# Texture preference in the eastern desert grassland scorpion: *Paruroctonus utahensis* (Scorpionida, Vaejovidae).

*A biology cornerstone manuscript by:*
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## Summary

Anatomically and physiologically, scorpion pectines appear to be elaborate chemo-tactile sensory organs. Behavioral studies show that scorpions use their pectines to detect ground-based chemical signals. However, it is uncertain if scorpions use their pectines to discern and discriminate different textures. A first step in assessing the significance of the pectines in texture discrimination is to develop a reliable behavioral assay. To do this, we tested animal preferences in arenas containing four different substrates (sand, gravel, stone, and potting mix). We used a specially written MATLAB program to track scorpion movements and determine the time animals spent atop each substrate. We found that scorpions significantly preferred the gravel substrate while significantly avoiding the soil substrate. This is different from our prediction that these animals would prefer sand from their natural habitat. These results suggest that scorpions can discriminate texture when encountering very different substrates. Furthermore, scorpion stings are a medical concern in many parts of the world and our finding that animals avoided the potting mix substrate opens the door to further research directed at developing materials for deterring scorpions from human habitats.

## Introduction

All scorpions possess unique, paired ventral organs called pectines. Pectines are comb-like, chemo-tactile structures that brush the ground as the animal walks. Behavioral and physiological research reveals that pectines are responsive to multiple stimuli: moisture (Gaffin 1992, Gaffin & Brownell 1997), chemicals (Knowlton & Gaffin 2011, Taylor et al. 2012), and mechanical contact (Gaffin & Brownell 1997).

One area of study that has not received much attention is texture or substrate-based discrimination. Abushama (1964) reported that when tested on substrates with different sizes of sand grains, deathstalker scorpions (*Leiurus quinquestriatus*) preferred finer substrates over coarser ones. A similar experiment was performed on the northern scorpion, *Paroctonus boreus* (Boyden 1978). However, in this experiment where animals were tested against three different sediments, the scorpions preferred the coarser substrate. Boyden inferred that this was because the natural habitat of *P. boreus* consists of rocky hillsides. Also, it was found in both studies that the discriminatory preference disappeared when the pectines were removed. Taken together, this suggests that the pectines are tactile sensory organs that help guide a species' substrate preference.

The eastern desert grassland scorpion, *Paruroctonus utahensis*, is a scorpion found in the Chihuahua Desert of southwestern United States and northern Mexico. In their natural habitat, *P. utahensis* may encounter many different textures, such as stone, dirt, loose gravel, or rocks. We tested *P. utahensis* to see if it can detect differences in substrate texture when put in an arena with textures simulating the natural substrates the animal may experience. We found that scorpions showed a significant preference for a gravel substrate and a significant avoidance of a potting mix substrate.

## Methods

*Animals*

We used 40 adult female desert grassland scorpions (*Paruroctonus utahensis*, Williams 1968) collected near Monahans, Texas for the experiment. The animals were kept in separate jars (6 cm radius x 30 cm height) filled with sand from their native habitat to ~3 cm depth, watered with approximately 5 ml of water three times a week, and fed one small cricket every other week. The scorpions were kept on the same natural light-dark cycle that they had in the wild, which meant that they were most active at night.

*Apparatus*

Our experiment involved putting scorpions in circular plastic arenas (13.5 cm radius, 10 cm tall) broken into four quadrants that each contained a different ground texture (Figure 1). The four textures were dune sand (from Monahans Sandhills State Park, Texas), gravel (Pavestone decorative stone pea pebbles), potting mix (Miracle-Gro), and white porcelain tile (Ivetta). We started by placing the arenas on top of the tiles to form the floor of exactly one quadrant of the arena that measured 13.5 cm radius. The tile was then taped to the bottom of the arena, and a paper towel was taped to the bottom of it to serve as a base for the other substrates. We then filled in the three remaining quadrants of each arena with the correct substances taking care that each was level with the stone so the entire base of the arena was the same height. Substrate materials were changed after every trial to control for any chemicals or footstep patterns left by test animals. We offset each arena by 90° to control for factors such as geomagnetic cues, sounds in the room, or visual biases from objects in the behavioral chamber. Around all four arenas, we placed a larger cardboard enclosure where the cameras rested; we placed a light-blocking sheet on the cardboard above the cameras to reduce visual cues.

