

Examining textural familiarity in scorpion homing.

A biology cornerstone manuscript by:

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Summary

Scorpions have unique abdominal organs called pectines that have chemosensory and mechanosensory properties. These organs potentially assist scorpions in navigating to their burrows after nightly excursions for hunting. Our purpose was to investigate whether scorpions use chemotextural familiarity when returning to their burrows. Tests occurred in rectangular, sand-filled arenas with provided pottery shards intended to function as burrows. Testing occurred in an isolated room with lights on a 15:9 hour light-dark cycle. Scorpions were introduced to their habitats and tests were performed the following week over a two-day period. Scorpions were inconclusively more likely to return home when the sand in their environment was changed. We also developed better techniques for successfully getting scorpions to establish a burrow. This behavioral information could be useful in future attempts to have scorpions adopt burrows in a lab setting.

Introduction

Scorpions are predatory arachnids found on every continent except Antarctica. While their modified pedipalps and stingers are among their more recognizable appendages, scorpions also have unique mid-ventral organs called pectines. Morphological evidence (Foelix & Müller-Vorholt 1983) and observation of scorpion behavior lend support to the hypothesis that pectines function as both chemoreceptors and mechanoreceptors. Scorpion pectines have a comb-like appearance, with dense patches of minute sensory peg sensilla on the ventral surface of each “tooth” (Gaffin & Brownell 1997). The pectines of most scorpions are sexually dimorphic, with males normally having more teeth on their pectines and more than twice as many peg sensilla than females in some species (Gaffin & Brownell 1997). Pectines can assist males in the detection of female substrate-borne chemicals, a useful tool for locating distant females when it is time to mate (Gaffin & Brownell 1997). Numerous studies also suggest pectines play an important role in substrate recognition. However, little is known about the specific role that these organs play in scorpion navigation.

Desert grassland scorpions, *Paruroctonus utahensis*, are nocturnal hunters that emerge from their burrows to search for food. It is currently unknown how these

scorpions navigate back to their established burrows after a night spent hunting. Path integration is a method whereby an animal, when venturing from some significant starting point such as a nest, keeps record of its vector distance and direction from its origin; consequently, the animal can return by a direct path (Muller & Wehner 1988). Numerous homing insects and a few arachnids use path integration to aid in navigating back to their burrows or nests after foraging (Collett & Collett 2000), and one study suggests that scorpions may also use path integration to return to their burrows (Gaffin 2011). Yet, another study suggests that scorpions could be using textural scene familiarity to navigate home (Gaffin & Zhao 2014). Honeybees and desert ants employ scene familiarity when returning to their nests, using visual cues to determine which scenes are familiar and thus in which direction they should proceed (Baddeley et al. 2012). A textural interpretation of this hypothesis would mean that scorpions use tactile and/or chemical familiarity (instead of visual) to determine which direction to proceed to their burrows. Scorpions are often observed to “sweep the sand” with their pectines, a behavior that could be used to familiarize themselves with the texture and chemical make-up of the sand surrounding their burrows, thus aiding in navigation. We hypothesize that the pectines may play an important role in assisting scorpion navigation, but we do not know the extent to

which the physical information received from the pectines influences homing behavior. To address this question, we developed a laboratory procedure to test the effects of changing the textural cues leading to scorpion burrows by altering the surface of the sand when the scorpions are displaced from their burrows.

Research shows scorpions exhibit a negative phototactic response to light and use visible light to escape to comparatively darker regions (Camp & Gaffin 1999). Based on these data, we used light to induce a response in our scorpions so that they would attempt to seek shelter in their arenas. Our expectation was that the scorpions would seek shelter by attempting to return to their burrows, and we planned to time the returns so that we could later alter textural cues and compare scorpion return times. However, a small pilot study showed the scorpions did not exhibit the expected escape behavior. As such, we altered our protocol to measure the position of the scorpions in relation to their burrows over an extended amount of time. We did this by assigning each part of the arena a number and then scoring scorpion position over the course of an hour.

If scorpions rely on textural cues to navigate, and if we disturb the sand leading back to their burrows while they are away from their burrows, then the scorpions should have more difficulty navigating home than when no change occurs because the recognized textural cues would be disrupted by the sand disturbance. If there is no difference between scorpion behavior when sand is changed versus when the sand is not changed, then the null hypothesis would be supported and we could posit that scorpions do not heavily rely on sensory information from their pectines to return to their burrows.

