

Scientific Note: Evaluating potential aposematic signals in caterpillars using a fluorescent microscope and spectrometer

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Abstract: While fluorescence induced by UV light is fascinating and useful in studying animals, it is not immediately clear how much it correlates with the UV spectrum of reflected light, which, while invisible to humans, is visible to many other animals, including predators. Here, I illustrate several caterpillars, *Danaus plexippus* (Nymphalidae), *Eumaeus atala* (Lycaenidae), *Isochaetes beutenmuelleri* (Limaconidae), and *Asbolis capucinus* (Hesperiidae), contrasting their appearance under white light with that when illuminated with UV light in near darkness. All but the last species are known to be toxic to predators. In all four species, some parts of their body appeared to be fluorescent under UV light, which is rarely the case in cryptically colored caterpillars. In a stinging, poisonous, but normally cryptic caterpillar of *I. beutenmuelleri*, fluorescence is localized to the dangerous tips of the caterpillar's projections, in *D. plexippus* to the narrow white stripes, in *E. atala* to the dorsal yellow spots, and in *A. capucinus* to the last abdominal segment. While in the first three fluorescence appears to enhance the aposematic pattern, in the last, where the caterpillar inhabits a tube-shaped leaf shelter, the last segment is the one that 'plugs' the entrance and may deliver a warning signal to a predator. I demonstrate with the help of reflectance spectrometry that the fluorescent dorsal spots of *E. atala*'s caterpillar do not reflect more UV compared to the rest of the caterpillar's body, except for short-wave UV around 200 nm, where while the difference is barely significant, the signal is weak. Spectrometry also demonstrates that the fluorescent spots of *E. atala* reflect significantly more light than the rest of the body in yellow through near-infrared spectra, but in the green (500 nm) spectrum, the reverse is true. Based on these examples, it may be hypothesized that fluorescence may be a potentially valuable indicator of otherwise unrecognized warning signals encoded into caterpillar color patterns. However, field studies are required to confirm its function as a signal.

There is a strong selective pressure for defended caterpillars to appear both cryptic and aposematic, with the cryptic function working from a distance to avoid detection, and aposematism prevailing after detection to ward off imminent attack. To achieve this dual function, some animals may engage in specialized displays. For instance, Bluetongue Skink display the backs of their tongues, which are more luminescent than the front, only after they are discovered by a predator and attack becomes imminent (Badiane *et al.*, 2018). In caterpillars, however, which are less equipped to detect an approaching predator and have fewer specialized organs and behaviors than vertebrate prey, in only a few cases (such as the eversible osmeteria of swallowtail (Papilionidae) caterpillars, e.g., Damman (1986)), or sounds produced by various Bombycoidea (e.g., Bura *et al.*, 2009, 2016) do caterpillars employ active defensive displays. Otherwise, the change in display to predators by caterpillars tends to be passive, encoded into a color pattern and its function depending on the viewing distance.

It is known that avian UV vision enhances contrast in the forest environment, likely improving searching for caterpillars (Tedore & Nilsson, 2019). Comparing photographs that simulate bird vision in Tedore and Nilsson (2019) with the way leaves appear under the UV light of, for example, a Dinolite Edge AM4115T-CFVW 10X-220X UV fluorescence

digital microscope, leads me to believe that other objects, such as caterpillars, could also be visualized through bird eyes by using photography under UV light. Fluorescence is the form of luminescence in which certain pigments in some animals emit light usually of a longer wavelength than the light absorbed. Fluorescence is especially noticeable to a human eye when it is instigated by UV light in dark conditions, producing sometimes unexpected bright patterns.

While in most edible, cryptically colored caterpillars, fluorescence is nearly or totally absent (pers. obs.), in some aposematically colored caterpillars some of the pattern elements appear to be quite fluorescent. For instance, in Figure 1, I illustrate several caterpillars and contrast their appearance under white light with that when illuminated with weak 400 nm UV light in near darkness. In *Danaus plexippus* (Nymphalidae) (Fig. 1A), which gains its chemical protection from cardenolides ingested with Milkweed leaves (Roeske *et al.*, 1976), the narrow white stripes and white spots on the prolegs fluoresce, enhancing the overall striped warning appearance. In the caterpillar of *Eumaeus atala* (Lycaenidae) (Fig. 1B), which is toxic due to ingesting cycasin from Coontie Palm leaves (Bower & Larin, 1989), the dorsal yellow spots that make it resemble a toxic glow-worm are fluorescent. In a stinging, poisonous, but normally cryptic caterpillar of

