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NOTES AND OBSERVATIONS ON COURTSHIP AND MATING IN *Tityus* (*Atreus*) *magnimanus* POCOCK, 1897 (Scorpiones: Buthidae)

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ABSTRACT: Courtship and mating behaviors of the scorpion *Tityus* (*Atreus*) *magnimanus* are herein described, consisting of various components that pertain to four distinct behavioral stages. The courtship and mating rituals of *Tityus* (*Atreus*) *magnimanus* are similar to those of other scorpions. Behavioral components are presented in an ethogram to demonstrate their occurrence during mating sequences. The current report is presented as observational data that were acquired during life history studies of this species.

KEY WORDS: scorpions, Buthidae, *Tityus (Atreus) magnimanus*, courtship, mating, *promenade á deux*, sperm transfer.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Courtship and mating among scorpion species, on which such data are available, comprise a series of complex, ritualized and characteristic behaviors that involve a courtship period of stereotyped acts leading up to sperm transfer from male to female via an external stationary spermatophore (1-5). Courtship in scorpions is usually divided into four distinct behavioral stages (different terminologies have been applied to individual stages by various authors): initiation, *promenade á deux*, sperm transfer and separation (3, 4, 6).

In the majority of scorpion species, courtship is initiated by males and typically involves a male approaching a female and grasping her chelae within his own (in some instances, a chelicerae-to-chelicerae grasp may be initiated by males and used simultaneously with the chelal grasp to control female movements). After the chelal grasp, the male initiates and guides the female in a series of coordinated movements termed *promenade á deux*. This courtship "dance" may last from five minutes to several days, with most courtship and mating sequences lasting from 30 to 60 minutes (3).

During the *promenade*, the male seeks out a suitable surface or structure upon which to deposit his spermatophore. Once the spermatophore has been extruded and attached to a suitable surface, the male then directs the female movements until her genital opening is positioned above the spermatophore. A brief struggle ensues and the female rocking motion assists in engaging the spermatophore with the genital operculum, and the sperm is released into this opening (7). Once sperm transfer is complete, the male usually initiates separation of the participants (3).

Courtship and mating behaviors have been described in approximately thirty scorpion species (3, 4), representing seven of the 18 extant families (8). Based on data reviewed by Polis and Sissom (3), both rituals have been described in more species (n = 12) from the family Buthidae than for any other scorpion family. *Tityus* C. L. Koch, 1836 is the largest genus within this family, represented by 181 species (9, 10). *Tityus* and the genus *Centruroides* Marx, 1890 (Buthidae), represent the only scorpion genera recognized as being medically significant in the Nearctic and Neotropical regions (11-13).

Despite the large number of species among *Tityus*, its abundance in many urbanized and rural human occupied areas, and the medical significance of many of its

component species, courtship and mating in members of this genus are few in number (1, 14, 15).

The following contribution is based on laboratory observations of 12 mating sequences in the Venezuelan endemic scorpion, *Tityus* (*Atreus*) *magnimanus* Pocock, 1897 (Buthidae), conducted during February and March 2008. *Tityus* (*Atreus*) *magnimanus* inhabits tropical altitudinal forests in the states of Falcón and Lara in north-central Venezuela, and is considered to be of medical importance, with all severe and near-fatal envenomations in Falcón state attributed to this species. The present work is part of a continuing study on the life history of *Tityus* (*Atreus*) *magnimanus* and represents the first report on this medically significant species.

MATERIALS AND METHODS

In order to acquire female specimens of known age for life history studies, specimens of *Tityus* (*Atreus*) *magnimanus* were mated in the laboratory and observational data were recorded, where specimens of *Tityus* (*Atreus*) *magnimanus* (n = 84) were reared to maturity. Upon attaining sexual maturity, unrelated adult males (n = 12) and laboratory reared females (n = 12) were randomly selected and transferred to individual containers.

Mature females were housed in circular 9 x 23 cm clear plastic containers, provided with a 50 mm layer of moist potting soil, a piece of cork or shelter and several flat rocks. As the containers of females would also serve as mating arenas, the addition of flat rocks provided a surface structure for spermatophore deposition by males.

