# Assay of Shelter-Seeking Behavior in Desert Grassland Scorpions

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Spring 2013

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## Summary

The desert grassland scorpion (*Paruroctonus utahensis*) is a nocturnal arachnid. These animals venture into the night searching for food and mates, exposing themselves to predators such as owls and rodents. All scorpions have the peculiar property of a fluorescent cuticle that emits green light (~500 nm) when exposed to ultraviolet light (~395 nm). Many researchers have speculated on the functionality of this fluorescence; a recent idea is that it serves as a whole body light detector. Scorpions have been found in the field stationed under twigs and other small overhangs, which led to the hypothesis that scorpions use the supposed cuticular light perception to detect shelter. We designed circular sand-lined experimental arenas, each with an ultraviolet LED suspended above and a 9.5 mm diameter dowel rod laid across the rims of the arena to cast a shadow on the sand. Scorpions were placed in the arenas and exposed to the experimental light treatment for 30 minutes while an infrared security camera recorded their behavior. Each of 16 tested scorpions was exposed to three light levels: no light, the ultraviolet intensity of moonlight, and the ultraviolet intensity of dusk. We calibrated the irradiances of the moonlight and dusk levels with a spectrophotometer to match recorded levels of UV light at those times of night. We recorded how much time each animal spent in the shadow of the rod. Although one subject spent significantly more time in the shadow during the dusk trial, there was no overall trend in the data, leading us to reject our initial hypothesis of scorpion shelter seeking. Subjects did, however, pause under the rod while near the arena wall more often than predicted.

## Introduction

Scorpion fluorescence has been a subject of study ever since the property was discovered over 50 years ago (Pavan 1954). All extant scorpion species fluoresce blue-green when exposed to ultraviolet light, suggesting that fluorescence is an ancestral trait that has been preserved throughout the evolutionary history of the Scorpiones order. One hypothesis is that the fluorescent molecules are vestigial and may have served as a sunblock against UV light for scorpions' ancient diurnal ancestors (Frost et al. 2001). Another idea proposes that the fluorescence could be a prey lure (Polis 1979). Other hypotheses for the function of fluorescence include recognition of conspecifics, insect attraction, and that there is no function at all (Kloock 2005, 2008; Wankhede 2004).

Both sets of scorpion eyes have sensitivity peaks in green (500 nm) and ultraviolet (350-400 nm) wavelengths, which are nearly identical to the wavelengths of scorpion fluorescence and excitation, respectively (Machan 1968; Gaffin et al. 2012). It is therefore possible that scorpion vision and fluorescence are related, but little evidence has been brought forth to support this relationship. Kloock found that the difference in fluorescent wavelengths of two sympatric species was not large enough to be a basis for species recognition (Kloock 2008).

Recent evidence has suggested the possibility of the scorpion cuticle functioning as an extraocular light sensor. Scorpions have previously been shown to have extraocular light detection capabilities in their tails (Zwicky 1968, 1970). More recent studies suggest that scorpion fluorescence does affect their behavior and is used as a form of light detection (Blass & Gaffin 2008;

**Fig. 1:** Artistic rendition of a scorpion fluorescing green from UV light hiding under a thin shelter. Scorpions are occasionally found in the field under small overhangs such as grass or twigs.