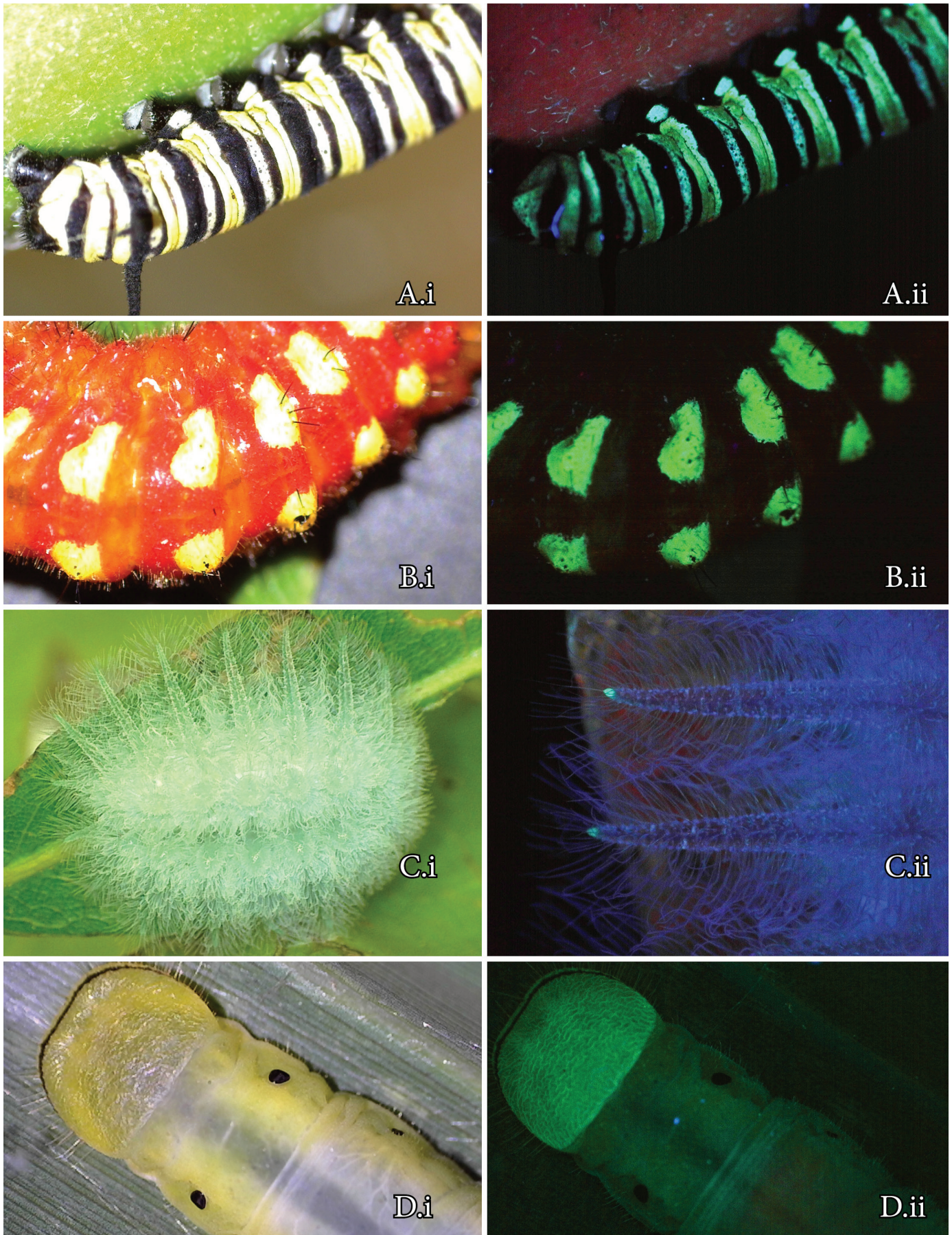


Figure 1. Caterpillars under white light (i) and UV light in near darkness (ii): A. *Danaus plexippus* (Nymphalidae). B. *Eumaeus atala* (Lycaenidae). C. *Isochaetes beutenmuelleri* (Limacodidae). D. *Asbolis capucinus* (Hesperiidae).

