

# **Comparative Spectral Sensitivities of Six Species from the Noctuoidea**

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## **Introduction**

The Lepidoptera have some of the widest ranges of electromagnetic spectral sensitivity in insects. The distribution of peak responses to different wavelengths of light is very similar across species in this order, despite the many differences that exist in both behavior and habitat (Briscoe and Chittka, 2001a). Many moths respond to light from wavelengths of less than 400 nm to near 600 nm, with maximum sensitivities in the blue and green regions (450-520 nm and 520-570 nm, respectively). Several species of nocturnal moths have been demonstrated to possess sensitivities to UV light (< 400 nm). This is interesting considering the lack of UV light during nighttime (Eguchi et al., 1982). Along with UV, red reception is another trait that appears in behaviorally diverse moth species, such as in both diurnal and nocturnal moths. Briscoe and Chittka (2001a) concluded that red reception in moths evolved, in one instance, with the Noctuids.

There does not appear to be documentation of an adaptational advantage to having these traits (Briscoe and Chittka 2001a). It is still an unresolved question as to why spectral sensitivities are relatively similar across the Lepidoptera, and whether there is any significance to possible adaptive pressures that shape color vision across such a speciose and ecologically diverse order. Many have suggested that the evolutionary advantage of wide ranged spectral sensitivity in nocturnal moths is not to perceive color but to increase photon capture in low-light environments (Eguchi et al., 1982; Briscoe and Chittka, 2001b; Hariyama et al., 2001). It would be intriguing that this sensory trait could have come to fulfill two disparate adaptations, that of flower recognition in diurnal butterflies and efficient photon capture in nocturnal moths, while remaining isomorphic across such species. Briscoe and

Chittka (2001b) argued that it is instead the result of phylogenetic and molecular constraints, the same constraints which determine, for example, why all insects have six legs.

Unlike spectral sensitivity, there appears to be considerable variation between species in terms of their overall sensitivities to light. van Lengevelde et al. (2011) showed that artificial lights emitting smaller wavelengths (381.8 nm, 534.3 nm) attract a higher species richness and abundance of moths with larger eyes, wings, and bodies than lights with higher wavelengths. This implies that larger moths have a greater sensitivity to light in general. Their finding exposes a possible selective pressure in regards to overall light sensitivity, that larger moths might experience an increased risk of predation compared to smaller moths due to their level of attraction to artificial lighting.

Data regarding moth visual traits are an essential resource for answering unresolved evolutionary questions regarding insect vision. However, there are few surveys which examine the comparative ranges of spectral sensitivity and overall light sensitivity in multiple moth species. With this study I aimed to provide further documentation of the variation or lack thereof in spectral sensitivities of moths, specifically UV and red reception, and also of correlations between morphological characteristics and light sensitivity.

### **Materials & Methods**

I captured moths from artificially lit sites around the Bennington College campus and tested them the next day. Relying on Bennington County sightings data from the BAMONA database ([http://www.butterfliesandmoths.org/checklists?species\\_type=1&tid=3188](http://www.butterfliesandmoths.org/checklists?species_type=1&tid=3188)), and anatomical characteristics of captured specimens, I estimated that all moths belonged to the Noctuoidea superfamily, and likely the Notodontidae subfamily.

I set up my experiments based on a simplified version of previous experimental methods (Eguchi et al. 1982, Bandai et al. 1992, Silver and Smith 1999). I prepared each moth for an electroretinogram as follows: I severed the head with a razor blade and removed the

antennae and proboscis. I then placed the moth in a faraday cage for recording, inserted a ground electrode through the middle of its head, and a recording electrode into one of its compound eyes. I used a pre-amplifier at 100x amplification, and sent signal through a USB device into LoggerPro for data collection. I adapted the moth to dim light for 10 minutes prior to recording. I used a 365nm UV lamp, and a desk lamp with a 60 watt incandescent bulb along with blue and red LEE light filters as visual stimuli (Figure 3). Lamps were placed ~6 inches away from the moth. I flashed the lamps on for ~250 ms. The order in which I tested different wavelengths was randomized by a true random number generator (<http://www.random.org/sequences/>) to reduce the effects habituation and light adaptation had on the data. Each moth's response to the unfiltered bulb was used as a baseline response from which to calculate percentage-based blue and red light responses, so they could be compared across species regardless of the strength of response.

I used ImageJ to calculate both wingspan and eye diameter of each moth. I determined wingspan by measuring the length from the apex of the left forewing to the apex of the right forewing, and eye diameter by measuring the length from one point to its opposite on the eye from which I had recorded. I analyzed data in OpenOffice Calc and LoggerPro.

