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RESEARCH NOTE

ESCAPE BEHAVIOR MEDIATED BY NEGATIVE PHOTOTAXIS IN THE SCORPION *PARUROCTONUS UTAHENSIS* (SCORPIONES, VAEJOVIDAE)

Desert grassland scorpions, *Paruroctonus utahensis* (Williams 1968), are nocturnal, sand-dwelling arachnids that inhabit relatively open, easily accessible sand dune systems. They maintain individual home burrows from which they emerge at night to hunt and to which they return. In general, scorpions can orient in low light levels (Fleissner 1977b) and may be less active during a full moon (Warburg & Polis 1990).

Both positive and negative responses to light have been observed for a large number of arthropods, yet little is known about the effect of light on scorpion behavior. In an early study of vinegaroons (Uropygids), another group of arachnids, tests on photoreceptive behavior provided evidence of a negative phototactic response (Patten 1917, 1919). One of the first studies to examine this behavior in scorpions was by Abushama (1964), who used a simple two-choice behavioral test chamber with illumination directed from the side and found that the scorpion *Leiurus quinquestriatus* Erenberg 1828 (Buthidae) exhibits a negative phototactic response. Subsequent researchers (Jander 1965; Torres & Heatwole 1967; Zwicky 1970b) examined orientation behavior in several arthropods, including scorpions, and observed negative phototaxis. Apart from these studies and the effect of photoreception on circadian activity patterns (Fleissner 1977a, 1977b, 1985, 1986), the influence of light on scorpion behaviors, such as visually guided orientation, has received little attention.

Most scorpions have two sets of eyes, a medial pair and a lateral set, on their dorsal prosoma. The medial eyes are paired structures located on either side of a mid-sagittal plane through the carapace. The lateral eyes are located along the anterolateral margin of the carapace, can number from 0–5 (3 in *P. utahensis*) and usually, but not always, occur in equal numbers on the two sides (Hjelle 1990). Several studies have shown that the medial eyes have greater visual acuity and spatial discrimination, but lower absolute sensitivity, than the lateral eyes (Machan 1967, 1968; Fleissner 1974, 1977b). The average sensitivity of 8.6×10^{-7} candles·m^{-2}, suggested for the lateral eyes of *Androctonus australis* Linnaeus 1758 (Buthidae), rivals the sensitivity of the moths of the genus *Ephestia* (Fleissner 1977b).

We have observed that when *P. utahensis* are disturbed outside their burrows they often do not immediately return to their burrows, but rather run toward nearby bushes. Given that scorpions orient in extremely low light levels, and that they possess highly sensitive eyes, we consider here whether these animals may be using visual information to guide such escape movements.

In simulated natural habitats in the laboratory, we have been able to elicit escape behavior (a movement to the arena wall within 20 seconds of being dropped into the arena center) in *P. utahensis*. In this study we used a two-choice experimental apparatus, under both infrared and visible light conditions, and found that scorpions use visible light to escape toward dark regions.

The animals used in this study were collected at night in early 1997 from sandy regions south of El Paso, Texas. Voucher specimens (voucher #626) of *P. utahensis* used in this study have been deposited (by EAC) at Texas A & M University, Department of Entomology Insect Collection, College Station, Texas. Animals were measured, weighed, sexed and maintained in 3.8 liter glass jars containing 250 ml of sand collected from the animals' natural habitat. Animals were maintained and experiments conducted in the animal laboratory facilities at the University of Oklahoma under constant temperature and humidity (22 °C, RH 55–65%). The testing room was photoperiod reversed to allow for more convenient observations and video recording. The light-dark cycle was set as follows: dark 0840–1830 h and light 1830–0840 h. Animals were fed two small live crickets (*Acheta domestica*) per week and misted with water (20 ml/animal) twice a week.

