Scorpion light sensitivity and relation to fluorescence

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Spring 2015

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Summary

Scorpions display the mysterious property of fluorescing green under ultraviolet light. Several hypotheses have been proposed, but the function of the fluorescence remains enigmatic. One challenge is the inefficiency of current behavioral tests of scorpion sensitivity to light levels and hues. Scorpions appear to have peak physiological sensitivity to green and ultraviolet light in their eyes and in photosensitive elements in their tails. We set out to create an assay that would indicate the effect of these wavelengths on scorpion behavior. To do this, we created a simple rectangular Plexiglas arena with different wavelength LEDs on one end. Under darkness, scorpions were placed in these arenas and allowed to explore for 30 minutes while being exposed to UV, Green, Blue light or no light. To judge their behavior, we compared the percentage of time scorpions spent in the nearest half of the apparatus closest to the LED and the nearest fifth. This showed there to be no significant differences among light exposures. However, during post hoc analysis, we shortened the analyzed time to only the first five minutes of every trial, and loosened our criteria for judging appropriate trials. This revealed there to be a significant difference between green and control exposure, and also green and UV exposure. Our results indicate that scorpions were positively reacting to green light, preferring to be close to the LED. This contradicts earlier behavioral studies indicating a negative reaction to this type of light. Our analysis supports earlier studies showing scorpions to have maximum sensitivity to green light. Furthermore, the lack of noticeable change in behavior when exposed to UV light calls into question earlier hypotheses on the role of scorpion fluorescence.

Introduction

Scorpions are largely nocturnal creatures that prefer to mate and hunt under very low light conditions (Polis 1979). To detect light in this environment they have two different sets of eyes: median and lateral (Hjelle 1990). Much research has been done relating to scorpions light sensitivity and the function of these median and lateral eyes. It has been shown that these two different visual systems are used for different tasks, with the median ones largely dedicated to contrast and spatial discrimination, and the lateral dedicated to light detection (Schliwa & Fleissner 1980). Regardless, both sets of eyes have peak sensitivity to green light of about 500 nm as well as sensitivity to UV wavelengths in the range of 350 to 400 nm (Fleissner & Fleissner 2001). No response has been shown to light in the red and infrared regions above around 675 nm. Other studies have shown that scorpions are negatively phototactic in response to green and UV light (Camp & Gaffin 1999, Blass & Gaffin 2008).

Scorpions fluoresce bright green under UV light due to the presence of beta-carboline and 4-methyl-7-hydroxycoumarin in their cuticle (Stachel et al. 1999, Frost et al. 2001). Scorpions are one of several species of arthropods that exhibit this sort of fluorescence under UV; others include millipedes, dung spiders, and crab spiders (Kuse et al. 1995). Scorpions, though, are the only one of these arthropods whose fluorescence has been studied in detail. Several hypotheses have been put forward to explain this fluorescence, but none are well supported by experimentation. One hypothesis is that scorpion eyes are not actually sensitive to UV light, only to the green light emanating from their bodies when placed under UV light (Gaffin et al. 2012). They might use this to expand their spectral sensitivity into shorter wavelengths while detecting whether their bodies are out in the open and susceptible to predation.

To examine how scorpions respond to various types of light and how that relates to their fluorescence, I have designed a new behavioral test. This involves placing scorpions inside slim rectangular black Plexiglas arenas with various light exposures coming from one end of the apparatus. We tracked scorpion movements in response to UV, green, blue, or no light and compared them to see if there were any differences between them. After performing both an ANOVA and paired ANOVA on the data set, we found no significant differences between behaviors of the various light sources. Post-hoc analyses, however, showed a positive phototactic response to green light. This has never been shown before in a previous study, and goes against data showing scorpions are negatively phototactic to this light (Abushama 1964, Camp & Gaffin 1999).

Methods

Male and female scorpions (Centruroides vittatus) were collected from Lake Thunderbird State Park outside of Norman, Oklahoma in May and August of 2014 between 2000 and 2100 using hand held black lights (scorpion exoskeletons fluoresce bright green under UV light). Scorpions were taken back to the University of Oklahoma and housed individually in large glass jars with enough sand to cover the base and a shard of clay pottery to retain and moderate humidity and act as a source of cover. Scorpions were all placed in a single room kept at 22°C and a timer switched the room lights on at 0600 and off at 2000. Every scorpion was fed a small cricket every other week and misted with about 5 mL of water three times weekly.

