

October 18, 2020

To Whom It May Concern:

I write to review the doctoral dissertation of David Stella. Mr. Stella's dissertation work focuses on the evolution of ultraviolet (UV) coloration and patterning in butterflies, with a particular emphasis on geographic patterns of variation in UV color patterns. The thesis itself is composed of two sections: a synthetic review of topics important to understanding ultraviolet coloration, color perception, and signaling in terrestrial visual environments. This overview is followed by six published studies investigating geographic patterns of UV coloration in the butterflies *Pieris napi*, *Gonepteryx rhamni*, *Colias* spp., and *Araschnia elevana*. Together, these elements represent a strong body of work that is likely to motivate future work by researchers interested in butterfly coloration and evolutionary ecology.

Strengths:

The work, especially the published studies, are commendable in their rigor, employing careful characterization of wing patterns with appropriate multivariate analyses of geographic and environmental variables. Although there are similarities in both technique and outcomes between the group of published studies, each "story" of geographical variation in wing coloration is likely to be the result of selective pressures and ecological relationships specific to a given taxa. In this regard, it is rather striking that the overall message is that costly UV-related color traits appear to become more pronounced in more southerly and/or more productive habitats. This general finding is likely to motivate subsequent work to better understand the specific driving forces responsible for this consistent result.

I also commend Mr. Stella for advocating for a view that UV traits are by no means unique from the standpoint of the organisms that bear them, but are rather just a part of their color world. For a stretch of time, many researchers falsely claimed that UV signals were somehow unique, largely, as it turns out, because we can't see them. But I agree that for most animals, UV is simply a part of their visual Umwelt, and holds no specific meaning except as imparted by the particular visual "vocabulary" of their lives.

Lastly, I appreciated the thorough and broad scope of the introductory overview, which touched on subjects not necessarily considered in the published work. This communicated a broader understanding of the biology of visual perception and visual ecology, which complemented the more specific empirical work.

Weaknesses:

My general impression of the thesis is a strongly positive one. The published work largely left me with follow-up questions, which I describe later in my review, and are mainly motivated out of curiosity. Thus my discussion of weaknesses is focused mostly on small details in the introductory overview. There are times when this overview makes sweeping statements that are perhaps too overly general or are not representative of the current state of the field. In a couple of instances, there are also missed



opportunities to cite relevant work. I detail these rather specific criticisms in order of appearance, rather than in order of importance, below:

P2 – In the first paragraph, I would encourage a revisiting of the assertion that UV vision is least explored. There has now been decades of work on UV vision (see Tom Cronin's recent review), so this is now a well-established area of study. In the second paragraph, I wonder what is meant by UV reflectance having no functional role, given that significant portions of the thesis involve UV reflectances with functional roles at least in signaling.

P4 – I was confused by the reference to UV haze and UV camera filters as an explanation for Rayleigh scattering. Perhaps remove this reference, as it seems not to serve a clear purpose here? In the subsequent paragraph, I would note that there are many excellent models for the optical properties of aquatic environments that include estimates for the behavior of UV light.

P6 – Lambertian reflectors are only “more realistic” for diffusely scattering tissues. Many biological tissues are better modeled as specular reflectors! I'm also not convinced that the paragraph on albedo is necessary to include. Lastly, the second sentence of the last paragraph appears to equate signaling traits with traits that are subject to evolutionary hypotheses. While these two trait sets are overlapping, they are not equivalent, and this sentence should be adjusted to acknowledge this.

P7 – I'm not sure what is meant by “plant-host populations” in regard to bird-plant interactions.

P8 – UV reflectance in mammals DOES have biological functions, although it less often has signaling functions. Often, UV reflectance (or the lack thereof) is involved in photoprotection, camouflage, etc., in mammals.

P11 – I'm not sure what is meant by “meta-organismal” here. My understanding of metaorganisms is that they are “multigenomic” organisms, but this seems not to qualify.

