* **39**: 1–14.

SHILLINGTON, C. & VERRELL, P. 1997: Sexual strategies of a North American 'Tarantula' (Araneae: Theraphosidae). *Ethology* **103**: 588–598.

WILDER, S. M., DEVITO, J., PERSONS, M. H. & RYPSTRA, A. L. 2005: The effects of moisture and heat on the efficacy of chemical cues used in predator detection by the wolf spider *Pardosa milvina* (Araneae, Lycosidae). *Journal of Arachnology* **33**: 857–861.