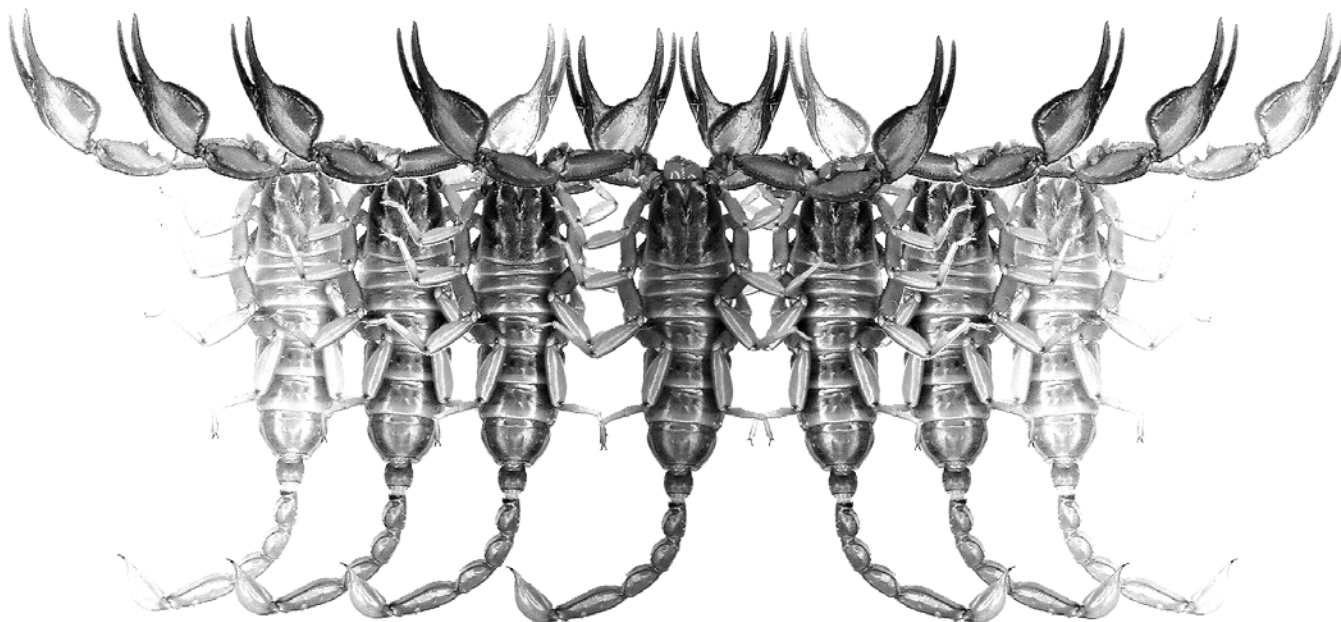


Euscorpilus

Occasional Publications in Scorpiology



**Response of Male *Centruroides vittatus* (Scorpiones: Buthidae)
to Aerial and Substrate-Borne Chemical Signals**

Steffany B. Steinmetz, Karen C. Bost, and Douglas D. Gaffin

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Response of male *Centruroides vittatus* (Scorpiones: Buthidae) to aerial and substrate-borne chemical signals

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Summary

Chemical signaling promotes mate location throughout numerous animal taxa. In this study we investigated the possibility that striped scorpions, *Centruroides vittatus*, use chemical signaling in the form of odor plumes or substrate-borne deposits as communication channels. A Y-shaped arena was constructed to test scorpions' use of air-borne chemical cues in the detection of potential mates. A second, circular behavioral choice chamber was used to test male scorpions' responses to female deposits by direct substrate contact. Male scorpions showed no tendency to move toward the female in tests of air-borne chemical transmission but they did demonstrate behavior associated with chemical detection when exposed to substrate-borne feminine deposits. Our experiments do not provide evidence that male *C. vittatus* use aerial pheromones to locate female scorpions but that they are highly sensitive to direct contact of substrate-borne chemical deposits.

