Can scorpions detect fluorescing scorpions?

An honors thesis by:
Andrea M. Jordan

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Approved by:
The Department of Zoology; University of Oklahoma; Norman, Oklahoma 73019

Committee members:
Douglas Gaffin, chair; Rosemary Knapp; Thomas Ray

Summary

Scientists have observed scorpion fluorescence under ultra-violet light for several decades, but the evolutionary reason for this phenomenon is still unknown. Due to their high optical sensitivity to green light, a wavelength similar to their own fluorescent hue, I hypothesized that scorpions may be able to detect and recognize the fluorescence of their conspecifics. This recognition may either lead to an avoidance of fluorescence, due to the cannibalistic tendencies of females, or possibly an attraction to fluorescence for mating purposes. To test these possibilities, I videotaped scorpion activity in clear, circular arenas. Outside of each arena was either a fluorescent box (similar in hue to natural scorpion fluorescence) or a black control box. I ran trials using both natural starlight and an artificial ultraviolet light directed only onto the box. I quantified scorpion activity by measuring the total amount of time the scorpions spent in each of the eight possible equal sections of the arena, with one randomized section adjacent to the box. For trials conducted under natural starlight, I found no significant difference between the control and experimental groups. Similarly, I found no significant difference between the control and experimental groups for artificial ultraviolet light; however, a statistically significant directional bias existed in both groups toward the direction of the box. These data suggest that scorpions do not respond behaviorally to other scorpions' fluorescence. Subsequent trials demonstrated that neither the boxes alone nor the ultraviolet light alone could elicit a significant behavioral response. Thus, I believe the ultraviolet light shining onto objects may resemble shelter for this species, which would explain the significant behavior in the lab. Consequently, the mystery of scorpion fluorescence is still unsolved, but answers may be found by expanding these experiments to different species, different times of year, and different sources of fluorescence.

Introduction

Scorpion fluorescence under ultraviolet (UV) light is a phenomenon that is full of intrigue; however, a lack of a definitive evolutionary explanation calls out for further exploration. The biological basis of this fluorescence lies in two chemicals in the outermost layer of the scorpion's exoskeleton. They include beta-carboline and 7-hydroxy-4-methylcoumarin, the latter being the same chemical found in human cataracts (Stachel et al. 1999; Frost et al. 2001). While scorpion fluorescence does not appear at its fullest intensity until the third instar, the fluorescence is permanent after this developmental stage, even after death (Stahnke, 1972).

The use of fluorescence to recognize and communicate within a species is known in the animal kingdom. For example, female jumping spiders have a UV-activated fluorescent patch that the males seek out for mating purposes (Lim et al. 2007). Fluorescence in parrot plumage is also attributed to sexual selection (Arnold et al. 2002). For scorpions, however, both males and females exhibit fluorescence, and no link indicating sexual selection has been established.

From an evolutionary perspective, the main problem is determining why these nocturnal animals fluoresce under UV light, since the sky emits significantly less UV light at night than during the day. More specifically, studies have shown that the UV irradiance of starlight compared to sunlight is approximately 1×10^{6} versus 1×10^{11} photons ($cm^{-2}\ s^{-1}\ nm^{-1}$), respectively (Johnsen et al. 2006). However, information about scorpion behavior and physiology may hold clues to this puzzle. Despite the low levels of UV light at night, evidence indicates that scorpion vision can be highly sensitive at this time and that scorpions can detect starlight (Fleissner & Fleissner 2001). It has also been shown that scorpions are behaviorally sensitive to UV and green light (Blass & Gaffin, 2008). Furthermore, different species of scorpions show a large range of intensities and wavelengths in their fluorescence (Stahnke 1972), but it appears the majority of species fluoresce a shade of green.

This fortunate coincidence could be evidence that scorpions are sensitive to the color of their fluorescence and has led us to propose that scorpions can recognize each other by their fluorescence. This ability may be advantageous for several reasons. Some species of scorpion are cannibalistic, and up to 45% of encounters between males and females result in cannibalism (Polis & Farley 1979). Avoiding conspecifics, or presumably their fluorescence, would be beneficial because it would help males reduce the deadly risk of female cannibalism or save energy by avoiding male competition. Also, the recognition of fluorescence could speed up the process of males finding mates. Thus, I have tested the hypothesis that mature male scorpions can detect and behaviorally react to a fluorescent object. The results may provide clues to the overall question of why scorpions fluoresce.

