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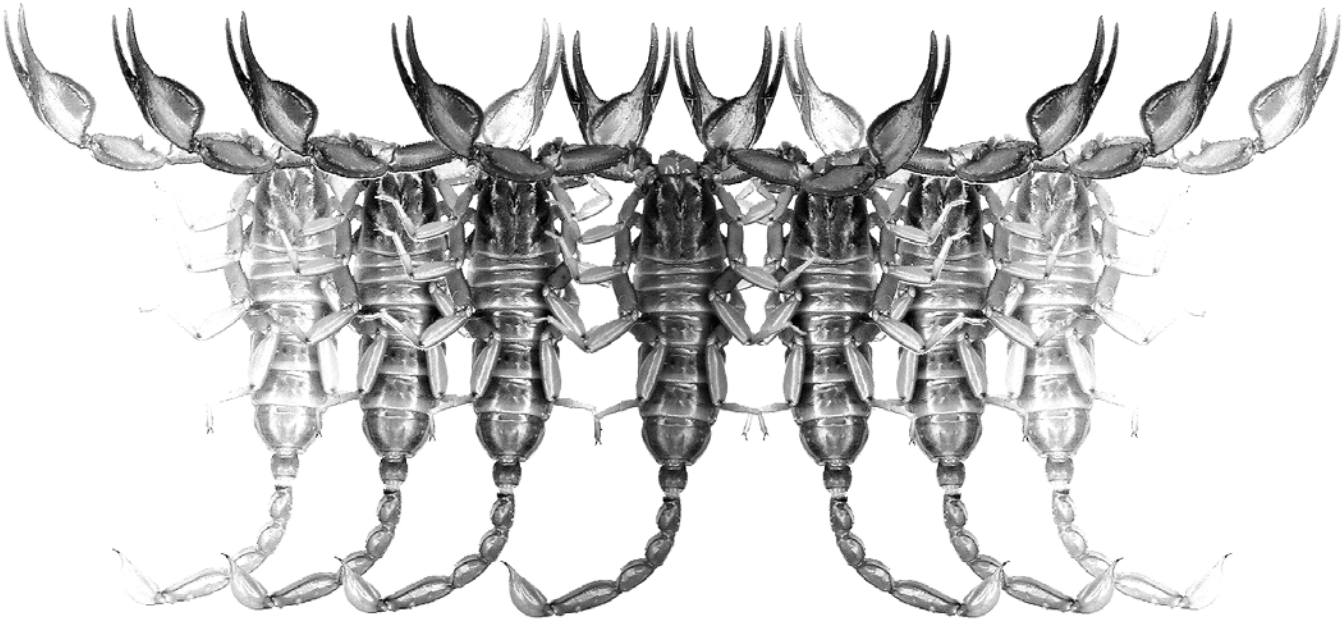

Courtship and Mating in *Heterometrus petersii* (Thorell, 1876) (Scorpiones: Scorpionidae)

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Summary

Courtship and mating in *Heterometrus petersii* (Thorell, 1876) (Scorpiones: Scorpionidae) was observed in the laboratory. In this paper the behavior components displayed in courtship and mating are identified, analyzed and discussed.

Introduction

Since the mid-1950s, courtship and mating behaviors have been described in different scorpion species (Polis & Sissom, 1990; Benton, 2001). Basically, the main structure of the courtship in different species is similar: the female usually follows the leading male in a classical *promenade à deux* until a suitable spot is found for spermatophore deposition. Then, the male assists the female in positioning her genital aperture over the spermatophore in order to permit sperm transfer; after that, the pair separates (Polis & Sissom, 1990).

The process of courtship and mating in scorpions consists of several characteristic behaviors. These behavior components are identified and their possible functions have been discussed in two reviews, by Polis & Sissom (1990) and Benton (2001). However, different terminologies for some behaviors are used in these two works. For example, "cheliceral massage" and "kiss" are different names for the same behavior in scorpions; Polis & Sissom (1990) use the former in their review, whereas Benton (2001) uses the latter. Generally speaking, different terminologies are applied to individual courtship stage by various authors. Courtship and mating behavior is similar among all families, implying that these reproductive behaviors appeared in scorpion evolution before the various modern families diverged. However, among families, genera, and even species in the same genus, to some extent, scorpions exhibit differences in their behavioral patterns (Polis & Farley, 1979; Polis & Sissom, 1990). Buthidae behavior is less complex than observed in other families. The reason could be that buthid scorpions separated early from all other living families (Polis & Sissom, 1990).