Adult males were kept in individual 15 x 15 x 15 cm clear plastic containers, with a 50 mm layer of moist potting soil and a single piece of cork for shelter. All specimens were maintained at temperatures from 26.6 to 29.0°C during the day and 23.8 to 25°C at night. The substrate was kept moist while ambient humidity levels were maintained in the range of 70 to 80%. Prey consisted of adult house crickets (*Acheta domesticus* L.) and cockroaches (*Blatta lateralis* Walker) with the type of prey alternated between *ad libitum* feedings. All observations were conducted under a low-intensity 60 watt red incandescent bulb in a parabolic reflector, suspended 50 cm above breeding arenas.

Mature virgin females were provided a 24-hour acclimation period before mating trials were initiated. A randomly selected mature male was introduced into each of 12 breeding arenas and all observed courtship and mating behaviors were recorded.

Once sperm transfer was completed, indicating termination of the mating sequence, males were removed from arenas and returned to individual containers.

RESULTS

In each of the 12 observed mating sequences, males immediately initiated courtship upon contact with females. As has been previously reported in scorpions (3), four distinct behavioral stages were observed: initiation, *promenade á deux*, sperm transfer, and separation. If specimens became separated during the *promenade á deux*, the behavioral sequence was immediately resumed by specimens without a return to a previous stage. The following ethogram demonstrates each behavioral component of courtship and the order in which they occurred during mating sequences (Figure 1).

Initial Contact **♂ active**
♂ positions body carapace to carapace and grasps chelae of ♀ ♀ active
♂ grasps single pedipalp and/or chela of ♀ ♀ passive
♂ grasps metasoma of ♀ ♀ passive
♂ initiates coordinated movements of the *promenade á deux* ♀ active
♀ resists and ♂ judders and forcibly pulls ♀ ♀ unreceptive
♂ directs ♀ in backwards movements ♀ receptive
♂ locates suitable surface for deposition of spermatophore ♂/♀ pause movements
♂ pulls ♀ in chelal grasp toward the spermatophore ♀ receptive
♂ pulls ♀ in chelal grasp toward the spermatophore ♀ unreceptive
♂ deposits the spermatophore
♂ judders and forcibly pulls ♀ over the spermatophore ♀ receptive
♀ accepts the spermatophore; after uptake, ♀ pulls away and moves from ♂
♂ severs flagellum of spermatophore and moves away

Figure 1. Ethogram demonstrating the behavioral components of the courtship sequence in *Tityus* (*Atreus*) *magnimanus*.

Stage I: Initiation

In all observed mating sequences, males initiated courtship after detecting the movements of females (n = 3; 25%) or during random encounters with stationary females (n = 9; 75%). When a male detected a moving female or encountered a stationary female, courtship was initiated by the male grasping and rapidly moving his chelae over the segments (including the chelae) of one (n = 8; 66.6%) or both (n = 2; 16.7%) pedipalps of a female.

Two males (16.7%) encountered immobile females and immediately grasped and moved their chelae over metasomal segments III to V of each female. After a brief period (less than 45 seconds) of metasomal or pedipalpal grasping behaviors, a male would grasp each chela of a female within his own and align his body with that of the female in a chelicerae-to-chelicerae position thus, making contact with the female and terminating the initiation stage of courtship.

After initial contact by males, females typically withdrew their pedipalps against their own bodies and remained immobile until males initiated pulling movements. If a female resisted the attempts of a male to begin directed movements during initial contact, the male would judder for 10 to 25 seconds, then vigorously and repeatedly pull the female toward him until she began moving.

Polis and Sissom (3) reported that juddering by male scorpions functions either as a highly ritualized sexual or species-recognition behavior, as an instigator of mating behavior so that the female is stimulated to cooperate, or as a simple byproduct of intense sexual excitation. The only instances in which juddering was exhibited by males was in direct response to resistant females during the initiation and *promenade á deux* stages and appeared to be used by males to suppress unreceptive behaviors and stimulate females into mating.

In two pairings that involved receptive females that never resisted males during courtship movements, juddering was not observed in either male throughout all stages of courtship. These observations suggest that juddering behavior may be used by courting males to stimulate unreceptive females into mating.

