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# Light wavelength biases of scorpions

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Scorpions are negatively phototactic animals, and physiological data suggests that their photoreceptors are differentially sensitive to light wavelengths ranging from red to ultraviolet. However, behaviour modification resulting from exposure to different wavelengths has not been established. We monitored behavioural responses of animals in small circular arenas while they were presented with different wavelengths of light (red, green, UV, or no light) matched for intensity. In the first experiment using desert grassland scorpions, *Paruroctonus utahensis*, half of each arena received the light treatment, while the other half was shaded. The results indicated that the amount of time that scorpions spent on the light-exposed side varied depending on the treatment and that avoidance was greatest for UV light followed by green light. In subsequent experiments using both *P. utahensis* and striped bark scorpions, *Centruroides vittatus*, the entire arena was subjected to the particular light wavelength while animal locomotory activity was monitored. We found no significant difference in animal responses to randomized, sequential 30 min presentations of all four light treatments. Scorpion activity was greatest during the first 10 min of the 30 min trials; in the first 5 min period, the highest activity levels were in the UV light treatments, followed by the green light treatments. In behavioural tests to either green or IR light, animals moved sporadically and significantly faster under green light compared to IR light treatments. Taken together, we conclude that different wavelengths of light affect scorpion locomotory behaviour differently.



*Keywords*: *Centruroides vittatus*; fluorescence; light; orientation; *Paruroctonus utahensis*; scorpion; sensory; vision

Sand scorpions are nocturnal predators that emerge from the seclusion of their burrows to hunt and mate under very low light conditions (Polis 1979, 1980). These animals have an impressive collection of well-adapted sensory structures that allow them to effectively detect and track cues in the dark. They use large, mid-ventral, chemosensory appendages, called pectines (Ivanov & Balashov 1979; Foelix & Müller-Vorholt 1983; Gaffin & Brownell 1992, 1997), along with tarsal taste hairs (Foelix & Schabronath 1983; Gaffin et al. 1992) to detect traces of substrate-borne chemical cues. Small pits called tarsal organs on the dorsal aspect of each tarsus are responsive to humidity changes and perhaps aid in locating water sources or detecting moisture gradients associated with their home burrows (Gaffin et al. 1992). Basitarsal compound slit sensilla and mechanosensory hairs on each leg allow scorpions to detect and locate the source of substrate vibrations produced by small arthropod prey (Brownell 1977). In addition, small, constricted hairs on their pedipalps, called trichobothria, are responsive to very near-field movements and allow scorpions to precisely locate their prey during capture (Hoffmann 1967).

However, of all scorpion sensory systems, vision has received limited attention for its potential role in orientation. Considerable work exists on the circadian activity of scorpion eyes relative to changing intensities of light (Fleissner 1974, 1986). Some studies have suggested the existence of an extraocular light sense on the scorpion tail (Abushama 1964; Zwicky 1968, 1970a, b; Rao & Rao

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1973). Camp & Gaffin (1999) used a behavioural assay to explore the scorpions' negative phototaxis in relation to a potential escape behaviour. However, little is known about how various light properties affect scorpion orientation behaviour within their natural environments.

Like many arthropods, scorpions have the ability to detect ultraviolet light (Machan 1968; Fleissner & Fleissner 2001). Arthropods use UV light during orientation (Brines & Gould 1982), navigation (Duelli & Wehner 1973), prey capture (Craig & Bernard 1990) and mate attraction (Lim & Li 2006). Curiously, the exoskeleton of all scorpions fluoresces green when exposed to UV light (Brownell 2001), and two of the responsible chemicals have been identified (Stachel et al. 1999; Frost et al. 2001). However, the function (if any) of scorpion fluorescence is still an open question. Some studies suggest that nocturnal animals use the natural shift of light towards shorter wavelengths during dusk and into the night as a method of synchronizing their activities as well as achieving better visual acuity (Nordtug & Melø 1988). Since there is a greater proportion of UV light during the night, it is possible that scorpions use their fluorescent exoskeleton to gather more information about their environment, recognize each other, or to avoid predators that use shorter wavelengths of light to find their prey.

Most scorpions have eight eyes categorized into two types: median and lateral (Hjelle 1990). Their median eyes have lenses and a daily oscillation of light intensity sensitivity, and may be capable of image formation. In contrast, the optical properties of the lateral eyes suggest that they detect only changes in light intensities (Schliwa & Fleissner 1980). Both sets of eyes are sensitive to low-light, nocturnal conditions. Neural responses have been recorded from scorpion median eyes using light stimuli comparable to moonless, starlight conditions (Fleissner 1977a, b, 1985; Fleissner & Fleissner 2001). Both sets of eyes have peak neural sensitivity to green light (about 500 nm; Machan 1968; Fleissner & Fleissner 2001). However, a plateau of sensitivity that is 60% of the peak electroretinogram frequency to green exists in the UV range of wavelengths (from 400 down to 350 nm). The researchers found no detectable neural response in the red and infrared regions (above about 675 nm) of the spectrum.

We developed a behavioural assay using the scorpion's natural negative phototaxis behaviour to determine whether a spectral light bias exists. We examined the behaviour of two species of scorpions while varying light wavelength. In the first experiment, we used a light shelter to mimic the use of burrows by *Paruroctonus utahensis*; however, many of the scorpions under the shelter did not move, so we removed the shelter in subsequent experiments. In the second experiment, we compared the behaviour of *Centruroides vittatus* scorpions in open arenas illuminated with three different wavelengths of light. In the third experiment, we compared two wavelengths that previous research indicated were the most physiologically different. In an attempt to reduce variation, we also dark-adapted the animals and reduced the intensity of the light treatments. However, these changes were not effective, so in experiments 4 and 5, we returned to the previous light levels and did not dark-adapt the animals. We used *C. vittatus* and *P. utahensis* in these two final experiments, which apparently represent the best testing conditions for both species of scorpions. We expected a behavioural difference upon exposure to different wavelengths of light of equal intensity. Our results show behavioural sensitivity to both UV and green wavelengths as compared to red light and infrared.