Tallarovic et al (2000) described for the first time the courtship and mating in *Hadrurus arizonensis* (Ewing, 1928) (Caraboctonidae). Recently, Ross (2009) conducted observations on courtship and mating in *Tityus (Atreus) magnimanus* Pocock, 1897 (Buthidae), and behavioral components were presented in an ethogram to demonstrate their occurrence during mating sequences. Courtship and mating behavior in these two scorpions were not remarkably different from that in other scorpions.

We studied courtship and mating in *Heterometrus petersii* (Thorell, 1876) in captivity. Courtship and mating behavior components were identified, and their possible functions were analyzed.

Material and Methods

Species studied

Heterometrus petersii is found in Cambodia, Laos, Philippines, and Vietnam (Kovařík, 2004). Specimens for this study were purchased from pet suppliers in China who obtain scorpions from Tay Ninh Province, Vietnam. *Heterometrus* scorpions are frequently bred as pets and for food, and have many common names, e.g. "tropical forest scorpion", "Asian forest scorpion" and "Malaysian forest scorpion" (Zhu & Yang, 2007).

Materials

Studied specimens (10 males, 7 females, all adults) ranged from 92 to 110 mm in length. Body color ranged from greenish-black to black. Scorpions were individually housed in terraria (40×20×30cm) with a loamy substrate. Water (misting) and mealworms

(*Tenebrio molitor*) as preys (20 specimens each time) were provided weekly. The room temperature was maintained at 25±1°C, while daylight span was 10-14 hours.

Experiment

Male–female pairs were placed in a mating arena (60×50×40cm) with a substrate of soil and several tree barks supplied for spermatophore deposition. The process of courtship and mating was observed directly at night using two 40W red bulbs, which apparently did not affect the scorpion behaviors (Machan, 1968; Peretti & Carrera, 2005). The time and frequency spent in a main behavior during courtship and mating were noted. Observations covered a four-month period (January-April 2009). In order to have a clearer sequential model of the reproductive process, it was divided artificially into three phases—introductory phase, promenade phase and spermatophore deposition phase (Benton, 2001). In the introductory phase (I), scorpions meet and the male pacifies the female. In the promenade phase (II), the male leads the female to move looking for a suitable spot for depositing the spermatophore. In the spermatophore deposition phase (III), the spermatophore is deposited and sperm transfer occurs (Benton, 2001). The terminology of behavioral components involved in the three phases was modified from Polis & Sissom (1990) and Benton (2001) (see Table 1).

Results

Introductory phase

Courtship beginning involved the female's pedipalps (chelae or other segments) being gripped by the male's pedipalps chelae. After gripping, the female typically attempted to push the male away with her metasoma, even by trying to sting, and the male sometimes protected itself from stinging using his own metasoma (clubbing). The initial grip, usually short (2-5 seconds), seemed to evaluate reactivity of the female. After the initial grip, the female quickly moved towards the male or both stayed motionless for 5-120 seconds. Subsequently, the male approached the female at a very short distance (about 2-3 cm), then stopped and juddered by rocking the body forward and backward and keeping his legs motionless (juddering). The male repeated these movements (walking-stopping-juddering) until the female started grasping with pedipalp chelae. The male rocked his body forward and backward 3-6 times for each juddering sequence. The second grip could also result in clubbing, but the female's pushing and stinging did not loosen the male's grip, because he now seemed to have a more persistent and powerful grip than at the first attempt. Sometimes the male combed the chela manus of the female with the chelicerae ("cheliceral massage" in Polis & Sissom, 1990; "kiss" in Benton, 2001) as a reaction to the pushing and stinging female during the second grip. After cheliceral massage, the female became more cooperative and less aggressive. After the second and successful grip, either the pair stayed motionless for 10-180 seconds and then began a male-led walk, or immediately began the male-led walk. During the whole introductory phase, the male's pectines were usually widely opened and sporadically swept across the substrate (pecten movement).