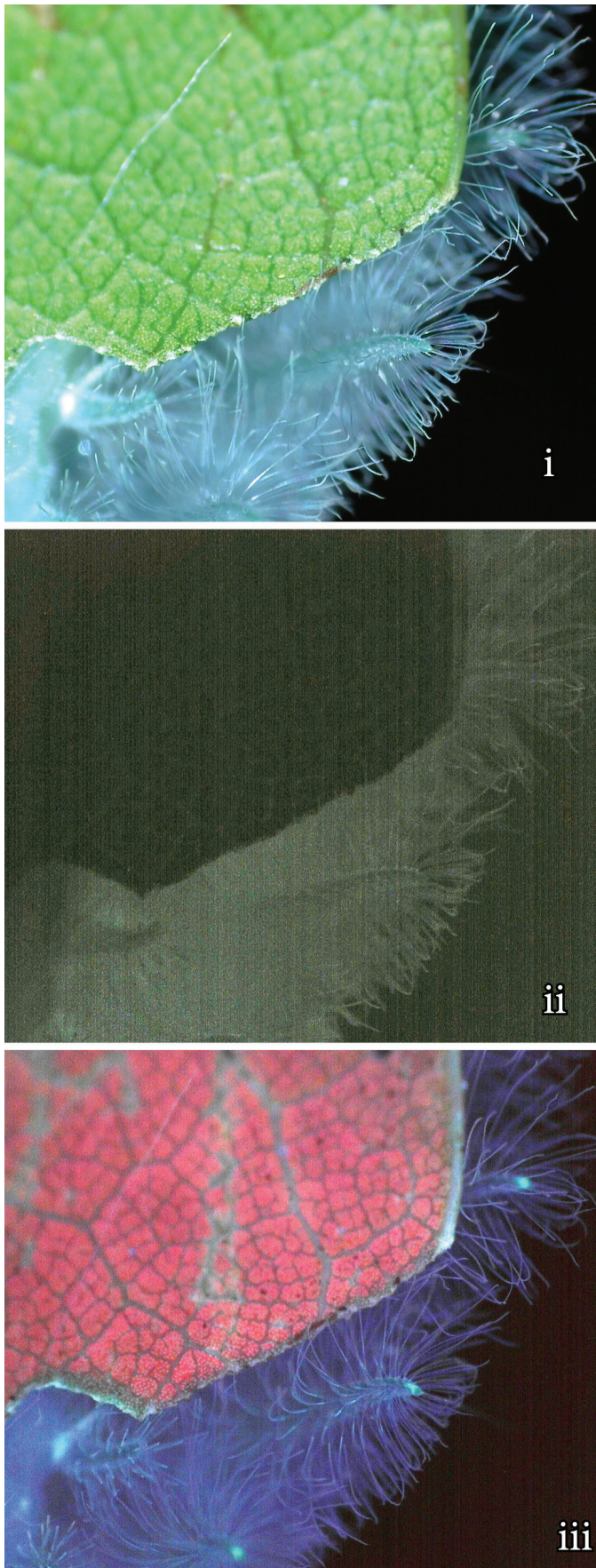


Figure 2. *Isochaetes beutenmuelleri* (Limacodidae) photographed in LED white light (i), near dusk conditions of daylight (ii), and under UV in near dusk condition (iii).

Isochaetes beutenmuelleri (Limacodidae), which gains its effective protection from synthesizing histamine and histidine, along with other substances (Fourie & Hull, 1980; Murphy *et al.*, 2009), the fluorescence is localized to the dangerous tips of the caterpillar's spines (Fig. 1C). For comparison, I provide three, otherwise identical, photographs of *I. beutenmuelleri* (Fig. 2): one taken in bright white light (simulating sunlight), one in dim light (simulating dusk or moonlight), and one in UV light (simulating birds' perception). While in the first two the caterpillar appears cryptic, in the third the tips of the caterpillar's projections are bright, and they are the ones that deliver in a syringe-like fashion the toxins together with, one presumes, a memorable message.

Finally, in *Asbolis capucinus* (Hesperiidae) (Fig. 1D), which is, to my knowledge, not known to be chemically defended, the fluorescence is localized to the last abdominal segment. While in the first three species the fluorescence seems to enhance the aposematic pattern, in *A. capucinus* the caterpillar inhabits a tube-shaped leaf shelter in a palm leaf, so that the last segment is the one that 'plugs' the entrance. In this case, the last segment may therefore have a possible warning pattern that is visible to predators, in the same way that shelter-feeding skipper caterpillars and their pupae frequently have eyespots and other warning patterns, on both ends of caterpillars (Janzen *et al.*, 2010). In the case of *A. capucinus*, the enlarged black posterior spiracles and a transverse line across the last segment may contribute to the impression that this is a potentially dangerous organism, rather than a caterpillar, hiding in the shelter.