Methods

Animals

I used male adult scorpions, *Centruroides vittatus*, that I collected at Lake Thunderbird State Park near Norman, Oklahoma in September 2009. The scorpions were kept in 2-liter circular glass jars containing about 600 ml of sand. Our lab fed the scorpions one early instar cricket (*Acheta domesticus*) every week and moistened the sand with distilled water twice a week. We housed these jars under constant air temperature of 22ºC and a humidity of 72% with a 15:9 hour light-dark cycle.

Basic Experimental Setup

I performed three experiments, all with a similar set-up. I used a tub or trough filled with enough sand to cover the bottom. Inside this large container, I placed a clear, plastic, circular tube (13.5 cm diameter and 7 cm deep) to form the experimental arena. To represent a scorpion, I made a 1 x 5 x 1 cm rectangular box made of green fluorescent paper, and placed it directly outside of the arena, with the longest side laying flat and at a slight angle. Most green fluorescent paper emits a wavelength around 510 nm at its highest intensity under UV light, which is reasonably close to the 490 nm emission of the exoskeleton of *C. vittatus* under UV light (Stachel 1999) and within the wavelength range that generates maximum response from their eyes (Fleissner & Fleissner 2001).

Experiment 1

For experiment 1, I wanted to test the scorpions in their natural environment with starlight being the only source of UV light. The location of Experiment 1 was within 2 kilometers of the area where the experimental scorpions were collected. The experiment took place on the evening of October 15th, 2009, between the hours of 20:30 and 00:30 on a cloudless, moonless night with temperatures at approximately 10° C. The location was an open field that was far away from any trees or other possibly distracting landmarks. I used a 45.7-cm diameter, 35.5-cm high circular, plastic tub ith an open top and the bottom covered in sand to hold the plastic arena.

Experiments 2 and 3

Experiments 2 and 3 took place in the laboratory so I could run the same experiment in a more controlled setting. Instead of a plastic tub, I used a large 95-gallon metal trough (91 cm diameter by 61 cm deep) to hold the sand and arena. I used a 5 mm, dome-shaped, LED light that emits UV light (395 nm) wavelength (*www.mouser.com*). The light was secured at a height of 40 cm above the arena and positioned to direct light only on the box. The intensity of UV light used was stronger than starlight to elicit a behavioral response. At this height, the measured intensity of the UV light was 197 lx when measured by a Pascophotometer 9152B. In comparison, starlight has been measured at around 2 x 10^{-4} lx (Fleissner & Fleissner 2001.

Procedure

All three experiments followed a similar procedure, with slight modifications for Experiment 3 (described below). 14 scorpions were used for Experiment 1, 20 for Experiment 2, and 14 for Experiment 3. The difference in scorpion numbers is due to male deaths between experiments. These deaths were among the *C. vittatus* collected in September 2009, so other *C. vittatus* scorpions collected in March 2009 were used as replacements. Every scorpion participated in both experimental and control trials, but half were randomly assigned to be in the experimental trials first, whereas the other half began with control trials. With the exception of Experiment 1, which was performed in one evening, no scorpion participated in both experimental and control trials within the same 48-hour period.

During the experimental trials, I placed the fluorescent or the black box outside the arena and recorded its cardinal position in relation to true north. The position of the box was randomly assigned and recorded for each trial. Before a trial, each scorpion was dark adapted in an opaque film canister for five minutes. Once I began video recording, I opened the film canister and placed the scorpion inside the center of the test arena. I recorded the scorpion for five minutes and then returned it to its jar. After each trial, I stirred the sand inside the large container and wiped the inside of the circular arena with ethanol to disrupt chemical cues that might influence scorpions in subsequent trials.