Experiments in the laboratory were conducted using four separate rectangular Plexiglas arenas aligned side by side. Black Plexiglas was used for the sides of each arena to prevent any unwanted light from entering adjacent arenas. The inside of each arena was also sanded to inhibit the reflectiveness of the black Plexiglas. This prevented the scorpion from perceiving the light as coming from both ends of the arena. The bottoms of the arenas were all made of clear Plexiglas. Each arena measured 5 cm in width, 35 cm long, and 7 cm tall. The apparatus was constructed so that pieces of black Plexiglas could be held upright within the arena at the 15 and 20 cm points. These were used to contain scorpions during the acclimation phase of the trial periods. An LED holder was placed in one end of each arena to hold a 5mm LED light. The four rectangular arenas were then placed atop a clear piece of Plexiglas and supported at a height of 1m by 4 PVC pipes. A Sony *Handycam* infrared video camera was placed facing upward at the base of the PVC pipes, and was used to record scorpion movement through the two clear Plexiglas layers (Figure 1).

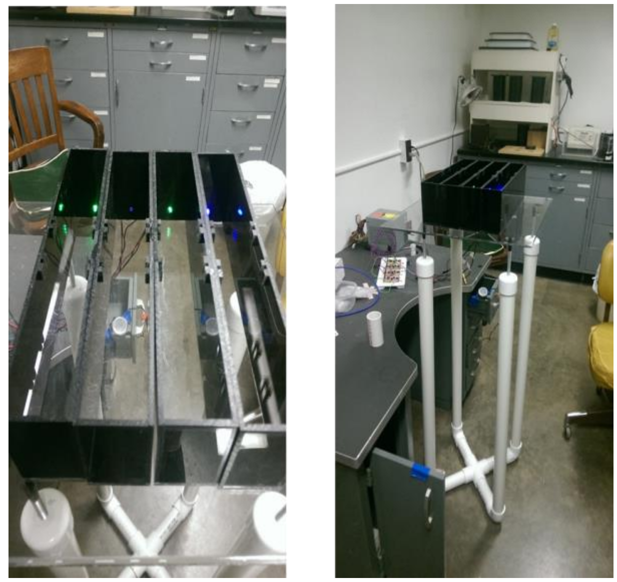

Fig. 1: Four behavioral arenas side by side. (a.) The top view of the behavioral apparatus. We used four different light treatments to gauge behavior. (b.) The side view. We used a clear platform of Plexiglas so filming could take place underneath with an infrared camera. We also used a light calibration circuit board to change the intensities of light.

All trials for this experiment were conducted between the hours of 2000 and 2400. Each scorpion was kept in the dark up until the trial began. The room in which the trials were conducted was kept dark with the exclusion of the red LED lamp the experimenter used to see in the dark (scorpions are not responsive to red light; Camp & Gaffin 1999, Blass & Gaffin 2008). Before each trial, the rectangular arenas were washed with 70% ethanol, rinsed with tap water, towel dried, and allowed to air dry. At the beginning of each trial, the four rectangular arenas were arranged on the clear Plexiglas surface with the long ends facing either East/West or North/South to control for possible influences of Earth's magnetic field. The four 5 mm LEDs were then placed into their respective LED holders without being turned on. Each of the scorpions was then carefully picked up and placed into the acclimation chamber of their rectangular arena using a pair of long metal forceps. Each trial began with a 10-minute period during which the scorpions were allowed to acclimate to the room with no light present. After this period, the LED lights were turned on, the walls of the acclimation chamber were lifted, and the scorpions were free to explore the arena for 30 minutes while being recorded from below.

Each animal was tested in four light treatments: green, UV, blue, and no light. 5 mm round LEDs were used to produce the green, UV, and blue light (3.0-3.4 v, 24 mA, 27000 mcd). An op amp circuit was used to adjust the LEDs to produce an intensity of approximately 0.01

	UV	Green	Blue	Control			UV	Green	Blue	Control	
Group 1						**Group 3**					
Sunday						Sunday					
0	14	37	8	6		0	35	4	11	22	
180	13	28	34	10		180	32	31	2	7	
Monday						Monday					
180	37	8	6	14		180	4	11	22	35	
0	28	34	10	13		0	31	2	7	32	
Tuesday						Tuesday					
0	8	6	14	37		0	11	22	35	4	
180	34	10	13	28		180	2	7	32	31	
Wednesday						Wednesday					
180	6	14	37	8		180	22	35	4	11	
0	10	13	28	34		0	7	32	31	2	
Group 2						**Group 4**					
Sunday						Sunday					
0	20	40	15	5		0	16	30	42	21	
180	24	9	18	1		180	36	23			
Monday						Monday					
180	40	15	5	20		180	30	42	21	16	
0	9	18	1	24		0	23			36	
Tuesday						Tuesday					
0	15	5	20	40		0	42	21	16	30	
180	18	1	24	9		180			36	23	
Wednesday						Wednesday					
180	5	20	40	15		180	21	16	30	42	
0	1	24	9	18		0		36	23		