Using reflectance spectrometry (OceanOptics system consisting of a Flame spectrometer, light source and 400 μm reflection probe combined with OceanView software), I demonstrate (Fig. 3) that the fluorescent dorsal spots of the *E. atala* caterpillar in most of the UV range through blue (400 nm) do not reflect more UV compared to the rest of the caterpillar's body, except for the short-wave UV around 200 nm where the difference is barely significant and the signal is very weak. However, spectrometry also demonstrates that the fluorescent spots of *E. atala* reflect significantly more light than the rest of the body in most visible spectra (yellow at 550 nm through near-infrared). In the green spectrum (500 nm), the reverse is true, so, from a distance and in the normally diffused greener light of the understory, *Atala* caterpillars should not appear more aposematic on a green background than if they were uniformly colored. However, at closer and closer range, the aposematic pattern should become more and more pronounced to an approaching bird, even in dim light. It is interesting to note that the spots reflect more than the body in the near-infrared spectrum, which may be useful in conveying the aposematic pattern to predators equipped with infrared vision.

Based on these limited examples, it may be speculated that fluorescence, while not a good indicator of patterns throughout the UV spectrum, may nevertheless be an indicator of some warning signals encoded into caterpillars' color patterns. Alternatively, it is of course possible that these patterns are merely a coincidental byproduct of some caterpillar pigments, or perhaps both are true depending on the species. Until field tests are conducted focused on possible functional significance, this note remains mere speculation about these hidden color

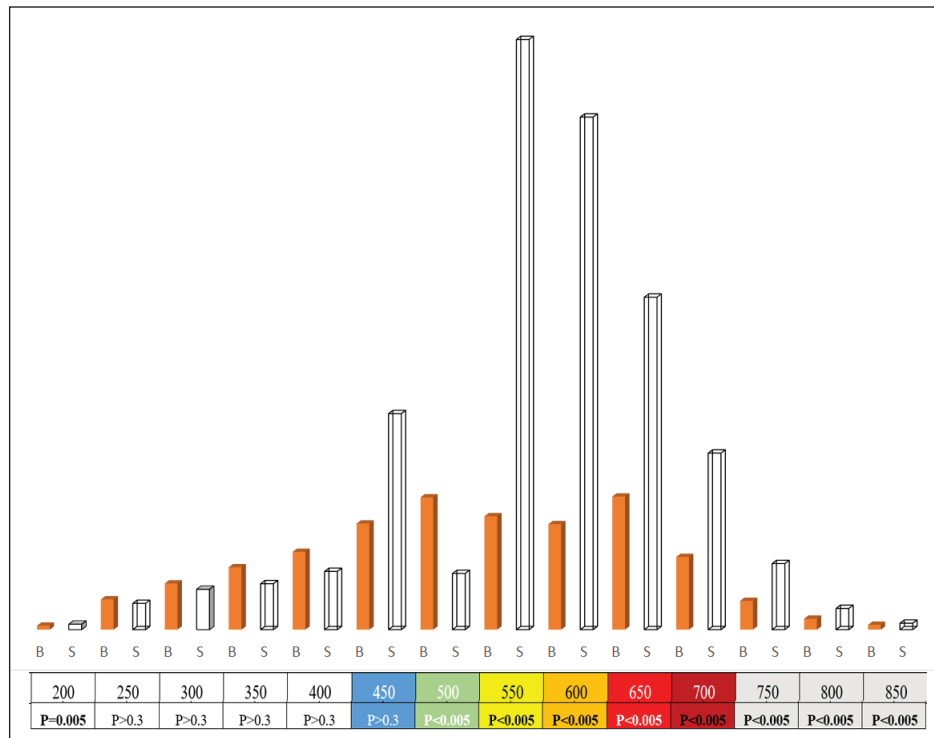


Figure 3. Relative amounts of reflections from body (orange bars, B) and spots (white bars, S) of *Eumaeus atala* caterpillar (see Fig. 1) by different spectra of light as measured by reflectance spectrometry; T-test is based on 10 measurements for each caterpillar region. Only in the green spectrum (500 nm) did the body have significantly higher reflectivity than the spots.

patterns of caterpillars, which, akin to secret writings with invisible inks, can only be revealed under special conditions.

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