Introduction

Sex pheromones are special signaling molecules that are important in directing mating behaviors among conspecifics (Pollard, 1994). Many animals use pheromones to attract mates, although the chemistry and mode of transmission vary among species (Birch & Haynes, 1982). Males have been shown to detect and respond to feminine substrate-borne chemical deposits in several species (Krapf, 1986; Gaffin & Brownell, 1992) with a tendency to follow conspecific chemical trails (Melville et al., 2003). However, few scorpion species have been tested for pheromonal communication and essentially no information exists concerning air-borne chemical communication in scorpions.

The primary chemosensory appendages of scorpions are the ventromedial pectines, organs that contact the ground as the animal walks (Foelix & Muller-Vorholt, 1983; Gaffin & Brownell, 1997, 2001). The pectines of male scorpions appear to be involved in the near-range detection of pheromones deposited on the substrate and may direct the orientation of males to female conspecifics (Gaffin & Brownell, 2001). Males that make contact with sand containing female extract initiate courtship behavior (Polis & Sissom, 1990; Gaffin & Brownell, 1992) while males with excised pectines do not elicit any response to treated sand (Gaffin & Brownell, 2001).

Published information concerning pheromonal detection in scorpions is scarce. However, field observations suggest that some species may use air-borne pheromones as attractants. For example, several male *Paruroctonus luteolus* may congregate near burrows of conspecific females (Polis, pers. comm.), suggesting the presence of a chemical attractant. Additionally, some researchers have noted the presence of unique odors during the prime mating season of several scorpion species in Baja California (Sissom, pers. comm.). The possession of an air-borne signal should provide a reproductive advantage by offering a broader detection range than that of direct substrate contact.

In this study, we conduct a series of experiments to investigate the response of male striped scorpions, *C. vittatus*, to air- and substrate-borne female cues. A Y-shaped behavioral choice chamber was used to test scorpions' use of odor in the detection of potential mates. Additionally, circular behavioral choice arenas were constructed to test male scorpion responses to female pheromones by direct substrate contact.

Methods

Animals

Specimens of *C. vittatus* were collected from various locations in Cleveland County, Oklahoma. In the

laboratory at the University of Oklahoma, the animals were maintained in 3.8 L glass jars filled with 250 mL of soil from their natural habitat, a pine cone, a piece of bark from local trees, and a piece of pottery clay. The scorpions were fed crickets and given water on a regular schedule. The experiments were conducted under constant temperature (22°C), humidity (RH 55%-65%), and light-dark phase (2000-0700 h dark and 0700-2000 h light). The trials of Experiments 1, 2, and 3 were conducted from September through March in the same laboratory facility. The trials of Experiment 4 were conducted July through August.

Tests for air-borne chemical communication

Behavioral apparatus: Experiments 1, 2, and 3 were designed to test for transference of air-borne chemical information. These trials used a Plexiglas Y-shaped arena (Y-maze) modified from a design by Melville, et al. (2003). The long arm of the maze was 25.0 cm long and 5.5 cm wide. The end wall of the long arm had 35 holes drilled in the lower 8 cm. The left and right arms of the maze were 15.5 cm long and 4.0 cm wide; all arena walls were 15.5 cm high (Fig. 1). The lower 5.5 cm portion of each arm's end wall was made of screen, affixed to the Plexiglas by "Rug Gripper" double-sided carpet tape. Two Plexiglas cubes with lengths of 5.5 cm were constructed to hold stimulant animals. Twenty holes were drilled into one side of each cube with the opposite side made of screen, supported by "Rug Gripper" double-sided carpet tape. One cube was placed at the end of each short arm so that the screened side of each cube was adjacent to the screen at the end of each arm.

