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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