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LIMITS ON THE WAVELENGTHS REPRESENTED IN ULTRAVIOLET IMAGES OF LEPIDOPTERA

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ABSTRACT. Ultraviolet (UV) imaging is commonly used in the study of plant and animal coloration, especially for visualizing the size and shape of UV reflectance patterns in butterflies and moths. We show that the spectral emission of light sources and the transmission spectra of the lenses and filters often used to make such images are not flat in the UV. As result the images are made with a narrow range of UV wavelengths as small as 360-390 nm in an inexpensive system with typical components. This limit on the wavelengths represented in the images can lead to various measurement errors that must be considered in using such images to characterize and study UV coloration.

Additional key words: coloration, photography, image analysis

Photographic images that are produced using only near ultraviolet (UV) wavelengths of light (300–400 nm) have often been used to visualize the size, shape, and brightness of UV components in the coloration of butterflies (recent e.g.'s: Obara and Majerus 2000; Knüttel and Fiedler 2001; Robertson and Montiero 2005; Rutowski *et al*. 2007; Fig. 1), moths (recent e.g., Lyytinen *et al*. 2004), and other organisms (recent e.g.'s: crustaceans, Ziel and Hofmann 2001; fish, Cummings *et al*. 2002; lizards, Thorpe and Richard 2000; birds, Bleiweiss 1994; plants: Yoshioka *et al*. 2005). Ideally such images are made with systems that are optimized for UV imaging with quartz lenses and filters, and broad-spectrum UV light sources (Eisner *et al*. 1969). However, such equipment is expensive and, in the case of quartz camera lenses, becoming rare, which has led those interested to seek less expensive options (Ferris 1972; Hill 1977). Indeed, relatively inexpensive digital still and video cameras with glass lenses have displayed a sensitivity to UV wavelengths that is adequate for producing such images with both sunlight and light from other sources (e.g. Acorn 2002; Rutowski *et al*. 2007).

Unfortunately, the specific wavelengths that contribute to these images have not been quantified. Given the variation in spectral output of different light sources and in the transmission properties of the lenses, filters, and fiber optics often used in such systems, there are good reasons to think that even with quartz optics, some UV wavelengths are better represented than others in these images, potentially confounding their interpretation. For example, and as Hill (1977) pointed out, glass does not transmit light at wavelengths less than 350 nm, which yields images that are based on a very biased sampling of UV wavelengths. This bias could impede accurate characterization of color pattern features in the UV that many invertebrate and vertebrate animals would be able to see (Briscoe and Chittka 2001; Bennett and Cuthill 1994).

Here we present information regarding the spectral properties of the output of several light sources we and others have used, and the transmission properties of lenses and filters employed in a non-quartz imaging system. Our goal was to assess quantitatively the spectral composition of light reaching the light-sensitive elements in such systems, and therefore the potential biases imposed by this equipment on UV images.

METHODS AND RESULTS

Characteristics of light sources. We evaluated three light sources for the intensity and spectral composition of the light they emit. The relative intensity of near UV light emission (300–400 nm) was measured using Ocean Optics OOIIRAD (v. 2 beta) software. We first calibrated our spectrometer (Ocean Optics USB-2000) for irradiance measurements with a bare 400 ϕm diameter optical fiber using a calibrated light source (Ocean Optics LS-1-CAL). For these measurements, we attached a collimating lens (Ocean Optics UV-74 adjusted to an acceptance angle of approximately 3.5°; flat transmission spectrum) to the optical fiber that had been used to calibrate the spectrometer, and positioned this fiber/lens so that it was 5 cm from and pointed directly at the surface of each bulb. The software was set to spectral graph mode ($\phi W/cm^2/nm$) and an emission spectrum was captured.

