SHORT COMMUNICATION

Is more better? Sexual confusion during courtship between two sympatric and synchronic tarantulas: *Acanthoscurria suina* and *Eupalaestrus weijenberghi*

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ABSTRACT. When two similar species co-occur in time and space, strong mechanisms isolating them from each other are expected. *Acanthoscurria suina* Pocock, 1903 and *Eupalaestrus weijenberghi* (Thorell, 1894) are two sympatric and synchronic tarantulas that inhabit burrows in Uruguay's meadows. Here we test how and when reproductive isolation operates between these species. We exposed females of each species simultaneously to two males: either one male of each species, or two males of the same species. Males courted females of both species. Contrary to expectations, however, females of *A. suina* responded more effusively to heterospecific than to conspecific males, whereas females of *E. weijenberghi* only responded to conspecific males. Clasping (prelude of mating) was only recorded for couples of the same species. Females of *A. suina* at first seem to prefer the stronger body vibrations performed by heterospecific courting males than by males of their own species.

KEY WORDS. Courtship; Neotropical tarantulas; reproductive isolation; sex pheromone; sexual selection.

Courtship has a key function in partner recognition. Signals from the male inform the female that he is conspecific with her, and that his physical condition potentially reflects his genetic potential (Zahavi 1975, Andersson 1994). Conspecific recognition is crucial to avoid hybridization, particularly between similar species that are sympatric and synchronic, (Fisher 1930, Krebs & Davies 1996). Theraphosid tarantulas have a limited sexual repertoire (Costa & Pérez-Miles 2002), which reduces the possibilities of early discrimination among similar species that co-occur in space and time. Theraphosidae is an ancient linage that includes the largest spiders in the world. They are distributed mainly across the tropics and the Southern hemisphere. Despite their remarkable size, the reproductive biology of these tarantulas is poorly known. Due to the fact that it is difficult to collect many individuals at a time, it is not easy to maintain large numbers of spiders in captivity for experimentation. Additionally, the sexually mature males have a very short life, typically a few months to a few years (Costa & Pérez-Miles 2002).

The first comprehensive studies on tarantula reproduction were carried out by Baerg (1928, 1958). More recently, several authors have conducted additional studies, mainly on North-American species (Minch 1979, Prentice 1997, Shillington & Verrell 1997, Punzo & Henderson 1999, Yáñez *et al.* 1999). Even though more than a half of the approximately 900 species of Theraphosidae occur in South America, their reproductive biology had been seldom studied until the last two decades (Costa & Pérez-Miles 1992, 2002, Pérez-Miles *et al.* 2005, 2007, Bertani *et al.* 2008, Ferretti & Ferrero 2008).

Eupalaestrus weijenberghi (Thorell, 1894) and *Acanthoscurria suina* Pocock, 1903, are sympatric and synchronic species that are common in the meadows of Uruguay. Males are active during a short sexual period (mainly in March), coinciding with the end of the summer and beginning of autumn in the Southern hemisphere (Pérez-Miles *et al.* 2005). Both species have similar size and sexual repertoires. Male courtship involves body vibrations and palpal drumming, and receptive females call males by tapping their legs (Costa & Pérez-Miles 2002, Quirici & Costa 2005). Body vibration is organized in bouts of consecutive movements separated by pauses, and is repeated throughout the courtship (Fig. 1). Mating occurs at the entrance of the female's burrow (Costa & Pérez-Miles 2002). Quirici & Costa (2007) reported that male courtship is more intense in *E. weijenberghi* than in *A. suina*, implying that in the former species body vibration bouts are longer and inter-pulse durations are shorter, and that these dif-

Figure 1. Male of *E. weijenberghi* courting at the entrance of the female's burrow. Note the dense whitish silk mat covering the soil.

ferences in seismic communication might contribute to maintain reproductive isolation between these two sympatric and synchronic tarantulas.

Previous observations on *E. weijenberghi* and *A. suina* suggest that males may misunderstand female sex pheromones and court heterospecifically (R.Postiglioni, F.G. Costa & F. Pérez-Miles unpub. data). Furthermore, occasional observations indicate that females of *A. suina* frequently call for courting males of *E. weijenberghi*. In the present study we determine in which phase of the courtship the confusion occurs between both tarantula species and test whether females of each species prefer conspecific or heterospecific courting males when they are simultaneously given the choice of both. This information might contribute to the understanding of the mechanisms underlying the sexual preferences and reproductive isolation between these remarkable tarantulas.

We used 10 females and 20 males of each species, collected in Salinas Norte, Southern Canelones, Uruguay. Adult males were collected during March 2009, whereas females had been reared in the laboratory for at least two years, had molted at the beginning of 2009, and were sexually receptive. We carried out the experiments during March-April 2009, coinciding with the reproductive period of the species. We designed our experiment with four groups, each with 10 trials. In each experiment, two males were exposed simultaneously to a female. In group AAA, a female of *A. suina* was exposed to two conspecific males; in EEE, a female of *E. weijenberghi* was exposed to two conspecific males; in AAE a female of *A. suina* was exposed to one male *A. suina* and other of *E. weijenberghi*; in EEA a female of *E. weijenberghi* was exposed to one male *E. weijenberghi* and other of *A. suina*. Each female was reused once after a week; in one trial we exposed the female to two conspecific males and in the other to two males, one of each species. Males were also reused once after a week, in one trial together with a conspecific male and in other with a heterospecific male. In order to prevent prior trials from influencing the results, half of the individuals had their first trial in conspecific combinations and

half in heterospecific ones, while the inverse combination was used for the second trials.