The testing chamber consisted of a round acrylic plastic (Plexiglas™) arena approximately 15 cm tall and 76 cm in diameter. The floor of the arena was covered with 800 ml of autoclaved sand (20 minutes at 130 °C). Encircling the arena was a posterboard shield measuring 74 cm tall and 85 cm in diameter; the part of the shield surrounding one half of the arena (the ''dark'' side) was black and the part surrounding the other half of the arena (the ''light'' side) was white. The height of the shield was designed to limit the radius of a test animal's aerial visual field. Test chambers were monitored by a low-light, infrared-sensitive camera (Panasonic CCTV camera, model #WV-BP314) mounted 1.83 m above the arena center. The camera was necessary to score an animal's choice during infrared light (IR) trials (the animal was not visible to the experimenter) but not necessary in the white light (WL) trials in which just enough light was available for the experimenter to score the trial. The camera was also needed to produce video records for analysis. A television monitor, used to view IR experiments via the low-light camera, was positioned outside the testing room. A voltage meter (Micronta Digital Multimeter 22-185A) and broad-band solar cell (Radio Shack, silicon solar cell 2 × 4 cm, max ratings 0.55 V, 0.3 amp) were used to ensure that WL and IR intensities remained constant and that IR intensity remained at least 10× the WL intensity. The WL (Radio Shack Krypton minilamp, model #272-1150, 2.5 V, 0.430 amp) and IR (Ultrac IR-50FL, 50 W) light sources were centered above the arena at a height of 1.83 m and gave off an illumination level of 0.25 lux (measured using a Pasco scientific photometer, model 9152, calibrated to a 2700 °K tungsten filament lamp). Black felt was hung over the top of the apparatus to reduce overhead visual cues.

To test the effect of visible light on the escape behavior of *P. utahensis*, 20 animals were randomly assigned to one of two groups, A and B (5♂ and 5♀ in each group). Group A was exposed to white light (WL) first and infrared light (IR) second and group B was exposed to IR first and WL second. To eliminate bias in the direction of placement, animals were dropped into the arena center by means of a cylindrical tube (13 cm long and 5 cm in diameter). If an animal did not move within approximately one minute it was removed from the arena and re-dropped. Animals were scored based on their first contact with the arena wall: '1' for contact with the dark side, '0' for contact with the light side. Once a movement was observed and scored, the scorpion was removed from the arena and returned to its jar and the substrate on the arena floor was mixed to eliminate chemical cues that could be used by subsequent animals. Halfway through each light regime (after 25 drops) the sand substrate was removed and fresh substrate was spread on the arena floor. Before each drop, the arena and shield were rotated 45° independently (arena counterclockwise, shield clockwise) so that no animal encountered the same arena/shield orientation twice. Each animal was tested five non-consecutive times per light regime (approximately 20–30 minutes between each drop) and given at least three days off between the two light treatments. This set of tests was conducted over a two-week period in January 1998 between the times of 0900–1700 h CST.

In order to have video records of movements, the above experiment was repeated under the same conditions except that IR was added to the WL. Groups were reduced to $n = 5$ (3♀, 2♂) due to the limited number of healthy animals available from the first experimental group. These tests were conducted over a two-week period in February–March of 1998, and daily video recording times were as in the first experiment.

For both experiments, the behavioral scores of animals, based on their five drops, were summed and averaged. Scores for each treatment group were compared to a theoretical random score of 2.5 (no light-dark preference) using Wilcoxon's Signed Rank Test.

To verify that animals were responding to the light conditions of our experiment and not to other sensory cues, such as geomagnetic information or chemical trails, the video records of the second experiment were reviewed and the animals' initial wall contacts were plotted in three ways: relative to the shield, relative to the arena and relative to the room. These contacts were transcribed to transparencies and then to computer-generated plots for analyses. We calculated the mean vectors (Batschelet 1981) for each animal ($n = 5$, five trials) and the average vector for the test group ($n = 10$, 10 animals). Vectors were calculated in this manner to permit consideration of the data as independent measures. The Rayleigh test for randomness was used to determine the statistical significance of the mean vector for each group (Batschelet 1981).

There was considerable variability in the behavior of animals in our experiments. Some animals moved quickly under both WL and IR light treatments, reaching the arena wall within 5 sec, while others took several minutes to reach the wall. The distance traveled was also variable; some scorpions took a direct route to the dark or light side while others had a more circuitous route. Only 15% of the animals needed to be re-dropped before they initiated escape behavior.

