PŘÍRODOVĚDECKÁ
FAKULTA
Univerzita Karlova

Katedra filosofie a dějin přírodních věd

PROSTOROVÁ A ČASOVÁ VARIABILITA UV REFLEKTANCE VE VZTAHU S PROMĚNNÝMI PROSTŘEDÍ U RODU *PIERIS* A *COLIAS*

Disertační práce

Mgr. David Stella MSc

Vedoucí: doc. Mgr. Karel Kleisner, Ph.D.

Praha 2020

FACULTY OF SCIENCE
Charles University

Department of Philosophy and History of Science

SPATIAL AND TEMPORAL VARIATION OF UV REFLECTANCE IN RELATION TO ENVIRONMENTAL FACTORS IN GENUS *PIERIS* AND *COLIAS*

Dissertation thesis

Mgr. David Stella, MSc

Supervisor: doc. Mgr. Karel Kleisner, Ph.D.

Praha 2020

Bibliographic record

STELLA, David. Spatial and temporal variation of UV reflectance in relation to environmental factors in genus *Pieris* and *Colias*. Prague: Charles University, Faculty of Science, Department of Philosophy and History of Science, 2020. 160p. Supervisor: Doc. Mgr. Karel Kleisner PhD.

Prohlášení

Prohlašuji, že jsem předkládanou práci *Prostorová a časová variabilita UV reflektance ve vztahu s proměnnými prostředí u rodu* Pieris *a* Colias vypracoval samostatně a za použití jen uvedených pramenů a literatury. Dále prohlašuji, že práce nebyla využita k získání jiného nebo stejného titulu na této ani jiné univerzitě.

Studie uvedené v této práci byly podpořeny Grantovou agenturou Univerzity Karlovy GAUK 764313/2013 a GAUK 964216.

V Praze dne Mgr. David Stella MSc

Declaration

I hereby declare that I worked on thesis *Spatial and temporal variation of UV reflectance in relation to environmental factors in genus* Pieris *and* Colias on my own, using only sources mentioned in the Bibliography section. Moreover, no degree, diploma, or distinction has been conferred on to me before, either at this or in any other university, for the present thesis which I am submitting to the Charles University.

Listed studies were supported by The Charles University Grant GAUK 764313/2013 and GAUK 964216.

Prague Mgr. David Stella MSc

Poděkování

Nejprve bych rád poděkoval mému školiteli Karlu Kleisnerovi, mému vedoucímu, ale i kamarádovi. Ačkoliv jsem si vědom, že jsem mnohokrát nenaplnil jeho očekávání, opozdil se a věnoval se jiné práci, byl schopen mě motivovat k další práci. Měl jsem to štěstí studovat pod jeho vedením již od bakalářského studia. Jeho cit pro jazyk, inovativní a zevrubné řešení vědeckých otázek mi budou vždy příkladem a motivací.

Dále bych rád poděkoval mým kolegům a spoluautorům, zvláště pak Pavlovi Pecháčkovi za jeho skvělé nápady a vytrvalost při dlouhých hodinách v muzejních sbírkách při sběru dat a psaní vědeckých publikací. Ostatní vědečtí kolegové mi byli vždy oporou a inspirací k další práci.

Děkuji také celé své rodině, především rodičům, kteří mě podporovali na cestě za mými sny i přes vzdálenost tématu vůči jejich zaměření a často složité rodinné situaci.

V neposlední řadě děkuji Karolíně za její snad bezmezné porozumění, podporu a rady. Přestože nemám vlastnosti nutné k soustavné a intenzivní vědecké práce, Karolína mi dokázala pomoct v potlačení těchto neduhů.

Acknowledgments

First of all, I would like to express my thanks to Karel Kleisner, my thesis supervisor and friend. Although I often failed to meet all of his demands and requests on time and got distracted by pursuing other research interests, his patience and efforts to motivate me always helped me to focus and not give up on this project. I have been lucky to study under him since my time as an undergraduate. His feeling for language and his innovative and comprehensive approach to scientific challenges will always remain an example and a huge source of motivation for me.

I would also like to thank all my colleagues and co-authors, especially Pavel Pecháček, for his outstanding ideas and patience during the many hours of collecting data in various museums and throughout the writing process and the innumerable drafts we collaborated on. Other scientific colleagues provided me with much-needed support and motivation for completing this work.