Table 1. Randomization of scorpion trials. We randomized the scorpion order and divided the animals into groups. Each scorpion completed all four trials within a one-week period.

uW/cm2/nm as measured with a spectrophotometer (Ocean Optics) at the middle of the arena. This intensity induced avoidance behavior in scorpions in previous studies (Gaffin & Barker 2014) and reflects the level of UV light at sunset. The light treatment, and the ordering of the scorpions was randomized so that each scorpion would have the same time period between trials, but would experience varying orders of light exposure. (Table 1)

To analyze the video footage, a MATLAB program was written to track the movement of scorpions throughout the arenas. The arenas were split up into 50 evenly spaced values as a function of their distance from the LED. The scorpions were then given a number value every second of their 30 minute trial corresponding to their position within the arena, which resulted in a total of 1800 values per trial. These data were saved to an Excel spreadsheet and transformed as follows. First, we eliminated the initial 10 values (seconds) from each trial. This was the period during which the experimenter was removing the blocks preventing scorpion movement, and could have interfered with the tracking software. Next, we screened trials based on the following criteria. To qualify as a "legitimate" trial, the scorpion had to move a total of 35 cm (the length of the apparatus). If they moved less than 35 cm, the scorpion had to be moving for a minimum of 60 seconds with at least 18 cm of total distance traveled. Trials that did not meet these requirements were removed from our analyses. For legitimate trials, we then identified any periods in which the scorpion did not move for more than 30 seconds. We removed these sections from our records to eliminate long periods of inactivity.

To analyze these data, we created frequency plots of the 50 values that divided the apparatus with a value of 1 being closest to the LED and 50 being farthest away. We looked at the percentage of the trial scorpions spent at each of the values, and compared them among light treatments using a repeated measures ANOVA and one way ANOVA. For the post hoc analysis, we still removed the first 10 seconds of each trial. For a trial to be eliminated though, the scorpion was required to remain inactive for the duration of the trial. If the scorpion moved at all during the 30 minutes, its trial was kept. Also, no values were removed from consideration even if scorpion activity did not change for longer than 30 seconds.

Results

Scorpions seemed to adapt well to the circumstances of the testing arenas and typically exhibited walking behaviors that went back and forth along the apparatus (Figure 3). Their activity did not seem obviously different between the light treatments. However, closer examination revealed subtle changes in scorpion behavior depending on their light exposures.

For our first set of criteria, we eliminated any trials in which the scorpion did not move the full length of the apparatus or failed to move for at least one whole minute. Any lack of movement beyond a period of 30 seconds was also removed from consideration. Using these criteria, we examined the differences in proportion of time scorpions spent in the near half of the apparatus in differing light exposures. Our results showed there to be high variability among the trials, with some scorpions spending upwards of 90% of their time in the near half of the apparatus while others spent 0%. An ANOVA analysis found no significant differences among the light exposures in the time that scorpions spent in the near half of the arenas (P=0.065). We also looked to see whether a repeated measures ANOVA might better reflect differences between light treatments for the near half of the apparatus. For this, we could only use scorpions with no illegitimate trials (12 out of a total of 30), and the analysis did not show any significant differences (P=0.077)

Next, we looked at the percentage of time scorpions spent in first ten sections of the arena (the fifth nearest the LED). Again, we saw a very large amount of variability among the individual scorpion trials. However, when we did an ANOVA, we found there was a significant difference among the trials as a whole (P=0.038). However, when we examined the individual differences between light treatments, we found no significant differences.

Post hoc analysis

In a post hoc analysis, we changed our criteria to include only the values of the first five minutes of every trial. Earlier data suggested that scorpion activity decreased over time, with the first five minutes being the period where they were most active (Blass & Gaffin

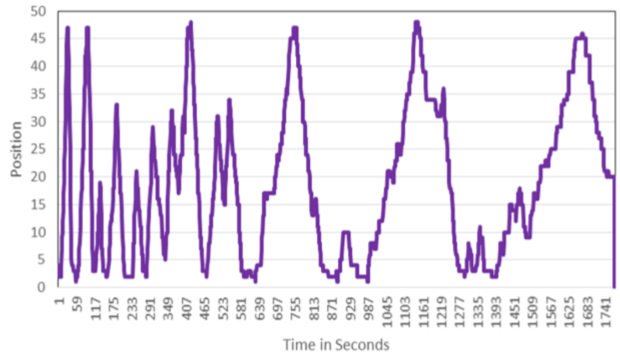

Fig. 3: Typical scorpion movement pattern over duration of 30-minute trial. Scorpions usually moved in back and forth motion across the length of the apparatus.