We conducted all trials in the female's cage (glass terrarium of 50 x 15 cm of base, with a soil layer of 8 cm depth). Each female occupied a handmade burrow, simulating those observed in the field (PÉREZ-MILES *et al.* 2005). The female was placed in the terrarium at least one month before each observation. The burrow was located in the middle of the cage, against the glass wall, allowing the individual inside the burrow to be observed. Two observers controlled each trial. We carefully and simultaneously released the males on the opposite extremes of the terrarium and began the observations when the males made contact with the substrate. The observations were terminated after male-female clasping, or 30 minutes after the beginning of the trial. We prevented copulation by separating the couple after clasping. We monitored male body vibration, male-male aggressive interactions, female rejection (piston behavior and other rejecting actions), female call and clasping behavior. We also determined which male a female called, knowing that the call usually occurs about a second after male vibration (QUIRICI & COSTA 2005).

We analyzed the results with Past Statistical Package (HAMMER *et al.* 2001. To compare frequencies, we used the Fisher exact probability test (two-tailed). We also used paired and non-paired Student t-tests to compare parametric data samples. We deposited voucher specimens of each sex and species at the Entomological Collection of Facultad de Ciencias, Montevideo, Uruguay.

Most males courted both intra and interspecifically after they made contact with the female's silk. Males of *E. weijenberghi* made equal numbers of courtship displays on the silk of *E. weijenberghi* (18 in 20 cases) and *A. suina* females (10 in 10), whereas males of *A. suina* displayed less vibration occurrences on interspecific (7 in 10) than on conspecific silk (20 in 20) (Fisher test $p = 0.03$). We observed females calling in the four experimental groups (Table I). The females called conspecific males (immediately after his vibration) in the same number of trials, regardless of whether they were together with heterospecific or conspecific males. Females of *E. weijenberghi* called and clasped with the same frequency, regardless of whether they were exposed to conspecific or heterospecific males. By contrast, females

Table I. Number of trials in which behaviors occurred (from total ten trials per row). (AAA) Female *A. suina* with two conspecific males; (EEE) female *E. weijenberghi* with two conspecific males; (AAE) female *A. suina* with a male *A. suina*, and a male of *E. weijenberghi*; (EEA) female *E. weijenberghi* with a male of *E. weijenberghi* and a male of *A. suina*. Within parenthesis is given the distribution of behavior by species.

	Female call	Clasping	Rejection	Male-Male Attack
AAA	3	1	1	8
EEE	7	6	1	5
AAE	10 (3A/7E)	0	0	8 (7A/1E)
EEA	7 (7E/0A)	6 (6E/0A)	0	6 (3E/3A)

of *A. suina* called more often in the presence of heterospecific males (Fisher test, p = 0.031). Females of *A. suina* called in response to the courtship of conspecific and heterospecific males, while females of *E. weijenberghi* only called after the courtship of conspecific males (Table I, Fisher test, p = 0.0098).

Females of both species called conspecific males that performed body vibration in high or low frequencies, with no significant differences (Table II). Males of *A. suina* vibrated in higher frequencies towards conspecific females than towards heterospecific ones (paired t-test, t = 4.52, p = 0.004), whereas males *E. weijenberghi* did not change their vibration frequencies when exposed to heterospecific females (paired t-test, t = 0.78, p = 0.46).

We did not observe interspecific clasping in the trials. Clasping was more frequent in groups that included *E. weijenberghi* females (EEE and EEA) (in the limit of significance) than in groups including females of *A. suina* (Fisher test, p = 0.057). When we compared the occurrence of clasping between AAE and EEA, females of *E. weijenberghi* clasped in more trials than females of *A. suina* (P = 0.01). Female rejection towards males was rare and was only observed in AAA and EEE (Table I).

Male-male attacks were frequent, but we did not observe injuries. Attacks consisted in assaults and pushes with the legs raised, as well as threats with open fangs, but no bites. Males of *A. suina* attacked other males in more trials than males of *E. weijenberghi*, but we did not observe significant difference in the global comparison (p = 0.35) or in the interspecific encounters (p = 0.25). No relationship was found between male attacks and clasping (Table I). Females did not attack any male.