Experiment 3

Based on the results of Experiment 2, I conducted additional trials to clarify whether the presence of the box or of the UV light influenced scorpion behavior. I conducted trials containing only a fluorescent or black box placed outside the arena, but with no UV light. This put the scorpions in complete darkness. I then conducted another set of trials without either type of box, but with UV light directed onto the sand outside of the arena where the box would have been placed. All 14 scorpions participated in both the box-only and UV-only trials, but 48 hours separated the two trials.

Data Analysis

In every experiment, a small number of trials were considered invalid due to technological reasons (i.e. tape unknowingly ending) or due to lack of scorpion movement during the entire 5-minute trial. For valid trials, I watched the tapes and recorded the amount of time the scorpion spent in each of 8 equal-sized sections, one of which was adjacent to the box. The total amount of time spent in each section was then averaged with all the other trials, and then expressed as a percentage of total time. This percentage represented the average percent time the scorpions spent in each section in relation to the box. For every experiment, a directional control was used to assure no orientation bias was present during the trials. I calculated this by measuring the total amount of time spent in each section in relation to Cardinal North. These data were then analyzed using the circular statistics toolbox in Matlab 2008.

Results

In all three experiments, the scorpions moved throughout the arena with no apparent overall changes in behavior. The scorpions tended to walk along the edge of arena, and only on occasion crossed its diameter. However, a change of direction was common. It is also noteworthy to mention the wall-climbing behavior that occurred with the majority of the scorpions in all three experiments.

Experiment 1

Of 28 possible trials, 11 experimental and 13 control trials were considered valid. I found that the amount of time spent near the box was not significantly different from the amount of time spent in the rest of the arena in either the experimental ($p > 0.10$) or control trials ($p > 0.10$). These results are represented in Figure 1A and 1B. The test for a directional bias in Fig. 1C was also not significant ($p > 0.10$).

Experiment 2

Of 40 possible trials, 19 experimental and 17 control trials were considered valid. In the experimental trials (Fig. 2A), I found that the scorpions spent a significantly greater amount of time in or near the section adjacent to the fluorescent box ($p < 0.005$); likewise, in the control trials (Fig. 2B), scorpions spent a significantly greater amount of time in or near the section adjacent to the black box ($p < 0.005$). No significance was found ($p = 0.10$) in the test for a directional bias (Fig. 2C).

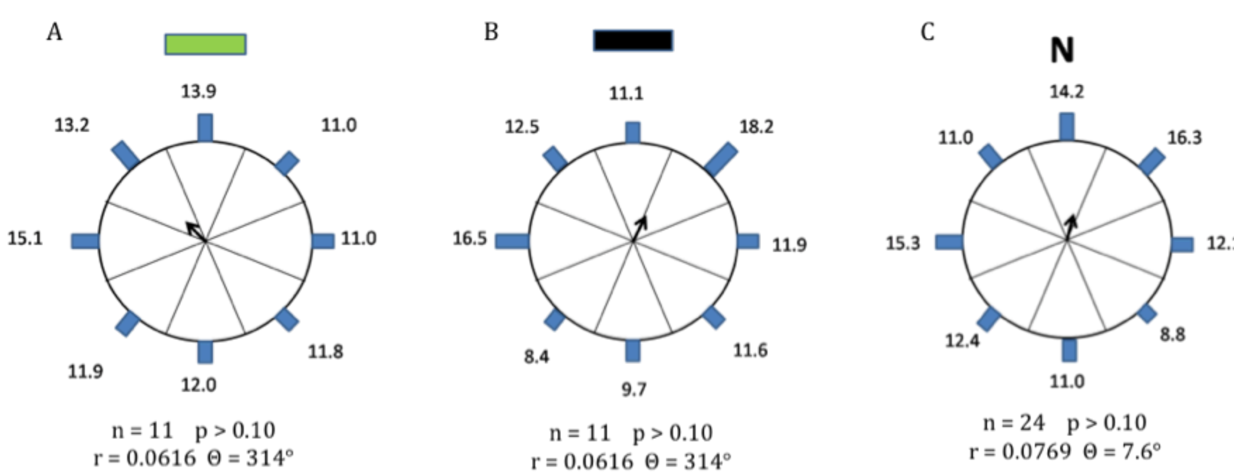

Fig. 1: Mean percentile (%) of scorpion orientation during Experiment 1. The experimental (A) and control (B) trials are shown along with directional biases (C), using the combined data of the experimental and control trials. Values in bold are considered significant. *Abbreviations*: n = the number of trials, p = the probability based on Rayleigh tests for randomness, r = the average length of the vector, and Θ = the average angle of the vector.