I want to thank my family and especially my parents for their support. They stood behind me all along the way as I tried to realise my dreams and goals, although the subjects were often outside the scope of their interest.

Finally, my heartfelt thanks go to Karolína for her endless understanding, support, and encouragement. Although I am incapable of systematic and intensive scientific research due to my natural inclinations, Karolína has always been there to help me overcome obstacles.

Abstrakt

Vlnová délka ultrafialového záření leží za oblastí viditelného světla, tedy nad (ultra = nad) fialovým světlem. Termínem *viditelné světlo* je míněno spektrum světla, které je viditelné pro člověka, jehož schopnost vnímání světla je v porovnání s mnoha jinými organismy velmi omezená. Proto je komunikace v UV světle často považována za skrytou, ačkoliv je s největší pravděpodobností důležitým prvkem ve vnitro i mezidruhové komunikaci různých organismů.

Cílem této dizertační práce je objasnit funkce a relativní význam UV vzorů s důrazem na skupinu denních motýlů, a to v komplexní perspektivě ekologické a evoluční biologie. I přes nepopiratelný význam UV reflektance v biologii je tento jev nutno chápat jako součást celé palety souvisejících jevů jako jsou světelné podmínky, optické vlastnosti přírodních objektů a vizuální a nervový systém studovaných organismů. Krátká část této práce se také věnuje UV fotografii v jejích historických a metodických souvislostech.

Výsledky první předložené studie ukázaly významný vztah mezi úrovní UV reflektance a dvanácti geograficko-environmentálními proměnnými u druhu *Pieris napi*. Méně vhodné životní podmínky vedou k silnější UV reflektanci u zmíněného druhu. Dalším výsledkem studie je i zjištění, že úroveň UV reflektance je pohlavně specifická: samci mívají celkově nižší UV reflektanci než samice daného druhu. Druhá studie ukázala, že variabilita tvarů UV vzorů na křídlech *Gonepteryx rhamni* koreluje s teplotou, srážkami a zeměpisnou šířkou místa sběru jednotlivých jedinců. Třetí studie analyzuje vztah mezi 106 taxony rodu *Colias*, jejich preferovanými stanovišti a oblastmi rozšíření. Další studie mapovaly UV vzory u rodu *Gonepteryx* a *Araschnia* v rámci jejich prostorové distribuce.

Závěrem můžeme konstatovat, že existuje vztah mezi UV reflektancí křídel u studovaných druhů a geograficko-environmentálními podmínkami v nichž žijí. UV vzorce na křídlech jsou významným prvkem v mezidruhové i vnitrodruhové komunikaci denních motýlů. Navíc se ukázalo, že UV vzory mohou sloužit jako klíčový znak při rozpoznávání druhů, a nabízí se tedy jejich využití jako potenciálních taxonomických znaků. UV reflektance je tak důležitým prvkem v životě mnoha druhů motýlů.

Klíčová slova

UV, UV reflektance, Lepidoptera, *Pieris*, *Colias*, Prostorová variabilita, UV fotografie

Abstract

Ultraviolet (UV) means 'beyond violet' (from the Latin – *ultra* – beyond), whereby violet is the colour with highest frequencies in the 'visible' light spectrum. By 'visible' we refer to human vision but it must be taken into account that human visual perception is in comparison to many other organisms rather limited in terms of wavelengths it can perceive. This is why communication in the UV spectrum is often called hidden, although it most likely plays a very important role in the communication of various kinds of information among a wide variety of organisms.

The aim of the present thesis is to elucidate the functions and relative importance of UV patterns mainly in Lepidoptera from a holistic ecological and evolutionary perspective. UV reflectance cannot be studied in isolation: important interactions among several other variables such as light conditions, general optic properties of natural objects, the visual system, and signal processing are thus discussed as well, but an overall emphasis on UV reflectance is maintained throughout. Moreover, this work also briefly touches upon the historical development of UV photography and its methodological background.

The first study traces a significant link between UV reflectance levels and twelve geographical and environmental factors in the distribution of *Pieris napi*, where it turns out that less suitable conditions predict a stronger UV reflectance. Moreover, males and females of this species significantly differ in their levels of UV reflectance. The second study demonstrates that shape variation of UV patterns on the forewings of *Gonepteryx rhamni* correlates with a number of large-scale environmental variables, namely temperature, precipitation, and latitude. The third study analyses the association between 106 taxa of *Colias* and their preferred habitat and area of distribution. Further studies then map UV patterns in genera *Gonepteryx* and *Araschnia* within their spatial distribution.