Methods

Animals

Twenty-five scorpions, 12 males and 13 females, of the species *P. utahensis* were obtained from the deserts of West Texas. The animals were kept on a 15:9 hour light/dark cycle, with dark hours from 1200 to 2100. Scorpions were watered twice a week and fed one or two crickets every other Thursday after experiments were completed. Scorpions were housed in 3.8 L glass jars with approximately two cm of sand from their natural habitat.

Experimental Arrangement

To eliminate interference from other light sources, our experiment was conducted in a closed room with no windows. Our experimental arrangement used a 92x92x183 cm metal scaffold (LxWxH). Attached to this metal framework were four lamps with 60-watt incandescent light bulbs, evenly spaced over ten experimental arenas to uniformly distribute light and eliminate shadows. These arenas were plastic tubs

(20.3x24.0x45.5 cm) filled with 3.2 L of sand that corresponded to a depth of approximately 5 cm. The sand in each arena was leveled and cleaned of sticks and other debris that the animals could potentially use as navigation cues.

We used distilled water to moisten the sand on one end of each arena, then used tongs to dig a small hole (roughly the size of a quarter) and covered this with a pottery shard approximately 3 cm in diameter. We then divided each container into three equal horizontal sections and gave each section a number to record the position of each scorpion in the arenas during the experiment. Section one contained the provided pottery shard intended to serve as a burrow. Section two (the middle third of the arena) was the site of textural alteration in the experiment, accomplished by brushing the top of the sand with a paintbrush. The third section of the arena was the site of scorpion displacement using film canisters (Fig. 1).

Pilot Study One

We first ran a small pilot study with five animals two weeks before our intended experiment. The pilot study was used to determine how the scorpions would react to our proposed experimental design of displacement using film canisters, using light to induce an escape response, and then timing the response. We discovered that the scorpions did not display the behavior we expected after displacement. Instead of trying to seek shelter by returning to the provided burrows as we predicted, the scorpions displayed varied escape behaviors. Some did not move upon their release, but stood frozen in place; some ran immediately to the nearest wall and walked around the walls of their arenas before becoming still;

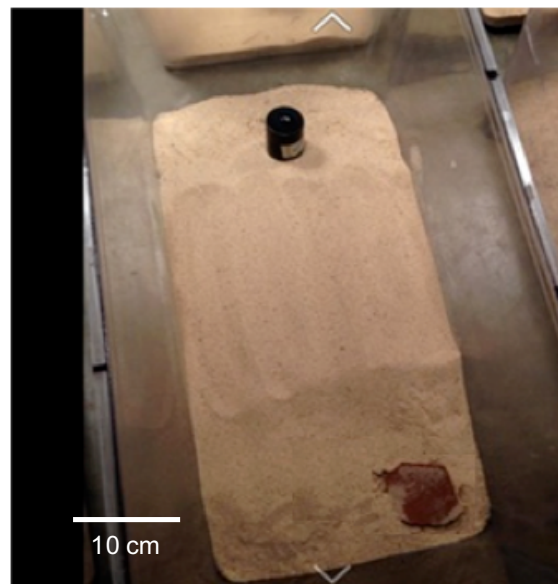


Fig. 1: Example of arena set up. This arena is shown with changed sand.

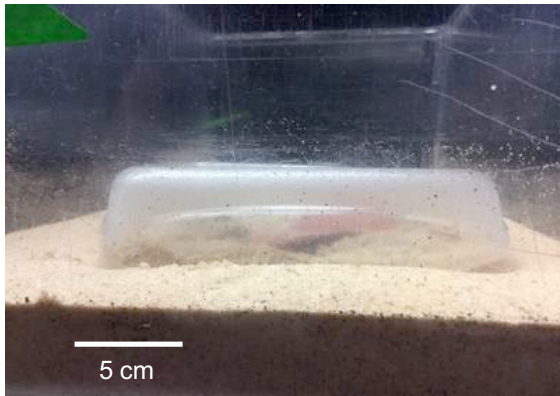


Fig. 2: Burrow covering. Image shows Tupperware covering burrow.

some simply sought refuge in the first corner they reached. None of our first pilot study scorpions attempted to return to the burrows we provided them. Considering these results, we altered our original experimental design and decided to score scorpion position in the arenas over the course of an hour instead of timing the scorpions' return to burrows.