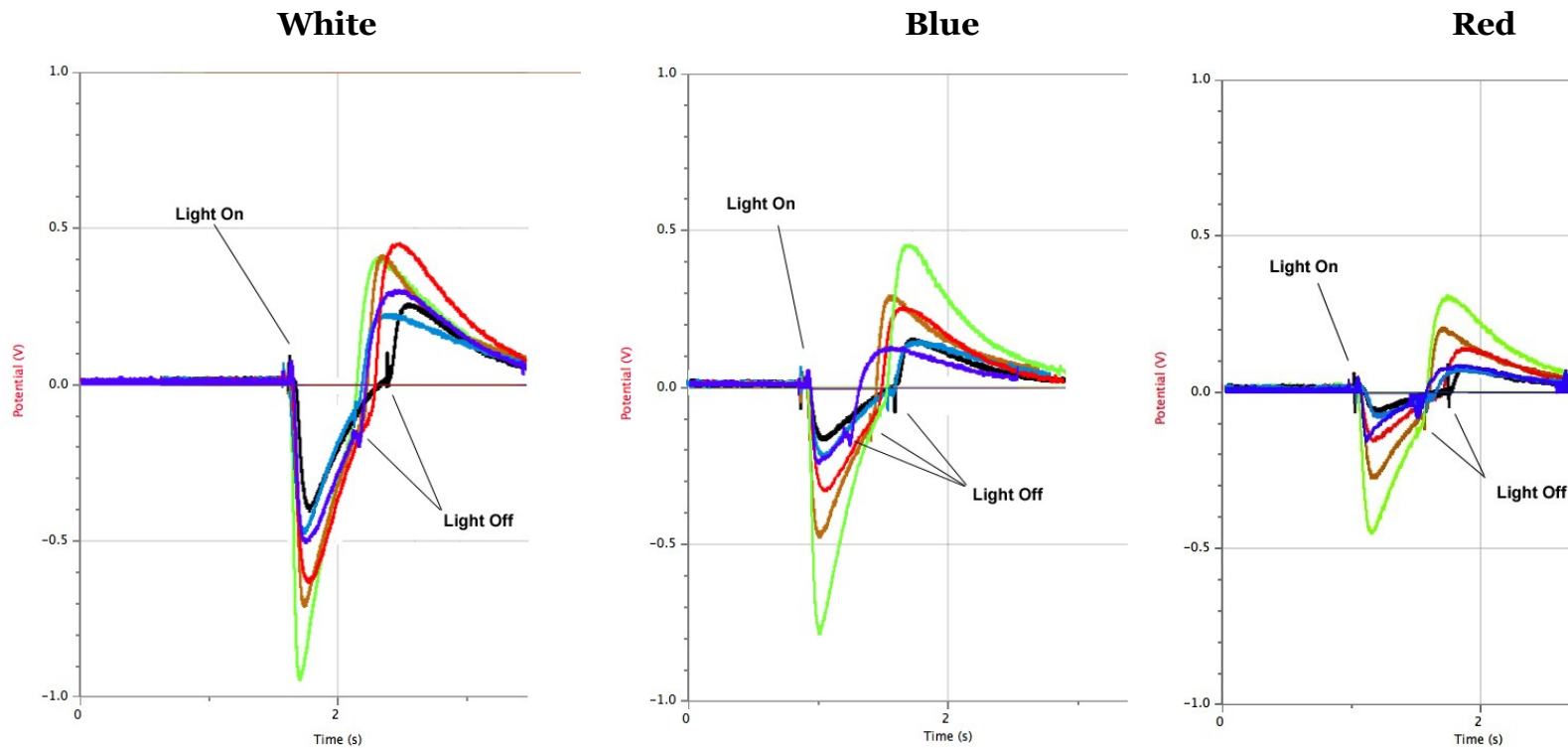
## **Results**

All moths tested showed a response to red light at ~575+ nm wavelengths, and none showed any response to UV light at the 365 nm wavelength (Figure 1). The lack of UV response is possibly accounted for by Eguchi et al. (1982), as they found that many Lepidoptera perceive UV at the 383 nm wavelength. There was a relationship between relative responses to blue and red light,  $r = .93$ ,  $p = .007$  (Figure 2), which were calculated for each individual as a percentage of their response to white light. The standard deviation of the differences between relative blue and red light responses for tested moths was only 6.08%, which shows that the proportion of red to blue light responses was similar between moths.