Alexander (1) reported metasomal grasping by courting males of *Tityus* (*Atreus*) *trinitatis* Pocock, 1897 and suggested that such behaviors by males may function as a means of identifying conspecific females through the sexually differentiated morphologies of metasomal segments. Repeated grasping of pedipalps and chelae

of females by courting males may serve as a similar means of identifying conspecific females via differences in these morphological structures.

Sexually mature males and females of *Tityus* (*Atreus*) *magnimanus* exhibit differing morphologies of the pectines, chelae and metasomal segments. As has been reported by Lourenço (9), females of some species within the subgenus *Atreus* Gervais, 1843 possess enlarged basal middle lamellae that are not present in males. The chelal manus of males is moderately inflated and serves to differentiate mature males from immature males and females. Additionally, females are larger and more robust than males and the metasomal segments of sexually mature females are thickened and well developed in comparison to those of males.

Stage II: *Promenade á Deux*

The *promenade á deux* occurs in all scorpion species for which courtship and mating data are available (2, 3, 4). As in other scorpion species, the males of *Tityus* (*Atreus*) *magnimanus* direct and coordinate the movements of the pair during the *promenade á deux*. In *Tityus* (*Atreus*) *magnimanus*, the *promenade* begins when a male firmly grasps the chelae of a female within his own and begins to guide the female in mutually backward, sideways and rarely, forward movements. The male begins *promenade* movements by elevating its body slightly above the ground surface with the metasoma elevated and dorsomedially flexed, and moving backwards, effectively pulling the female along and directing her movements.

During backwards movements, the male will periodically flex and straighten the metasoma while simultaneously raising and lowering it in response to the willingness of the female to follow his movements.

If a female resists or stops moving, the male will move closer to her, elevate his body, and begin pulling the female in vigorous backward movements while simultaneously elevating and waving his metasoma from side-to-side. Females typically keep their bodies close to the substratum with the metasoma flexed and held laterally.

During this stage of courtship, the male guides the female across the substratum until a suitable surface is located for spermatophore deposition. During *promenade* movements, male pectines are directed downward and are moved back and forth over the substratum and all encountered surface structures. During *promenade* movements, the female may resist male attempts to direct coordinated movements of

the pair by pulling and trying to move in a direction opposite that of the male or by ceasing movements, lowering her body to the substratum and becoming still.

Immobile females only resume moving after males exhibit juddering (6 to10 seconds) and vigorous pulling behaviors. In all observed pairings, the *promenade á deux* lasted from 4.5 to 9.0 minutes and was terminated when a suitable location for spermatophore deposition was selected by the male. A courting pair may pass over a structure several times from different directions until the male ceases their movements. Selection of a proper structure for spermatophore deposition finishes the *promenade á deux*.

Stage III: Sperm Transfer

As in other scorpions, sperm transfer in *Tityus* (*Atreus*) *magnimanus* is accomplished via a stationary spermatophore that is flagelliform in shape and structure (7). Males of this species are capable of producing their first spermatophore within seven days of maturing molts and can regenerate spermatophores in a very short period, 24 hours (Ross, personal observation).

Once a suitable surface is selected by a male, he pulls the female close and with his chelicerae grasps either the anterior edge of her carapace (n = 10; 83.3%) or chelicerae (n = 2; 16.7%). The courting pair remains still during the release of the spermatophore. The male lowers his venter against the selected surface and the pedicel is attached to the surface. Whereas the spermatophore is slowly extruded, the male gradually elevates his body while simultaneously moving backwards until the trunk and flagellum of the spermatophore are exposed.

Spermatophore expulsion is marked by the male elevating his entire metasoma vertically and slowly waving it from side-to-side (15 to 39 seconds). Once the spermatophore is deposited, male metasomal movements cease (n = 12; 100%) and he pulls the female toward him and directs her movements until her genital aperture is positioned directly above the erect spermatophore. Once the female genital orifice is positioned above the capsule, the male will rock the female back and forth (6 to 10 seconds) so that the spermatophore is engaged by her genital operculum. During the back-and-forth rocking movements, the female lowers her body upon the trunk of the spermatophore that houses the sperm vesicle and sperm duct, so that the sperm mass is released into her genital aperture. In this observation, as soon as the sperm

mass was transferred, females (n = 12; 100%) immediately separated and retreated 4 to 10 cm away from males.