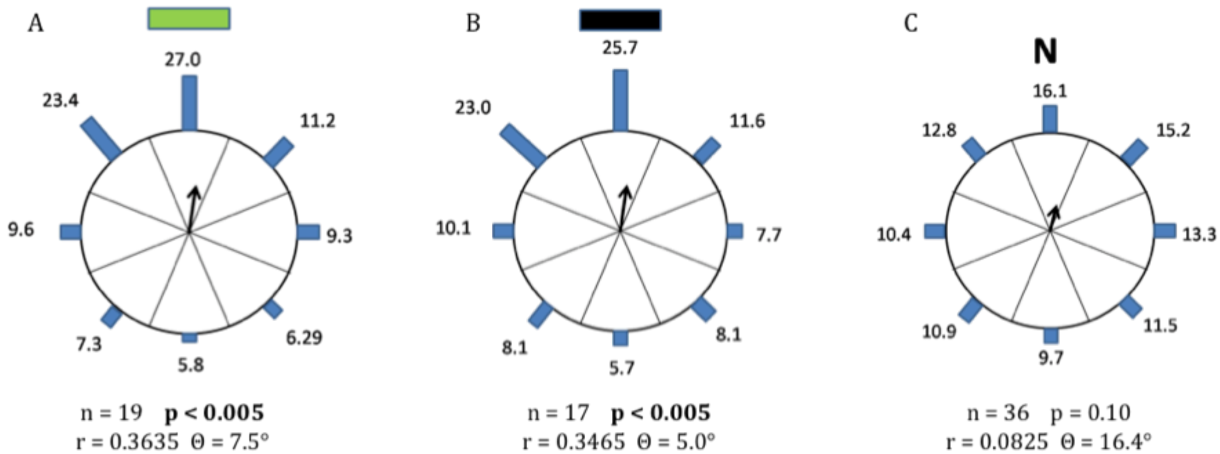

Fig. 2: Mean percentile (%) of scorpion orientation during Experiment 2. The experimental (A) and control (B) trials are shown along with directional biases (C), using the combined data of the experimental and control trials. Values in bold are considered significant. *Abbreviations*: n = the number of trials, p = the probability based on Rayleigh tests for randomness, r = the average length of the vector, and Θ = the average angle of the vector.

Experiment 3

Of 28 possible trials, 14 box-only trials and 13 UV-only trials were valid. No statistically significant differences in scorpion orientation occurred for the trials containing only boxes (Fig. 3A, $p > 0.10$), or for the UV-light only trials (Fig. 2B, $p > 0.10$). Directional tests were run separately for both types of trials (Fig. 2C, 2D), but neither directional test was significant ($p > 0.10$ for box-only and UV-only).

Discussion

The results of these three experiments produced some unexpected information about the behavior of scorpions in different environments. The purpose of Experiment 1 was to test if the natural amount of UV light in the night sky would be enough to stimulate fluorescence from the box and produce a behavioral response from the scorpions. The lack of significance in both the experimental and control trials showed that either the UV light was insufficient or that scorpions do not react to fluorescence, or more specifically the fluorescence of the green paper. This information alone is not enough to answer my questions, which is why I conducted a similar experiment, Experiment 2, in the laboratory with a stronger UV light intensity. In these trials, scorpions spent more time near both the fluorescent box and the black box control than in other areas of the arena. So while the intensified UV light elicited a behavioral response, the response did not seem to be caused by the fluorescence alone, but rather a common element in both the experimental and control trials.

Three likely influences could have influenced scorpion behavior in Experiment 2: 1) the UV light itself, 2) the presence of an object (the box), or 3) the combination of the UV light shining directly onto the box. To differentiate between the possible causes of Experiment 2's result, I conducted one more experiment (Experiment 3). The first part of Experiment 3 used only boxes located on the outside of the arena, just like the previous experiments, but without a UV source shining on it. The results of this test were not significant, suggesting the objects alone are not attracting the scorpions. Next, I used only UV light shining directly on the sand located outside of the arena, but without the fluorescent or black box. While the results showed a slight increase in time spent near the UV light, I still found no statistically significant differences in these trials. The elimination of these two influences on behavior suggests that it was the combination of intense UV light and its position on an object that was somehow attracting the scorpions in Experiment 2.