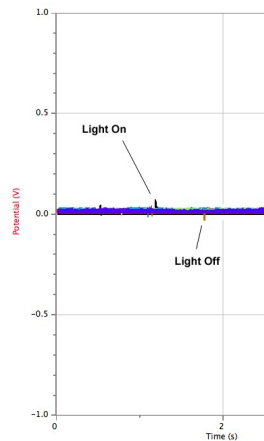
These data show that the directional and proportional relationship between blue and red reception was isomorphic in all of the tested species.

There were no significance correlations (using a significance level of .05) between either eye diameter or wing span and level of sensitivity to light (Figure 4). However, there appears to be a pattern in the data which suggests a trend that moths with eye diameters of less than 1.1 mm will have a decreased response, and moths with eye diameters of greater than 1.2 mm will have an increased response. A possible explanation for this is because of the thick diameter of the recording electrode that was used. Upon puncturing smaller compound eyes, the electrode deformed the surrounding area of those eyes similar to the way a stick would deform a partially deflated basketball. Larger eyes did not deform when punctured. These recordings could thus be considered more accurate if performed with a smaller recording electrode. Although at this point this is purely conjecture, it is nonetheless a methodological issue to keep in mind for future studies. Additionally, a sample bias exists in the data because if commonly used lights attract larger moths, then those are the only moths I would have collected, resulting in less variation in my data.

There was, however, variation in overall sensitivity to light. Spectral responses to white, blue, and red light had standard deviations of 0.2 volts, 0.23 volts, and 0.15 volts respectively (Figure 5). To provide a sense of the relative magnitude of these deviations: they were 21%, 29%, and 32% (respectively) of the magnitude of the strongest response out of all specimens for each color of light.



**UV**

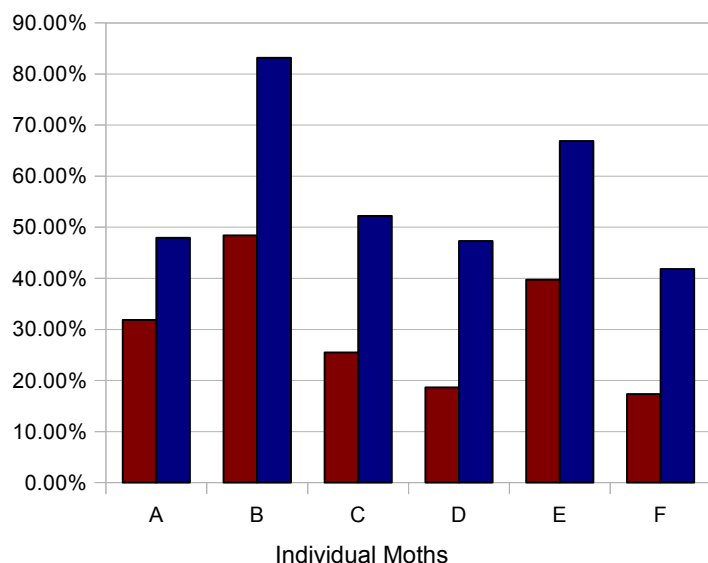


- Moth A
- Moth B
- Moth C
- Moth D
- Moth E
- Moth F

**Figure 1.**

Electrophysiological responses of individual moths to brief flashes of light (~0.25s) as determined by electroretinogram. Responses were strongest to white light, then blue, and then red, with no responses to UV at the 365nm wavelength.