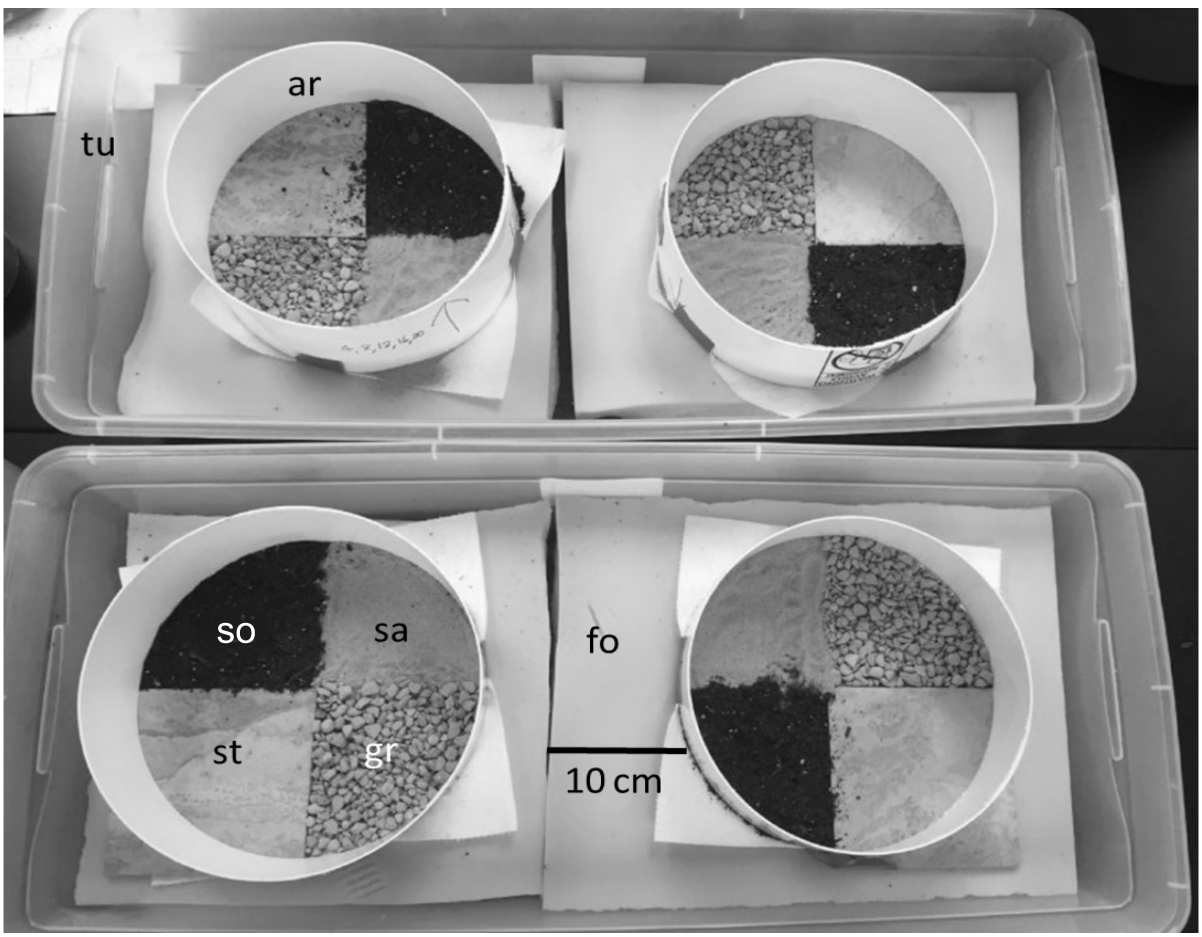


**Fig. 1:** Behavioral chambers. Each arena (ar) has four quadrants, each with different substrate textures (sa: sand, so: soil, gr: gravel, st: stone) that are offset by 90°. The arenas sit atop memory foam (fo) and are contained in larger tubs (tu) to trap any escaping animals.

We used two infrared surveillance cameras (Nest) to monitor the animals (one camera for two arenas). We also put memory foam beneath the arenas to mitigate vibrations. We kept the all substrates at a constant room temperature (21°C) before putting them in the arenas.