## GENERAL METHODS

### Animals

We used adult desert grassland scorpions, *P. utahensis*, collected from sandy areas east of Kermit and Monahans, Texas, U.S.A., in experiments 1 and 5. We used adult striped bark scorpions, *C. vittatus*, collected from a field within the deciduous forested area on the south side of Lake Thunderbird in Norman, Oklahoma in experiments 2–4. We kept the scorpions in 2-litre circular glass jars filled with 600 ml of sand. We moistened the sand with distilled water twice a week and fed each scorpion one cricket (*Acheta domesticus*) every 2 weeks. We changed the sand when its water-absorption capacity diminished significantly. We kept the *C. vittatus* jars partially covered with aluminium foil to retain moisture. The air temperature and humidity were kept constant at 24 °C and 60% RH, respectively, and the room lights were kept on a 15:9 h light:dark cycle to simulate summer photoperiod.

### Behavioural Apparatus

We constructed a polyvinyl chloride (PVC) frame to support a clear, rectangular (76 × 76 × 1 cm) Plexiglas stage (Fig. 1). Between the PVC frame and the stage were threaded metal rods that screwed into the top of each leg of the PVC frame. Turning these rods adjusted the height and the level of the stage. The stage contained 16 holes, 12.7 cm apart, arranged in a 4 × 4 grid.

The behavioural chambers of experiment 1 consisted of 8.75 cm diameter clear plastic petri dish bottoms fastened with black electrical tape to an identical, overturned petri dish bottom. For experiments 2–5, we used 8.75 cm diameter petri dish bottoms, each with a smaller petri dish bottom (5.40 cm diameter) glued to its inside centre and a petri dish lid to enclose the chamber. We covered the outside walls with black electrical tape.

In all experiments, PVC tubing (10 cm diameter × 15 cm height) was placed around each petri dish (Fig. 1b, c). Double layers of black plastic, sealed with strips of black electrical tape, wrapped each of the PVC enclosures. Each enclosure had a square piece of black Plexiglas (10 × 10 × 1 cm) completely covering the top and sealed to the walls with black silicon glue. A hole drilled into the centre of each square allowed the mounting of a light-emitting diode (LED). The LEDs were held by individual 8-pin integrated circuit (IC) sockets soldered to square perforate boards. The boards had wood posts that rested on the black Plexiglas covers, and rubber bands held the devices in place. We used a photocell (HOBO Light Intensity Data Logger) attached to a laptop

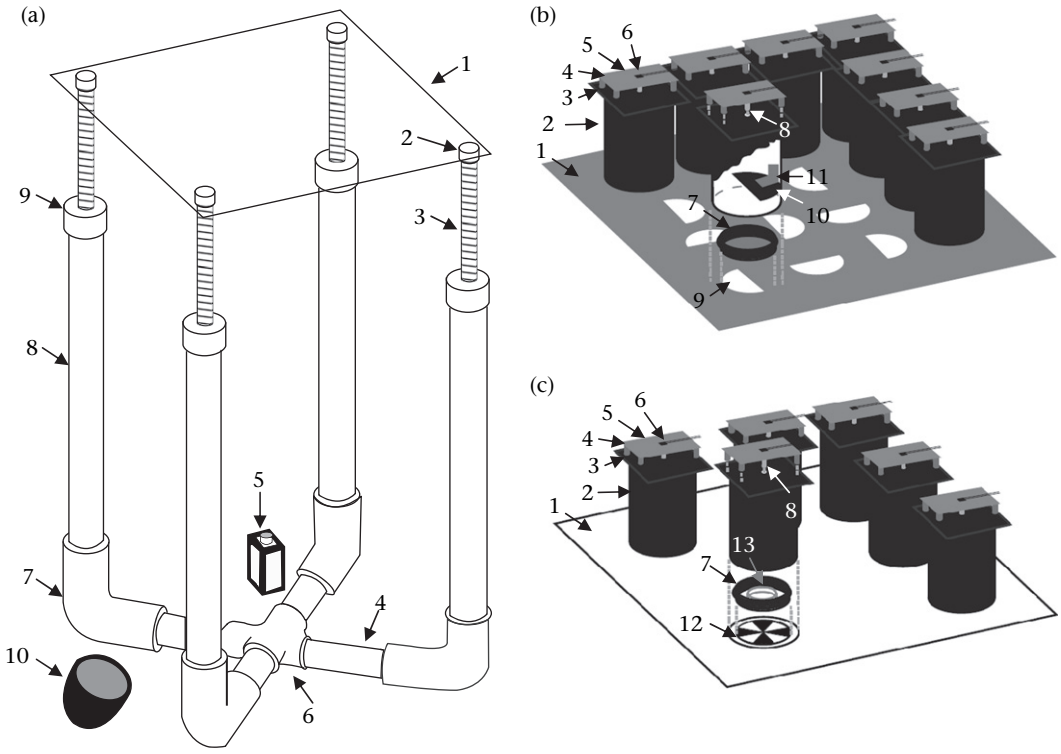


**Figure 1.** (a) Platform supporting frame of the behavioural apparatus used to test scorpions' sensitivity to light wavelengths in experiments 1–5: (1) Plexiglas platform, 76 × 76 cm; (2) PVC flat top cap; (3) 60 × 1 cm threaded rod; (4) 9 cm diameter PVC pipe; (5) infrared camera; (6) 9 cm diameter PVC 'X' connector; (7) 9 cm diameter PVC 'L' connector; (8) 60 cm height × 9 cm diameter PVC pipe; (9) 9 cm diameter PVC end cap; (10) infrared spotlight. Behavioural chambers for experiment 1 (b) and experiments 2–5 (c): (1) clear Plexiglas platform with black paper cover (b) and without a paper cover (c); (2) 10 cm diameter × 15 cm height PVC tube wrapped with two layers of black plastic (b, c); (3) 1 × 11 × 11 cm black Plexiglas (b, c); (4) 1.25 cm height × 1 cm diameter wood post (b, c); (5) 6 × 10 cm perforated circuit board (b, c); (6) two wires soldered to separate pins of an eight-pin IC socket underneath the perforated board (b, c); (7) scorpion petri dish container wrapped in black electrical tape (b, c); (8) 5 mm LED (b, c); (9) half circle cutout from the black paper platform cover (b); (10) half circle 10 cm diameter black paper (b); (11) Black electrical tape (b); (12) printed design on white paper with wedges cut out of the paper (c); (13) 5.40 cm diameter petri dish (c).

computer to determine and calibrate the light intensities of the LEDs.