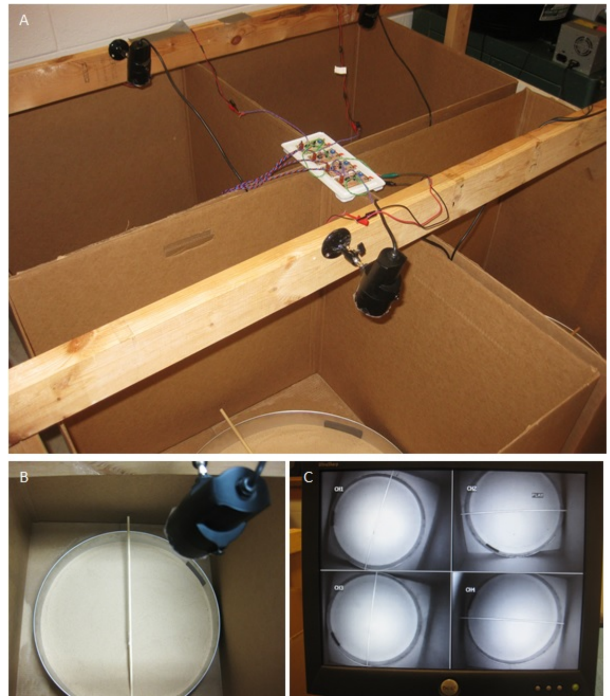

**Fig. 2:** Experimental arena set-up and recording. **A** Cameras and ultraviolet LEDs (connected to the operational amplifier unit) were mounted on a wooden frame. **B** The shadow of the dowel rod served as the artificial shelter. **C** We recorded and reviewed scorpion behavior on a security system program.

Gaffin et al. 2012; Kloock et al. 2010). Still, a function for this light detection has yet to be discovered.

We investigated the possibility of scorpion fluorescence serving as a nocturnal shadow detector. Scorpions are occasionally found in the field under small overhangs such as twigs or leaves, perhaps with the purpose of sheltering themselves from nocturnal predators (Fig. 1). While scorpions have a sensitive enough visual system to detect starlight (and, therefore, shade from starlight), they were not always found positioned with their eyes in the shade of the overhang (Fleissner & Fleissner 2001). We designed an experimental assay with a thin dowel rod suspended above a sandy arena to emulate a twig or branch in a natural desert environment. We shone UV lights down on the individual scorpions in these arenas to try to elicit the hypothesized shelter-seeking behavioral response, which we measured by timing the pauses scorpions made in the shadow of the rod. Scorpions did not exhibit a significant difference in behavior between the three treatment levels (no light and two intensities of UV light). Our secondary analysis, in which we categorized pauses as occurring near the wall or in the arena, showed that scorpions paused under the rod near the wall more often than away from the wall. We also found that they spent more time under the rod while near the wall than would be expected from the percentage of time they spend near the wall based on an ongoing study.

## Methods

### *Research Animal and Animal Care*

We used *Paruroctonus utahensis* males and females collected near the Sevilleta National Wildlife Refuge near La Joya, NM (34°35'N, 106.88°W) in October 2012. All animals were kept in 3.8 l glass jars lined with sand and including a terracotta potshard. We kept these jars in a room with constant temperature and humidity (22° C, 55-60 relative humidity) and lights were timed to mimic the natural day/night cycle (14:10 h L:D). We watered scorpions once a week and fed them a cricket every two weeks.

### *Arenas and Trial Recording*

Experimental trials were conducted in four circular, metal drain pans (68.6 cm rim diameter, 65.4 cm base diameter, 7.6 cm height) lined with sand, which was stirred between trials to disperse ground-based chemicals that may have been left by previous animals. We set each arena in a lidless cardboard box (76.8 cm cube) to block out any other potential light sources (Fig. 2a). A 73.4 cm long dowel rod (9 mm diameter) was placed across a diameter of each arena (Fig. 2b). We rotated the polar angle of the dowel rod to a different diameter of the arena between each trial to correct for potential arena orientation bias.

We suspended a 5 mm ultraviolet LED (398 nm; Super Bright LEDs Inc.) 88.5 cm above each arena. We built four operational amplifier circuits to which we attached each of the bulbs with alligator clamps. All four circuits were powered by the same power supply.

We recorded trials with a set of four infrared security cameras (Defender SN501-16CH-007) mounted approximately 94 cm above the arena floors. The cameras recorded all video data from trials to an external computer for later viewing and scoring (Fig. 2c).

### *Ultraviolet Light Trials*

We exposed 16 scorpions to three levels of ultraviolet irradiance, for a total of 48 trials. The three treatment levels were no light (LEDs off), the UV component of moonlight (0.0001 μW/cm$^2$/nm), and the UV component of dusk light (0.01 μW/cm$^2$/nm; based on measurements from Johnsen et al. 2006). We began trials every night at 18:30 (corresponding to the transition time from light to dark) and completed all 48 trials in three nights. Each scorpion underwent one trial per night, and trials were conducted so that all four arenas were at the same treatment level at any give time. The order of treatment presentation was randomized.