*Procedure*

Every night, we tested four scorpions in separate arenas from 20:00 to 21:00, which is the animals' normal active period (Polis 1980). We placed the scorpions in the arenas and waited five minutes for them to acclimate to their surroundings. After that period, our hour-long trials began. We only counted trials if the scorpion traveled to each quadrant at least once. We also discounted trials in which the scorpions escaped their arenas or if the camera failed. After the trial, we deconstructed the set-up and cleaned the arenas. Afterwards, we reviewed a time-lapsed version of the video.

*Analysis*

We used the Nest program to time-lapse the one-hour trials into two minute clips and saved them to our computer as .mp4 files. These clips were then put through a specially made MATLAB program that condensed the footage into 360 frames and used a frame-by-frame subtraction method to detect scorpion movement and plot each animal's X-Y locations. There were some animal movements that the computer did not catch and some extraneous points were added. We cleaned up these error points by subsequent visual inspection. After we extracted all the data from the footage, we determined the percent time each animal spent atop each substrate. We used InStat to analyze our results with a Friedman (non-parametric, repeated measure ANOVA) test, which provided us with a $p$ value and a sum of ranks. The sum of ranks was then used in Dunn's Multiple Comparisons Test to determine the significance between rank sum means to compare each substrate.

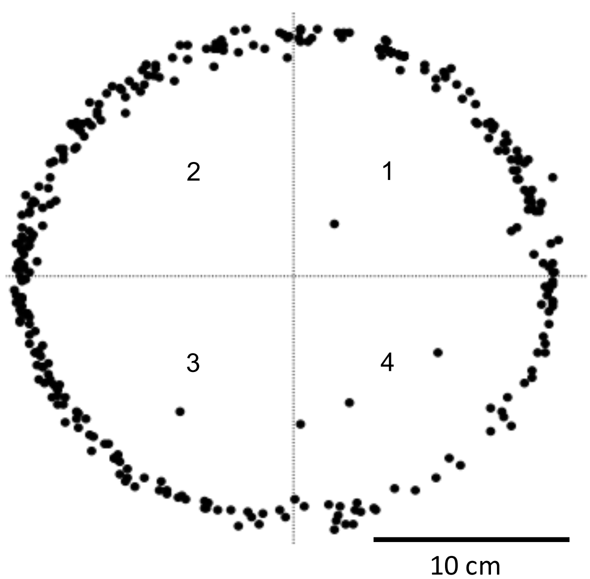


**Fig. 2:** Example of the activity tracked by the MATLAB software. Each dot represents a scorpion's position at a 10 second interval. The dots towards the center in quadrant 4 represent the scorpion moving away from the wall and towards the center or across to another quadrant. Quadrant 4 in this sample is gravel.

## Results

*Behavior*

During our trials, animals typically stayed or moved near the walls for long periods, occasionally walking across the center of the arena. Figure 2 shows a typical trial, with movements plotted every 10 seconds. We noted that animals sometimes concentrated their activity at the intersections between adjacent quadrants.

*Trial Results*

Of the 40 trials, 33 (82.5%) were considered successful (i.e., they reached every section at least once). Of the seven animals that failed, three were due to unforeseen camera problems, while the other four escaped from their arenas before the trial was completed. Of the 33 successful trials, the scorpions were more likely to spend time within the quadrant containing gravel (Figure 3A). The average preference in descending order was gravel, stone, sand, and soil. Of the 33 animals, 16 spent the most time in the gravel quadrant (49%), with 10 of the 33 (30%) spending most of their time in the stone quadrant. When analyzed based on cardinal direction (Figure 3B) there was a high $p$ value in relation to the difference in the quadrants. This served as a control for the influence of room-based or geocentric stimuli.

A normality test administered to our data failed for every substrate except for the gravel quadrant. As such, a Friedman (non-parametric, repeated measure ANOVA) test was conducted, revealing that there was a significance difference among the quadrant choices. These data were then put through a Dunn's Multiple Comparisons Test (Table 1), which showed significant differences between gravel and soil ($p<0.001$), gravel and sand ($p<0.05$), and stone and soil ($p<0.05$).