2008). We cut the trial time down to this duration and compared the proportion of time spent in the near half of the apparatus among light treatments, but found no significant differences (P=0.16). However, when comparing the proportion of time spent in the near fifth of the arena, we found significant differences among all treatments (P=0.004); pairwise comparisons showed green and control treatments were significantly different from one another (P<0.01).

Lastly, we experimented with softening up our criteria. We allowed any trial to be counted if the scorpion moved at any time during the 30-minute trials, and we did not cut any values out of the trial even if the scorpion remained stationary for longer than 30 seconds. We found no significant results when comparing whole 30 minute trials, but did see very significant results when only looking at the first five minutes and the nearest fifth of the arena (P=0.002). Furthermore, we found significant differences between green and control exposures (P<0.001) and green and UV exposures (P<0.05; Table 2).

Discussion

As a whole, these results paint a rather confusing picture about the exact wavelengths of light scorpions are sensitive to and the role that these sensitivities have in understanding scorpion fluorescence. Scorpions showed no significant preference for one wavelength over the other when examining full 30-minute trials. Narrowing the trial analysis to the first five minutes revealed a significant difference between green and control, and green and UV exposures (when using looser criteria). These differences also showed scorpions to be attracted to green light, which is contrary to earlier studies showing negative phototaxis for this wavelength.

For this experiment, we initially used a trial length time of 30 minutes to provide enough data points for us to draw reliable conclusions from. We compared the

Trial Type	Overall P Value	Comparative P Values	Specific P Values	Normal Distribution?
First Five Minutes w/normal criteria and Near Fifth	**0.0041**	**Only Green/Control Sig. Nothing else even close**	**Green/Control <.01**	**Yes**
First Five Minutes w/normal criteria and Far Fifth	0.4864	Nowhere near sig.		UV, Control not normal
First Five Minutes w/normal criteria and Near Half	0.1595	All above .05		Control not normal
First Five Minutes w/normal criteria and Far Half	0.1595	All Above .05		Control not normal
Whole Trial w/normal criteria and Near Fifth	0.0382	No outright sig. Green/Control, **Blue/Control** VERY Close		Yes
Whole Trial w/normal criteria and Far Fifth	0.4864	No sig		UV, Control not normal
Whole Trial w/normal criteria and Near Half	0.065	No outright sig. Blue/Control very close		No
Repeated Measures Whole Trial w/normal criteria and Near	0.0774	No outright sig. Blue/Control very close		Yes
Repeated Measures Whole Trial w/normal criteria and Far Ha	0.0774	No sig		Yes
Whole Trial w/loose criteria and Near Fifth	0.3229	No Sig		Green, Control not norma
Whole Trial w/loose criteria and Far Fifth	0.5815	No Sig		Nothing Normal
Whole Trial w/loose criteria and Near Half	0.5687	No sig		only control normal
Whole Trial w/loose criteria and Far Half	0.5687	No Sig		only control normal
First Five Minutes w/loose criteria and Near Fifth	**0.0015**	**Green/UV, Green/Control sig**	**UV/Green <.05, Green/Control <.00**	**all normal**
First Five Minutes w/loose criteria and Far Fifth	0.4909	No sig		UV, Control not normal
First Five Minutes w/loose criteria and Near Half	0.099	No sig		Control not normal
First Five Minutes w/loose criteria and Far Half	0.099	No sig		Control not normal

Table 2. Statistical analyses using various scoring criteria. We examined a host of different ways to analyze the data collected using ANOVA and repeated measures ANOVA analyses. This included shortening the trial, and loosening up the criteria for judging what was a valid trial.

proportion of time scorpions spent in the half of the arena nearest the LED. However, no significant differences were found among any light exposures. When we examined the whole trial and compared the proportion spent in the nearest fifth of the apparatus, we did find an overall significant value of 0.038. This did not translate, however, into any significant differences among the specific light treatments. To run a repeated measures ANOVA, we could only use those scorpions that had four legitimate trials. This reduced our sample size down to twelve scorpions and still yielded no significant differences. For our post-hoc analysis, we found evidence that indicated scorpions reduced their activity levels when exposed to light after the first 5 minutes of each trial (Blass & Gaffin 2008). This led us to reduce our selection criteria to include only that period of time. We were also interested in which values our specific criteria were forcing us to throw out. We thought we might potentially be eliminating data points that were indicative of scorpion light preference. To examine this, we loosened up our criteria and allowed any trial to count as long as the scorpion moved *any* distance. In addition, we did not throw out position values when a scorpion remained stationary for longer than 30 seconds. We thought that even non-movement at various distances from the LED might be indicative of a preference for that wavelength of light. When examining those first 5 minutes of every trial, we found that keeping our normal criteria and looking at the proportion of time in the near fifth of the arena resulted in a significant difference between green and control light exposures. Furthermore, this revealed a positive preference for green light, with the scorpion spending a lot of time as close to the light as possible. Using the looser criteria and only the first five minutes showed a similar significant difference between green and control treatments. This analysis also revealed a surprising difference among green and UV treatments, again showing that green exposure caused scorpions to be attracted to the LED.

