A comparison of fluorescence in two sympatric scorpion species

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A R T I C L E   I N F O

Article history:
Received 26 November 2007
Received in revised form 17 February 2008
Accepted 27 February 2008
Available online 4 March 2008

Keywords:
Scorpions
Fluorescence
Spectroscopy
Gender
Discrimination

A B S T R A C T

In order to test the feasibility of scorpion fluorescence as an indicator of gender and/or species identity, a comparison of the fluorescence spectra between genders across two sympatric species of scorpions (Vaejovis confusus Stahnke 1940 and Paruroctonus shulovi Williams 1970) was conducted. Each spectrum was represented in a simple multivariate analysis by its peak wavelength and width at 90% of peak intensity. No difference between genders was found, but a statistically significant difference between species was detected. The potential of fluorescence as a basis for species recognition based on this difference is discussed in the context of what is known about scorpion vision and discriminatory abilities.

1. Introduction

There is no known function of fluorescence in scorpions [1–3]. Several functions have been proposed, but very few have been tested. Lourenço and Cloudsley-Thompson [1] and Frost et al. [2] speculated that fluorescence functions as a protection against ultra-violet light, although they consider this a relict function given the usual nocturnal habits of scorpions. A test of the hypothesis that scorpion fluorescence acts as a prey attractant suggested that aerial insects are more likely to avoid fluorescing scorpions than be attracted to them [4]. Fasel et al. [5] point out that in other organisms, photoluminescence is often associated with mating strategies, though they reject this possibility for scorpions on theoretical grounds. However, no empirical evidence exists that bears on the potential for fluorescence to function in mating or other scorpion behaviors.

Given that scorpions are well known for both inter-specific (intra-guild) predation [6] and cannibalism [7], it would be advantageous for both male and female scorpions to determine, at a distance, whether a nearby scorpion is likely to be a conspecific and/or of the opposite gender. This information could help a scorpion choose whether or not to approach closely (males) or allow a close approach (females). In terms of reproductive behavior in particular, fluorescence could function in two different but potentially compatible ways. If males and females within a species differ, then scorpions could use these differences to determine the sex of a nearby scorpion. At close range, chemical and vibratory cues would likely take precedence [8], but visual cues could initiate approach for further investigation or retreat from a potential predator. Even if no gender differences exist, differences between species in a single locality could potentially serve as a species recognition mechanism while maintaining a safe distance. If scorpions can detect differences at a distance, they could save time and energy they might otherwise spend on ultimately futile courtship, which would benefit both species directly. Detecting differences at a distance could also reduce the frequency of potentially dangerous encounters, especially when the size difference between the scorpions is small. If a large size difference exists, it could conceivably be in the interest of the larger species to “mimic” the signal of the smaller species in order to lure prey. Of course, this assumes that scorpions are actually capable of detecting each other in their environment due to fluorescence. Evidence suggests that scorpions possess sufficient sensory abilities to accomplish this.

Fleissner [9] reported that night-adapted scorpion eyes can detect illuminance as low as 7.0 × 10−6 lux (median eye) and 2.2 × 10−6 lux (lateral eye), and that the extreme sensitivity of scorpion eyes should allow them to readily detect objects such as light-colored stones and shadows even on moonless nights. Machan [10] studied the spectral sensitivity of the scorpion eye and found a sensitivity peak near 509 nm for both median and lateral eyes. Measured values for fluorescence [2,3,5,11,16] fall near this region of peak sensitivity. Unfortunately, no measurements of fluorescence under natural illumination have been made, so we do not know definitively that scorpions can detect each other by fluorescence. However, the fact that they emit light near the same region

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1011-1344/$ - see front matter Published by Elsevier B.V.
doi:10.1016/j.jphotobiol.2008.02.008
in which their eyes exhibit peak sensitivity at low-light levels suggests that scorpions may detect one another via their fluorescence, even under very dimly illuminated conditions [3].

In order for fluorescence to function as an indicator of gender or species identity, differences between genders and/or sympatric species must be present: if differences do not exist, fluorescence cannot have this function. Thus, a simple, indirect way to test the potential for fluorescence to function in gender or species identification is to measure the fluorescence of two sympatric species and look for differences in the fluorescence spectra across both gender and species.