Stage IV: Separation

In all sequences in the present study, mating terminated when the female pulled away and moved a short distance from the male immediately following sperm transfer. As soon as a female started to struggle and pull away, a male simply released his grasp upon female. Once a female moved away from a male, she either rubbed her venter upon the substratum (n = 7; 58.3%) or used one or both pairs of anterior walking legs to vigorously rub her genital operculum (n = 5; 41.7%).

The male remained with a spent spermatophore and rapidly moved the tarsi of the first pair of walking legs over the base and trunk of the spermatophore (n = 9: 75%) (4 to 8 seconds) or remained stationary without exhibiting any activity (n = 3; 25%) (30 to 120 seconds).

After the recuperative stage, males (n = 12; 100%) moved slightly backwards and used their chelicerae to sever the flagellum near its base on the spermatophore. After severing the flagellum, males rested for a brief period (2 to 6 minutes) before becoming vagile and moving away from spermatophores. Consumption of the spermatophore by male or female was not observed in any mating sequence. Post-mating males and females do not exhibit agonistic or aggressive behaviors toward each other and will aggregate in large, mixed gender groups in laboratory enclosures.

DISCUSSION

The courtship and mating behaviors of *Tityus* (*Atreus*) *magnimanus* are similar to behaviors described in other scorpions including several species within the genus *Tityus*. However, in order to explain and more thoroughly understand patterns of phylogeny, ecology, biology, reproductive evolution and diversification among scorpions, a precise knowledge of the reproductive biology and associated behaviors in scorpions is required (12).

The lengthy and time consuming process of the *promenade á deux* has been traditionally interpreted as the time necessary for a courting male to locate and select a suitable surface upon which he will deposit his spermatophore. However, when a courting male engaged in the *promenade á deux* is presented with two different

surface structures (a stick and a flat rock) during successive mating trials, the male will select the flat rock instead of the stick for spermatophore deposition during one trial and when introduced into another mating arena with a new female, chooses the stick instead of the rock.

Additionally, a male may direct the female over a surface dozens of times before finally selecting the surface as the site of spermatophore deposition. Francke (7) reported that males of *Centruroides sculpturatus* Ewing, 1928 (Buthidae) when maintained overnight in plastic bags with pieces of crumpled newsprint for cover would deposit spermatophores upon the newsprint.

Overall, a courting male does not appear to be overly selective in his choice of site for spermatophore deposition. Personal observations of additional mating sequences in *Tityus* (*Atreus*) *magnimanus* and *Tityus* (*Atreus*) *trinitatis* Pocock, 1897 have revealed that a courting male will select any solid surface encountered for spermatophore deposition including compacted substrate, fragments of clay plant pots, pieces of peat seedling pots, rocks, sticks, various tree barks, dried leafs, sections of palm root, quarter dollars, plastic bottle caps, cardboard and construction paper.

Male juddering has been reported to occur in 18 species, from eight families, and has been reported to occur during all stages of courtship (3, 4). Juddering behavior during the *promenade á deux* was observed in ten pairings of *Tityus* (*Atreus*) *magnimanus* and was utilized by a male in response to a female that became unresponsive to male-directed movements or attempted to move in an opposite direction of male during courtship. In two pairings, females did not resist male-directed movements or attempt to pull away and move in an opposite direction. In both pairings, juddering was not displayed by either male during any stage of courtship. These observations suggest that juddering by male *Tityus* (*Atreus*) *magnimanus* may be used in direct response to resistance behaviors exhibited by females during the *promenade* and may act to stimulate mating.

Juddering by the male during the initial stage did not occur in any of the 12 pairings observed during this study. Males introduced into mating arenas containing females either sensed their movements and moved directly to them or encountered immobile females during random movements. Males did not exhibit pre-contact mate recognition behaviors and readily approached moving and immobile females.

