Prey localization by scorpions in the absence of a vibrational stimulus.

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Summary

Scorpions have two symmetrical organs called pectines on the underside of their body. These comb-like structures are lowered to the ground while the scorpion walks and pick up chemical signals from the substrate. Scorpions can also sense vibrations traveling through the substrate using the pectines and tarsal hairs and compound slit sensilla on their legs. It is believed that scorpions may use all of these organs in the localization and tracking of prey. We tested this hypothesis by creating a chemical extract from a cricket in a volatile 2:1 chloroform methanol solvent and a control solvent of just 2:1 chloroform methanol. Scorpions were placed in an arena divided into four quadrants with either the extract or control solvent placed into one of the quadrants. Scorpions were then observed over a period of ten minutes. We found that the scorpions spent on average 2.699 minutes out of 10 in the control solvent quadrant and 3.423 minutes out of 10 in the quadrant with the extract. However, a t-test analysis of these results was not significant ($P = 0.586$). We noted and described an interesting behavior we observed in one animal during an extract trial.

Introduction

Arthropods possess highly evolved sensory organs that aid in tasks such as localization and tracking. Species in the order Hymenoptera use a variety of methods for accurate localization. For example, ants can follow where other ants have been by using chemoreceptors in the tips of their antennae to detect pheromones (Narendra et al. 2013). Alternatively, in absence of such a chemical trail, they navigate by other means, such as landmark recognition. Arachnids have similar sensory abilities and may be capable of switching to secondary organs to optimize a specific task. Of interest are animals in the order Scorpiones. Since their origin, they have remained relatively the same, making them an ideal model for evolutionary study (Kjellesvig-Waering 1986). Scorpions can detect chemicals and vibrations for a variety of functions (Gaffin and Brownell 1997). Like ants, we suggest that scorpions may have selectivity over their use of chemo- and mechano-sensory organs to optimize localization of prey.

Scorpions have primary organs for both mechanoreception and chemoreception that may play a role in prey localization. The mechanoreceptive organs are the slit sensilla and tarsal hairs. The slit sensilla are located on the basitarsus near to its articulation with the scorpion's tarsus (foot). The tarsal hairs are located on the ventral, ground-facing portions of the tarsi. These structures can detect physical strain emanating from vibrations on the substrate. Scorpions use these organs to detect the vibrations of a cricket in sand up to 50 centimeters away (Brownell 1977). The slit sensilla are used mostly for fast and accurate capture of prey. The primary chemoreceptive counterparts are called pectines. The pectines are remarkable chemical sensors used for mating, pheromone detection, and possibly navigation to their home burrow (Gaffin & Brownell 1997; Knowlton & Gaffin 2011). They consist of two ventrally located comb-like structures with variable numbers of teeth. On each tooth reside dense patches of sensors called peg sensilla. These pegs are rapidly lowered by the spine, to obtain chemical information about the environment, but are also able to transduce mechanical information as well. Structural and physiological analysis has shown that the peg sensilla are responsive to chemicals and are likely used in combination with mechanoreceptive organs that detect substrate vibrations (Wolf 2008).

Although both organs have distinct functions, they both provide information for localization. There has been very limited research detailing the abilities of scorpions to use these organs interchangeably for an alternative method of localization. However, a paper published in 2006 by Mineo et al. explored the functionality of the pectines during hunting – a behavior that is believed to be reliant on mechanical cues received by the slit sensilla. They found that covering the pectines caused the scorpions to have less success at prey localization. This suggests that the pectines, a primarily chemoreceptive organ, may have a role in prey capture. A different study indicated that they may be responding to chemicals in the cuticles of prey insects (Krapf 1986). In this regard, there has been no further research into whether scorpions can use their pectines for chemoreception of prey as a tool for localization. We hypothesize that scorpions use chemosensory organs to detect prey. We tested this idea by recording the time that a scorpion spent in each of four quadrants in a circular arena. For each trial, only one quadrant was treated with either a control solvent or cuticle extract. We expected the scorpions would spend more time in the quadrant with the extract than the quadrant with the solvent.

Methods

Research Animals

We began our trials with 15 *P. utahensis* (1 male, 14 females) collected from a sandy region 30 km SE of Monahans Texas, USA. In the laboratory, the scorpions were kept in 3.8 L glass jars with a bottom diameter of 15 cm. The jars were filled with 3 cm of sand from their natural habitat in west Texas. The scorpions received approximately 20-30 mL of distilled water every Monday, Wednesday, and Friday during the trial process. To encourage the scorpions to exhibit hunting behavior, the scorpions were not fed throughout the six-week trial process. Before the trials, they were given one medium sized cricket every two weeks. The scorpions had a 12L:12D light cycle. The cycle was re-established over the course of a week and the dark was 1300-0100 and the light was 0100-1300. The temperature was kept at 21° C using two space heaters. During the trial, the scorpions were exposed to red lighting for periods up to two hours to aid in location and care of the animals in a dark room. Scorpions do not show behavioral changes to light above 675 nm, the red and infrared spectrum (Blass & Gaffin 2008). We darkened the trial room by covering all the windows with blackout tarps.