In experiment 1, twenty animals were scored as they made initial contact with the arena wall. The order in which animals were exposed to light (i.e., IR or WL first) did not affect scorpion behavior, and the results for the two groups of animals were therefore combined. The averaged scores for animals in each light treatment group were compared to a hypothetical random score of 2.5. Under WL, animals showed a significant preference for the dark region of the arena ($\bar{x} = 4.18 \pm 0.28$ SE, $P < 0.01$), whereas under IR, the animals did not exhibit a preference ($\bar{x} = 2.65 \pm 0.42$ SE).

The second experiment utilized WL + IR as the visible light source and IR served as the non-visible light source. The scores from this experiment were processed as in the first; again, there was a statistically significant preference for the dark side under WL + IR ($\bar{x} = 3.70 \pm 0.21$ SE, $P < 0.01$) but not IR alone ($\bar{x} = 2.10 \pm 0.43$ SE).

In the second experiment we recorded scorpion movements under both light treatments using the low-light, IR-sensitive camera. Using the video records we noted the animals' initial wall contacts and plotted these in three different ways: relative to fixed points on the black and white shield, to fixed points in the arena, and to fixed points in the room. When plotted relative to a fixed point on the shield, the data reveal a distinct preference toward the dark side under WL + IR ($P < 0.05$) but not under IR. In contrast, the initial wall contacts did not show any apparent distribution relative to fixed points in the arena or room (Fig. 1).

Our study presents strong evidence that *P. utahensis* can use light to orient. When provided with a choice between black and white sides of an arena, animals demonstrated a significant preference to move toward the dark region under WL but showed no significant preference for either region under IR.

Researchers have previously established negative phototaxis (Angermann 1957; Abushama 1964; Jander 1965; Torres & Heatwole 1967) for several species of scorpion. Scorpions are also known to have chemosensory organs which might contribute to orientation (Abushama 1964; Foelix & Schabronath 1983; Gaffin & Brownell 1992, 1997). We evaluated other cues that were potentially available for orientation by plotting initial wall contacts relative to fixed points in the arena and room. In both cases, initial wall contacts were not significantly grouped along the arena wall, suggesting that neither chemical cues from previous trials nor geomagnetic forces played a role in the scorpion's orientation behavior in our study.

The photoreceptors responsible for mediating the orientation responses we have observed are unknown, but possible candidates include the medial and lateral eyes and metasomatic (tail) photoreceptors. Previous researchers (Zwicky 1970b; Geethabali & Rao 1973) have shown that scorpions with their eyes masked could still orient to darkened regions of test chambers, thus suggesting that the tail photoreceptors are sufficient to guide this behavior.

We found no indication of negative phototaxis under IR illumination which is in line with physiological responses recorded by previous researchers who examined spectral sensitivity in various species of scorpion (Machan 1968; Geethabali & Rao 1973; Fleissner 1985; Zwicky 1968, 1970a). The medial and lateral eyes have been shown to have a peak response in the blue-green (450–500 nm) region of the spectrum, with the lateral eyes