Once contact was made with a female, the male would immediately grasp a single pedipalp of the female and make repeated grasps upon the various segments. In two pairings, the male made repeated grasps upon metasomal segments III to V of the female.

Alexander (1) noted that there is a marked sexual dimorphism of the metasomal segments in males and females of *Tityus* (*Atreus*) *trinitatis* and that courting males would approach females and repeatedly grasp various metasomal segments. Furthermore, the author suggested that males utilize grasping behaviors to identify the sexually dimorphic characters of the metasomal segments in females. The exhibition of grasping behaviors in courting males of *Tityus* (*Atreus*) *magnimanus* and *Tityus* (*Atreus*) *trinitatis* propose that differences in the morphology of various structures (chelae, pedipalps, metasomal segments) are important for mate recognition by males of both species during courtship.

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REFERENCES

1. Alexander AJ. Courtship and mating in the buthid scorpions. Proc Zool Soc London. 1959;133:145-69.

2. Lourenço WR. Reproduction in scorpions, with special reference to parthenogenesis. In: Toft S, Scharff N, editors. European Arachnology 2000. Proceedings of the 19th European Colloquium of Arachnology; 2000 July 17-22; Åarhus, Denmark. Åarhus: Åarhus University Press; 2002. p. 71-85.

3. Polis GA, Sissom WD. Life History. In: Polis GA, editor. The Biology of Scorpions. Standford: Stanford University Press; 1990. p. 161-223.

4. Tallarovic SK, Melville JM, Brownell PH. Courtship and mating in the giant desert hairy scorpion, *Hadrurus arizonensis* (Scorpionida, Iuridae). J Insect Behav. 2000;13(6):827-38.

5. Weygoldt P. Arthropoda-Chelicerata: sperm transfer. In: Adiyodi KG, Adiyodi RG, editors. In: Reproductive biology of invertebrates. Part B. v4. New Delhi: Oxford & IBH Publishing Co.; 1990. p. 77-119.

6. Peretti AV, Acosta LE. Comparative analysis of mating in scorpions: the post-transfer stage in selected *Argentinian bothriurids* (Scorpiones, Bothriuridae). Zool Anz. 1999;237:259-65.

7. Francke OF. Spermatophores of some North American scorpions (Arachnida, Scorpiones). J Arachnol. 1979;7:19-32.

8. Prendini L, Wheeler WC. Scorpion higher phylogeny and classification, taxonomic anarchy, and standards for peer review in online publishing. Cladistics. 2005;21(5):446-94.

9. Lourenço WR. Nouvelle proposition de découpage sous-générique du genre *Tityus* C. L. Koch, 1836 (Scorpiones: Buthidae). Bol Soc Entomol Aragonesa. 2006;39:55-67.

10. Scorpion Files [homepage on the Internet]. Trondheim: Norwegian University of Science and Technology; c2001-2009 [updated 2008 Dec 16; cited 2009 Jan 5]. Available from: http://www.ub.ntnu.no/scorpion-files.

11. Barona J, Batista CV, Zamudio FZ, Gomez-Lagunas F, Wanke E, Otero R, Possani LD. Proteomic analysis of the venom and characterization of toxins specific for Na+ - and K+ -channels from the Colombian scorpion *Tityus pachyurus*. Biochim Biophys Acta. 2006;1764(1):76-84.

12. Dehesa-Davila M, Possani LD. Scorpionism and serotherapy in Mexico. Toxicon. 1994:32(9):1015-8.

13. Freire-Maia L, Campos JA, Amaral CFS. Treatment of scorpion envenomation in Brazil. Toxicon. 1996;34(2):153-4.

14. Bücherl W. Escorpiões e escorpionismo no Brasil. V. Observações sobre o aparelho reprodutor masculino e o acasalamento de *Tityus trivittatus* e *Tityus bahiensis*. Mem Inst Butantan. 1956;27:121-55.

15. Lourenço WR. La biologie sexuelle et le développement post-embryonnaire du scorpion Buthidae: *Tityus trivittatus fasciolatus* Pessôa, 1935. Rev Nordestina Biol. 1979;2:49-96.