Pilot Study Two

Pilot study two was originally intended to serve as part of our main study, and thus was similar in arrangement to our final experiment. We analyzed the behavior of 10 scorpions, split into a control group and an experimental group, each consisting of five specimens. The arenas had the same environmental design with an artificial burrow provided in the form of a clay shard placed medially

approximately 5 cm away from the wall of the arena. The scorpions were placed in the arenas and confined with the pottery shards inside a 14x10x6 cm Gladware container. We cut the flared top off the container so it could be placed upside down and reach completely through the sand to the bottom of the enclosure to prevent the animals from digging underneath the container. Studies show scorpions may be more likely to adopt a burrow if they are confined in a smaller area (Bost and Gaffin 2004). Each container had two to three air holes cut in its bottom (Fig. 2). After two days of confinement, the containers were removed and each scorpion could roam free in its arena for one week before experiments took place, allowing it to familiarize with its environment.

After the acclimation period of ten days we used tongs to place the scorpions in film canisters (3.2 cm in diameter and 5 cm tall), and then displaced the scorpions to the side of the arena opposite the provided pottery shards. The film canisters were placed roughly in the middle of section three, about 3 cm from the end wall and 10 cm from the side walls. We left the animals in the canisters for approximately two minutes to randomize their positions upon release. While the scorpions were adjusting, we turned the lights on over their arenas to induce a homing response (Camp & Gaffin 1999). We began displacement of scorpions at 1345 each day and released scorpions from the canisters at 1357, whereupon we commenced timing. Over the next hour, we marked the position of each scorpion at 5-minute intervals (whether in section 1, 2, or 3 of the arena). Once the hour was up, we turned off the lights and returned the scorpions to their regular dark cycle. This controlled trial was performed on Wednesdays, while the experimental trials

Displacement	Displacement	Displacement	Displacement	Displacement	Section 3
F 264 (# 2)	M 225	F 275	M 219	F 265	Section 2
					Section 1
Burrow	Burrow	Burrow	Burrow	Burrow	
Burrow	Burrow	Burrow	Burrow	Burrow	Section 1
F 276	M 221	F 266	M 218	F 264	Section 2
Displacement	Displacement	Displacement	Displacement	Displacement	Section 3

Control

Experimental

Fig. 3: Experimental set up, burrow, and displacement locations.

were performed the following day. During the experimental trials, we manipulated half of the scorpion enclosures by lightly brushing the top of the sand in section two with a paintbrush. The control group had nothing altered in their environments from the day before. Then we repeated the displacement procedure, the only difference from the day before being the brushed sand in the experimental group.

Textural Familiarity Experiment

Our experiment used the same experimental arrangement as pilot study two, with some slight modifications. We shifted the sand in the arenas between pilot study two and our textural familiarity experiment to rid the sand of any residual chemicals from the previous scorpions. Due to scorpion tendency to seek shelter in corners, we placed the pottery shards in a corner of section one to encourage burrow adoption, rather than in the middle of the section as in pilot study two. Figure 3 shows all experimental configurations. We moistened the

sand around the pottery shards every other day so that the moisture would stiffen the sand and make it easier for the scorpions to burrow. We conducted the experiments on Wednesdays and Thursdays beginning at 1430, and we collected quantitative data using the same technique as in pilot study two, by scoring scorpion position every 5 minutes for an hour.

Results

First, during the week of free-roam time, every scorpion from the textural familiarity experiment had evidence of burrowing. They each had at least one hole, roughly the size and shape of a normal scorpion burrow under or near the clay shard. Each scorpion also either partially buried or completely buried its clay shard, suggesting that they had all made the clay shard a “home.”

Scorpions that experienced changed sand appeared more likely to return to section one than scorpions who did not experience changed sand (Fig. 4). Over time,