In experiment 1, we wired each LED to one of two circuit boards. We connected each pair of wires to 680 Ω resistors in parallel and all were in series with a 1000 Ω variable resistor. For experiments 2–5, four independent circuit boards regulated the intensity of each light treatment. Each circuit board had a 1000 Ω variable resistor attached to eight 480 Ω, 0.5 V resistors attached in parallel. We attached the other end of each resistor to separate pins on one side of an IC socket. We joined and attached the pins on the other side of the socket to the variable resistor. Each circuit board attached to separate 12 V power sources of the power supply.

An infrared camera (Panasonic WV-BP314) focused on the stage was positioned 120 cm below the stage. We positioned on the ground an infrared spotlight (Ultrank UL-IR-50-FL) to the side of the PVC frame to illuminate the entire bottom of the stage. The camera connected to a television monitor located in an adjacent room to minimize light contamination of the experimental set-up. In experiment 1, we sent the signal to a time-lapse recorder (Panasonic AG-RT600). In experiments 2–5, we sent the signal to a laptop computer by way of a USB capture card to record video of the scorpions' movements in mpeg 1 format using the software included with the capture card.

## Procedure

Before each recording, we plugged the appropriate colour LED into every IC socket and visually tested for light. We wiped each petri dish with 70% ethanol and allowed it to dry. We then placed the PVC light enclosures over their designated petri dishes and turned on the TV, recording system, infrared lights and infrared camera. After each trial, we returned the scorpions to their glass jar habitats and turned off all electrical equipment. We chose nonparametric statistics in our experiments because of the high variability observed in scorpion response to light conditions in previous studies (Camp & Gaffin 1999).

## Analysis

We used the Friedman test in experiments 1 and 2 because the scorpions were exposed sequentially to all

treatments. We did not reuse the scorpions in experiments 3–5, so the Mann–Whitney *U* test was used instead.

## Experiment 1

### *Behavioural apparatus*

We covered the top of the stage with paper that was black on top and white on the bottom (Fig. 1b). We cut a half circle (3.81 cm radius) out of the paper around each hole; the half circles alternated in four orientation patterns. We arranged 16 petri dishes, each with a Plexiglas rod (0.30 cm diameter × 2.50 cm length) extending from their bottom centres, on the stage by threading their rods through the corresponding holes on the stage. We attached a semicircle piece of black construction paper (10.16 cm radius) to the inside of each PVC enclosure using black electrical tape so that it rested on the petri dish cover and was positioned directly over the cut out portion of the paper on the stage. Three colours of LEDs (3 mm diameter) were used as three of the four light treatments: red (630 nm), green (525 nm) and UV (405 nm), with the fourth being no light.

### *Procedure*

This study had three trials, each one using a different set of 16 scorpions (*P. utahensis*, all female), with each trial lasting four consecutive nights. We used a different treatment for each night within a trial. All trials had infrared light (from the spotlight below), but the first night did not have any other light sources and was used as a control. The second night, one of the three treatment lights was used, a different treatment for each trial. On the third and fourth nights, eight scorpions received a different treatment from that of the other eight. In this way, we used every possible treatment by the end of the study, thereby reducing the bias associated with presentation order (Table 1). We adjusted the variable resistors to regulate all 16 LEDs to a common intensity (0.9 lx) as gauged by the photocell.

Three and a half hours after the room lights turned off, we transferred the 16 test animals to their petri dish arenas and placed the arenas on the Plexiglas stage by sliding their attached rods into the stage holes. We turned the tape recorder on 4 h after lights off. We quickly moved each scorpion to the light-exposed side of their individual arenas by turning the rods under the Plexiglas stage and checking their placement with the TV monitor in the adjacent room. Once every scorpion was on the light-exposed side, we turned the treatment lights on and recorded the scorpions behaviour for 1 h.

**Table 1.** Treatment order for experiment 1

| Day | Trial 1 | Trial 2 | Trial 3 |
|---|---|---|---|
| 1 | None | None | None |
| 2 | Red | Green | UV |
| 3 | Green or UV | UV or Red | Red or Green |
| 4 | UV or Green | Red or UV | Green or Red |

### *Analysis*

We used the video recordings to determine the time that each scorpion spent on the light-exposed side of its arena, 30 min after the LED treatment started, and during a 10 min sample period that began with the first time it entered the treatment side (scorpions on the treatment side at the 30 min mark were required to exit and return to the treatment side to begin their sample). In this and all subsequent experiments, we used the point where the scorpion mesosoma joins the metasoma to determine position. For statistical analyses, we excluded all bouts of inactivity and all excursions into the treatment side that lasted fewer than 10 s. We used the Friedman test in SPSS (Chicago, IL, U.S.A.) to analyse the data.

## Experiment 2

### *Behavioural apparatus*

In this and subsequent experiments, a circular paper stencil divided into eight segments of alternating open and closed areas (Fig. 1c) was taped to the bottom of each arena to block light interference to the camera from the LEDs and to serve as markers for quantifying scorpion activity. Four LEDs (5 mm, 30° viewing angle) were used: infrared (880 nm, 20 mW/sr), red (630 nm, 8000 mcd), green (525 nm, 7500 mcd), ultraviolet (405 nm, 20 mW/sr). We calibrated each LED to 0.9 lx light intensity using the photocell.