Promenade phase

This phase was characterized by the male-led walk. The male usually moved backward with the female following, and sometimes backward and forward. Four other main behaviors occurred in this phase: pause, cheliceral massage, chelicera grip, and sand scraping. While leading the female in a pedipalp to pedipalp grip, the male sometimes outstretched his chelicerae to comb the partner's pedipalp chelae, prosoma edge, or chelicerae ("cheliceral massage"). If the female also stretched out her chelicerae, the male immediately grasped them with his chelicerae. After that, the male led the female, walking in a chelicera to chelicera grip, often with an assisting pedipalp grip ("chelicera grip"). Once the chelicera grip was less effective for control, another pedipalp grip began. The male-led walk was not continuous either with a pedipalp grip or a chelicera grip, and often the pair stopped moving and stayed motionless after a short distance walk. This motionless behavior named "the pause" (after Benton, 2001) lasted 10-300 seconds and consumed most of the time during this phase. Sometimes the male did not pull the female to walk together after the pause, then the pair continued to stay motionless or the male displayed the cheliceral massage behavior: if the male grasped the female only with a pedipalp grip during the pause, he outstretched his chelicerae to comb the pedipalp chelae and/or chelicerae of the female; if the male grasped the female with a chelicera grip and a pedipalp grip during the pause, he continued to grip one of her chelicera with his own and combed her other with his own chelicera. After cheliceral massage, females became more cooperative and less aggressive. Usually, during the pair walk, male's first pair of legs quickly swept the rough substrate ("sand scraping"), probably in order to clear a suitable spot for spermatophore deposition; this behavior was displayed some times around the spermatophore deposition spot. Also, during this phase, male's pectines were usually opened widely and sporadically swept across the substrate. The frequency of sweeping over the substrate increased as the male approached a suitable spot for spermatophore deposition. When the suitable

Behavior	Sex	Phase	Description	Possible Function
Pedipalp Grip	♂-♀	I, II & III	The male usually grasps the female in a pedipalp-to-pedipalp grip. The strength of the first pedipalp grip is only slight, seeming to be an attempted or investigating grip, while the subsequent pedipalp grip is stronger.	Evaluating reactivity of the female (first grip); leading the female as the pair moves together (second grip)
Clubbing	♀-♂	I	The female strikes the male with the metasoma while the sting is tucked away	A threat behavior to the male
Juddering	♂	I	Rapidly rocking movements of the male's body backward and forward while keeping his legs immobile	A species recognition behavior
Pecten Movement	♂	I, II & III	Pectines are widely spread and sporadically sweep across the substrate	Receiving information from the environment, such as the female, substrate, etc.
Cheliceral Massage	♂-♀	I, II & III	The male combs the chelicerae, the edge of the prosoma and/or pedipalp chelae of the female with his chelicerae	Making the female more docile and receptive
Chelicera Grip	♂-♀	II & III	The male leads the female walking in a chelicera to chelicera grip, often with an assisting pedipalp grip.	Leading the female as the pair moves together
Pause	♂ & ♀	II & III	The pair stays motionless for a while.	An apparent appeasement behavior to the female
Sand Scraping	♂	II	The male sweeps the substrate rapidly using the first pair of legs.	Clearing a spot suitable for spermatophore deposition
Tail Waving	♂	III	Male's metasoma swings over the substrate continually up and down and/or left to right	Displaying arm pulling behavior; maybe a ritualization of arm pulling behavior
Arm Pulling	♂-♀	III	The male keeps its legs motionless and its pedipalps move forward and backward, causing the female's pedipalps to do likewise	Assisting the female in completing uptake of the sperm as soon as possible

Symbols: ♂, behavior performed by the male; ♀, behavior performed by the female; ♂ & ♀, behavior performed by the pair; ♂-♀, behavior initiated by the male needing female's cooperation; ♀-♂, behavior initiated by the female needing male's cooperation.

Table 1: Behavior components involved in courtship and mating of *Heterometrus petersii* and their descriptions, modified from Polis & Sissom (1990) and Benton (2001).

spermatophore deposition spot was found, the spermatophore deposition phase began.

Spermatophore deposition phase

The male-led walk reduced as a suitable site for spermatophore deposition approached. Once a suitable spot was encountered, the male lowered his mesosoma until the genital aperture touched the ground, and then the entire spermatophore was extruded as the male moved his prosoma upward and backward as the sticky basal plate just contacted the surface of substrate. Subsequently, the male pulled the female onto a proper position over the spermatophore, in order to permit sperm collection with genital opercula. The spermatophore was bent slightly under female's weight and sperm transfer occurred thanks to male's pushing of the female backward. The male repeated movements, pulling the female with his pedipalps until their chelicerae or the anterior edges of prosoma touched each other (arm pulling). Before this movement, the male usually scratched the substrate several times with legs II, III and IV, and then pushed against the substrate with these legs using much more strength; sometimes, this behavior was also observed in females. Before the female's pulling, or simultaneously, male's tail swung continually up and down and/or from left to right over the ground (tail waving), which seemed to be a signal of arm pulling. If the male did not pull the female during sperm transfer, the cheliceral massage was usually