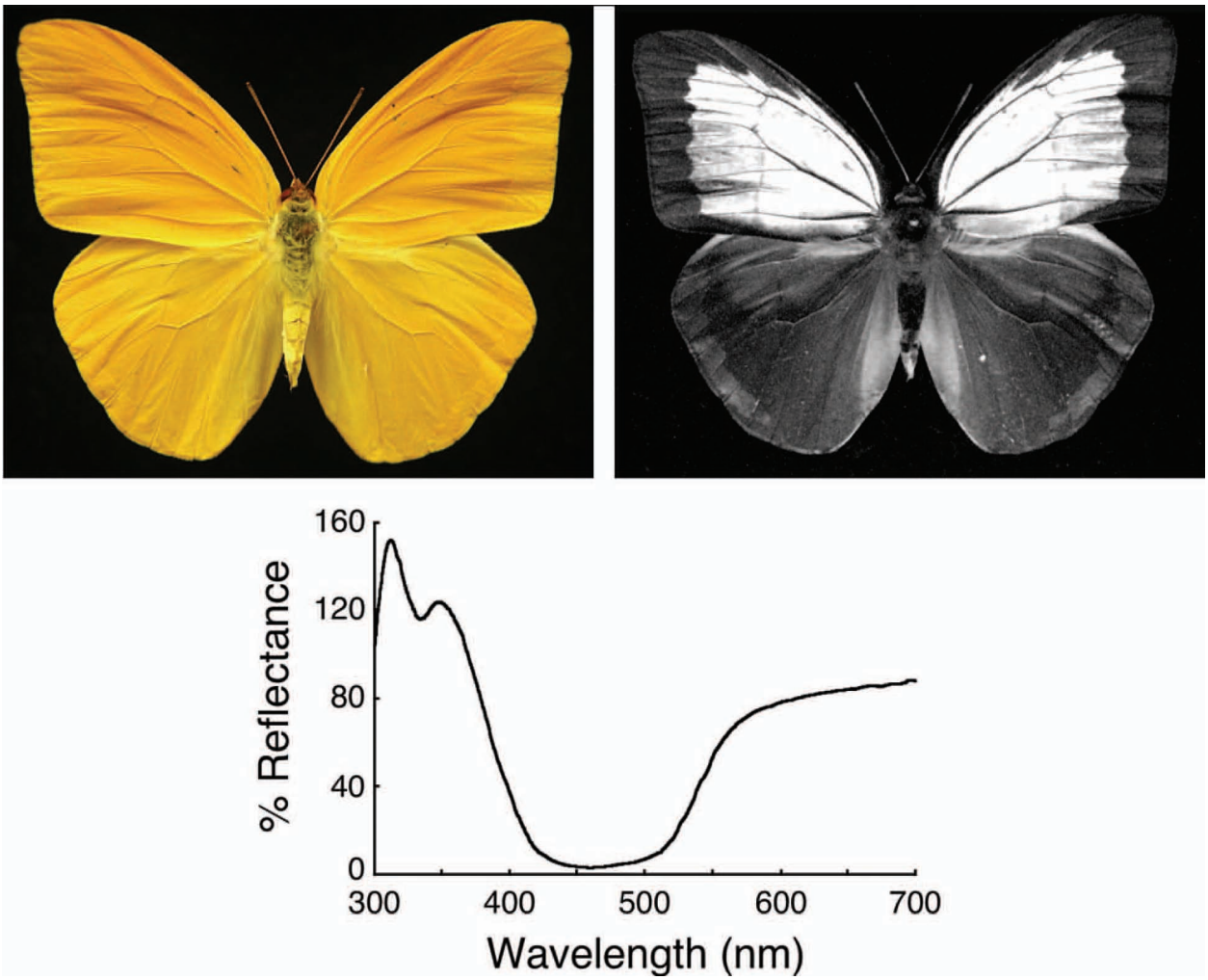

FIG. 1. Images of a male Phoebis agarithe made (above, left image) with wavelengths of light visible to humans (400–700 nm) and (above, right image) with near UV wavelengths using the system described in Fig. 4 with the straight tube fluorescent light source. The graph below shows the reflectance spectrum relative to a white standard taken from the central UV-reflectant area of the forewing.