Apparatus and Trial Set-up

To record each trial, we used two infrared Defender© cameras (model 21320). For optimal recording of each trial, both cameras were attached to a wooden frame above the trial arenas. We built the wooden frame to be 122 cm tall, 148 cm wide, and 236 cm long. We attached two crossbars to the wooden frame with wood screws and then mounted one camera on each crossbar for a bird's-eye view of each trial arena 50 cm above the sand (Fig. 1). The four Pyrex© trial arenas (15 cm diameter, 7.5 cm deep) were filled to 3 cm with a layer of sand. Camera cords were run outside of the trial room to a computer monitor and DVR to record the trials.

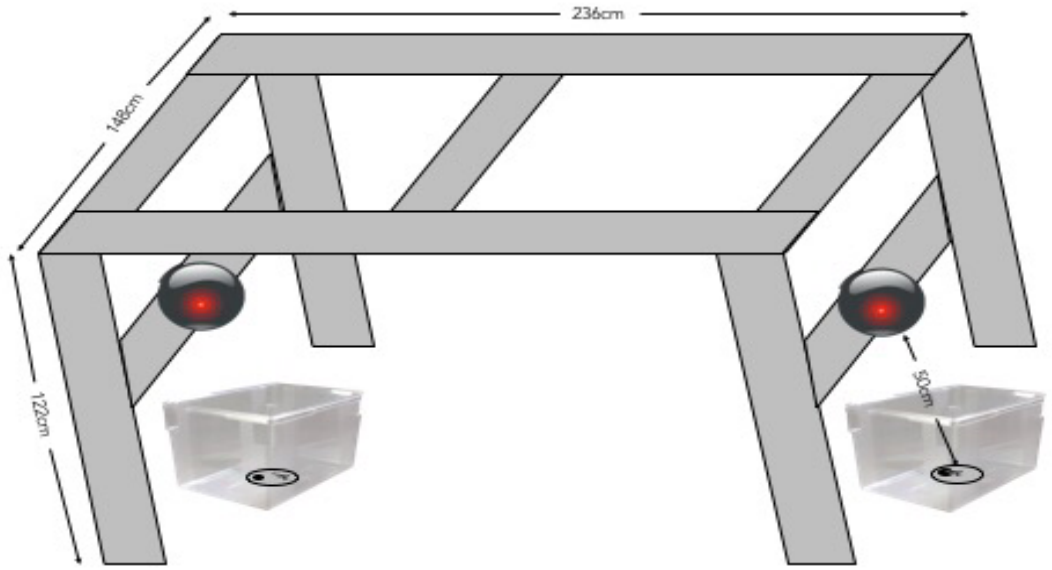

Fig. 1: Drawing of the apparatus used for trials.

Extract Preparation

To extract a variety of compounds from the crickets, we dissolved ground crickets in a polar solvent. We purchased 50 large crickets (approximately 2.5 cm in length) from Petsmart in Norman, OK. After the crickets were euthanized by freezing with dry ice, they were ground up using a glass mortar and pestle. The cricket residue was washed in the mortar with a 2:1 chloroform methanol solution (100 mL total). The solution was transferred to a 500 mL glass beaker and stirred for 10 minutes on a magnetic stirrer. Next, the solution was separated into 15 mL centrifuge tubes and centrifuged for 4 minutes at 2000 RPM. The liquid portion was pipetted to 15 mL test tubes to separate the solution from the organic solids. The test tubes were stored in a freezer until the trials began. Five crickets were used for every 10 mL of extract.

Trial Protocol

Scorpions were allowed to adapt to their glass jars for at least one week before beginning trials. Trials began by placing a circle (3 cm in diameter) of solution (approximately 2 mL for 1 cricket per trial) into one of the four quadrants of the arena and allowing it to air dry for 3 h before placing an animal in the arena. We released the scorpions in the middle of the arena with no acclimation period other than for their glass jars, which were the same diameter. The scorpions were recorded for the first 10 mins after exposure. Individual trials were considered legitimate if the animal moved during the 10-minute period. Each animal experienced an arena with the extract and with the control solution. Paired trials were only legitimate if both the control and extract trials for a scorpion met criteria for legitimacy. The order of exposure was randomized throughout the trials.

