Notes on the courtship behavior of the parthenogenetic scorpion *Tityus stigmurus*

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Abstract — Scorpions can adopt different reproductive strategies (e.g., sexual or parthenogenesis) depending on the environmental conditions. In this study, we described the courtship repertoire of the parthenogenetic scorpion *Tityus stigmurus*, a species with inconspicuous sexual populations distributed throughout northeastern Brazil. Specimens of both sexes (10 males, 10 females) were obtained from field expeditions in three locations in northeastern Brazil and were paired under laboratory conditions in an intrapopulation design. All observations were conducted at night using red light positioned above an experimental arena. From the 10 courtship interactions observed in this study, only one pair successfully completed the reproductive repertoire that included the insemination process. The incomplete interactions were abruptly terminated by the females immediately after deposition of the spermatophore. Overall, the reproductive repertoire of *T. stigmurus* was similar to that of other scorpions, especially its congeners, possibly because this reproductive behavior emerged at a basal point in the evolution of scorpions. The acquisition of new insights into the reproductive biology of different scorpion species may improve the theoretical background for applications ranging from evolutionary biology to public health strategies.

Key words — Arachnida, Buthidae, courtship behavior, parthenogenesis, reproductive biology, Scorpiones

Introduction

The most frequent form of reproduction in scorpions involves the indirect transfer of male gametes via spermato- phores, and occurs after an elaborate and ritualized courtship (Polis & Sissom 1990; Stockmann 2015). However, some species can also reproduce by parthenogenesis, in which females generate offspring without the genetic contribution of males (Francke 2008; Lourenço 2008). For these species, males are considered rare (Lourenço 2008, 2015), and parthenogenesis is assumed to be the main method of reproduction (Warburg 2011; Ayrey 2017; Seiter & Stockmann 2017). Consequently, behavioral studies addressing the courtship repertoire in scorpions that can reproduce by either method (i.e., sexual reproduction or parthenogenesis) are extremely rare, and this in turn leads to incorrect generalizations about the reproductive biology of these species. The study of the sexual populations of these arachnids has historically been useful in enhancing our understanding of their life histories, especially for specious genera such as *Tityus* C. L. Koch 1836 (Bücherl 1956; Lourenço & Cuellar 1995, 1999; Lourenço & Cloudsley-Thompson 1999; Dionisio-da-Silva et al. 2018). This is particularly important for species such as *Tityus stigmurus* (Thorell 1876), one of the most medically important species in South America (Albuquerque et al. 2013; Pucca et al. 2015).

Populations of *T. stigmurus* distributed in urban environments are assumed to reproduce by parthenogenesis (Lourenço & Cloudsley-Thompson 1999). In theory, this can lead to a rapid demographic expansion in such newly created environments due to density-independent processes (Cuellar 1977, 1994; Lourenço & Cuellar 1995). However, the inconspicuous sexual populations of *T. stigmurus* occur in a more restricted geographic range (Lourenço 2015) and are associated with undisturbed environments (Lourenço & Cloudsley-Thompson 1999). This, in turn, could explain the absence of any knowledge concerning the reproductive behavior of this species. Many arthropod groups are excellent models for the study of
mating system evolution due to their stereotyped patterns of reproductive behavior (Polis & Sissom 1990; Tallarovic et al. 2000). Therefore, we provide here the first description of courtship and mating behavior in sexual populations of the scorpion *T. stigmurus*. This information may be useful in obtaining a better understanding of the evolution of behavioral traits related to reproduction in scorpions.

**Materials and methods**

Behavioral trials were performed in December 2019 using a plastic circular arena of 20 × 15 cm (diameter × height) containing white filter paper as the substrate. For these trials, we used 20 adult scorpions (10 males, 10 females) collected from undisturbed environments in Buique (8°40'S, 37°9'W; n = 1 couple), Crato (7°21'S, 39°31'W; n = 1 couple), and Tríunfo (7°52'S, 38°7'W; n = 8 couples), in northeastern Brazil. We avoided pairing individuals from different locations in order to avoid any possible interference in the courtship repertoire due to interpopulation divergence, as this has been reported in other scorpions (Olivero et al. 2015, 2017). All observations were conducted at night (19h00min – 23h00min) using a red light (Machan 1968) positioned 60 cm above the arena. Meanwhile, temperature and relative air humidity were maintained at 27°C and 70%, respectively. Movements were recorded using the *ad libitum* method (Altmann 1984), which is characterized by the observer freely noting information, allowing opportunistic annotations and the recording of rare events that may be a significant part of their behavior (Martin et al. 1993). The terminology used in this study was based on Tallarovic et al. (2000) and Jiao & Zhu (2010).

**Results**

From the 10 courtship interactions observed in this study, only one pair, collected from Buique, completed the reproductive repertoire that successfully included the insemination process. Aside from this, the frequencies of each behavior were nearly identical for all pairs. The behavioral sequences adopted by *T. stigmurus* are shown in Fig. 1. Courtship in the remaining nine pairs was interrupted by the aggressive behavior of females triggered by the deposition of the spermatophore by the male. Overall, the courtship repertoire of *T. stigmurus* was executed in an average of 39 ± 7 minutes (mean ± standard deviation), ranging from 27 to 49 minutes.