Dark noise readings were taken prior to each measurement and subtracted from the bulb emission recordings.

Fluorescent UV light, ring tube (Sylvania 350 Blacklight 22W). Peak output of this bulb was around 340 nm but fell to 0 at 300 nm and below (Fig. 2). Two narrow, low amplitude spikes occurred in the spectrum between 400 and 450 nm. Among the light sources we examined, this source produced the highest level of UV emissions for the area of bulb sampled.

Fluorescent UV light, straight tube (length: 30 cm; Lunalite® Blacklight, IMS Corporation). The output of this tube was very similar to that of the ring tube in the UV but with only a few small peaks greater than 400 nm (Fig. 2).

Tungsten filament light with fiber optic guides (Fiber-lite Model 180 with twin gooseneck Fiber-light guides and 21V 50W EKE bulb, Dolan Jenner Industries, Inc). The output of this light source peaked around 650 nm and declined rapidly on the long wavelength side of the peak (Fig. 2). There was very little UV light in the output with essentially none below 360 nm.

Light path transmission properties. In the set up we have often used to create UV images (e.g., Rutowski *et al*. 2007), the path from the light source to the specimen and then to the photodiode in the camera includes two filters and a lens (Fig. 3). One filter ("UV pass") is mounted at the front of the camera lens and should pass only wavelengths below 400 nm (UV) and above 700 nm (infrared (IR)). The other filter is placed between the light source and the specimen and should block IR wavelengths. Our experience as well as that of

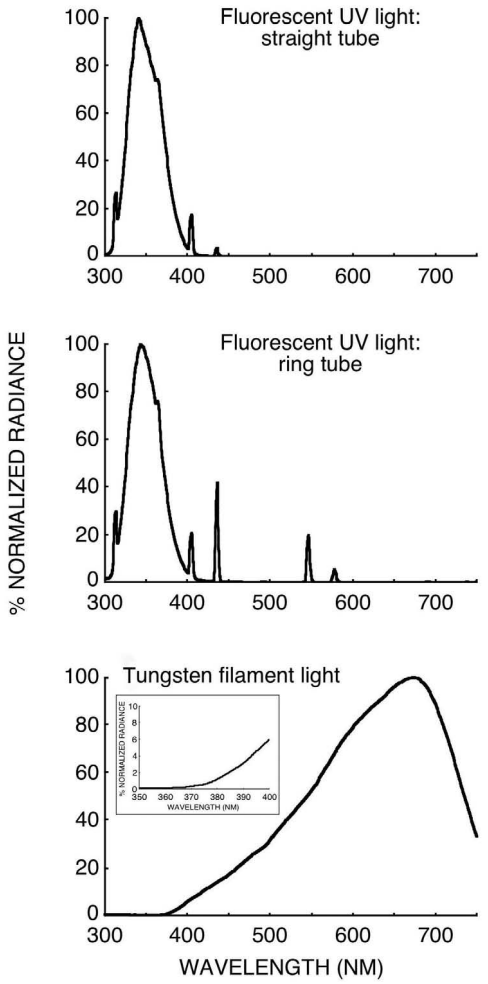

FIG. 2. Spectral properties of the output of the three light sources used in UV imaging. The inset for the tungsten filament light source shows its output in the UV with the intensity scale expanded. See text for details.

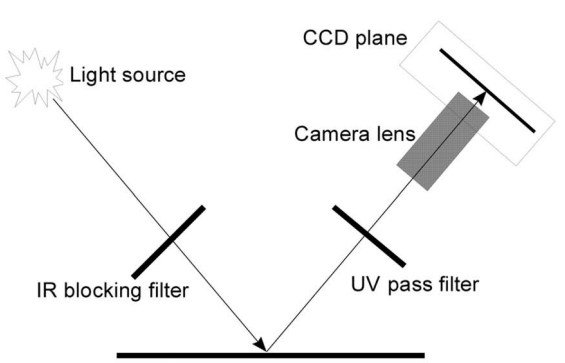

FIG. 3. Light path diagram for a typical UV imaging system.

others (http://dpfwiw.com/filters.htm#ir; last accessed 21 January 2008) suggests that the IR wavelengths passed by the UV pass filter, especially from IR rich light sources such as tungsten filament lamps and the sun, interfere with making images using only UV light. Hence, at least with the tungsten filament source, we place a filter in the light path to remove infrared energy. Others place it on the camera lens with the UV-pass filter.