Variation in the level of UV reflectance and UV pattern shape is significantly linked with large-scale environmental factors within studied species. UV patches may thus play a significant role in intraspecific and/or interspecific communication of butterflies. Furthermore, because it has been demonstrated that UV patterns are an important factor in species identification, it is proposed that they can and should be used as a prospective taxonomical trait. Based on these studies, we can conclude that UV reflectance plays an essential role in the life of lepidopteran species.

Keywords

UV, UV reflectance, Lepidoptera, *Pieris*, *Colias*, Geographic variability, UV photography.

Content

Part 1

Introduction

Biologists are physically handicapped. In comparison with the organisms they study, their senses are usually rather limited. This is most certainly the case of vision, whereby the study of animal vision began quite early and revealed a number of unexpected phenomena. Perhaps the most interesting and least explored aspect of vision has to do with the part of the electromagnetic spectrum that is normally invisible to humans but most other organisms are visually sensitive to – the ultraviolet (UV) part of the light spectrum.

Because UV reflectance is often the function of structural elements of bodies and does not play a functional role, UV perception is rather separate and distinct. UV visual sensors are frequently shaped by evolution and are thus importantly linked to the environment in which a particular organism lives. A crucial feature of UV light is 'how colour and other properties of light' appear to organisms. The 'visual merkwelt' (perceptual world) of an organism includes four basic aspects (Silberglied 1979) and this thesis is structured so as to follow the sequence of natural processes in which UV signals are formed, transmitted, and perceptually processed, that is:

1) *Light environment*: the spectral quality and quantity of light vary across time and space.
2) *Optical properties of natural objects:* light can be reflected, absorbed, or transmitted.
3) *Visual system:* light can be refracted, filtered, and absorbed. Optical properties of a particular eye (mainly receptor pigments) determine the information that is transferred in the form of an electrical signal to the brain.
4) *Central nervous system processing of UV visual information including the brain:* How the world appears to an organism depends on the way in which its brain processes and interprets the information sent to visual receptors. The visual system and the brain are key factors in determining an organism's ability to perceive a specific spectral range, including UV light. To understand how the world appears to an organism, we must therefore understand its visual world and take into account all available information about that species and its total colour vision system. It makes no sense to consider vision in UV as a completely separate subject.

The following thesis conforms to the structure outlined above and mostly focuses on optical properties of natural objects: butterfly UV patterns and their properties.

1 Light environment: UV as a variable

To start dealing with UV light in the context of biology, one must first of all understand the factors which alter the characteristics of UV light. The process of discovery of UV radiation started in 1614, when Angelo Sala noticed and documented that sunlight turned silver nitrate crystals black. In 1801, German physicist Johann Wilhelm Ritter observed that invisible rays just beyond the violet end of the spectrum were especially effective at darkening paper soaked in silver chloride. He called them 'deoxidizing rays' to express their chemical reactivity and to differentiate them from the 'heat rays' at the other end of the visible spectrum. With this observation, the existence of UV radiation, its properties and its relationship with sunlight were gradually established (Hockberger, 2002). A major breakthrough then came with photon physics in 1865, when Maxwell proposed a theory according to which light and sound are part of a larger spectrum of energy with wave-like properties. He called them electromagnetic waves.

Nowadays, ultraviolet light is defined as electromagnetic radiation of wavelengths between 10 and 400nm described as field rather than particles (Hobson, 2013). The discussion over the wave–particle duality of light is, however, ongoing and the phenomenon is still not fully understood (Johnsen, 2012). That, however, is not crucial for biological research. Approximately 10% of overall radiation takes the form of UV light and only about 3% of sunlight reaching the ground is of wavelengths shorter than 400nm (Haigh, 2007). Nevertheless, this UV light that does reach the Earth surface has more energy per quantum than other solar radiation (Liley & McKenzie, 2006). UV can be subdivided in a number of ranges, for example UVA (315nm–400nm), UVB (280nm–315nm), and UVC (100nm–280nm). This classification is derived from ISO 21348 (Tobiska & Nusinov, 2007) and used mainly by biologists (Hockberger, 2002). Of the UV radiation that reaches the Earth's surface, over 95% is in the longer UVA wavelengths of UVA, with a small remainder in the UVB range and essentially no relevant amount of UVC (Haigh, 2007). Of the various kinds of UV radiation, UVA received the greatest amount of attention from biologists due to its direct biological effects on organisms and its importance in their communication systems (Silberglied, 1979). There is nothing special about UV and most vertebrates do not have eyes adapted to its perception. Wavelengths shorter than 400nm are usually blocked by pigments in the lens and the cornea (Mainster, 2006). Humans lack colour receptor adaptations for UV rays but photoreceptors of the human retina are sufficiently sensitive to UVA to allow people lacking a lens to perceive it as whitish blue or whitish violet (Lynch et al., 2001). For further detail, see Chapter 3.