We introduced the scorpion to its arena 15 minutes before the beginning of the trial. After the acclimation period, we turned on the LEDs (except in the case of no light trials) and began recording for the one-hour trial duration. Afterward, we scored each trial for the number of seconds the scorpion paused in the shadow of the dowel rod. A pause was only counted if the scorpion stopped moving for one second or more. A scorpion's pause data was only considered valid if it paused under the rod at least once across the three light treatments.

### *Light Calibrations*

We carefully calibrated the irradiances for each bulb by recording the voltage in the individual circuits while confirming the correct irradiance with an Ocean Optics spectrophotometer attached to a fiber optic cable and cosine corrector. For the dusk light level of irradiance (0.01 μW/cm$^2$/nm), we held the end of the fiber optic cable at the center surface of the arena (with the dowel rod removed) to calibrate the irradiance from the scorpion's perspective. The moonlight level of irradiance (0.0001 μW/cm$^2$/nm) was too dim for the spectrophotometer to register from 88.5 cm; we therefore employed the inverse-square law to calibrate the expected irradiance (0.0016 μW/cm$^2$/nm) from one-fourth the distance (22.125 cm). All trials included infrared light from the security cameras, but scorpions do not exhibit behavioral changes when exposed to that wavelength of light (Blass & Gaffin 2008).

### *Analysis*

We scored each trial for the total duration in seconds the scorpion spent paused with any portion of its body in the shadow of the dowel rod. We considered a scorpion paused only if it stopped moving for one second or more. We subsequently reanalyzed the data, categorizing pauses under the rod as next to the wall (within approximately 5 cm of the wall) or in the arena. We ran a repeated measures ANOVA test to analyze our data (SPSS software, version 21.0.0).

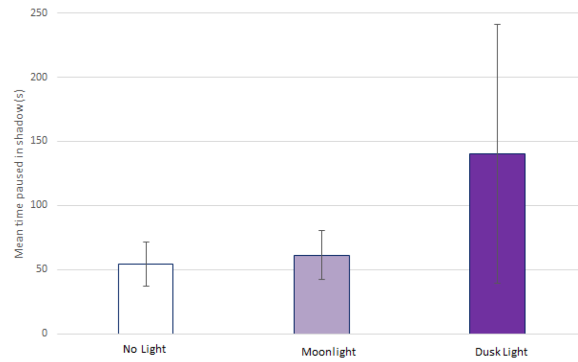


**Fig. 3:** Mean amount of time scorpions spent paused in shadow of dowel rod for each treatment level. The comparison of means ± SD of time in seconds between the three treatment groups showed no significant differences. A repeated-measures ANOVA test indicated no differences for between-groups comparisons (p=0.635).

## Results

Scorpion movements during these trials was characterized by short bursts of movement interrupted by brief pauses. Many of the animals spent the majority of the time along the wall of the arena, while some forayed into the central area of the arena for significant periods of time.

We added the durations of all pauses in the shadow of the dowel rod for each scorpion. The means of these summed pause durations show a positive correlation between shelter-seeking behavior and ultraviolet light intensity (Fig 3). However, this apparent correlation can be attributed to one outlying datum, as one individual spent 1446.5 s in the shadow of the rod during the dusk treatment. Of the 16 scorpions that we used, 14 met the criteria for trial validity. One scorpion did not pause under the dowel rod during any of the three light trials, and another subject died during the recording process.

As a secondary measure of shelter-seeking behavior, we calculated the amounts of time an individual would be expected to spend under the dowel rod next to the wall and under the dowel rod in the arena based on a measure of scorpion wall-preference concluded from another study. According to observations of the same species in the same experimental arena (without the dowel rod and UV light), scorpions spend about 96% of their time near the wall (Vinnedge pers. comm.). Using this percentage, along with the area of the dowel rod shadow (65.4 cm$^2$) in comparison to the area of the arena, we determined that scorpions should spend 33.6 s in the shadow near the wall and 3.3 s in the shadow away from the wall if the shadow was not affecting their behavior. We found that subjects paused in the shadow near the wall more than expected for the moonlight and dusk light treatments. When scorpions were away from the wall, they paused in the shadow more than expected during the no light and moonlight treatments (Fig. 4).