### *Procedure*

This study had three trials, with each trial using a different set of eight scorpions (*C. vittatus*: 4 males, 4 females). Each trial was done on a separate day and lasted no more than 3 h. We exposed each scorpion to its own unique presentation order of lights, thereby using all possible presentation orders. We put each scorpion for that night's trial in its petri dish chamber 30 min before the first light treatment. Fifteen minutes before the light treatment, we placed the chambers on the Plexiglas stage in a 3 × 3 grid formation (excluding the centre square) and placed their light enclosures over them. The chambers were oriented so that one of the dark areas on the printed paper that was attached to each chamber was pointed towards the centre of the camera's field of vision. The calibrated LEDs, connected to their circuit boards, were inserted in the light chambers and secured by rubber bands. Each treatment lasted 30 min. We exchanged the lights during a 15 min period between each light treatment. We used a flashlight with a white xenon bulb during the exchanging of lights.

### *Analysis*

We viewed the video recordings using Nero ShowTime video player. We recorded the number of times a scorpion crossed from one region into another marked by the paper stencil for each of the 30 min recordings. We analysed the data using the Friedman test in SPSS.

## Experiments 3–5

### *Behavioural apparatus*

The same apparatus as in experiment 2 was used, except only IR and green light treatments were provided. For experiment 3, the light intensities were set to 0.4 lx. For experiments 4 and 5, the intensities were increased to 0.9 lx.

### *Procedure*

We used 48 *C. vittatus* (half male, half female) in experiments 3 and 4 and 48 *P. utahensis* (half male, half female) in experiment 5. In experiment 3, scorpions were kept in complete darkness for 9 days before the experiment; in experiments 4 and 5, animals were kept in their normal light/dark maintenance conditions.

We grouped scorpions in six sets of eight, each with four males and four females. We placed scorpions into their petri dish chambers 4 h before the start of the experiment. Half of the males and females received the IR treatment, while the other half received the green light treatment in each of the six 10 min video sessions. Each stage position alternated between the sexes through the six groups. Each 10 min period was followed by a maximum of 5 min to set up the next group of scorpions. We used low levels of red light when setting up the experiment.

### *Analysis*

We used QuickTime to review the recordings and note the exact time of line crossings during the 10 min sessions. We transformed the records to determine the frequency of intercrossing intervals (by 1 s intervals) for each record. We excluded Scorpions that did not meet a minimum of five line crossings and all intercrossing intervals (ICI) greater than 90 s from the analyses. We made statistical comparisons between treatments of the mean, median and mode intercrossing interval frequency using the Mann–Whitney *U* test.

## RESULTS

Scorpions displayed a wide range of activity levels throughout all recordings in these experiments. Some animals moved frequently and continuously, while others did not move at all. Both *P. utahensis* and *C. vittatus* adapted well to the test chambers and moved in what appeared to be normal, nonriled movements. In general, *P. utahensis* was more active in the test chambers than *C. vittatus*. No significant difference was observed between the activity levels of males and females (Mann–Whitney *U* test: experiment 2: $U = 55$, $N_1 = N_2 = 12$, $P = 0.347$; experiment 3: $U = 83$, $N_1 = 12$, $N_2 = 14$, $P = 0.98$; experiment 4: $U = 44$, $N_1 = 11$, $N_2 = 9$, $P = 0.710$, experiment 5: $U = 183.5$, $N_1 = 16$, $N_2 = 23$, $P = 0.989$).

## Experiment 1

In experiment 1, half of each arena was exposed to the light treatment while the other half was sheltered. Some animals ventured into the light-exposed side often and throughout the recording, while others displayed no activity for most of the 30 min. This variability created highly skewed data that limited the statistical analysis. Despite this variation, the scorpions' behaviour differed significantly between treatments (Friedman test: $\chi^2_3 = 11.603$, $P = 0.009$). Scorpions spent the least time in the treatment side with UV, slightly more time with green light, and the most time with red light or no light (Fig. 2).

## Experiment 2

In experiment 2 and all subsequent experiments, the entire arena was illuminated by the LED treatment and scorpion activity was monitored based on the number of lines that the animals crossed as they moved within their circular 'track-like' arenas. Again, several scorpions did not move at all during any of the treatments, while others were active for most of the 30 min. However, the active

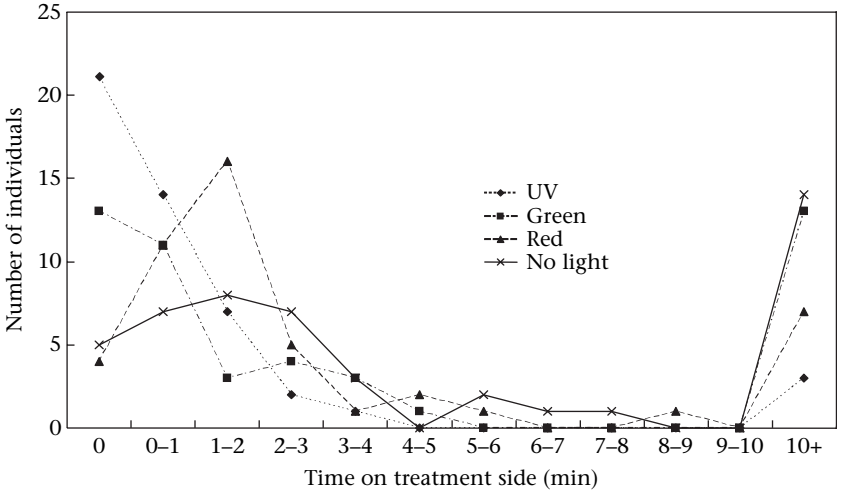


**Figure 2.** Categorization of *P. utahensis* individuals based on time spent on light-exposed side of arena in experiment 1. The 48 animals were categorized based on the number of minutes that each spent on the light-exposed side of the test chamber during the 10 min sample period.

scorpions showed a consistent decrease in movement both within the 30 min treatments and from the first to the fourth light treatment.