**Stage I: Initiation.** *Tityus stigmurus* males took the initiation after random encounters with standing females. Before these encounters, males walked slowly through the arena, adopting a “searching” posture characterized by the opening of the chela, the extension of the pedipalps, and the intermittent contact of the pectens with the substrate. Males touched different parts of the females’ bodies (mesosoma, legs, or metasoma), and both sexes reciprocally touched and grasped the fifth segment of each other’s metasomas. Metasoma grasp was always followed by body shaking pre-courtship behavior (juddering) by males. However, in six observations, the male exhibited the juddering behavior before the first physical contact with females. When it occurred, this behavior preceded the engagement of the pair by their pedipalps, which was always initiated by the males (Fig. 1). The *initiation* stage lasted from four to nine minutes (mean ± standard deviation: 6 ± 2).

**Stage II: Promenade à deux.** After the first physical contact, the males initiated courtship by holding both of the females’ pedipalps, causing their bodies to be aligned in the chelicerae-chelicerae position. The males of *T. stigmurus* directed and coordinated the movements of the couple during the *promenade*. In this species, the *promenade* was initiated when the male firmly grasped the female pedipalps and began to guide her through backward and forward movements. When the females resisted or stopped moving, the males pulled them more vigorously and balanced their metasomas with “forward to back” movements, interweaving this behavior with juddering movements. Throughout this stage, the males held their metasomas elevated and guided the females through the substrate in search of a suitable place to deposit the spermatophore. The males’ pectens were always directed downward, touching the substrate. Across all observed pairings, the *promenade à deux* lasted between 18 and 38 minutes (mean ± standard deviation: 28 ± 7), and was completed with the males finding a suitable place to deposit the spermatophore.

**Stage III: Sperm transfer.** Once an appropriate site for depositing the spermatophore was found, the males’ chelicerae protruded and held the females’ chelicerae in a “kiss”. During this act, the males kept their metasomas high and erected. As the spermatophore was slowly deposited, *T. stigmurus* males quickly moved their metasomas to the sides. All courtship interactions presented the same behavioral repertoire until spermatophore deposition. After that, courtships were divided into successful (n = 1) and unsuccessful (n = 9) groups, based on the occurrence or absence of the insemination process. For the single successful act, the male groped the female’s gonopore with his first pair of legs and pulled her vigorously toward the spermatophore. Once positioned on the spermatophore, the female remained on top of it for 35 s; the whole act lasted for two minutes.

**Stage IV: Separation.** For the single pair that successfully completed the courtship interaction, separation occurred immediately after insemination. The female remained standing near the site where the spermatophore was deposited, while the male walked slowly around the borders of the arena. For the remaining nine courtship interactions in which insemination did not occur, the females adopted aggressive behavior immediately after spermatophore deposition (Fig. 1). This consisted of a series of repulsive movements by the females, in which they vigorously retracted their pedipalps and vertically shook their metasomas in the direction of the males. Such behavior forced the disengagement of both sexes and led to an abrupt interruption of the courtship process. The males then adopted defensive behavior by keeping distance from the females and trying to escape from the containers. Separation lasted for two minutes in the successful courtship, and ranged between one and three minutes for the re-
mainly nine unsuccessful courtship interactions.

**Discussion**

This study describes, for the first time, the courtship behavior of the parthenogenetic species *T. stigmurus*. Overall, we noted that their reproductive repertoire is similar to that of other species in the Buthidae family, especially the congeneric species *T. bahiensis*, *T. fasciolatus*, *T. magnimanus* Pocock 1897, *T. pusillus*, *T. trinitatis* Pocock 1897, and *T. trivittatus* Kraepelin 1898 (Polis & Sissom 1990; Ross 2009; Lira et al. 2018). Juddering behavior, for instance, was observed in the courtship trials in our study, as well as in the congenic *T. magnimanus* and *T. trinitatis* (Alexander 1959; Ross 2009). In *T. stigmurus*, this behavior occurred during the first two stages of the courtship (Fig. 1), suggesting that it may be related to both the identification of the male and female prior to initial contact, and the reduction of resistance in females during the promenade phase. Previous studies have demonstrated that juddering is a behavior usually performed by males to induce the female to cooperate during courtship (Alexander 1959; Polis & Sissom 1990). Moreover, Ross (2009) observed the absence of juddering in males of *T. magnimanus* when females did not offer resistance during the promenade. Females of *T. stigmurus* often resisted male movements during the promenade and subsequent stages, and this may explain the presence of juddering after the initiation of courtship. Nevertheless, although the role of juddering behavior as a recognition mechanism in the initial phases of courtship has been reported in the literature (Polis & Sissom 1990), it is not clear if it is triggered by...
physical contact between sexes and/or if it depends on the actuation of pheromones as well. In our trials, we observed males exhibiting juddering behavior before the first physical contact with females in six cases, suggesting that chemical clues may be related to the identification of sexes in *T. stigmurus*, as is observed in other scorpions (e.g., Gaffin & Brownell 1992; Taylor et al. 2012; Pordeus et al. 2019).

The overall similarity observed in the courtship behavior of *T. stigmurus* and other species within the order Scorpiones may reflect the emergence of this reproductive behavior at a basal point in the evolution of scorpions (Polis & Sissom 1990). However, more studies are needed to answer fundamental questions about the population and reproductive biology of *T. stigmurus*, especially regarding the mechanisms of sexual recognition as well as the evolutionary and environmental factors that lead to the different strategies of reproduction in this species. This is especially true for medically important species such as *T. stigmurus*, which is one of the most harmful scorpions in South America. For example, in this species, parthenogenetic populations are expected to be more dispersive and proliferative, in part due to density-independent demographic processes (Lourenço 2008). Hence, an understanding of the reproductive biology of such understudied species can provide basic scientific knowledge for the establishment of public health strategies aimed at mitigating recurrent problems related to scorpion envenomation (Lourenço 2015).

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