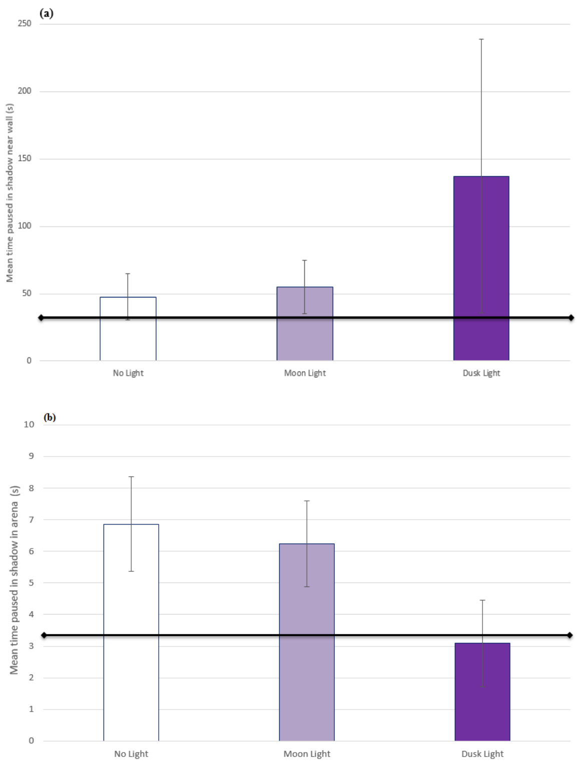


**Fig. 4:** Scorpion pauses categorized by location in arena relative to wall. The mean amount of time scorpions spent paused in the shadow of the dowel rod for each treatment level while **A** near the wall and **B** away from the wall in the arena is compared to the expected times we calculated. Black lines indicate the expected time spent under the rod for each spatial division of the arena.

## Discussion

Although our data were not conclusive in support of our original hypothesis that scorpions take shelter in response to light detection, there were some clues that pointed to its validity. The fact that scorpions paused more than expected during the light trials while near the wall (where they spend most of their time) could indicate a preference for the shadow. Scorpion pausing behavior away from the wall was not in line with our hypothesis (the highest mean amount of time paused under the rod was for the no light treatment), but this may have been due to their general avoidance of the middle of the arena under the light treatments.

While thigmotaxis is a known phenomenon in most arthropods, it is also possible that the individuals were seeking the lowest level of light in the arena. Because the LED bulb was suspended above the center of the arena, the highest irradiance was in the middle of the arena and the lowest irradiance was along the perimeter. Scorpions have been shown to be negatively phototactic, especially to UV light (Abushama 1964; Blass 2008). In a potential future study, scorpions could be confined to a perimeter track of the arena (maintaining a relatively constant irradiance) and provided with a similar stick-like shelter under which they could hide. This would demonstrate shadow preference and eliminate the confounding factors of thigmotaxis and negative phototaxis.

The large difference between expected time under the rod near the wall and mean recorded pauses near the wall could lend credence to our hypothesis. Further scrutiny of the video data would be helpful for drawing further conclusions, as we did not analyze the data in a way that allowed us to compare how much time a scorpion spent along the wall (disregarding the rod) to time spent away from the wall. The preliminary data gathered from Vinnedge's ongoing study provided us with a general ratio of time scorpions spend near the wall and away from the wall. However, his data have been processed for only one individual and therefore may not indicate the general behavior of scorpions as accurately as possible.

Scorpion locomotion patterns were not unique for each light treatment level, but there were some differences worth noting. A score of 0 s, meaning that a scorpion did not pause under the rod at any point, usually indicated an inactive, stationary animal. One individual had two 0 s scores on the moonlight and dusk light trials, and another animal had a 0 s score on the dusk light trial. This leads us to hypothesize that scorpions may be generally less active under high levels of UV. This finding is in direct contrast with a previous study, and may therefore be worth examining further (Gaffin et al. 2012).

Our results did not support our hypothesis, but we do not think that this single experiment rules out the whole-body hypothesis of light detection. Illuminating the arena with a uniform level of irradiance is likely not feasible, but confining scorpions to a circular track would solve this problem. Future researchers may benefit from observing scorpion response over a longer period of time. One hour per treatment may not have been long enough to measure true scorpion preference; additionally, the subjects might abandon their thigmotactic tendencies if given a longer time in the arena As referenced above, Vinnedge is tracking the positions of scorpions in a large arena over 24 hours; combining the ultraviolet light assay of our present study with the software used in the position tracking study would provide a larger sample across time for assessing scorpion preference for light. Demonstrating that scorpions use their cuticles to detect light would be among the first findings of extraocular vision in all arthropods.