No significant difference was found in total line crossings for the different treatments (Friedman test: $\chi^2_3 = 6.361$, $P = 0.095$). Some patterns emerged when we parsed the 30 min treatments into 5 min increments. During the first 5 min increment, scorpions had a higher mean activity under UV ($\overline{X} = 3.8$ min) than under green ($\overline{X} = 2.3$ min), red ($\overline{X} = 1.8$ min) or IR ($\overline{X} = 0.6$ min) light. Scorpions in all treatments showed an overall decrease in activity as time progressed, and the separation of means became less pronounced across the 30 min (Fig. 3).

## Experiment 3

In experiments 3, 4 and 5, only green and IR light treatments were used and recordings were restricted to 10 min. We tested 48 *C. vittatus* in experiment 3 (24 under IR, 24 under green). The lights were set to 0.4 lx and the animals were dark-adapted for 9 days before testing. Thirteen scorpions in each group met the minimum criterion of crossing at least five lines without any ICI greater than 90 s (Fig. 4). Although the mean (IR: 11.1 + 5.3 s; green: 8.7 + 4.6 s), median (IR: 7.6 + 3.3 s; green: 6.2 + 3.9 s) and mode (IR: 6.2 + 3.1 s; green: 5.5 + 5.5 s) ICIs were greater for IR compared to green light treatments the differences were not significant (Mann–Whitney $U$ test: mean ICI: $U = 59$, $N_1 = N_2 = 13$, $P = 0.102$; median ICI: $U = 58.5$, $N_1 = N_2 = 13$, $P = 0.093$; mode ICI: $U = 52$, $N_1 = N_2 = 13$, $P = 0.051$).

## Experiment 4

We tested 48 *C. vittatus* in experiment 4 (24 IR, 24 green), however the light levels were increased to 0.9 lx and the animals were not dark-adapted as in experiment 3. Fifteen scorpions in the green light treatment group met the minimum five line-crossing criterion while only five scorpions in the IR group met the minimum movement criterion (Fig. 5). The mean (IR: 20.5 + 6.4 s; green: 13.7 + 6.1 s), median (IR: 17.1 + 4.1 s; green: 9.6 + 4.7 s) and mode (IR: 12.4 + 5.7 s; green: 4.9 + 2.9 s) ICIs were all significantly greater for IR than for green light treatments (Mann–Whitney $U$ test: mean ICI: $U = 18$, $N_1 = 5$, $N_2 = 15$, $P = 0.049$; median ICI: $U = 12$, $N_1 = 5$, $N_2 = 15$, $P = 0.01$; mode ICI: $U = 11$, $N_1 = 5$, $N_2 = 15$, $P = 0.01$).

## Experiment 5

We tested 48 *P. utahensis* in experiment 5 (24 IR, 24 green); light levels were set to 0.9 lx and the animals were not dark-adapted (similar to experiment 4). Eighteen scorpions in the green light group and 21 scorpions in the IR group met the minimum five line-crossing criterion (Fig. 6). The mean (IR: 14.8 + 7.5 s; green: 9.1 + 3.4 s), median (IR: 12.6 + 7.2 s; green: 6.4 + 3.3 s) and mode (IR: 10.7 + 10.1 s; green: 5.5 + 4.1 s) ICIs were all significantly greater for IR than for green light treatments (Mann–Whitney $U$ test: mean ICI: $U = 93$, $N_1 = 21$, $N_2 = 18$, $P = 0.003$; median ICI: $U = 67$, $N_1 = 21$, $N_2 = 18$, $P < 0.001$; mode ICI: $U = 63.5$, $N_1 = 21$, $N_2 = 18$, $P < 0.001$).

## DISCUSSION

Taken together, these results suggest that scorpion visual systems may be differentially sensitivity to various light wavelengths, and that it is possible to detect their spectral sensitivities using behavioural observations. This is the first study to show a behavioural difference in scorpions during exposure to different wavelengths of light.

Under the conditions of these five experiments, scorpions behaved differently depending on the wavelength of light to which they were exposed. In the first experiment, where scorpions were given a retreat to 'escape' the light treatment, avoidance was greater for UV and green light wavelengths compared to red and no light. This was expected since scorpions are negatively phototaxic and they have little physiological sensitivity to red light. In experiments 2–5, no retreat was offered, a circular barrier was placed in the centre of each chamber and movement was monitored (based on line crossings in the circular arena). We made these changes to restrict the behaviour of the scorpions and increase the accuracy of measurements. While no significant results were found across the 30 min trials, scorpion activity was greatest for UV and green light wavelengths compared to red and IR wavelengths at the

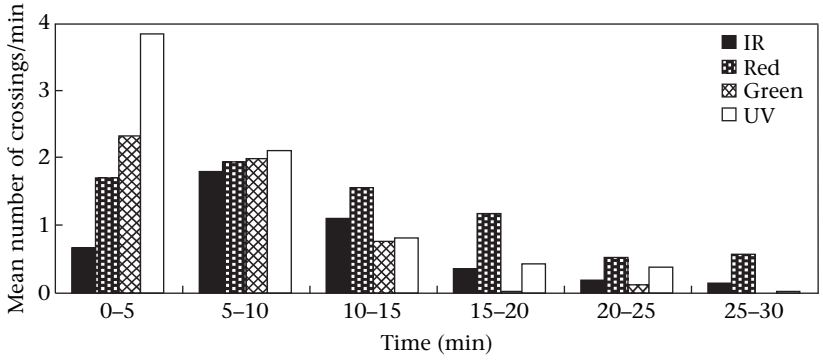


**Figure 3.** Activity level of *C. vittatus* by treatment and time of recording in experiment 2. The mean number of line crossings within each 5 min bin is shown for the 48 animals for each type of light exposure.