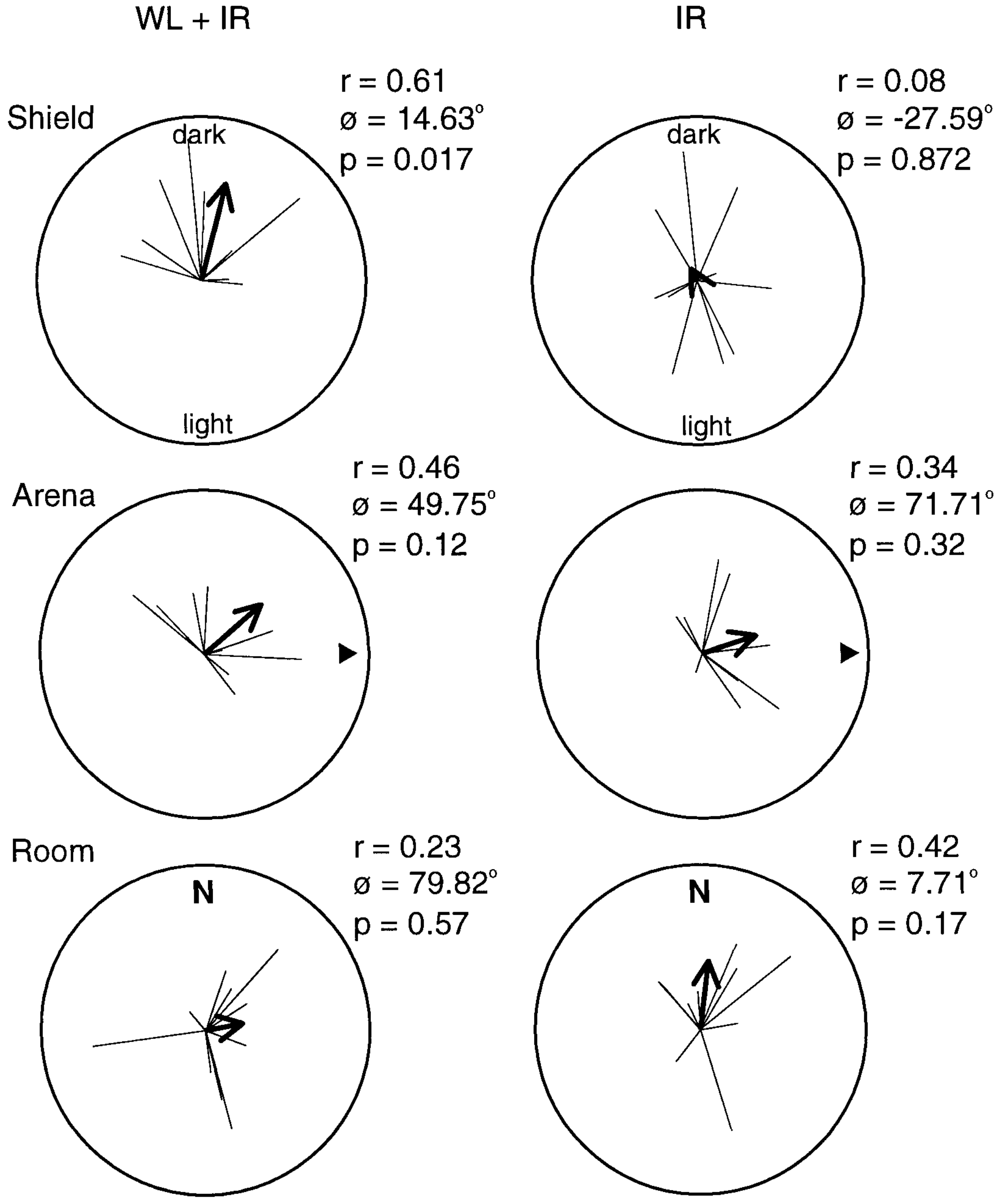

Figure 1.—Scorpion orientation behavior under white light plus infrared light (WL + IR) and infrared light (IR). Animals' initial wall contacts have been plotted relative to the shield, the arena and the room. In each plot, the thin lines indicate the mean vector for each animal, while the thick arrow indicates the mean vector for each group. The light and dark halves of the shield, the arbitrary reference point within the arena (▶) and the northerly direction (N) in the room are noted. *Abbreviations:* r = mean length of vector, ϕ = mean angle of vector and *P* = probability based on Rayleigh tests for randomness.

showing an additional peak in the long ultraviolet (300+ nm) region (Machan 1968). The metasomatic photoreceptors also show sensitivity in the same spectral range (300–500 nm) (Geethalbali & Rao 1973; Zwicky 1970b). Furthermore, it is interesting to note that scorpion cuticle fluoresces green under ultraviolet illumination peaking around 450–500 nm (Fasel et al. 1997). Taken together, it is tempting to offer the hypothesis that scorpion cuticle is acting as a light amplifier and that animal movements in our experiments may have been directed by the neural integration of light intensity across the animal's entire cuticular surface.

In this study we have presented evidence of a distinctive, naturalistic behavior that could be used as an assay to resolve some longstanding questions concerning scorpion vision. In particular, this assay could be used to determine which types of photoreceptors are required for orientation and the light intensity threshold necessary for the occurrence of this behavior.

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