P13 – What is meant by “a weakness in the orange and red colour range”? Is this meant to communicate an inability to see in these wavelengths? Also, I disagree with the sweeping assertion that “UV reflectance in the Lepidoptera is more associated with nocturnal rather than diurnal lifestyle”. Indeed, most of the work on UV signaling in Lepidoptera is in diurnal butterflies, including the work in this thesis.

P16 – Here and in several other places, there is a discussion of higher “whiteness” as being a preferred trait in *Pieris* species. However, this not only ignores the more holistic perspective of integrating UV and non-UV as a continuous color space, it also misses work by Morehouse and Rutowski (2010, American Naturalist) that specifically evaluates these wing colors as colors and provides evidence for female preferences for more colorful males as mates. I apologize for the self-serving nature of this suggestion, but I encourage revisiting the 2010 paper mentioned above because it is relevant to the discussion here and elsewhere (e.g., P18) with regard to female preferences driving the evolution of colors involving distinct UV patterns.

P19 – It seems unlikely to me that tropical butterflies in general do not use UV in their color signals, but rather that Crane's assertions were due to a lack of broad sampling. We know, for example, that *Heliconius* butterflies often have distinct differences in the UV (e.g., work by Bybee, Briscoe, etc.)

P20 – Is the contrast in results from *Bicyclus anynana* described midway through the first paragraph resolved by the seasonal polyphenism in mate choice described by Prudic, et al. cited later in the thesis? Also, in the second paragraph, there is a missed opportunity to cite Jeff Oliver and Antonia Monteiro's



paper in Proc B from 2009 on the compartmentalization of naturally and sexually selective forces on the ventral and dorsal surfaces of butterfly wings respectively.

P23 – It is more typical to refer to “prey-predation interactions” as “predator-prey interactions”. Also, as noted before, the assertion by Lyytinen et al that UV patterns are more common in nocturnal Lepidoptera does not, I don’t think, hold up to broader taxonomic scrutiny, even within the Lepidoptera.

P24 – My understanding is that while avian predators do not learn to fully avoid “white” butterflies like *Pieris*, they tend to take other non-“white” butterflies first, suggesting an overall aversion to them.

P25 – The middle paragraph would benefit from citing Morehouse and Rutowski, 2010 (*American Naturalist*) where this question of countervailing selection on sexually selected traits by predators is directly considered.

P27 – I’m not sure what is meant by “achromatic UV signal”. Also in this section, a number of authors, perhaps most notably Richard Prum, have argued that the nanostructure assembly underlying structural coloration is not particularly costly because it is the result of self-assembly processes that are not under any immediate control during development. In other words, if you just put the right amounts of the right ingredients in the right location, these nanostructures emerge based on self-assembly processes. It might be useful to acknowledge this as a possibility during this discussion.

P28 – Pigment types here are listed with family associations in parentheses afterwards, which implies family-level associations, but many of these pigments are expressed by several butterfly families (e.g. melanins are not restricted to nymphalids, flavonoids are not restricted to lycaenids, etc.)

P29 – The sentence starting with “Illumination of the upper wings...” is confusing, as it is not a general property of all butterflies. Also, the “glass scale” cover scale description is, as far as I understand, something rather unique to *Morpho* butterflies, rather than being a generally observed phenomenon across the Lepidoptera. Finally, UV-reflectant structural colors are not restricted to pierids. For example, see the nymphalid *Hypolimnas bolina*, which has a violet (to us) structural color that peaks in the UV.

P33 – What is meant by “more prominent UV patterns”? Patterns with greater UV contrast? Would be helpful to clarify. Also, although I agree that the *Gonepteryx* study concluded that there was no evidence for condition dependence, this evidence was limited to condition dependence as assessed by measuring fluctuating asymmetry. In other words, these colors may still be condition dependent in some other way, and this portion of the discussion should acknowledge this limitation.

P34 – I don’t agree that the Kemp work indicated that UV patterns were correlated with UV light in the environment. Instead, this work indicated the female responses to male UV patterns were contingent upon access to ambient UV light (in order to see them, which seems only natural). The third paragraph (starting with “Although a number of direct...”) misses an opportunity to discuss the extensive work on human skin coloration and its relationship to UV irradiance.