UV light varies in relation to time and location. It is therefore essential to note the factors which determine the characteristics of UV light. Spectral quality and quantity of UV light vary with the time of the day, season, latitude (Madronich et al., 1998), altitude (Chadyšiene & Girgždys, 2008), and meteorological conditions. UV light is strongest at high altitudes (every 1000m in altitude increases the intensity of UV radiation by about 11%) where the air layer is thinner and the atmosphere tends to be cleaner than at lower elevations or in the tropics, where ozone concentrations are the lowest (Liley & McKenzie, 2006). In polar and temperate regions, UV radiation is at its maximum during the summer months, while in the tropics, there is less seasonal variation (Fitzka et al., 2012). Snow cover increases UV irradiation of the earth by scattered radiation by up to 15% (Eliseev et al., 1998).

The daily variation of UV light follows a pattern similar to changes in the visible part of the light spectrum, while its nocturnal intensity depends on the lunar phase. Since lunar spectral reflectance is lower in the UV than in the visible part of the spectrum, nocturnal illumination contains less UV light than visible light (Biberman et al., 1966). Furthermore, different types of cloud cover may block or enhance UV rays. While thick and dark clouds can block UV radiation, puffy or thin-layered clouds do not (Eliseev et al., 1998). On hazy days, the values of UV light reaching the surface can be as high as on clear days. Some clouds may even increase UV radiation by reflecting and refracting solar rays or the shine of the sky back to the ground (Mainster, 2006). With decreasing visible light intensity, the general loss of UV intensity is lower than of the visible light (Brown & Wilson, 1956). This is due to a scattering of sun-light off the molecules of the atmosphere, a phenomenon called Rayleigh scattering (reduction of UV haze by UV filters in cameras). Under these conditions, UV light can therefore become a much more important visual feature in a specific organism's environment.

Optical properties of UV light in aquatic habitats are curiously difficult to estimate. For instance in the case of the sea, due to the molecular properties of water and suspended particles, as the wavelength of light penetrating the sea decreases, the scattering of photons increases. The optical path over which UV light is transmitted shortens in comparison with the blue region of the spectrum, and the veiling brightness simultaneously increases (Lythgoe, 1979). Even in coastal waters and many freshwater habitats, where particles and dissolved organics usually attenuate UVA light further, the number of UV photons at moderate depths remains well above the visual threshold of most fish.

Moreover, UV radiation ought be viewed as a non-stable or inconstant variable over relatively short time, mainly due to ozone depletion caused by human activities. Despite the success of the Montreal Protocol, whose aim was to reduce ozone depletion, over the past three decades, the phenomenon is not fully understood and this climatic change over a relatively short period of time may alter the distribution of organisms by exposing them to different levels of UV radiation with all the attendant negative and positive effects (Häder et al., 2007; Williamson et al., 2014; Beckmann et al., 2014). All this should be taken into consideration in studies of various organisms in the context of UV reflectance.

2 Optical properties of natural objects

Because in the previous chapter we avoided any discussion of or reference to the physical nature of light, this chapter is dedicated to the description of the terms and concepts most frequently used in the context of this thesis. They are presented without a mathematical description, since that is for our intents and purposes unnecessary.

The existence of any visual pattern is based on an interaction between a quantum electromagnetic field and an electron field (Feynman, 2006). The theory behind these interactions is called quantum electrodynamics (QED) (Del Giudice et al., 2005). Within the framework of this theory, all optical mechanisms are described by three basic events:

1. A photon goes from one place and time to another place and time.
2. An electron goes from one place and time to another place and time.
3. An electron emits or absorbs a photon at a certain place and time.