2. Methods

We collected 81 live Vaejovis confusus Stahnke and 33 live Paruroctonus shulovi Williams from Kern County, CA, over a three-week period using black lights to find scorpions engaging in surface activity. The collection site consisted largely of Saltbush (Atraphlex sp.) with a variety of grasses mixed in at low densities and is described in detail by Germano et al. [12]. Scorpions were identified as V. confusus using the description in [13] and as P. shulovi using [14]. Scorpion collections took place in a fairly homogeneous site, with no obvious habitat differences, although small differences in vegetation are recorded by Germano et al. [12]. Scorpions were identified to species within 50 cm of each other, though no interactions between all scorpions and the reflectance probe remained constant at 1 cm. A hand-built light source using six ultra-violet (UV) light emitting diodes (LED) and powered by a 9 V battery (patterned after [15]) supplied UV light. The LED’s peak wavelength of 395 nm (range: ~370–410 nm) produced readily visible fluorescence in all scorpions examined and is in the same range as excitation spectra measured on several other species of scorpions [2,5,11,16]. All spectrum measurements used the same light source, with the battery replaced daily to maintain power levels. The light source, positioned at a constant distance of 20 cm and angle of 45° to the scorpion, shone through the slit in the squeeze cage to induce fluorescence (Fig. 1b). The reflectance probe was positioned at 90° to the scorpion. The light, probe, scorpion and squeeze cage were all enclosed in a metal box with the interior walls covered in light absorbing fabric. The box was sealed to eliminate stray light during all measurements, with only a fiber optic cable connecting the reflectance probe to the spectrometer and transmitted through a snug hole sealed by a rubber washer leaving the box.

A visual light spectrometer (BWTech BRC111A using BWSpec 3.23 for Windows software, B&W Tek Inc.) analyzed light transmitted from the scorpion via the reflectance probe. All measurements used a 250 ms integration time and an average spectrum was calculated from 100 samples for each scorpion. All spectra used the dark subtraction method, with an empty squeeze cage serving as the “dark” source to control for any reflection from the squeeze cage that may have occurred. The spectrometer has a listed resolution of 0.66 at 546.11 nm, but the output actually reports values every 0.2–0.3 nm. To deal with the discrepancy between listed precision and output, all spectra were smoothed using the mean filter algorithm [17] with a window of nine data points (approximately 1 nm to either side of the smoothed point given the spectrometer output). To conservatively account for loss of precision involved in the smoothing process, all wavelength measurements were rounded to the nearest nanometer.

Two measurements were made on each spectrum. We measured the peak wavelength simply by finding the wavelength at which the peak intensity for each spectrum occurred. The actual intensity value was not used as a variable due to the fact that we observed a strong correlation between size and intensity in preliminary measurements. As a measure of the width of the spectrum, the highest and lowest wavelengths yielding intensities greater than 90% of the peak intensity for each spectrum were determined. The difference between these values yields a range (at 90% of the peak intensity) that provides a standardized measure of spectrum width. For convenience, I use the abbreviation WNPI (width at ninety percent peak intensity) to refer to this measurement. Ninety percent was chosen a priori primarily for convenience: it was considered to be far enough below the peak to reflect width and high enough that it was unlikely that noise from floor values would impact the measurement.

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Fig. 1. Schematic diagram showing the basic measurement set up. (a) Squeeze cage, as seen from above, showing slit to allow light. (b) Relative positions of the light, squeeze cage, and reflectance probe, as viewed from the side. The slit’s long axis pointed towards the light source, with scorpion facing towards the light source. The entire setup was enclosed in a box allowing no other light into the measurement area.
In our collections, measurements include all scorpions collected in these two species, as well as providing the sample sizes of each species and gender.

3. Results

After analysis, the smoothed spectra were further modified for the purpose of making a visual comparison of the average spectrum shape. The intensity of each point in each spectrum was divided by the maximum intensity of that spectrum. Thus, the maximum intensity of each spectrum was standardized at 1.0. These standardized spectra were then averaged for display of overall spectrum shape (Fig. 2).

To determine if gender and/or species differ in peak wavelength and WNPI, I conducted a multivariate analysis using species and gender as independent variables and peak wavelength and WNPI as dependent variables. All statistical analyses used SPSS for Windows [18].

Because no difference between genders was found, normalized spectra were averaged by species with genders pooled. The result of this process is displayed in Fig. 2 which shows that the entire spectrum for V. confusus is shifted slightly to the right compared to P. shulovi, with a more pronounced effect on the right side of the peak than on the left. As shown by the significant difference in WNPI, the average spectrum for V. confusus is also slightly broader than that of P. shulovi.

Table 2 provides summary statistics for both peak wavelength and WNPI.

Table 1 summarizes the results of the tests for normality and the regression analyses of carapace width by species and gender as well as providing the sample sizes of each species and gender. Measurements include all scorpions collected in these two species. In our collections, V. confusus outnumbered P. shulovi, and in both species, males outnumbered females. All data sets exhibit normal distributions according to the Kolmogorov–Smirnov tests; inspection of normal probability plots also revealed no major deviations from normality. No significant effects of size appeared (all p > 0.05), so size is discounted as a factor in all subsequent analyses. Inspection of Table 1 reveals that for V. confusus, the p-value for male peak wavelength by carapace width approaches 0.05. However, the low r² value suggests a poor overall effect. Further inspection of these data shows a single data point having significant influence. Removal of this data point results in a regression p-value of 0.420 for male V. confusus. Because the presence of this outlier increases the variance of males, inclusion of it in the analyses is conservative, in terms of finding spurious differences, so it is retained in subsequent analyses. However, removing this outlier does not change any of the conclusions presented.