With spectrophotometric equipment we evaluated the transmission properties of each of these elements in turn (Fig. 4). A light beam from a xenon lamp (Ocean Optics PX-2) was passed through the optical fiber/collimating lens arrangement described above, and was oriented normal to and 5 mm above the element whose transmission was to be measured. The element was held with a clamp 10 cm above a white standard (a slide coated with magnesium oxide), such that the beam from the PX-2 passed through and was focused onto the standard. An identical optical fiber/collimating lens setup was positioned 45° relative to and focused on a spot within the circumference of the PX-2 beam striking the white standard. This collected light was passed into the spectrometer and measured using Ocean Optics OOIBASE32 software. The element (lens or filter) then was removed from the light path and a second reading was taken from the beam striking the standard. Dark noise was removed prior to taking each measurement. We calculated the transmission characteristics of the element by taking the difference between these two reflectance spectra.

UV pass filter (Tiffen Series 7 18A). The transmission spectrum displayed a clear peak around 360 nm but dropped to essentially zero at 300 and 400 nm (Fig. 4). No measurable light was transmitted by this filter between 400 and about 710 nm. However, some infrared wavelengths were passed as is evident from

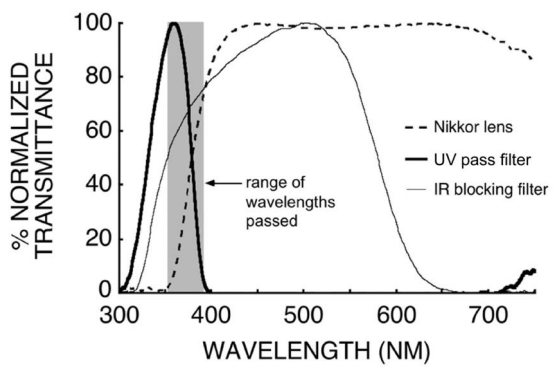

FIG. 4. Transmission spectra for elements in the light path used for UV imaging. Shaded range of wavelengths is that that would be passed through a system with these elements. See text for details.

10% transmission for this filter at 740–750 nm.

IR cut filter (Edmund Scientific). This filter displayed a single broad transmission peak with maximum transmission around 500 nm (Fig. 4). However, essentially no light energy was transmitted below 320 nm or above 650 nm.

Camera lenses. We have used two lenses for UV imaging. One lens is an AF MicroNikkor f-2.8 (Nikon), whose transmission rose quickly above 350 nm to reach approximately 95% at 420 nm, and remained largely flat at nearly 100% transmission to about 685 nm before declining slowly to approx. 85% at 750 nm. The other lens we examined was a Takumar f-1.8, 55 mm (Pentax screw mount, Asahi Optical Co.), whose transmission spectrum (not figured) was similar to that of the Nikkor lens, except that transmission rose more slowly above 350 nm, did not reach 100% transmission until about 525 nm, and began to decline slowly from 550 nm to approx. 85% at 750 nm.

From the graph summarizing the transmission characteristics of all these elements, we can see how the intensity of transmitted of light might vary with wavelength in the light path containing these elements (Fig. 4). Little or no energy at wavelengths below 350 or above 400 were passed by this combination of filters and lens. In fact, the peak of light transmission occurred between about 370 and 380 but transmission dropped rapidly to zero on either side (Fig. 4, shaded area).