This broad description can be applied to all optical mechanisms. Processes such as reflection, refraction, scattering, diffraction, absorption, fluorescence, and transmission can all be derived from the various properties of photons and electrons (Feynman, 2006). The processes mentioned above are usually combinations of the abovementioned basic events and each other rather than distinct phenomena, which is also why absorption and reflectance or structural and pigmentary colouration are closely related. With respect to UV communication, the three most prominent processes are absorption, reflectance, and fluorescence.

Absorption of electromagnetic radiation is the way in which the energy of a photon is taken up by matter, typically by atom's electrons. Electromagnetic energy is thus transformed into the internal energy of the absorber, for instance in the form of thermal energy. Spectral absorption is the absorption of a specific wavelength, e.g. ultraviolet (Gates et al., 1965). Compounds capable of such specific absorption are called pigments: they are the basic elements which form chemical or pigmentary colouration (Grether et al., 2001). The process of absorption is also a necessary precondition of any vision, whereby vision relies on photoreceptor cells are transformed pigments – carotenoids.

Reflectance of surface of a material is defined as its effectiveness in reflecting radiant energy. Reflectance is expressed as the fraction of incident electromagnetic output that is reflected at an interface. In other words, it is a special and mathematically tractable case of scattering (Feynman, 2006). Biologists use this term to designate an object's ability to reflect incident light. In general, there are two types of reflectance, specular reflectance (where the angle of incidence equals the angle of reflection) and the more realistic Lambertian reflection (so-called 'diffuse reflection', where the brightness of light is invariant to observer's angle of view). Reflection strongly depends on the wavelength of light, i.e. on its colour (Johnsen, 2012). Important for our topic is interference, a special case of reflectance where photons interact with one another. In this process, two or more waves of the same frequency either reinforce or cancel out each other, whereby the amplitude of the resulting wave is equal to the sum of amplitudes of the waves which combine. Interference is the main mechanism by which structural colouration is formed. Patterns on the surfaces of organisms are the product of multiple reflections or interference caused by the structure of animal surface, its layers, and even particular organelles.

Fluorescence is a two-stage chemical process which involves the absorption of light of shorter wavelength by a chemical fluorophore, such as a protein or carotenoid (excitation), followed by the release of some of the absorbed energy in the form of light of longer wavelength (emission) (Marshall & Johnsen, 2017). The UV range is often the excitation range but it is not the only one. Blue and yellow light can, thanks to fluorescence, also produce yellow and red hues, but fluorescent is not a topic related to this text.

A related process is albedo, which also pertains to reflectivity of surfaces. In the past, the terms reflectance and albedo were used interchangeably, but a new study (Turner & Parisi, 2018) suggests that analysis of the conditions and assumptions underlying each of these terms would help us tell them clearly apart. In general, the authors recommend that albedo be used to measure the natural reflectance of mostly horizontal surfaces in conditions where reflection does not significantly change during the day. Reflectance, on the other hand, should be viewed as a property of non-natural surfaces and non-horizontal surface measurements which do change depending on various factors including the geometry of incoming and reflected irradiance or the intrinsic nature of the surface itself (Turner & Parisi, 2018). This qualification, however, does not significantly influence the terminology used in this study or in biology in general, because there the term reflectance is tends to be used in the study of signalling in an ecological and evolutionary context.

2.1 UV reflectance

In general, there is nothing special about UV reflectance (for more on this subject, see Chapter 2). UV reflectance has been found in most types of signalling objects, that is, in objects which tend to be the subject of evolutionary hypotheses. Such objects include bird plumage (Burkhardt 1989), plant fruits and seeds (Burkhardt, 1982; Altshuler, 2001; Siitari et al., 1999), lizard dewlap (Fleishman et al., 1993;

Arribas, 2012), spiders (Heiling et al., 2005; Lim et al., 2007; Hsiung et al., 2019), and most importantly for us, also moths, butterflies, and other insects (Petersen et al., 1951; Eisner et al., 1969; Silberglied, 1979). We should note that since UV reflectance is sometimes viewed as a type of scattering, some authors use the term UV scattering instead of UV reflectance (Johnsen, 2012; Kemp, 2007b).