A variable autotransformer fan, type 3PN1010, was placed at the end of the long arm to draw air through the cubes and the maze. A constant voltage of 140V powered the fan. A sheet of Plexiglas was placed over the top of the maze to direct and confine airflow through the maze. Smoke from a burnt match was used to verify that air flowed evenly from holes on the ends of the short arms of the maze, across the arena floor, and out the end of the long arm. The maze was placed on a metal sheet that measured 58.5 cm long and 55.0 cm wide. The sheet of metal was fixed on a 'lazy-susan' turntable to allow for rotation of the maze, controlling for room and/or Earth-related cues. The metal sheet was then placed on a piece of Styrofoam (42.5 cm x 42.5 cm x 2.5 cm), which was placed on a piece of plywood (90.5 cm x 45.5 cm x 2.0 cm) to maintain a level surface. A level was used to insure the arenas were flat.

All experiments were filmed using a Sony Handycam video camera recorder with Night Shot capability (model #CCD-TRV16). The camera emitted and received infrared light in order to film in the dark. The camera was mounted on a tripod straddling the apparatus at 105

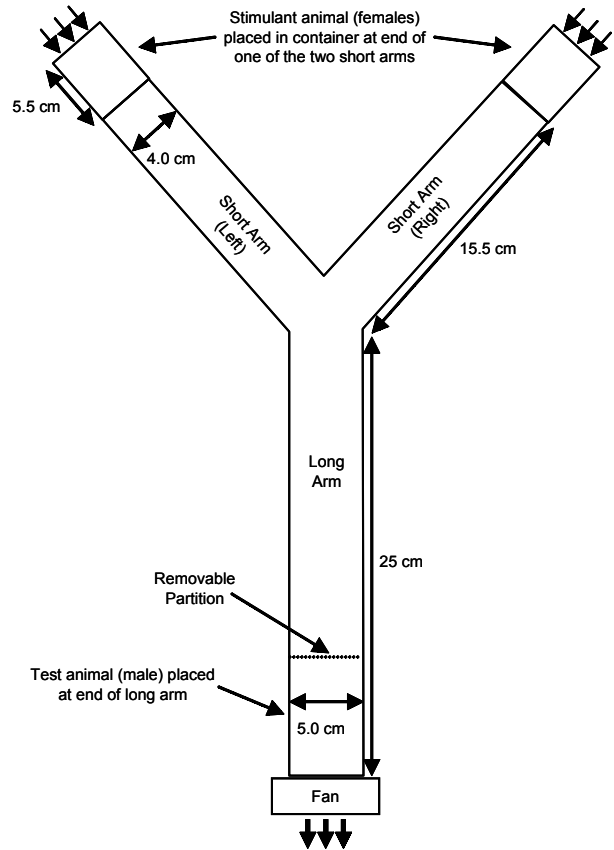


Fig. 1: Y-shaped arena for Experiments 1, 2, and 3 to test air-borne chemical communication in *C. vittatus*.

cm above the surface and was connected to a color television monitor located in a separate room.

Experimental protocol: Experiments 1 and 2 were conducted on consecutive nights, beginning at 2000 h. Prior to testing, each of the 16 males and four females were placed in separate black, light-resistant film canisters (the canisters were several years old and free of any obvious odor). The order of test and stimulant scorpion use was randomized. A piece of Plexiglas was placed 5 cm from the end of the long arm to form a chamber for the placement of the test (male) scorpion. The fan was turned on and the maze was rotated so the long arm faced north. When the experimental preparation was complete, the lights were turned off and the experiments began. Meanwhile, an assistant monitored the television in an adjacent room.

Experiment 1: Female 1 was placed in the cube at the end of the left arm of the Y-shaped arena for use in trials 1-4. Each trial was run using a different male scorpion. Following the fourth trial, Female 1 was returned to her canister and her cube was wiped with 70% ethanol. These procedures were repeated for the other three

females except female position varied. Female 2 was placed in the right arm cube for trials 5-8, Female 3 was placed in the left arm cube for trials 9-12 and Female 4 was placed in the right arm cube for trials 13-16.