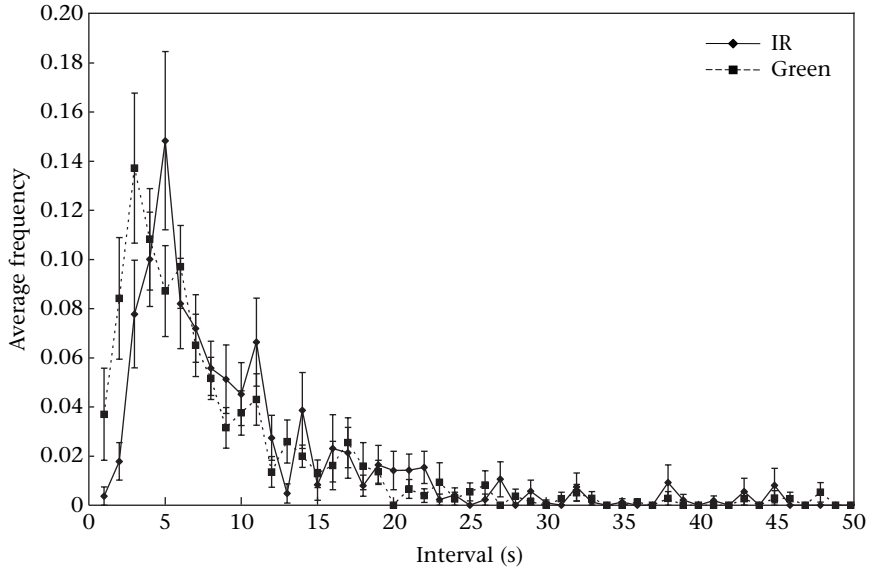


**Figure 4.** Locomotory response of *C. vittatus* to 4.0 lx IR and green light treatments in experiment 3. Shown are mean frequencies of interline intervals (±SE) for scorpions meeting the criteria in all IR and green light trials during the 10 min records.

beginning of the trials (especially in the first 5 min of the trial period). This information allowed us to cut the trial periods of experiments 3–5 to 10 min. Furthermore, we did not reuse scorpions in experiments 3–5 to reduce confounding effects of experience with the arena environment. To increase activity and reduce variability, we tried lowering light intensity and dark adapting scorpions for experiment 3. However, these changes did not improve animal responsiveness, so we returned the conditions of experiments 4 and 5 to those of experiment 2. We noticed that scorpions moved in more sporadic bursts of movement under green and UV light than under IR and red light. As such, we felt that parsing the data into intercrossing interval frequencies would better characterize the pattern that we observed between treatments. Under these conditions, we found significant differences between the mean, median and mode of the ICI frequencies of both *C. vittatus* and *P. utahensis* exposed to 0.9 lx levels of IR and green light (experiments 4 and 5). Furthermore, *P. utahensis* showed higher activity levels than *C. vittatus* under similar conditions.

Our behavioural findings are at odds with what has been reported for the scorpion's retinal sensitivities. Previous physiological studies have indicated that scorpion eyes are maximally sensitive to green wavelengths with a lesser, although pronounced sensitivity to UV wavelengths (Machan 1968; Fleissner & Fleissner 2001). Response to red and IR was negligible. In our first two experiments, peak behavioural activity occurred under UV, followed by green wavelengths. Sensitivity to red light

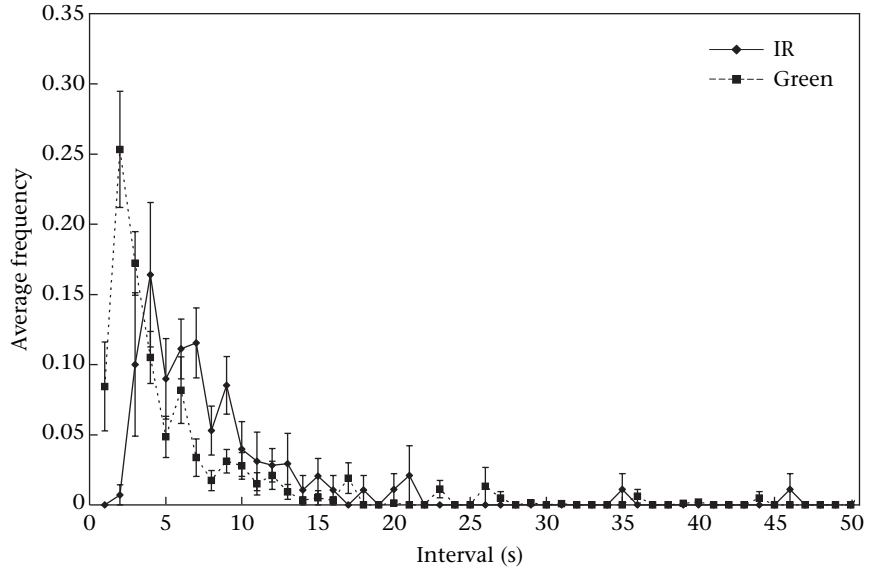


**Figure 5.** Locomotory response of *C. vittatus* to 9.0 lx IR and green light treatments in experiment 4. Shown are mean frequencies of interline intervals (±SE) for scorpions meeting the criteria in all IR and green light trials during the 10 min records.