## Discussion

These finding seems to compare with those of Boyden (1978), which showed that *P. boreus* preferred a coarser substrate. Although in our study the substrates used were very different and not necessarily placed in positions of r anking coarseness. Boyden noted that *P. boreus* is naturally found on rocky hillsides, which may explain the observed preference in a coarser substrate. However, *P. utahensis* is naturally found in a substrate like the sand they were kept in during this experiment. The animals in our experiment did not show a preference for sand compared to the other substrates, even though this was the same sand the scorpions make their burrows in. It is still not clear as to why the scorpions prefer gravel over their native sand, but the disparity between the two substrates is significant.

Movement difference across the various substrates could suggest animal preferences or aversions for certain textures. Across all 33 trials, only three scorpions spent most of their time atop the soil substrate. This could be because it the animals did not like moving in the substrate, or because of something in the soil, such as the specific chemicals or materials that it contained. The only certainty is that the scorpions did not move around in this soil as much as the other substrates on average.

This experiment could be improved upon in several ways. One way to better this study includes the use of a bigger sample size. For feasibility and time constraints this study was limited to 40 animals. The camera setup could have also been more precise and clear. The setup included two cameras for four arenas, which resulted in portions of some arenas being partly obscured by the arena wall. Using one camera per arena would likely improve the overall quality of the experiment.

To determine if the pectines are responsible sensing the textural difference of this experiment, a follow-up to this study using scorpions with their pectines impaired would be needed. Boyden found that scorpions with their pectines clipped did not discriminate texture compared to i ntact animals. Due to the lack of time, producing a whole new study and finding a way to impair the pectines in a humane way was not an option for this study. This could be done by covering the pectines in wax or plastic.

This study shows that whatever was in potting soil mix was not liked by *P. utahensis*. Further tests using other types of dirt could help to solidify that the potting mix is repellant. Doing a similar test to our experiment would help show the significance of the potting mix. If there were a way to produce a form of substrate consistent with the contents of the top soil mix, but amplified, there might be a way to create a scorpion repellant formula. This would also require testing against other species, but finding a way to keep scorpions out using a substrate could be useful in keeping people safe in scorpion dense regions of the planet.

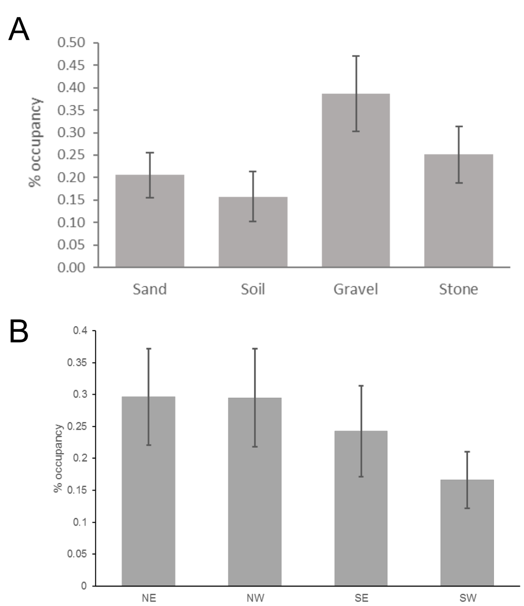


**Fig. 3:** Summary of scorpion behavior. **A:** Mean movements / occupancy of the 33 scorpions per substrate. Error bars set at +/-1.96 times the standard error (denoting confidence interval). **B:** Mean movements/ occupancy of the 33 scorpions per cardinal direction. Error bars set at +/-1.96 times the standard error (denoting confidence interval).

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**Table 1.** Dunn's comparison test results. Number of *: level of significance, ns: no significance. If the difference between rank sum means is greater than 27.681, then the *p*-value is less than 0.05.

| Comparison | Rank Sum Difference | Significance | P Value |
|---|---|---|---|
| Sand vs. Dirt | 14.000 | ns | p>0.05 |
| Sand vs. Gravel | -30.000 | * | p<0.05 |
| Sand vs. Stone | -18.000 | ns | p>0.05 |
| Dirt vs. Gravel | -44.000 | *** | p<0.001 |
| Dirt vs. Stone | -32.000 | * | p<0.05 |
| Gravel vs. Stone | 12.000 | ns | p>0.05 |

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