Groups	1	2	3	4	5	Mean±SD
Clubbing	1	2	2	2	3	2±0.7
Juddering	7	6	7	5	8	6.6±1.1
Cheliceral Massage	5	6	7	6	8	6.4±1.1
Chelicera Grip	2	2	2	1	1	1.6±0.5
Arm Pulling	9	8	8	7	6	7.6±1.1
Tail Waving	8	9	6	5	6	6.8±1.6
Total Time (min)	40	59	81	134	126	88±41.1

Groups 1, 2, non-virgin females; Groups 3, 4 and 5, virgin females**.**

Table 2: The number of times each behavior occurred with five mating pairs and the total time elapsed for courting and spermatophore uptake.

	Pedipalp Grip	Clubbing	Juddering	Pecten Movement	Cheliceral Massage	Chelicera Grip	Sand Scraping	Mating Cannibalism
Heterometrus scaber	+			+	+			+
Heterometrus petersii	+	+	+	+	+	+	+	

Data for *H. scaber* taken from Polis & Sissom (1990).

Table 3: A comparison of main courtship and mating behaviors between *Heterometrus scaber* and *H. petersii.*

displayed, and after that the female became more co-operative.

After sperm transfer accomplishment, either the pair immediately parted and stayed motionless, or the female attacked the male so that the male released the female and receded. The female became more aggressive if the male tried grasping her after courtship. Behaviors displayed in courtship and mating processes and their possible functions are summarized in Table 1, while frequencies of single behaviors are shown in Table 2.

Discussion

Most of the mating behaviors observed in *Heterometrus petersii* were not remarkably different from those displayed in other scorpion families, such as *Hadrurus arizonensis* described by Tallarovic et al. (2000) or *Tityus (Atreus) magnimanus* described by Ross (2009). However, courtship and mating behaviors of *Heterometrus petersii* were obviously different from those of the congeneric *Heterometrus scaber* (Polis & Sissom, 1990) (see Table 3). Juddering, clubbing, chelicera grip, and sand scraping behaviors displayed in the process of courtship and mating in *H. petersii* were not observed in *H. scaber*, whereas mating cannibalism behavior was observed in *H. scaber* but not in *H. petersii*. In most cases in *Heterometrus petersii*, the female stung the male causing the male to release her, and fled after sperm transfer, but no attempts to eat the male were observed. Once the pair met again after parting, sometimes the male tried grasping the female and the female only stung him and did not attack further more. In conclusion, the female did not attack the male actively after parting.

Benton (2001) observed that the male of *Centruroides margaritatus* (Gervais, 1841) (Buthidae) began thrilling before initiating the "kiss", and thus its legs moved forward and backward for a few seconds over the soil surface (sand scraping) before the female approached. Perhaps, then, sand scraping behavior is a ritualization of the kiss rather than working to clear the area for spermatophore deposition (Alexander, 1959). However, we observed that sand scraping behavior was displayed frequently during the promenade phase and it was not related to the kiss. So we suggest that sand scraping behavior maybe has a species-special function. Benton (2001) also observed that tail waving behavior in another species of Buthidae, *Leiurus quinquestriatus* (Ehrenberg, 1828), becomes more stereotyped as spermatophore deposition approached, whereas this behavior was displayed when the male assisted the female accomplishing sperm transfer using arm pulling in our observations of *H. petersii*. Therefore, we think that this behavior possibly was a signal to display arm pulling behavior or a ritualization of it. Arm pulling behavior in *L. quinquestriatus* was observed as sperm transfer approached, which seemed to be associated with the male finding the exact spot on which to deposit the

spermatophore (Benton, 2001). In our observations of *H. petersii*, the arm pulling behavior was frequently observed during the sperm transfer, suggesting that the possible function of this behavior is assisting the female to complete uptake of the sperm as soon as possible. Thus we think that it is an additional function of arm pulling behavior. Two functions of this behavior could be used to assist females to the right place and assist them in sperm uptake in some scorpions.

According to experimental data shown in Table 2, we can conclude that the total time elapsed for courting and spermatophore uptake of non-virgin females was shorter than that of virgin females, suggesting that probably the non-virgin female was familiar with the process of courtship and mating and more cooperative than virgin females. The small amount of scorpions observed (five pairs) indicates that further studies are required to obtain more detailed information on courtship and mating behavior mechanisms in *H. petersii.*

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