Results

When introduced to the arena, the animals generally walked along the perimeter of the circular arena and attempted to climb the walls. This occurred in both the control and extract trials. A representative example of this behavior is shown in (Fig. 2). Some of the scorpions exhibited unusual behavior to different degrees when introduced to the arena. Of interest was #264. During the first minute of the extract trial, the scorpion oriented and moved toward the extract area. When it was over the extract, it shuffled backwards and sideways in a circle with its tail raised until it was oriented opposite of the extract. Then, it backed up until it was over the extract and remained motionless for the last nine minutes of the trial. The MatLab plot for this trial is shown in (Fig. 3). This behavior (the circling and back up motion) was not seen in any of the other trials. Animal #264 did not display these behaviors during its control trial, even though it walked directly over the control solvent (Fig. 2).

Of our 17 paired trials, nine met our criteria for legitimacy (53%). The average time spent by the animals in the control quadrant was 2.70 ± 0.54 min (mean $\pm$ SE) compared to 3.42 ± 1.00 in the extract quadrant. The distribution of times that the animals spent in the extract

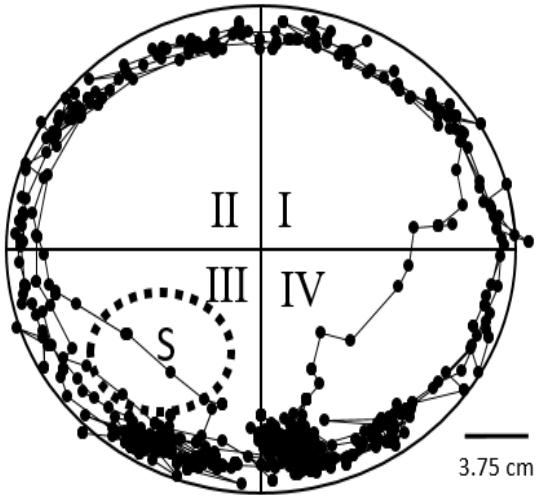

Fig. 2: Control trial tracking. Shown is the MatLab tracking of scorpion #264 during a trial with the control solvent. The solvent was placed in quadrant III. Individual points show the scorpion's position in 0.70 second intervals in a 600 second trial.

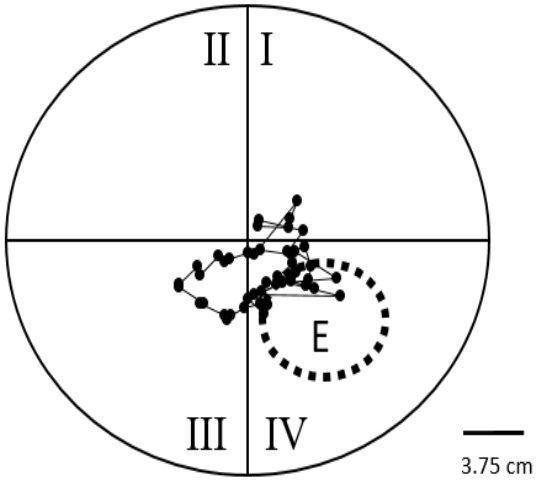

Fig. 3: Special animal response in extract trial. Shown is the MatLab plot for scorpion #264 during a trial with cricket extract. The extract was placed in quadrant IV. Individual points show the scorpion's position in 0.70 second intervals in a 600 second trial. The unusual behavior occurred during the first minute. The scorpion was motionless for the remaining nine minutes.

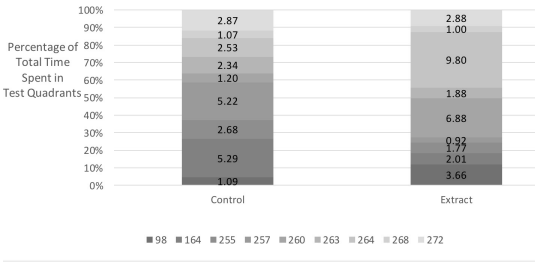

Fig. 4: Control versus extract variance. Shown is the amount of time in minutes that each scorpion spent in the control versus the extract quadrant. The difference in size of identically colored sections depicts the relative amount of variance in each of the paired trials.

and control quadrants is shown in Fig. 4 for all nine legitimate trials. The sample variance was 2.59 for the control trials and 9.01 for the extract trials. With only nine trials, a paired t-test (two-tailed) yielded a P-value much greater than 0.05 (P = 0.58).

Trial arenas were oriented so quadrants I and II were at the top of our video playback screen. The average time spent in each quadrant for both the extract and control trials were plotted (see Fig. 5). It should be noted that this is the average time for all the trials – the quadrants containing test solutions were randomized. Taking standard error into account, the average time in the control trials were relatively stable across the quadrants. Interestingly, the means are different between the extract and control, and quadrant III's extract mean was lower than all other quadrants.