Each trial was initiated by dropping a male scorpion into the confined portion of the long arm, removing the Plexiglas partition, and sliding the Plexiglas cover completely over the arena. During each trial, the male scorpion was allowed five minutes to move into the left or right arm of the arena. We considered a trial to be complete if the male crossed into either the left or right arm of the arena. A trial was considered invalid if the male did not enter either arm within five minutes. Once the male scorpion had made a decision, or five minutes had passed, the observer monitoring the TV informed the conductor that the trial was complete. After each completed trial, the male was located using a black light and returned to its canister. The floor, walls, and screen of the arena were wiped with 70% ethanol and the entire apparatus was rotated 90° clockwise.

Experiment 2: The above procedures were repeated for Experiment 2 with the following exceptions. Only 15 males were tested due to the death of one of the animals. In addition, a single female was used for all 15 trials; this female remained in one cube, which was interchanged with the other cube after every four trials. The cube containing the female was placed on the left arm for Males 1 - 4 and 9 - 12 and on the right arm for Males 5 - 8 and 13 - 15. Between trials, the entire arena was wiped with Kimwipes and 70% ethanol and the apparatus was rotated 90° clockwise.

Experiment 1 and 2 trials were reviewed and scored during videotape playback. The time and direction of the male scorpions' movement into either the left or right arm of the Y-shaped arena were scored. All males made a decision within the allotted five minutes. The animal choices were then reported relative to the arm containing the female and the binomial distribution (Snedecor and Cochran 1967) was used to test for significant departure from a 50:50 random choice.

Experiment 3: To create a more natural environment for the male scorpions, the arena floor was covered with a soil substrate normally used in the scorpions' home containers. Six males were tested against one female. The female was kept in a single cube, along with soil taken from her home jar. This experiment began at 2000 h. Each male was placed in a black, light-resistant film canister; their order of use was randomized. Before each trial, a male scorpion was placed into the confined chamber at the end of the long arm and given two minutes to adjust. After two minutes, the Plexiglas barrier was removed and a sheet of Plexiglas was pulled completely over the arena to initiate the trial. During each trial, the male scorpion was allotted ten minutes to move freely throughout the arena. Following the ten-minute trial, the male scorpion was located with a black

light and returned to his canister. Between each trial, the walls were cleansed with 70% ethanol and the arena was rotated 90° clockwise. Each of the six males was tested twice. In the first six trials, the female-containing cube was connected to the left arm with the blank cube connected to the right arm. The cubes were exchanged between the arms for the second set of six trials. The amount of time each male scorpion spent in the long arm, the left arm, and the right arm of the Y-maze was scored for the allotted ten minutes. These results were then summarized relative to female placement.

Tests for substrate-borne chemical communication

Before the substrate-borne chemical communication experiments began, one preliminary trial was conducted. Using the Y-maze, we confined a female to the entire left arm for a period of 24 hours. After the female was removed, a male was dropped at the end of the long arm and was allowed to move freely throughout the Y-maze for a ten-minute period. His movements throughout the maze were monitored and recorded. The male spent 7 min and 27 sec in the left arm, 6 min and 10 sec of which were spent at the location where the female had spent most of her time. This observation suggested some bias toward female deposits and guided the subsequent experiments.

Experiment 4: These trials were designed to test for substrate-borne chemical communication. At 2200h each night, four circular Plexiglas arenas (13.5 cm diameter, 7 cm height) and removable Plexiglas partitions (7 cm height) were cleansed with 70% ethanol, placed atop a sheet of filter paper (Watmann 15.0 cm diameter, cat. # 1001 150) and arranged in a square pattern under the video camera. A total of 12 females and 12 males were used for these trials. Two arenas served as no-stimulus controls. In each of the other two arenas, a female was confined each night behind a partition delimiting half the arena's area. The placement of the females and the orientation of the partitions were randomized each night. Finally, the four arenas were covered by a square piece of Plexiglas.