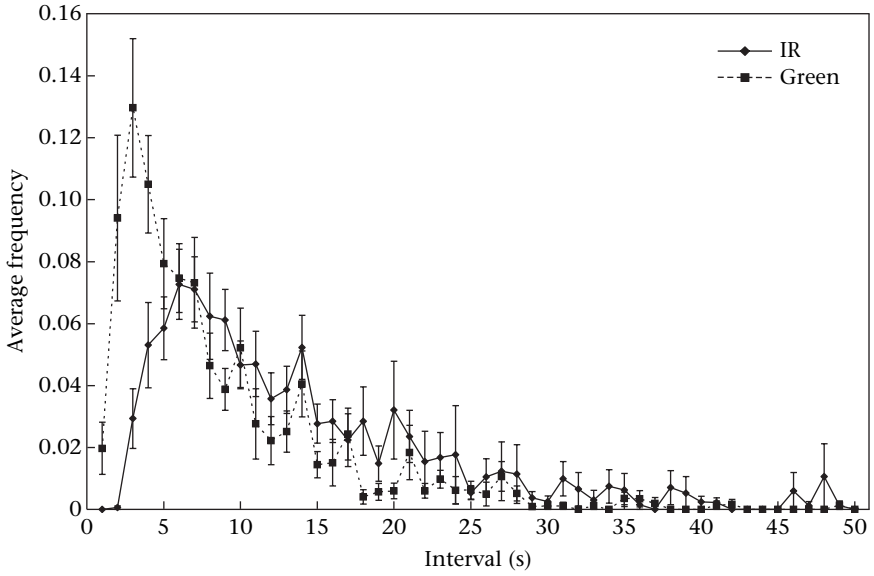


**Figure 6.** Locomotory response of *P. utahensis* to 9.0 lx IR and green light treatments in experiment 5. Shown are mean frequencies of interline intervals (±SE) for scorpions meeting the criteria in all IR and green light trials during the 10 min records.

appeared low and similar to activity in IR. Red light and IR light sensitivity may amount to essentially the same stimulus level as 'no light' to these animals. This apparent inversion of sensitivity to UV and green wavelengths in behavioural trials compared to retina physiological tests warrants further investigation. If this holds consistent, it suggests that scorpions have a method beyond retinal sensitivities for discriminating spectral wavelengths.

We detected some behavioural differences between *C. vittatus* and *P. utahensis* in our study. Under similar conditions, *P. utahensis* were more active than *C. vittatus* (especially under IR light). This is interesting because although *P. utahensis* is restricted to sandy regions, *C. vittatus* are broadly distributed and can be found within the same dunes as *P. utahensis*. When locating these animals with portable UV lamps, they are readily distinguishable: *C. vittatus* is typically found walking while *P. utahensis* is found stationary. We have considered this behaviour related to the sit-and-wait, seismic detection hunting method of psammophilic scorpions like *P. utahensis* (Brownell 1977). However, use of high intensity portable UV lamps to locate these animals may affect the behaviour of *P. utahensis* and *C. vittatus* differently.

The discrimination of different wavelengths of light could be important to scorpions in their natural habitats. Since the scorpion median and lateral eyes have only homogenous photoreceptors (Brownell 2001), discrimination of spectral wavelengths would require input from other areas of the scorpion. The exoskeleton of all scorpions fluoresce green under UV (Brownell 2001), and this phenomenon may contribute to their vision (Camp & Gaffin 1999). Scorpions may use the combination of environmental UV light and their own UV-induced green fluorescence to make them behaviourally more sensitive to UV than to green light. This could be accomplished by scorpions detecting the green fluorescence of their exoskeleton with habituation to longer wavelengths increasing their sensitivity to UV. In addition, photosensitive ganglia and unidentified tail photoreceptors (Zwicky 1968; Rao & Rao 1973) with different wavelength sensitivities (Zwicky 1970a) from the median and lateral eyes may allow the scorpion to compare intensities, thereby identifying a range of wavelengths present. The comparison of wavelengths of light would allow scorpions to synchronize their activities during the day, without requiring long-term memory necessary when using only the changing light intensity (Nordtug & Melø 1988).

The green fluorescence of scorpions may be used for more specific behavioural activities such as the detection of prey or mates, or they may use it to avoid detection from predators that use reflected UV cues to hunt. Using their peak sensitivity to green light, the scorpion's green fluorescence could increase the green light beyond the surrounding environment that matches the scorpion's peak sensitivity, thereby increasing their visibility to other scorpions as potential mates. Likewise, scorpions may be able to identify animals that are not fluorescing green as prey. First-instar scorpions do not fluoresce until after their first moult (Polis & Mohnac 1990), which may contribute to their predisposition for cannibalism. The green fluorescence of scorpions could be an adaptation for avoiding detection from prey and predators. Scorpions are sit-and-wait predators in environments that may not provide any cover for concealment. Since nocturnal animals have a sensitivity shift towards shorter wavelengths of light (Euguchi et al. 1982), having an exoskeleton that does not reflect UV but converts it to the longer wavelength of green light may provide a type of camouflage and increase the scorpion's chance of capturing prey and/or decrease detection by predators.

The unique aspects of scorpions as nocturnal predators found in a wide range of habitats with all species having a green fluorescent exoskeleton makes these animals useful organisms for a variety of further studies. The

behaviour of scorpions under different wavelengths of light can be a valuable tool when mapping the scorpion's visual system in lower and higher visual processing. Whether the curious phenomenon of UV fluorescence relates to scorpion visual perception is still unknown, but this study provides a useful behavioural assay for testing animal sensitivities to various spectral stimuli. Further manipulations, such as selective ablation studies within the context of this assay, could be useful for a comprehensive understanding of scorpion visual perception.

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## References

**Abushama, F. T.** 1964. On the behavior and sensory physiology of the scorpion *Leiurus quinquestriatus*. *Animal Behaviour*, **12**, 140–153.

**Brines, M. L. & Gould, J. L.** 1982. Skylight polarization patterns and animal orientation. *Journal of Experimental Biology*, **96**, 69–91.

**Brownell, P. H.** 1977. Compressional and surface waves in sand: used by desert scorpions to locate prey. *Science*, **197**, 479–482.

**Brownell, P. H.** 2001. Sensory ecology and orientational behaviors. In: *Scorpion Biology and Research* (Ed. by P. H. Brownell & G. A. Polis), pp. 159–183. Oxford: Oxford University Press.