## Acknowledgments

I thank Dr. Douglas Mock and Dr. P. L. Schwagmeyer for acting as committee members for the defense of this thesis. Without their instruction in the field of animal behavior and its practical applications in the lab, I would not have been able to complete this thesis. Special thanks to Dr. Mariëlle Hoefnagels, who has likely edited every scorpion research paper and presentation that I've written. I have grown so much as a science writer by learning from you. I thank Jay Vinnedge for allowing me to use the arenas from his experimental design, as well as his painstakingly gathered data. Thanks to Danielle Vinnedge for maintaining the animals, despite what may have seemed like attempts on my part to increase our lab's scorpion mortality rate. And, undoubtedly, my largest "thank you" goes to Dr. Douglas Gaffin. Coming into college, I had plans to study music and looming doubts about my dedication to the art. Thanks to the wisdom of my advisor, I had the incredible opportunity to take Introduction to Zoology during my first semester, taught by Dr. Gaffin. His friendly disposition, dorky humor, and absolutely inspiring teaching style led me to the decision to switch to studying science. Getting to work in his lab as an undergraduate researcher has been nothing short of an honor. The amount that I have learned about writing, teaching, presenting, and science in general from him is immeasurable.

## References

Abushama, F. T. 1964. On the behaviour and sensory physiology of the scorpion *Leiurus quinquestriatus* (H. & E.). Animal Behaviour, 12:140-153.

Blass, G.R.C. & D.D. Gaffin. 2008. Light wavelength biases of scorpions. Animal Behaviour. 76:365-373.

Fleissner, G. & G. Fleissner. 2001. Night vision in desert scorpions. *In* Scorpions 2001: In Memoriam Gary A. Polis. (V. Fet & P.A. Selden eds.). British Arachnological Society, Burnham Beeches, Bucks.

Frost, L. M., Butler, D.R., O'Dell, B., & V. Fet. 2001. A coumarin as a fluorescent compound in scorpion cuticle. *In* Scorpions 2001: In Memoriam Gary A. Polis. (V. Fet & P.A. Selden eds.). British Arachnological Society, Burnham Beeches, Bucks.

Gaffin, D. D., Bumm, L.A., Taylor, M.S., Popokina, N.V., & S. Mann. 2012. Scorpion fluorescence and reaction to light. Animal Behaviour 83:429-436.

Johnsen, S., A. Kelber, E. Warrant, A.M. Sweeney, E.A. Widder, R.L. Lee Jr. & J. Hernández-Andrés. 2006. Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. Journal of Experimental Biology 209:789–800.

Kloock, C. T. 2005. Aerial insects avoid fluorescing scorpions. Euscorpius 21:1-7.

Kloock, C. T. 2008. Journal of Photochemistry and Photobiology. 91:132-136.

Kloock, C. T., Kubli, A. & Reynolds, R. 2010. Ultraviolet light detection: a function of scorpion fluorescence. Journal of Arachnology 38:441-445.

Machan, L. 1968. Spectral sensitivity of scorpion eyes and the possible role of shielding pigment effect. Journal of Experimental Biology 49:95-105.

Pavan, M. 1954. Presenza e distribuzione di una sostanza fluorescente ne tegumento degli scorpioni. Bollettino di Societa Italiana Biologia Sperimentale 30:801-803.

Polis, G.A. 1979. Prey and feeding phenology of the desert sand scorpion *Paruroctonus mesaensis* (Scorpionida: Vaejovidae). Journal of Zoology 188:333-346.

Wankhede, R.A. 2004. Extraction, isolation, identification and distribution of soluble fluorescent compounds from the cuticle of scorpion (*Hadrurus arizonensis*). M.S. thesis, Marshall University, Huntington, Virginia.

Zwicky, K.T. 1968. A light response in the tail of *Urodacus*, a scorpion. Life Sciences 7:257-262.

Zwicky, K.T. 1970. The spectral sensitivity of the tail of *Urodacus*, a scorpion. Experientia 26:317.