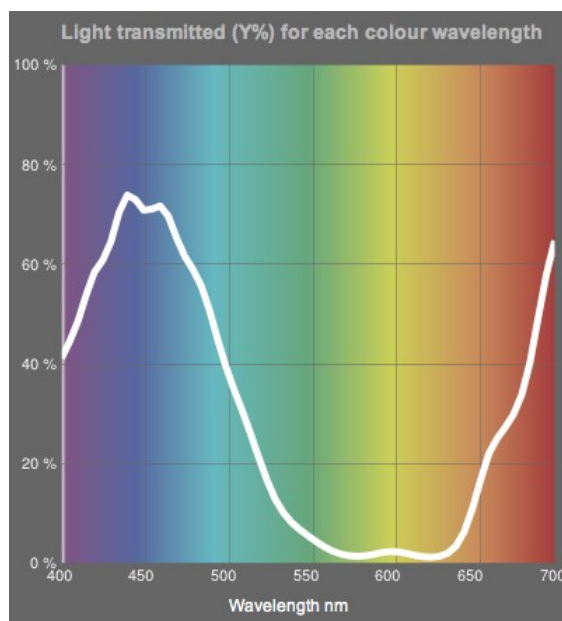
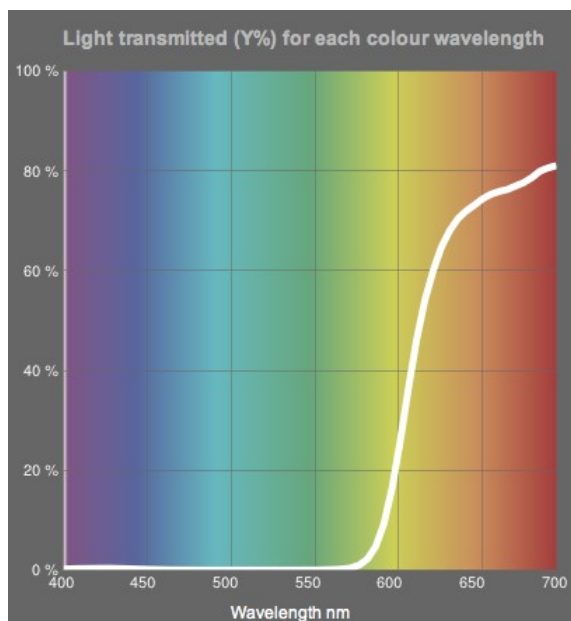
**Red & Blue sensitivity**  
relative to baseline



**Figure 2.**

A comparison between species of the strength of response to blue and red light relative to the baseline response (response to white light). I calculated relative blue and red responses as a percentage of the baseline response, which provided the greatest response in all subjects, in order to exclude the effects of eye diameter for comparison across species. Blue and red responses relative to baseline were strongly correlated,  $r = .93$ ,  $p = .007$ .

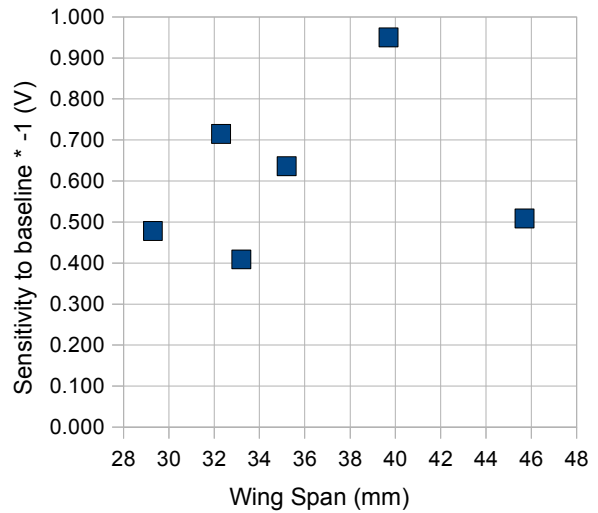
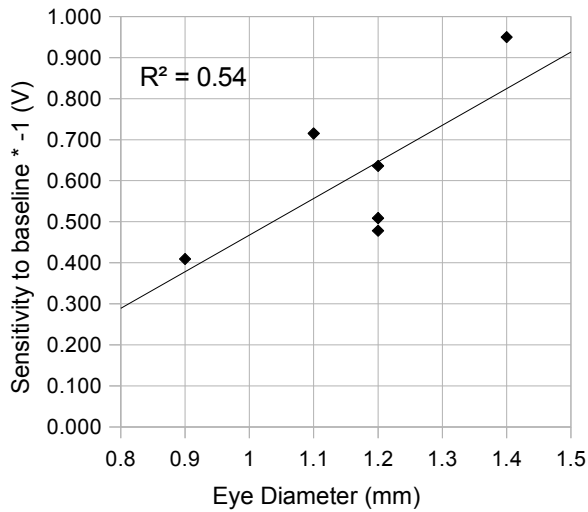
The standard deviation of the differences between red and blue light responses was 6.08%.