At 2000h the following night the females were removed from the arenas and returned to their home jars. The camera was turned on in infrared "night shot" mode, the room lights turned off, and the partitions removed. Circular PVC tubes (5 cm diameter) were placed in the center of each arena. Four test males were placed individually into black plastic film canisters and then transferred to each of the PVC tubes in the dark. PVC caps were then placed over the PVC tubes (tube and cap total 8 cm height) to prevent escape. Once all four males were in position, the tubes and caps were removed to initiate the trials. The trials were videotaped for 2 h, after which the males were returned to their home jars.

This procedure was repeated for the remaining five nights. Each male was run twice to account for 12 experimental arenas and 12 no-stimulus controls, yielding a total of 24 trials.

A reviewer unaware of the test situations scored the videotapes of Experiment 4. Partition locations were observed at the beginning of each video recording and traced onto the television screen before their removal. Male behaviors were recorded throughout the first three, five, and seven minutes of movement. Trial time was paused during male inactivity persisting more than 30 sec and then resumed with male movement. The amount of time the male spent in either side of each experimental arena was recorded for each trial. These data were analyzed via χ^2 analysis to determine whether or not these times were significantly different from expected amounts of time in each side of the arenas (50:50). The obtained χ^2 statistics were pooled to find the heterogeneity χ^2 . The amount of time males spent attempting to climb the arena wall was also recorded in order to determine the most appropriate trial time (3, 5, or 7 minutes) since we presume that climbing scorpions are not seeking contact chemical communication.

The occurrence of distinct behaviors we term 'backward lunges' were plotted by their location in the arena. This behavior is characterized by a sudden backward body movement simultaneous with an apparent brushing of the pectines against the substrate. The side of the arena ("stimulus" versus "non-stimulus") of the initial backward lunge was noted in each trial and assigned a value of 1.

The binomial distribution was used to test for significant departure from a random 50:50 distribution of those trials in which the behavior was observed. Assuming a random choice, scorpions should perform their initial lunge in the experimental "stimulus" side in $\frac{1}{2}$ of the trials and perform their initial lunge in the "non-stimulus" side in $\frac{1}{2}$ of the trials.

Results

Experiments 1, 2, and 3 were conducted to detect male response to aerially transmitted pheromones in *C. vittatus*. Under the conditions of the experiment, the male scorpions showed no response to aerial transport of pheromones. During Experiment 1, males advanced towards the Y-maze arm containing a female scorpion in 8 out of 16 trials (left: 4 out of 8; right 4 out of 8). The binomial probability function of $x=8$ with $n=16$ yields a probability of 0.196, considerably larger than α at 0.05 indicating that male scorpions showed no arm preference.

Experiment 2 was similar to Experiment 1, except we used only one female rather than four. Male scorpions advanced towards the Y-maze arm containing the female

scorpion in 9 out of 15 trials (left: 6 out of 8; right: 3 out of 7). The binomial probability function of $x=9$ with $n=15$ yields a probability of 0.153, again indicating that male scorpions showed no arm preference.

In Experiment 3, 12 individual trials were run for a duration of ten minutes each. The female was placed in the right arm of the Y-maze during six trials and in the left arm during the remaining six trials. Therefore, the female was located in the left arm for a total of 60 minutes and in the right arm for 60 minutes. While the female was in the left arm, the males spent a total of 13 minutes in the left arm, 6 minutes in the right arm, and 41 minutes in the long arm. While the female was in the right arm, the males spent a total of 4 minutes in the right arm, 23 minutes in the left arm, and 33 minutes in the long arm. Thus a considerable amount of the time was spent in the long arm, the initial drop location. Male scorpions spent all of the trial time (10 min.) in the long arm in 4 of the 12 trials. One male did not leave the long arm until the ninth minute and another remained until the seventh minute. Combined trial data shows that the males spent 14% of the time in the female arm, 24% in the blank arm, and 62% in the long arm.