The multivariate analysis of variance on peak wavelength and WNPI (using Hotelling’s Trace statistic, df = 2, 109) reveals a significant difference between the two species (F = 6.93, p = 0.00146), but no difference between genders (F = 0.652, p = 0.523) and no interaction effect (F = 0.0881, p = 0.916).

Given the significant result of the multivariate analysis for species, it is appropriate to proceed to univariate ANOVA of each dependent variable [19]. This analysis shows that peak wavelength differs between species (F = 6.64, df = 1, p = 0.0113) as does WNPI (F = 9.844, df = 1, p = 0.00219). Univariate analysis on gender was not performed due to the lack of a significant multivariate effect of gender.

Because no difference between genders was found, normalized spectra were averaged by species with genders pooled. The result of this process is displayed in Fig. 2 which shows that the entire spectrum for V. confusus is shifted slightly to the right compared to P. shulovi, with a more pronounced effect on the right side of the peak than on the left. As shown by the significant difference in WNPI, the average spectrum for V. confusus is also slightly broader than that of P. shulovi.

Table 2

Summary statistics (mean, standard deviation, standard error and sample size) for each species and gender for peak wavelength and width at 90% peak intensity (WNPI)

<table>
<thead>
<tr>
<th>Mean</th>
<th>s</th>
<th>SE</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak wavelength (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. confusus (all)</td>
<td>500</td>
<td>3.91</td>
<td>0.434</td>
</tr>
<tr>
<td>Females</td>
<td>499</td>
<td>2.76</td>
<td>0.602</td>
</tr>
<tr>
<td>Males</td>
<td>500</td>
<td>4.23</td>
<td>0.546</td>
</tr>
<tr>
<td>P. shulovi (all)</td>
<td>498</td>
<td>3.23</td>
<td>0.562</td>
</tr>
<tr>
<td>Females</td>
<td>497</td>
<td>4.13</td>
<td>1.14</td>
</tr>
<tr>
<td>Males</td>
<td>498</td>
<td>2.56</td>
<td>0.572</td>
</tr>
<tr>
<td>WNPI (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. confusus (all)</td>
<td>26.1</td>
<td>2.46</td>
<td>0.273</td>
</tr>
<tr>
<td>Females</td>
<td>26.3</td>
<td>2.42</td>
<td>0.541</td>
</tr>
<tr>
<td>Males</td>
<td>26.0</td>
<td>2.48</td>
<td>0.320</td>
</tr>
<tr>
<td>P. shulovi (all)</td>
<td>24.4</td>
<td>3.00</td>
<td>0.522</td>
</tr>
<tr>
<td>Females</td>
<td>24.3</td>
<td>1.84</td>
<td>0.510</td>
</tr>
<tr>
<td>Males</td>
<td>24.4</td>
<td>3.60</td>
<td>0.805</td>
</tr>
</tbody>
</table>

Table 1

Results of independent normality tests (Kolmogorov–Smirnov) and regression analyses on carapace width (CW) for peak wavelength and width at 90% peak intensity (WNPI) in each subgroup (species × gender)

<table>
<thead>
<tr>
<th>Normality</th>
<th>P. shulovi females (n = 13)</th>
<th>P. shulovi males (n = 20)</th>
<th>V. confusus females (n = 21)</th>
<th>V. confusus males (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak wavelength</td>
<td>0.283</td>
<td>0.988</td>
<td>0.859</td>
<td>0.604</td>
</tr>
<tr>
<td>WNPI</td>
<td>0.857</td>
<td>0.620</td>
<td>0.438</td>
<td>0.858</td>
</tr>
<tr>
<td>Regression vs. CW</td>
<td>0.631</td>
<td>0.022</td>
<td>0.071</td>
<td>0.170</td>
</tr>
<tr>
<td>Peak wavelength</td>
<td>0.512</td>
<td>0.040</td>
<td>0.076</td>
<td>0.164</td>
</tr>
</tbody>
</table>
4. Discussion

The data reveal no statistically significant difference between males and females in either species, but a significant difference between the species is evident. This result falsifies the hypothesis that fluorescence functions in gender identification in these species, but not the possibility that fluorescence could function in species identification. However, the differences between species are very small (~2 nm; Fig. 2, Table 2). Discrimination of species based on differences of this magnitude would require extremely fine discriminatory abilities of the scorpion eye.