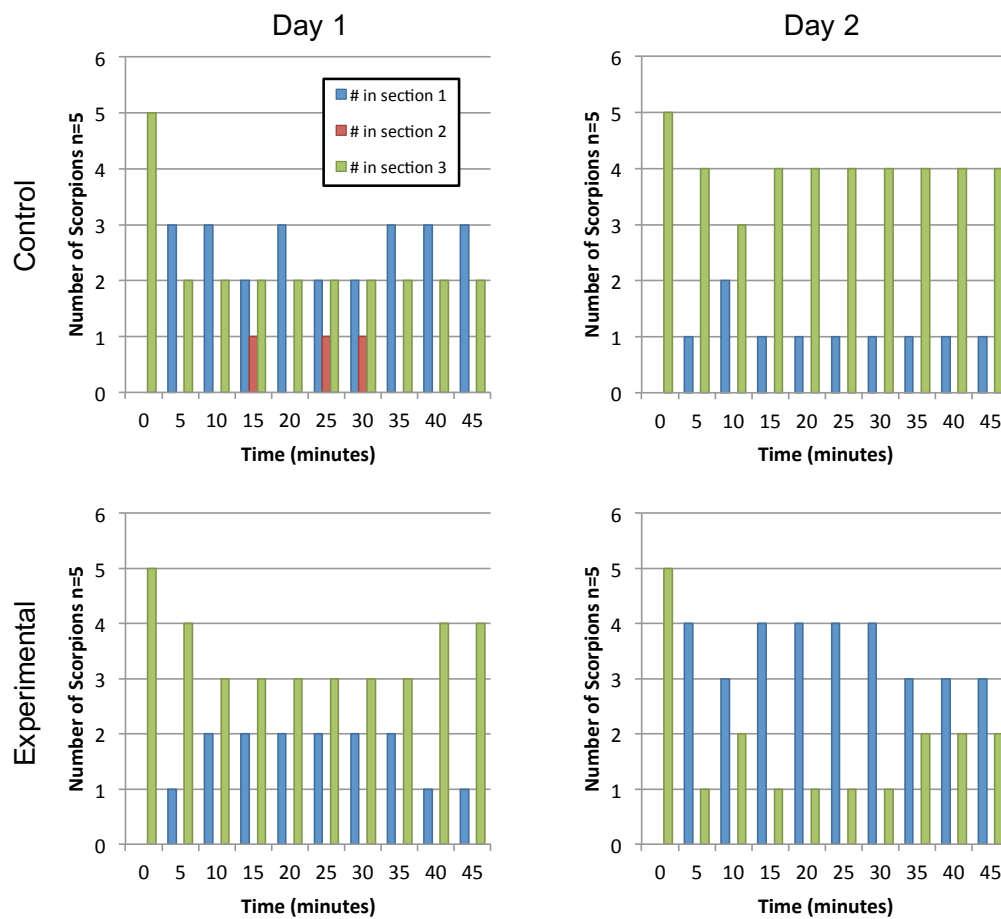


Fig. 4: Scorpion locations over time. A) Baseline data for sham treatment. B) Sham treatment. C) Baseline data for changed sand treatment. D) Changed sand treatment.

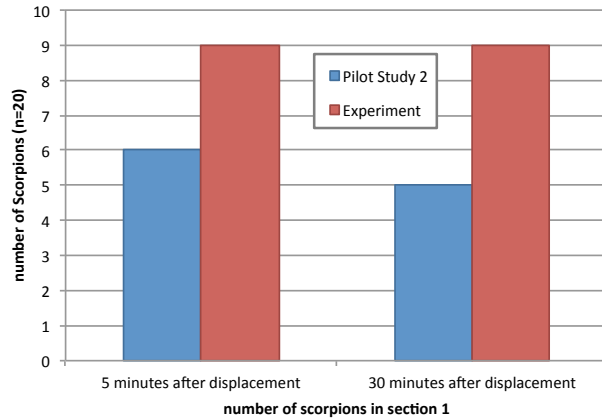


Fig. 5: Scorpion evidence of burrowing. Only scorpions located in section one are shown.

scorpions were more likely to be found in section one when the middle third of the sand was changed (part D, Fig. 4) than when sand went unchanged (parts A, B, and C, Fig. 4). This shows that more scorpions returned home when the sand was changed (Fig. 4D) and fewer returned home when the sand was not changed (Fig. 4B), compared to the baseline data (Fig. 4A,C). The sample size of this study was too small to run any statistical tests. We also obtained no evidence that the changed sand affected the scorpion's perception of its current location or how to get home.

We found evidence that changes between pilot study two and the actual experiment led to increased scorpion burrowing. Not only did we notice scorpion-created burrows from every animal in the experiment, but we also noticed an increase in their return rate to section one in the actual experiment compared to the pilot study trials (Fig. 5). Thirty minutes after displacement, scorpions were almost twice as likely to have returned to section one in the actual experiment than in the pilot study trials. Furthermore, most activity occurred within the first five minutes following displacement.

Discussion

The design of this experiment evolved from two successive pilot studies. Originally, we designed the experiment to have much smaller arenas with more pronounced corners and the dependent variable was the time it took a scorpion to return to its burrow. After preliminary testing, we noticed several cogent flaws. No scorpions returned to the vicinity of their burrows in the first pilot study, let alone re-entered them. This forced us to redesign the arenas and change the variable being measured. To avoid the ambiguity of the term "return to burrow" we instead used the system of scoring the scorpion based on its location in the arena thirds. From

then on, we used larger arenas with less pronounced corners.