Within the holistic approach of this thesis, it is important to mention Lepidoptera are not the only organisms which have and use UV patterns for their ecological and evolutionary traits. This is also why in the following, we also offer a brief excursion into UV reflectance and its adaptive functions in birds, lizards, amphibians, fish, and mammals.

Birds play an important role in the visual ecology of other organisms. They are probably one of the most frequently studied groups in connection with UV patterns and UV signalling. Majority of research on UV patterns in birds focuses on feathers (Stevens & Cuthill, 2007), although several studies looked at horned body parts, such as beaks (Jouventin et al., 2005). Over the course of decades of study, there emerged a number of topics in this area, such as the role of UV signalling in the context of intrasexual selection and sexual dichromatism (Hastad et al., 2005; Cuthill et al., 1999; Eaton, 2005; Hausmann et al., 2003; Hunt et al., 2001; Jouventin et al., 2005; Alonso-Alvarez et al., 2004; Doucet & Montgomerie, 2003; Burns & Shultz, 2012), intersexual communication (Bleiweiss, 2004; Hausmann et al., 2003; Meyer-Rochow & Shimoyama, 2008; Gamero et al., 2015), the role of UV patterns in sexual recognition and as a taxonomical tool (Andersson & Andersson, 1998; Hunt et al., 1998), UV pattern in the context of predator–prey detection and foraging strategies (Hastad et al., 2005; Church et al., 1998; Koivula & Viitala, 1999; Koivula et al., 1997; Lyytinen et al., 2001; Maddocks et al., 2001; Siitari et al., 1999; Church et al., 2001; Viitala et al., 1995; Dugas, 2009), controlled breeding experiments to shift the quality of the UV pattern (Liu et al., 2007; Delhey et al., 2006), physical and chemical mechanisms underlying the generation of UV reflectance (Vorobyev & Osorio, 1998; Vorobyev et al., 1998), and modification of UV pattern by a shift in the composition of uropygial gland secretions (Delhey et al., 2008). Mullen and Pohland (2008) described the results of a study on the existence of plumage regions with high proportions of UV-reflective feathers in 968 bird species covering the vast majority of orders. In general, research shows that UV patterns and UV signalling plays an important role in nearly all avian families (Eaton, 2005; Burns & Shultz, 2012), but research tends to focus on inter- and intraspecific communication in birds (Bennett & Cuthill, 1994; Stevens & Cuthill, 2007).

Given that many natural objects such as barks, soil, or leaves have been described as UV non-reflective, the contrast between any food item and its background could rapidly increase. This is also why studies dealing with foraging strategies of birds conclude that UV reflectance plays an important role in the dynamics of plant–host populations. One should not, however, overestimate the importance of UV light and its perception in comparison with wavelengths visible to the human eye, and it is crucial to be aware of natural light conditions in the design of a study (Honkavaara et al., 2002; Altshuler, 2001). Moreover, it is important to relate the visual sensitivity of foragers' cones to the reflectance pattern (reflectance spectrum) of the potential forage item (Cuthill et al., 1999).

In contrast to avian studies dealing with UV reflectance, investigations of reptile and amphibian species are relatively scarce. The main objects of studies in this area are lizards, with just a handful of studies on turtles (Rowe et al., 2012; Steffen et al., 2019) and frogs (Ries et al., 2008). The focus of these studies is very similar to studies on birds: researchers looked at how UV patterns function as intraspecific visual communication signal among a range of species of lizards or frogs (Stoehr & McGraw, 2001; Fleishman et al., 1993; Stapley & Whiting, 2006; Ries et al., 2008; Lebas & Marshall, 2001), how variation in UV reflectance promotes a reproductive barrier between two populations (Thorpe & Richard, 2001; Font & Molina-Borja, 2004), or studied UV patterns' maintenance function as aposematic/protective signalling (Font et al., 2009; Garcia et al., 2013). In several cases, researchers described a variation in UV reflectance within a species (i.e. morphs or subspecies) caused by various environmental variables, mainly light conditions and temperature (Thorpe, 2002; Macedonia, 2001). Moreover, because reptile species are more-habitat specific it has been suggested that in reptiles, the configuration of UV pattern is linked to the light conditions typical of particular (micro)habitats (Fleishman et al., 1993), thus reflecting the animals' ecological requirements (Thorpe & Richard, 2001). It is thus clear that in reptiles and amphibians, the role of UV reflectance is not yet fully understood. This is attested by the fact that in these classes of animals, UV reflectance is said to be part of a species-specific multiple signalling complex (Whiting et al., 2006; Bonnaffé et al., 2018; Martin et al., 2019; Romero-Diaz et al., 2019).