DISCUSSION

Our analysis of the emission of several commonly-used light sources and the transmission properties of filters and lenses that might be used in UV photography suggests that images produced with relatively inexpensive systems are made with a very narrow range of wavelengths, namely 360–390 nm. Internal features of the camera used may also set limits on the range of wavelengths that will contribute to the image. In digital cameras, the light-sensitive diode array has its own spectral sensitivity function (Stevens *et al*. 2007). However, because this sensitivity is generally broad and extends into the UV and IR, many manufacturers cover the diode array with filters that block these wavelengths, especially those in the IR, to reduce chromatic aberration and make the picture clearer. We did not take these filters into consideration in our analysis and for the most part they are not thought to have much impact on the UV wavelengths (http://www.astrosurf.com/buil/d70/ircut.htm; last accessed 8 January 2008). In film cameras, the various films available vary significantly in their sensitivity to UV wavelengths (Ferris 1972; Hill 1977).

What sorts of problems could arise from failing to take into consideration this variation in light source UV emissions and the UV filtering properties of the light path elements? Errors would arise if the wavelength of peak UV reflection of the specimen is some distance from the wavelength of peak transmission of the imaging system used. We propose three problems that might result from light source and equipment transmission biases in recording UV reflectance of biological materials: 1) failure to detect a bright UV signal that is present, 2) underestimation of relative signal brightness, and 3) misrepresentation of the area and shape of UV pattern elements.

As a case in point, we imaged the iridescent UV reflectance from the dorsal wing surface of a male sulphur butterfly, *Eurema candida*, which led us initially to conclude that the UV reflectance was quite weak. However, subsequent spectrophotometric studies showed clearly that the UV signal was bright with a high peak (>60%) at about 340 nm, but exhibited only about 20% reflectance at 370–380 nm (Rutowski *et al*. 2007), namely, those wavelengths used to make the images. We note that even if a grayscale reference were included in the image (e.g. Knüttel and Fiedler 2000, 2001), it would be subjected to the same filtering as the butterfly image and so would still underestimate the brightness of the male's coloration. The highly unequal transmission of light across the UV wavelengths by imaging systems also needs to be carefully considered when using images for color analyses, such as those recently outlined by Stevens *et al*. (2007).

Quartz optics are transparent to UV wavelengths with a flat transmission spectrum between 300 and 400 nm. This will help broaden the range of wavelengths available in the UV to make images. However, the UV-pass filters that are required to block longer wavelengths such as the Tiffen filter we used, the Hoya UV360 (e.g. Obara and Majerus 2000; for transmission spectrum, see: http://www.hoyaoptics.com/pdf/U360.pdf; last accessed 8 January 2008), and the Schott UG1 (Knüttel and Fiedler 2000; for transmission spectrum, see: http://www.schott.com/optics_devices/filter/english/index.html; last accessed 8 January 2008) do not have a flat transmission spectrum in the UV but show a peak in transmission with steep sides in the middle of the 300–400 nm range. This limits the benefits of using quartz optics.

Also, light sources such as those used here do not emit equal intensities of UV at all wavelengths. Even sunlight contains a decreasing amount of ultraviolet energy as wavelength decreases from 400 to 300 nm. Moreover, the spectral quality of sunlight varies with moment-to-moment atmospheric changes in cloud

cover and to a lesser degree changes in sun position (List 1951).

In summary, UV imaging is certain to remain one of the primary techniques used for assessing the shape and size of UV color pattern elements. Even with the limitations discussed here UV imaging is a powerful and quick qualitative technique for assessing characteristics of UV color patterns in animals and plants. However, for those using it to quantify color signals, we make three recommendations. First, the spectral properties of the light sources, filters, and lenses used should be carefully taken into consideration in interpreting any aspect of resulting images. Second, as much as is practically and economically possible, we recommend the use of equipment that maximizes the range of UV wavelengths that are contributing to the resulting images. Finally, any effort to quantify the reflectance properties of UV signals of interest should couple the use of images with full-range spectrophotometry.

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