However, during our second pilot study we observed that the scorpions did not show evidence of burrow fidelity. During the gathering of baseline data in this study, only 10% of the scorpions returned to the section with their burrow in it. Prolonged observation of the subjects in this study suggested that the majority had not formed burrows or were not loyal to the potshard mock burrow. Based on these observations the design was again changed. For the experimental study the potshards were placed in proximity to a corner and the sand was moistened. We found that this incentivized burrowing and seemed to induce burrow fidelity. During the gathering of baseline data for the experimental study, 60% of scorpions returned to the section containing their burrow. There was a correlation between burrow quality and scorpions returning to their burrows.

Major drawbacks to the experiment included the shape and layout of the arenas. The fact that they were rectangular meant that a flight reaction would inevitably lead the scorpions back to their burrows. Additionally, the arenas were small enough that any sustained movement by the subjects would have brought them to the section with their burrows. Most of the scorpions were observed to move quickly in a seemingly random direction until they encountered a wall, at which point they "wall walked" until they reached a corner. Ultimately, they showed very little preference for the section of the arena that the corner was located in. Throughout all trials, we noticed that the scorpions either remained motionless over the whole 60 minutes, or they moved rapidly at the beginning then stopped at approximately 5-10 minutes. These latter scorpions almost universally arrived at a wall or a corner and remained there for the rest of the trial. It was rare to see a scorpion change its position after the 30-minute mark.

A large source of error in this experiment likely came from the displacing of the scorpions at the beginning of each trial. The burrows in the experimental study appeared to be better formed and the scorpions spent more time in them, but this meant that often the scorpions were found in their burrows during the preparation for displacement. As a result, many of the burrows were damaged or destroyed in the displacing of the scorpions and the scorpions were likely traumatized in the process. Several scorpions were chased around the arena and picked up several times before they were placed in the film canister. This may have influenced the subjects' incentive to return to their burrows and it is possible that they no longer recognized the burrows as a place of safety or shelter. During the experimental study, only one scorpion in one trial, representing 5% of all trials, re-entered its burrow.

Our trials were run during the subjects' dark cycle to decrease the likelihood of them being in their burrows.

Nevertheless, scorpions were repeatedly observed to be in their burrows during the dark cycle and outside of them during the light cycle, possibly suggesting that the light was not as much of a deterrent as previously thought. In fact, 25% of subjects in the experimental study showed little or no signs of movement over the 60 minutes. Perhaps the introduction of light to the environment was not enough of an incentive for the scorpions to return to their burrows.

Overall our data were inconclusive as far as lending support to our hypothesis. The manipulation of sand in the middle third did not hinder scorpion navigation back to their burrows in the arenas. In fact, it might have caused a greater number of scorpions to return to their burrows. Perhaps the most useful data gleaned from this experiment was the difference in number of scorpions that returned to their burrows during the gathering of baseline data from the pilot study to the experimental study. We showed that by manipulating the mock burrow we could induce more scorpions to return to their burrows after being displaced. These data should be useful in further assays in scorpion navigation.

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References

- Baddeley BP, Graham P, Husbands P, Phillipides A (2012) A model of ant route navigation driven by scene familiarity. *PLoS Computational Biology* 8(1): e1002336
- Bost KC, Gaffin DD (2004) Sand scorpion home burrow navigation in the laboratory. *Euscorpius* 17:1-5
- Camp EA, Gaffin DD (1999) Escape behavior mediated by negative phototaxis in the scorpion *Paruroctonus utahensis* (Scorpiones, Vaejovidae). *Journal of Arachnology* 27:679-684
- Collett TS, Collett M (2000) Path integration in insects. *Current Opinion in Neurobiology* 10:757-762
- Cruse H, Wehner R (2011) No need for a cognitive map: decentralized memory for insect navigation. *PLoS Computational Biology* 7(3): e1002009
- Foelix RF, Müller-Vorholt G (1983) The fine structure of scorpion sensory organs. II. Pecten sensilla. *Bulletin of the British Arachnological Society* 6:68-74
- Gaffin DD (2011) *In situ* infrared videography of sand scorpion nighttime surface activity. *Euscorpius* 122:1-13
- Gaffin DD, Brownell PH (1997) Response properties of chemosensory peg sensilla on the pectines of scorpions. *Journal of Comparative Physiology A* 181:291-300
- Gaffin DD, Zhao K (2014) A new hypothesis for scorpion navigation: chemo-textural familiarity. Annual Meeting of the American Arachnological Society, June 20-24, 2014, Ohio State University, Newark, Ohio
- Muller M Wehner R (1988) Path integration in desert ants, *Cataglyphis fortis*. *Proceedings of the National Academy of Sciences* 85: 5287-5290