UV reflectance in mammals has no biological function because most mammals have either no or very limited UV vision (see Chapter 3). There are some exceptions, such as rodents (Jacobs et al., 1991), marsupials (Jacobs, 1992), and bats (Winter et al., 2003). Nevertheless, despite the absence of UV vision, several studies described interesting phenomena connected to UV reflectance in mammals. For instance, UV reflectance plays a role in enhancing the visibility of scent marks of voles, whereby the UV reflectance of urine marks differs between reproductive categories, with for instance the scent marks of mature male voles being the brightest. These UV cues are also used as foraging signals in birds of prey, such as kestrels (see Chapter 2.1), and they function as important visual intraspecific cues (Peichl et al., 2005). In mammals, UV reflectance may have some highly specific functions: it is possible that in reindeer, their sensitivity to UV reflectance helps them detect the white fur of predatory polar bears in a snowy landscape (Hogg et al., 2011), and it has also been proposed that it plays a role in the communication of New World flying squirrels (Kohler et al., 2019). Another non-biological study proposed that UV reflectance could be used in remote sensing tools with a broad spectral range to discriminate polar bears and other mammals from clean snow, because animal fur has markedly low reflectance in comparison to the highly reflective snow (Leblanc et al., 2016).

In insects, it has been observed that some dragonflies exhibit a wax-based colour change and thus produce UV reflection (Holmes & Keiller, 2002). Interestingly, the waxy secretion responsible for this phenomenon varies over dragonflies' life and is produced only during specific life stages, especially during mating (Futahashi et al., 2019). In Odonata in general, UV reflection may be related to numerous aspects of mate recognition, intraspecific competition, UV protection, and anti-desiccation strategies (Corbet, 2004).

With respect to fish, studies related to UV reflectance are scarce. Although UVA rapidly scatters in water, in clear aquatic environments it is present in biologically useful amounts to a depth of at least 100m (Losey et al., 1999). The strong scattering of UV radiation should produce unique imaging conditions in the form of very bright UV background in the horizontal view and a marked veiling effect which with increasing distance obscures the image. UV reflectance patterns have been described in hundreds of species of fishes and it is generally believed that about 60% of fish species living in a UV-rich environment have UV patterning (Lythgoe, 1979; Marshall, 1996). It is worth noting that all categories of fish colours described by humans as, for example, blue, green, yellow, orange, or red exist both with and without a UV component. This is a distinction we cannot perceive and it is suggestive of a function that relies on UV vision (Lythgoe, 1979). In fish, UV patterns are present in all body regions but most frequently, they are found in three particular parts of the body: on the fins, the head, and on the flanks as spots or stripes. Such patterns are thought to play a role in foraging (Leech & Johnsen, 2006), in short distance intraspecific signalling (Endler, 1991; Shashar, 1994; Cummings et al., 2003; Rosenthal et al., 2002; Kodric-Brown & Johnson, 2002; Sabol et al., 2017; Siebeck, 2004), in predator detection (Shashar, 1994; Kodric-Brown & Johnson, 2002), sexual dimorphism (Cummings et al., 2003; Hankison & Palmer, 2016), and in navigation and orientation using UV polarization patterns (Parkyn & Hawryshyn, 1993; Leech & Johnsen, 2006; Hawryshyn, 2003). Because UV light conditions vary significantly with water quality and depth, it has been suggested that UV reflectance and its receptors are tuned to species-specific conditions, i.e. to the fish's specific merkwelt (Cummings et al., 2003; Osorio & Vorobyev, 2008). For our intents and purposes, we define merkwelt as the set of all environmental factors significant to a species, regardless of whether they are perceived or even perceptible by the species in question. In other words, it is the set of things and factors an organism might care about if it knew about them or the objective universe that impinges on its existence.

In the following, while we occasionally do refer to examples of UV reflectance pattern in various animals, our aim is not to provide a comprehensive overview of this phenomenon in all animals. This thesis focuses on Lepidoptera.