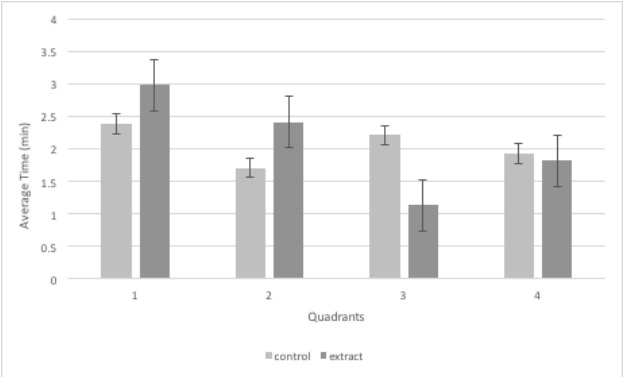

Fig. 5: Average time spent in each quadrant. The average time (minutes ± SE) the animals spent in quadrants I, II, III, and IV are shown for both the extract and the control trials.

Discussion

When scorpions were placed in a trial arena, they typically exhibited escape behavior in which they attempted to climb the walls and continuously circled the arena walls. This behavior is evident in our MatLab tracking program of animal 264 in the control trial as it stayed mostly along the perimeter wall of the arena (Fig. 2). Originally, we wanted to negate this behavior by trying to incite hunting behavior with a vibrational stimulus. We would then remove this stimulus so that the scorpion would only be exposed to the chemical trail or control solution after the initiation of hunting behavior. We would then see if the scorpion still attempted to hunt the "cricket" with only its chemical trail. We abandoned this idea because we could not consistently produce a vibrational stimulus that would attract the scorpion. We tried using a piezoelectric crystal to record the vibrational output of a cricket at a certain distance, but could not accurately reproduce and record these vibrations with the time and resources available. We then tried a live cricket that we would manipulate with fishing line tied around its thorax. Using a variety of "fishing" methods we tried to lure a scorpion with no consistent results across all animals. A few scorpions showed interest when the crickets were buried, though most scorpions still engaged in circling behavior and avoided the cricket. Interest in the lure could not be consistently reproduced so we abandoned the idea of using the lure.

Once we moved on from the idea of using a lure, we decided to focus on whether the scorpions would show interest in our cricket extract without a vibrational stimulus. This would indicate that the animals were sensing the chemical trail of the cricket on the substrate and possibly have more implications for scorpion hunting behavior. If the scorpions stayed near the chemical extract for significantly more time than the control, that could imply that the scorpions were tracking the cricket using a chemical trail without vibrations. Our data show that on average the scorpions spent 2.699 minutes in the control quadrant and 3.423 minutes in the extract quadrant during a 10-minute trial (Fig. 4). While, these data show that the scorpions did spend 0.724 minutes more, on average, in the extract quadrant compared to the control, they also failed to produce a significance level high enough to rule out chance as the reason for this time difference in arena quadrants.

There were many possible sources of error for our experiment. We estimate that we would need a total of 50, paired trials, instead of nine. Due to time constraints, scorpions that were brought in to replace scorpions that died (n=3) during pilot trials were fed too recently to be adequately starved to the same level of the other scorpions for accurate trials. Also, if a scorpion did not move during the 10-minute trial duration, it was considered a null trial.

Two scorpions performed null trials so both of their paired trials had to be ignored.

During our experiment, we made two interesting observations that led us to believe that behavioral patterns should have been analyzed. For example, the standard errors for the average time spent in each of the four quadrants were smaller for control trials compared to extract trials (Fig. 5). This implies that during control experiments, the animals engaged in circling behavior and generally spent close to equal times in the four quadrants, whereas in the extract trials they tended to spend different amounts of time in different quadrants. Our second observation was our extract trial with animal 264 in which it oriented itself towards the extract placed in the extract quadrant through a series of circular, backwards, and sideways shuffling movements that were not observed in any of the other animals (Fig. 3). Animal 264 also did not display these behaviors in the control trial. This suggests that instead of time spent in a control or extract quadrant, behavior near a concentrated area of extract should have been scored using specific objective characteristics.

In his experiment, Krapf surmised that scorpions can locate prey that is not moving (Krapf 1986). However, a lack of detail in the methods portion of his experiment makes it difficult to decide whether the results he observed during his experiment are due to the scorpion randomly wandering across the unmoving prey during the trial. Due to our low sample size, we cannot support or refute Krapf's claims, but we do believe additional experiments are needed to accurately ascertain whether scorpions can hunt by following a chemical trail in the absence of a vibrational stimulus. Experiments have been done in which the pectines were covered or removed and the scorpions' ability to localize prey was tested (Mineo et al.). We elected not to use this method in our experiment due to possible deleterious behavioral effects from incapacitating the pectines.

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