It is difficult to make sense of these data when closely examining past studies. The median scorpion eyes have been physiologically shown to peak in sensitivity to green light, with UV light coming in second. Their lateral eyes have shown to be more sensitive to UV light (Fleissner & Fleissner 2001). This is despite indications that scorpions have homogenous photoreceptors, bringing up questions of how they could be sensitive to both UV and green. (Fleissner & Fleissner 2001). In addition, behavioral data have shown that scorpions are most active under ultraviolet exposure, followed closely by green light (Blass & Gaffin 2008, Gaffin et al. 2012). Our data did not reflect any significant preferences for UV, but we also had no direct way of measuring overall activity level, which is the indicator those studies relied upon. It is also intriguing that we found green and UV light preferences to be significantly different when comparing them in the nearest fifth of the arena during the first five minutes. In this case, UV exposure was not different from control. Previous studies have postulated that ultraviolet induced fluorescence of the cuticle would explain why scorpions have only one type of photoreceptor. This would expand the spectral sensitivity of this arachnid while also serving as a way for the scorpion to detect shelter within a UV light rich environment (e.g. during a full moon; Gaffin et al. 2012). Scorpions show reduced activity levels under UV after their fluorescence is removed with a photobleaching method (Kloock 2009, 2010). Our apparatus did not allow us to reliably measure activity level, only preference. This might explain why we saw no significant results when comparing ultraviolet exposure with a control. Yet, one would think that our apparatus should elicit a similar response to UV and green if the ultraviolet was being transduced into green as a way to aid in its detection. As our LEDs were pointed horizontally at the scorpion, it is worth asking whether their perceived fluorescence would also give an indication of what direction to move toward. If so, then it appears scorpions might either have a much lower sensitivity to UV, or their reaction to this type of light might elicit a different type of phototaxis. One wonders what the behavioral implications of this might be, and how it would help them in a normal environment.

Another striking result of our experiment is the prominent positive reaction scorpions had to green light. Numerous previous studies have shown only negative phototaxis to be present in scorpions (Abushama 1964, Camp & Gaffin 1999). However, after looking at only the first five minutes, scorpions demonstrated a robust difference in time spent closest to the green LED as compared to control. This seems to be a pretty clear preference, which makes it remarkable that no studies have shown evidence of it thus far. The importance of this is not clear. The shelter hypothesis of fluorescence states that its greenish glow under UV light is used as a way to detect when its body is out in the open or under shelter. From this, one would think that seeing a similar green light in our apparatus would result in them moving away, not toward it.

Regardless of the implications for this hypothesis, our arena and trial methods were able to find a high sensitivity to green light over UV, control, and blue. Furthermore, the attraction to green light has not been documented in any previous published studies. Future experiments using a similar apparatus might benefit from the use of longer wavelengths like orange and red as a means to provide other controls, as scorpions have been shown to be unresponsive to these wavelengths (Fleissner & Fleissner 2001). Yet, because our apparatus was not able to show reliable sensitivities to either blue or UV, which they have been shown to be sensitive to, it leaves the question of whether our arena is sensitive enough to detect anything but the wavelength scorpions are most responsive to.

Acknowledgments

I want to thank Dr. Ola Fincke, Dr. Doug Gaffin, and Brad Brayfield for being committee members for the defense of this thesis. I also want to thank all members of the Gaffin lab for an incredible amount of help designing and executing my experiments. Thank you, Jay Vinnedge, Randall Proctor, and Megan Mont. I especially want to thank Brad Brayfield for giving me invaluable advice throughout this process. Dr. Marielle Hoefnagels also provided support as an expert editor on virtually all my written material. I want to thank Dr. Gaffin one more time for being a fantastic mentor over the past two years. I have learned far more participating in his lab than I thought I ever could. The experiences I've had and the opportunities that await me are a direct result of the guidance and understanding he freely provides. He has made my two years in his lab a truly transformative experience.

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