Males of both tarantula species courted heterospecifically, suggesting that they are not able to discriminate between the sex pheromones and/or silk support of conspecific females and females of the sympatric species. Early isolation mechanisms, which are expected in sympatric species, were not detected under lab conditions. Also, *A. suina* females were seduced by heterospecific courtship and, surprisingly, called males of *E. weijenberghi* more frequently than conspecific males. Given that no heterospecific clasping occurred, we can deduct from our data that chemo-tactile recognition ensuring reproductive isolation occurs only when the mating pairs make physical contact. Nevertheless, two main questions remain to be answered: 1) Why do these sympatric, syntopic and synchronic species

have not developed earlier and more efficient isolation mechanisms?, and 2) Why do males of *E. weijenberghi* elicit more intense receptive response in females of *A. suina* compared to females from their own species?

The chemical characteristics of female sex pheromones are generally conservative in closely related groups of spiders (SCHULZ 2004, GASKET 2007). However, theraphosid sex pheromones have not been extensively studied. Although the species belong to different genera, their pheromones seem to be similar. Nevertheless, this is a supposition, because we have not exposed males to pheromones from females of both species simultaneously. Behavioral confusion was unexpected considering the cost and risk of courtship for these animals. Still, some *A. suina* males did not perform body vibration towards the heterospecific stimulus, and the others performed it at a lower frequency, suggesting that some kind of species-specific discrimination is in place. This was not the case for *E. weijenberghi* males. Males of both species have a very limited courtship repertoire, which involves body vibration and palpal drumming. Body vibration is more intense in *E. weijenberghi*, whereas palpal drumming is frequent only in *A. suina* (COSTA & PÉREZ-MILES 2002).

The mechanisms mentioned above do not seem to be sufficient for an early isolation, at least for females of *A. suina*. Body vibration is a long-distance signal, whereas palpal drumming operates at short distances (QUIRICI & COSTA 2005). It is possible that both species have evolved in different environments and have distinct signal transmission requirements. While *A. suina* is found in meadows (homogeneous substrate) and in rocky hills (heterogeneous substrate), *E. weijenberghi* occurs only in meadows. The absence of early isolation barriers also suggest that these species have recently come in sympatry and synchrony, but there is no historical evidence to support this idea. The second question, regarding the intense response of *A. suina* females to heterospecific males, could be explained by the fact that body vibrations are more intense in males of *E. weijenberghi* (QUIRICI & COSTA 2007) and they could represent a supernormal stimulus for females of *A. suina*. Vigorous courtship is interpreted as an honest indicator of male genetic quality, which usually has a selective value for females (ZAHAVI 1975, ANDERSSON 1994).

Although males might be confused by the calls of heterospecific females, females seem not to use it to capture interspecific males. Sexual cannibalism is unusual in the two

Table II. Frequencies of bouts of body vibration (bouts per minute) performed by males during the first five minutes of courtship in the experimental groups (E = *E. weijenberghi*; A = *A. suina*). Data are presented as mean ± standard deviation and data number between parentheses. We discriminate between the frequencies of body vibration which determined female call and those which did not. (In EEE two males did not perform body vibration and in EEA three males *A. suina* did not perform body vibration).

Female (group)	Males *A. suina*			Males *E. weijenberghi*		
	Total	Call	No call	Total	Call	No call
A (AAA)	1.58 ± 0.83 (20)	2.67 ± 1.17 (3)	1.39 ± 0.61 (17)	–	–	–
A (AAE)	1.42 ± 0.94 (10)	1.60 ± 0.30 (2)	1.38 ± 1.05 (8)	1.76 ± 1.19 (10)	2.17 ± 1.30 (6)	1.15 ± 0.77 (4)
E (EEE)	–	–	–	1.31 ± 0.50 (18)	1.61 ± 0.34 (7)	1.11 ± 0.50 (11)
E(EEA)	0.69 ± 0.34 (7)	0 (0)	0.69 ± 0.34 (7)	1.98 ± 0.64 (10)	1.91 ± 0.50 (7)	2.13 ± 1.01 (3)

species, either in the field or in the laboratory (Costa & Pérez-Miles 2002, Pérez-Miles *et al.* 2005, 2007). We did not record any case of it in hundreds of personal observations, but there is a single literature report of female cannibalism on a conspecific male of *A. suina* in the field (Costa & Pérez-Miles 2002). Both species are similar in size, and the risk of interspecific predation is low; consequently, we do not expect a strong selective pressure to avoid interspecific encounters. Taking into account that females of *E. weijenberghi* remain unreceptive after copulation (Pérez-Miles *et al.* 2007), a strong competition among males would be expected; however, we did not find evidence of such competition. The female-biased sex ratio in both species (Pérez-Miles *et al.* 2005) might decrease competition for mates because females are widely available. Male-male attacks consisted of innocuous displays, which were easily and rapidly resolved, and did not seem to affect the ulterior behavior of males. Probably, scramble competition among males could operate in both tarantulas, as suggested by Shillington & Verrell (1997) for the tarantula *Aphonopelma* sp. It is possible that courting heterospecific females is not very costly for males, considering the high density of female burrows and mixture of both species, as well as the low risk of predation (Pérez-Miles *et al.* 2005).

Our unexpected results in sexual recognition mechanisms may help elucidate evolutionary processes such as speciation mechanisms. Heterospecific response of females to sexy males seems to support a classical statement in sexual selection: more is better.

ACKNOWLEDGEMENTS

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