P37 – The number of types of ommatidia in a butterfly eye depends on the species. Certainly the Arikawa work cited here provides an example of a species where there are three main types, but there are other examples in the work of Mike Perry, Kyle McCulloch, etc. that provide alternative arrangements in other butterfly species. Also, many if not most butterfly species have 9 photoreceptors in each ommatidium, rather than the 6 cited here.



P38 – The characterization here of chromacy levels in butterflies is not correct. There are many examples of butterfly species with many more photoreceptor types (e.g. *Papilio xuthus* has 8, *Pieris rapae* has 6, etc.). The more pertinent question is whether the color vision itself utilizes all photoreceptor types in a butterfly species or a subset. For example, *P. xuthus* only uses 4 of its 8 photoreceptor types during color vision tasks, making it a tetrachromat. The functional chromacy level has not been conclusively established for most other butterflies. Lastly, here and a few other places in the introduction, the fused rhabdom of a single ommatidium is referred to as a retina, but the retina is generally considered to be the sum contributions of all ommatidia across the entire eye, rather than any single ommatidium.

P39 – The statement at the end of the page is not up-to-date. We now have spectral sensitivities for dozens of lepidopteran species. These results are summarized in a review by Casper van der Kooi and colleagues in the *Annual Review of Entomology* published this month.

P41 – Funny quip about Rudolph! 😊

P42 – The definition of hue here leaves something to be desired. Hue is generally defined as the wavelength of maximum reflectance, which often corresponds to the perceived dominant color of an object or light source.

P43 – It is not true that neural systems mediating color vision have not been studied. There is a lot of work available. Work on the neurobiology of color vision in primates goes back decades and is well developed. In invertebrates, recent work in *Drosophila* by Schnaitmann and colleagues is relevant here, as is recent work by Kentaro Arikawa, Gregor Belusic, and Michiyo Kinoshita (in butterflies).

P45 – ERGs do not measure the effect of visual signals on the central nervous system, but rather measure the population response of photoreceptor cells in the retina (a peripheral, rather than central, nervous system response).

P46 – I don't necessarily agree that UV reflectance, UV phenomenon, and UV iridescence all describe exactly the same phenomenon. For example, UV reflectance is often used to describe differences in UV absorbance of diffusely scattering tissues (e.g., pterin-pigment-based colors), which an entirely different phenomenon from the UV iridescence produced by multilayer thin films on the wings of, for example, *Colias* butterflies.

P48 – I found the sentence that starts with "Due to a different wavelength" to be confusing. What you're referencing here is linear chromatic aberration, but you don't orient the reader to which wavelength you're talking about (it is presumably UV, which would focus closer to the lens system than longer wavelengths). I would encourage revisiting this portion to rephrase.

P49 – I was a bit confused by why it was relevant to note that improperly imaged UV photographs were not aesthetically appealing. It seems that the relevant point here is that they're scientifically misleading.

Questions:

Many of your results indicate that northern populations and/or species have either reduced structural UV coloration (*Gonepteryx rhamni*, *Colias* spp.) and/or reduced UV-absorbing pigmentation (*Pieris napi*). How might these patterns interact with latitudinal changes in voltinism? Changes in voltinism have



important implications for developmental timing and the length of a growing season, which might impact patterns of resource acquisition and allocation relevant to color production.

Throughout your empirical work, you highlight that increases in structural UV coloration and/or UV-absorbing pterin pigments appear to be associated with populations/species living in more productive habitats. You then draw the logical conclusion that such habitats might provide greater access to dietary resources needed to fund these colors. However, another potential consequence of higher primary productivity in these environments is increased population sizes and population densities. How might such changes to population dynamics play a role in the evolution of the patterns of wing coloration that you have observed?

One geographic pattern relevant to Western Europe in particular is differences in the history of industrialization, and associated patterns of anthropogenic nitrogen deposition.

Sincerely,

A handwritten signature in black ink, consisting of a series of loops and a long horizontal stroke extending to the right.

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