**Figure 3.**

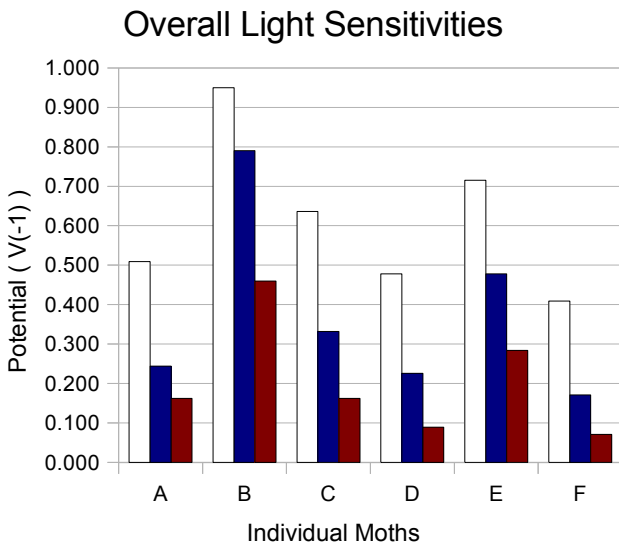
Wavelength distribution of light filters. Blue and red are shown. UV light was at the 365nm wavelength, and white light was at wavelengths across the spectrum from a 60 watt incandescent bulb. The 640+nm wavelengths present in the blue filter are inferred to have no effect given that the maximum perceived wavelength in many nocturnal moths is in the low 600nm range.

Filters and graphics from <http://leefilters.com/>



**Figure 4.**

Spectral response to white light plotted against eye diameter,  $r = 0.74$ ,  $p = .09$ , and wing span,  $r = 0.23$ ,  $p = 0.66$ . Neither correlation is statistically significant.



**Figure 5.**

Magnitude of individual responses to different colors of light. Standard deviations in response for white, blue, and red (in volts) were 0.2, 0.23, and 0.15, respectively. The standard deviations were 21%, 29%, and 32% (respectively) of the magnitude of the maximum response out of all specimens for each color of light.

## Discussion

The data are consistent with previous research (Eguchi, et al., 1982), showing that nocturnal moths possess a wide range of spectral sensitivity. The data are also consistent with Briscoe and Chittka's (2001a) findings that it is not environmental selective pressures, but rather molecular and genetic constraints which have played a dominant role in the evolution of color vision for the Lepidoptera, due to the consistent proportional distributions of spectral sensitivity I found in the tested species.

Although the relationship between light sensitivity and eye diameter was not statistically significant, future studies incorporating larger sample sizes may show a positive correlation in line with the findings of van Langevelde et. al (2011). The lack of correlation between morphological characteristics and light sensitivity is likely a result of non-substantial variation in eye diameter and wing span due to sample bias and methodology rather than a convincing instance of contradictory data.

Variation in overall light sensitivity between species was an average of 4.5 times greater than variation in spectral sensitivity (Figure 2, Figure 5). White, blue and red light responses varied by a standard deviation of 21%, 29%, and 32% of the maximum response between species, respectively, while the proportion of red to blue light response varied by only 6.08% between species. This strongly suggests that the pressures acting on the evolution of these traits have been very different, since overall light sensitivity has a much higher level of variability than spectral sensitivity.

Using color vision as a first example, theoretically there cannot be selection for specific color perception traits over others because there is not enough variation in spectral sensitivity between species, and because these species are limited genetically from developing any variations. There could, however, be enough variation in overall light sensitivity to allow for natural selection of such sensitivity, with attraction to artificial light as a case in point. The



fact that specific traits have been shown to correlate with overall light sensitivity in previous research suggests that those traits can be selected for. This could mean that in the evolutionary history of the Lepidoptera, there has been significantly more selection in regards to overall light sensitivity than color vision. My data partially supported this hypothesis, although it may have supported it more strongly without the experimental issues mentioned above.

My analysis showed that there is greater variation in overall light sensitivities than in spectral sensitivities. I hypothesize that overall light sensitivity is not a molecularly or phylogenetically constrained trait, as it has been shown to vary along with eye diameter, wing span, and body mass, traits which obviously have high variability even across individuals from the same habitat. One way to test this hypothesis further would be to compare the amount of variation in spectral sensitivity between butterflies and moths to the amount of variation in overall light sensitivity between butterflies and moths. Comparing species with vastly different behaviors and habitats, e.g. diurnal and nocturnal species, is useful for exposing conserved and non-conserved traits, because the variation of non-conserved traits becomes even greater.

From an evolutionary perspective, it is important to consider the possible selective pressures that act upon various elements of moth vision because it can aid conservation efforts, especially if the identified selective pressures, such as artificial lighting, are determined to be caused by humans. Analyses of the above questions and those related to them benefit strongly from an abundance of data regarding diverse species, because they make it possible for broader patterns and trends to be identified, as Briscoe and Chittka (2001a) proved with their phylogenetic and molecular analysis of insect color vision. The data I produced in this study provide evidence for the conservative nature of color vision, and the non-conservative nature of overall light sensitivity in the Lepidoptera, and also provide a direction for further research into the evolutionary development of such sensitivities.

## Literature Cited

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