**Camp, E. A. & Gaffin, D. D.** 1999. Escape Behavior mediated by negative phototaxis in the scorpion *Paruroctonus utahensis* (Scorpiones, Vaejovidae). *Journal of Arachnology*, **27**, 679–684.

**Craig, C. L. & Bernard, G. D.** 1990. Insect attraction to untraviolet-reflecting spider webs and web decorations. *Ecology*, **71**, 616–623.

**Duelli, P. & Wehner, R.** 1973. The spectral sensitivity of polarized light orientation in *Cataglyphis bicolor*. *Journal of Comparative Physiology*, **86**, 37–53.

**Euguchi, E., Watanabe, K., Hairyama, T. & Yamamoto, K.** 1982. A comparison of electrophysiologically determined spectral responses in 35 species of Lepidoptera. *Journal of Insect Physiology*, **28**, 675–682.

**Fleissner, G.** 1974. Circadiane Adaptation und Schirmpigmentverlagerung in den Sehzellen der Medianaugen von *Androctonus australis* L. (Buthidae, Scorpiones). *Journal of Comparative Physiology*, **91**, 399–416.

**Fleissner, G.** 1977a. Scorpion lateral eyes: extremely sensitive receptors of zeitgeber stimuli. *Journal of Comparative Physiology*, **118**, 101–108.

**Fleissner, G.** 1977b. The absolute sensitivity of the median and lateral eyes of the scorpion, *Androctonus australis* L. (Buthidae, Scorpiones). *Journal of Comparative Physiology*, **118**, 109–120.

**Fleissner, G.** 1985. Intracellular recordings of light responses from spiking and nonspiking cells in the median and lateral eyes of the scorpion. *Naturwissenschaften*, **72**, 46–48.

**Fleissner, G.** 1986. Die innere Uhr und der Lichtsinn von Skorpionen und Käfern; zur neurobiologischen Analyse der Circadianen Uhr der Arthropoden. *Naturwissenschaften*, **73**, 78–88.

**Fleissner, G. & Fleissner, G.** 2001. Night vision in desert scorpions. In: *Scorpions 2001; In Memoriam Gary A. Polis* (Ed. by V. Fet & P. A. Selden), pp. 317–324. Burnham Beeches, Bucks: British Arachnological Society.

**Foelix, R. F. & Müller-Vorholt, G.** 1983. The fine structure of scorpion sensory organs. II. Pecten sensilla. *Bulletin of the British Arachnological Society*, **6**, 68–74.

**Foelix, R. F. & Schabronath, J.** 1983. The fine structure of scorpion sensory organs. I. Tarsal sensilla. *Bulletin of the British Arachnological Society*, **6**, 53–67.

**Frost, L. M., Butler, D. R., O'Dell, B. & Fet, V.** 2001. A coumarin as a fluorescent compound in scorpion cuticle. In: *Scorpions 2001; In Memoriam Gary A. Polis* (Ed. by V. Fet & P. A. Selden), pp. 363–368. Burnham Beeches, Bucks: British Arachnological Society.

**Gaffin, D. D. & Brownell, P. H.** 1992. Evidence of chemical signaling in the sand scorpion, *Paruroctonus mesaensis* (Scorpionida: Vaejovidae). *Ethology*, **91**, 59–69.

**Gaffin, D. D. & Brownell, P. H.** 1997. Response properties of chemosensory peg sensilla on the pectines of scorpions. *Journal of Comparative Physiology A*, **181**, 291–300.

**Gaffin, D. D., Wennstrom, K. L. & Brownell, P. H.** 1992. Water detection in the desert sand scorpion *Paruroctonus mesaensis* (Scorpionida, Vejovidae). *Journal of Comparative Physiology A*, **170**, 623–629.

**Hjelle, J. T.** 1990. Anatomy and morphology. In: *The Biology of Scorpions* (Ed. by G. A. Polis), pp. 9–63. Stanford, California: Stanford University Press.

**Hoffmann, C.** 1967. Bau und Funktion der Trichobothrien von *Euscorpius carpathicus* L. *Zeitschrift für Vergleichende Physiologie*, **54**, 290–352.

**Ivanov, V. P. & Balashov, Y. S.** 1979. The structural and functional organization of the pectine in a scorpion *Buthus eupeus* Koch (Scorpiones, Buthidae) studied by electron microscopy. In: *The Fauna and Ecology of Arachnida. Vol. 85* (Ed. by Y. S. Balashov), pp. 73–87. Leningrad: Trudy Zoological Institute.

**Lim, M. L. M. & Li, D.** 2006. Behavioural evidence of UV sensitivity in jumping spiders (Araneae: Salticidae). *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, **192**, 871–878.

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

**Nordtug, T. & Melø, T. B.** 1988. Diurnal variations in natural light conditions at summer time in Arctic and subarctic areas in relation to light detection in insects. *Holarctic Ecology*, **11**, 202–209.

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

**Polis, G. A.** 1980. Seasonal patterns and age-specific variation in the surface activity of a population of desert scorpions in relation to environmental factors. *Journal of Animal Ecology*, **49**, 1–18.

**Polis, G. A. & Mohnac, M.** 1990. Mating by female scorpions while still carrying young. *Journal of Arachnology*, **18**, 363–364.

**Rao, G. & Rao, K. P.** 1973. A metasomatic neural photoreceptor in the scorpion. *Journal of Experimental Biology*, **58**, 189–196.

**Schliwa, M. & Fleissner, G.** 1980. The lateral eyes of the scorpion, *Androctonus australis*. *Cell and Tissue Research*, **206**, 95–114.

**Stachel, S. J., Stockwell, S. A. & Van Vranken, D. L.** 1999. The fluorescence of scorpions and cataractogenesis. *Chemistry & Biology*, **6**, 531–539.

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

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

**Zwicky, K. T.** 1970b. Behavioral aspects of the extraocular light sense of *Urodacus*, a scorpion. *Experientia*, **26**, 747–748.