Experiment 4 tested male detection of female chemical deposits via contact chemoreception. This experiment consisted of 12 stimulus trials and 12 no-stimulus control trials. Chi-square analysis for heterogeneity indicated that male scorpions show no preference (in terms of time) between the "stimulus" and "non-stimulus" sides in experimental arenas after 3 minutes ($\chi^2_{act}=120.332$, $DF=11$, $P\ll 0.05$), 5 minutes ($\chi^2_{act}=147.503$, $DF=11$, $P\ll 0.05$), or 7 minutes ($\chi^2_{act}=170.880$, $DF=11$, $P\ll 0.05$). We determined that a three minute trial duration was the most appropriate due to (1) the occurrence of the first backward lunge as well as the majority of subsequent lunges (Fig. 2, top) and (2) the least amount of time spent attempting to climb the arena walls relative to the total time of the trial occurred within this period (Fig. 2, bottom). These data suggest that male scorpions become less receptive to female chemical deposits over time.

Although male scorpions spent the same amount of time in each side of the experimental arenas, their movement appeared to be dependent upon stimulus location within the 3-minute trials. Scorpions moved steadily in the "no-stimulus" side. When scorpions crossed into the "stimulus" side, they generally moved more slowly and their movements appeared more deliberate. Figure 3 shows a representative plot of scorpion movement during a 3-minute trial.

Backward lunges occurred in nine of the experimental trials and did not occur in any of the control trials. These observations provide evidence that the lunges occur in response to contact chemoreception of female deposits. Similar observations by Gaffin & Brownell (1992) were reported in another scorpion species,

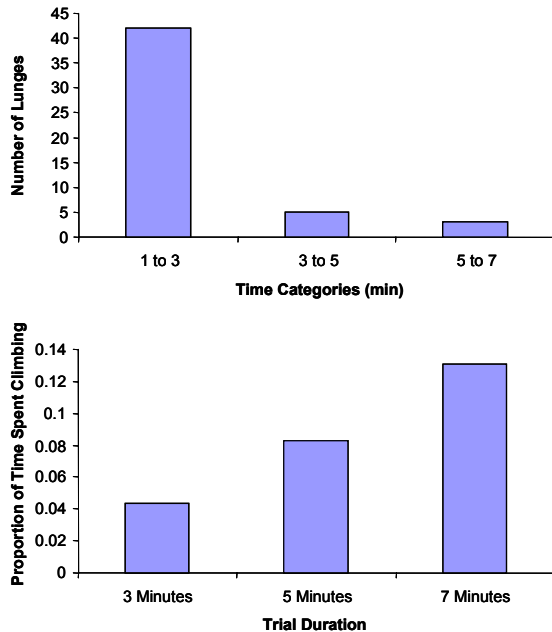


Fig. 2: (top) Total number of lunges observed in all trials within 3 minutes, between 3 and 5 minutes, and between 5 and 7 minutes. (bottom) Proportion of time spent wall climbing in the first 3 minutes, 5 minutes, and 7 minutes. These values include total observed climbing time (sec) in all trials divided by the total time of the trial (sec).

Smeringurus mesaensis. Of all lunges noted during these trials, all except one occurred in the stimulus side. Furthermore, the initial lunge in each trial occurred in the "stimulus" portion of all nine experimental arenas where we observed the response. A trial was determined successful and assigned a value of one if the initial lunge occurred in the stimulus portion of the arena. The resulting binomial probability equation yielded a p-value of <0.002 . This represents a highly significant difference in response between the stimulus and no-stimulus side when compared to $\alpha=0.05$.

Discussion

The use of pheromones in mate recognition and detection is likely crucial to successful mating in many if not all scorpion species. The detection of air-borne chemical cues would be useful in species where males and females do not always occur in close proximity (Polis et al., 1985). However, the experiments executed here (Experiments 1, 2, and 3) do not provide evidence that *Centruroides vittatus* use aurally transmitted pheromones. Under the conditions of our experiments, male choices were not influenced by the presence or absence of a female. Abushama (1964) reported no bias in movement relative to conspecifics in a similar study