Work on light detection by scorpion eyes [9,10] provides evidence of extreme low-light sensitivity. The issue of discrimination requires more than just detection, however. In order for fluorescence to function in a species identification capacity, scorpions would need to discriminate between these two very similar spectra (Fig. 2). Such fine discrimination seems extremely unlikely. However, although there are a number of studies on scorpion detection abilities (see [20] for a comprehensive treatment), none of these address the issue of discrimination. Locket’s review of scorpion vision [20] makes no mention of wavelength discrimination abilities, reflecting the lack of studies on this aspect of scorpion vision. When it comes to assessing the likelihood of scorpions making fine-scale discrimination we are dependent on indirect information.

Dichromatic vision is considered to be a minimum requirement for wavelength discrimination [21], and humans with dichromatic vision (as opposed to normal, trichromatic vision) have been shown to be capable of discriminating monochromatic light that differs by as little as 2 nm under controlled laboratory conditions [22]. So if scorpions have dichromatic vision, they may be able to discriminate between these signals, which differ by ~2 nm. Machan [10] suggested that scorpion’s lateral eyes could be dichromatic due to the existence of two distinct peaks in sensitivity (509 nm and 371 nm; the 371 nm peak does not occur in the median eye). However, scorpions would need to be capable of making this fine distinction in the field, under much noisier conditions, and scorpion fluorescence is far from monochromatic (see Fig. 1). Even if scorpions do possess dichromatic vision, this is a difficult discrimination task.

Light bright is also considered necessary for accurate wavelength discrimination [21], which probably explains why there has been no work on color discrimination in scorpions. However, Brownell [3] suggests that the presence of visual sensitivity peaks in the same regions as both the emission and excitation peaks of fluorescence may result in a contrast-enhanced image of fluorescing scorpions. If true, this enhancement could have significant ramifications in terms of discriminatory capability tuned specifically to fluorescence, even with low ambient light levels. The combination of fluorescence, extreme low-light sensitivity and contrast enhancement could act to provide a relatively high contrast, “bright” (in terms of the ratio of available light to sensitivity) signal, potentially enhancing discrimination. Such an imaging system could also potentially enhance species or gender specific features not directly associated with fluorescence such as size or shape indicators of sexual or species identity. More work on the potential of contrast enhancement in discrimination tasks is needed to evaluate this possibility.

Despite the presence of statistically significant differences between the sympatric scorpions observed here, the difficulty of the discrimination task and lack of knowledge about scorpion discrimination abilities may make it difficult to argue strongly that these differences are biologically significant. Although the similarities between fluorescence and spectral sensitivity are intriguing, it appears unlikely that scorpion fluorescence functions directly in species identification in this species pair. Thus, although I cannot say what the function of scorpion fluorescence is, I can at least start to say what it is not, and base that assessment on empirical evidence. In this pair of sympatric scorpion species, fluorescence does not function directly as a means of gender identification, and it probably does not function as a species recognition mechanism.

Given the results here, I cannot conclusively rule out the possibility that fluorescence functions in species recognition, regardless of how unlikely it seems. More work on scorpion discriminatory abilities, especially the idea of contrast enhancement [3], will be needed to fully address this possibility. Fluorescence could have this function in other scorpions, but more work on documenting the variation in fluorescence across species and in different habitats must take place before conclusions beyond this pair of species can be made. Existing information across species [2,3,5,16] suggests that scorpion fluorescence has a fairly narrow range. However, testing the hypotheses presented here requires detailed measurements and comparisons between genders within species, and between species within habitats. These data have not yet been collected for any other species. Future work on the function of scorpion fluorescence should not only include expanding the available data on fluorescence values of various species, but should also focus on the remaining hypotheses. These include: fluorescence may be a byproduct of other potential functions of the fluorescent compound, such as stiffening of the cuticle [11]; fluorescence may be a relict trait conferring protection against excess UV light [1,2]; fluorescence may enhance vision or act in other intra-specific communication contexts [23]; fluorescence may act as a light amplifier to mediate responses to light in the environment [24], or fluorescence may function as an aposematic signal to potential predators [4]. Of course, there is also the possibility that fluorescence has no function and inter-specific variation is the result of phylogenetic history. More empirical studies testing these hypotheses are needed to get an answer to the basic question of why scorpions fluoresce.

Acknowledgements

The work was funded by a CSUB URC Grant and a Chevron Corp (REVS-UP) Grant to CSUB. Access to the field site was provided by the Kern Water Bank Authority. Thanks to the research assistants funded by the Chevron Grant: M. Arellano, M. DeGuzman, B. Espinosa, and R. Rostain. The paper also benefited from the comments of three anonymous reviewers.

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