There are several possibilities as to why UV light shining on an object is creating significant behavioral responses from these scorpions. My favored hypothesis is based on their natural habitat choice. This species of scorpion tends to spend their day hiding under leaves and bark (McReynolds 2008). Perhaps in my experiments, the shining of the UV light on the box was creating a shadow. This shadow might resemble the shelter normally sought out by these scorpions during the daytime. Being able to recognize shelter quickly during high-UV (or similarly, daylight) conditions may be beneficial to this nocturnal animal. However, because this was not what I was originally testing for, future experiments and data will be needed to support this

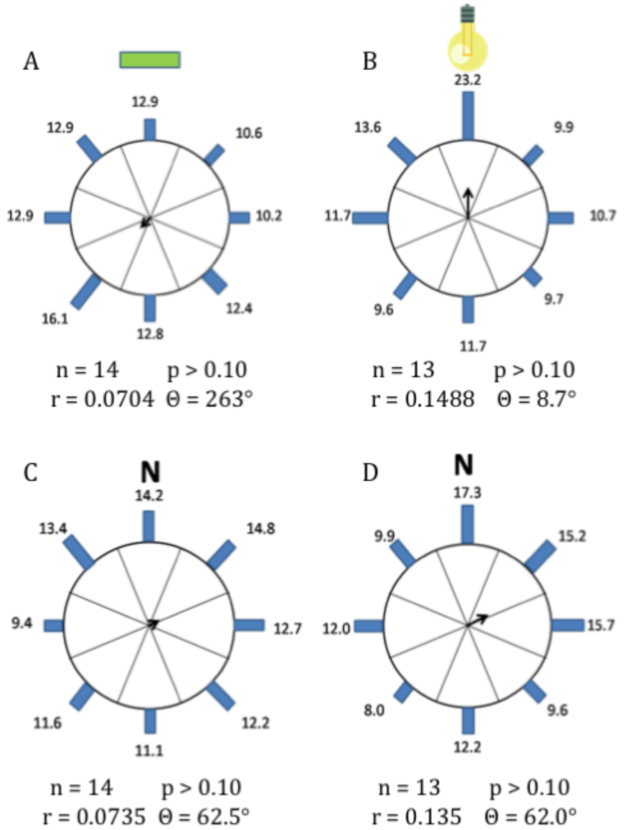

Fig. 3: Mean percentile (%) of scorpion orientation during Experiment 3. The experimental (A) and control (B) trials are shown along with directional biases for the boxes only (C) and the UV only (D), using the combined data of the experimental and control trials. Values in bold are considered significant. *Abbreviations*: n = the number of trials, p = the probability based on Rayleigh tests for randomness, r = the average length of the vector, and Θ = the average angle of the vector.

claim. Future research may also include trials that eliminate my experiments' weaknesses. This includes using the actual scorpion fluorescent compounds for stimuli rather than the green fluorescent paper. This would make the settings more natural and would eliminate any question as to whether the fluorescence was similar to what these scorpions emit. Future research would also involve more species of scorpions, including a species that burrows in the sand. If scorpions are indeed using the shadows to look for shelter, then a scorpion that finds shelter by digging in the sand should not give the same results as in my study. Research should also be conducted during the mating season, which may produce different results because my trials took place during the non-mating season.

While this research has raised interesting questions, it did not bring me much new information as to why scorpions fluoresce. My data indicate that *C. vittatus* do not respond to fluorescence as presented in the conditions of these experiments. However, a lack of an orientation response does not necessarily mean that the scorpions cannot see or detect this fluorescence. Thus, the detection of fluorescence by scorpions is still possible but may need to be tested in a non-behavioral way to obtain more information. However, until then, my only safe conclusion is that if scorpions can detect fluorescence, they are not showing any orientation-related behavioral tendencies because of it.

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