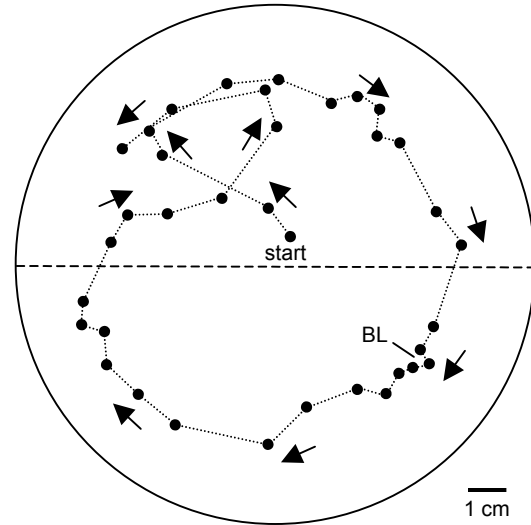


Fig. 3: The movement of a male scorpion during a 3-minute trial. Scorpion position was marked by a dot every 5 seconds of the trial. In general, the movement path has more space between dots in the "no-stimulus" side (top) versus the "stimulus" side (bottom). The average distance (+/- standard deviation) traveled between 5 second plots: no-stimulus side = 15.3 ± 7.8 mm (median = 12.5 mm), stimulus side = 10.8 ± 5.9 mm (median = 8.0 mm). "BL" indicates occurrence of a backward lunge.

using *Leiurus quinquestriatus*. However, Abushama did observe that scorpions move away from compartments treated with caustic chemicals suggesting that they have some ability to detect air-borne chemicals. Furthermore, scorpions with painted pedipalps did not avoid the chemicals, suggesting that air-borne chemical detection may occur on the pedipalps.

In Experiment 4 we focused on assessing the ability of male *C. vittatus* to detect substrate-borne female chemicals. A preliminary trial using the Y-maze arena used in olfactory studies suggested that males might be able to detect female chemicals by direct contact. Additionally, Gaffin and Brownell (1992) reported strong male behavioral responses to feminine chemical cues in another species of scorpion, *Smeringurus mesaensis*.

Experiment 4 yielded several important results. Male scorpions spent the same amount of time in each side of the arenas in the experimental trials but they demonstrated markedly different behavior between the "stimulus" and "no-stimulus" sides. The walking style and rate of movement was generally slower in the "stimulus" side of the arenas. In the "stimulus" side, male movement appeared to be considerably more deliberate and careful. This qualitative observation suggests that males obtain substrate-borne chemical stimulation and engage in substrate investigation to acquire more sensory input.

Interestingly, backward lunges only occurred in experimental trials (9 out of 12). Additionally, all lunges initiated in the "stimulus" side of the experimental arenas. Furthermore, of all lunges noted, all except one occurred in the "stimulus" side. Lunging appears to be a strong, relatively consistent behavior indicating its potentially high importance.

Lunging can presumably be compared to "sniffing" in that a single lunge, in response to chemical detection, was commonly followed by a series of subsequent lunges in the same area. It appears that the detection of a chemical cue stimulated male scorpions to "sniff" around the adjacent area to increase the probability of detecting more chemical. This "sniffing" pattern may be necessary in the detection of potential mates, especially without evidence that males respond to odor plumes as indicated by this study. If these scent trails are only detected directly on the substrate, "sniffing" should aid male scorpions in finding and following a feminine chemical trail.

There is evidence that other species of scorpion follow chemical scent trails in search of female conspecifics. *Hadrurus arizonensis* have been shown to prefer a Y-maze arm that a female has walked down over an unexposed Y-maze arm (Melville et al., 2003). The authors argue that random movement alone is unlikely to account for the observed amount of mating successes in desert scorpions. In addition to the difficulty of finding a mate, searching male scorpions are at a higher risk of predation.

We cannot rule out seasonality as a variable in pheromone production and/or detection. We conducted a preliminary test of contact chemoreception in February (data not shown) using a circular arena assay similar to that described here. In tests of four males and eleven females, we saw no tendency for male scorpions to detect female deposits. The significant results obtained in Experiment 4 during July and August may suggest a seasonal component exists in this pheromone system.

Acknowledgments

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