Interestingly, one might suppose that UV signals may be present in similar colours. Oftentimes, however, that is not the case, even among various species with the same UV pattern generation mechanisms. For instance in bird plumage, peak blue reflectance often includes a UV colour component (Hunt et al., 1998). Similarly, blue patches in the skin of some lizards (Macedonia et al., 2002; Macedonia, 2001) possess a highly reflective UV pattern, whereas blue patches of other lizards display no UV reflectance (Stoehr & McGraw, 2001), although in both examples above the formation of UV reflectance is based on structural means and the optical properties of spectra visible to humans are nearly the same. It thus seems that the colouration of any pattern as visible to human eyes is not a reliable criterion for differentiating between UV-reflective patches and non-UV- reflective patches. This clearly demonstrates the need to objectively assess the colour of patterns, because colour patches that look similar to us may look differently to others. A comparison of various colours within similar genera or even families might shed some light on this hypothesis. In the following, we focus on UV pattern in butterflies and flowers.

2.1.1 UV patterns in flowers

One of the most studied kinds of natural objects in the history of UV research are UV patterns in flowers. The fact that flowers stand out so distinctly among the surrounding vegetation or in the landscape thanks to their UV-reflecting properties was first reported by Lutz (1933, 1924). Since that time, research on UV patterns in flowers had blossomed (Brown & Wilson, 1956; Rutowski & Gilchrist, 1986; Warzecha & Egelhaaf, 1995; Rutowski et al., 1987; Hazel, 1990; Murali, 2018).

UV patterns in flowers are based on structural colouration and pigmentation just like in other organisms (Graham et al., 1980). Flowers frequently reflect UV light more strongly than their background but the reverse is sometimes the case as well (Brown & Wilson, 1956; Rutowski et al., 1987; Peach et al., 2019). Another general phenomenon often found in flowers is that there is little correlation between the visible colour and the UV pattern (Vane-Wright, 1984). In plants, UV reflection can serve a number of functions. Many flowers have detailed UV patterns composed of reflecting and/or absorbing regions. The centres of UV-reflecting flowers or inflorescences often feature conspicuous UV-absorbing marks – a ‘bull’s eye’ – which are specific to that species of plant and serve to particular species of animals as ‘nectar guides’ or ‘honey guides’ or even inform about the flower’s readiness for foraging or pollinating (Hirota et al., 2019; Peach et al., 2019). Several studies demonstrated that this is significant especially for signalling at relatively long distances where UV reflectance is an important feature which reduces the time needed to find sufficient nourishment (Rutowski & Gilchrist, 1986; Koski & Ashman, 2016). Sometimes one petal may contrast with other petals while in other cases, all petals may be UV-reflecting (Silberglied, 1979; Papiorek et al., 2016). The relationship between flowers and pollinators is an example of meta-organismal communication (Boggs & Gilbert, 1979; Klomberg et al., 2019).

Not only petals but also other parts of a flower can be UV-reflective, for instance the glands, style, stigma, and anthers (Silberglied, 1979). In most cases, petals are UV-absorbent, which suggests that their function may be to protect the pollen against high levels of UVB irradiance (Koski & Ashman, 2015). Since plants often use UV light to attract insects, it is assumed that the same mechanism is used in prey attraction by various carnivorous plants specialised in insect predation (Kurup et al., 2013; Carney et al., 2019). Furthermore, a mature flower has a different UV pattern than an immature one, which indicates that UV pattern may also signal the degree of maturity of flowers and blooms (Hazel, 1990).

There is no general trend in UV pattern configurations in within flower genera, but it seems that larger flowers reflect more often and this is independent of whether they display a bilateral or radial symmetry (Warzecha & Egelhaaf, 1995). Observations of diverse floras suggest that yellow flowers are more likely to reflect UV radiation and possess a UV pigmentation pattern than white or green flowers (Dyer, 1996), a process driven most probably by biochemical constraints or correlated selection (Koski & Ashman, 2016). It is estimated that UV patterns are present in app. 30% of flowering plants (Primack, 1982). A macroevolutionary pattern of changes in floral UV patterning dependent on environmental conditions is often found within a species rather than among various species (Wallace, 1865). For instance, temperature, altitude, and UV-B irradiation predict the configuration of the UV pattern (Koski & Ashman, 2016) following Gloger’s rule for this kind of floral colour variation (Koski & Ashman, 2015). Although the evolution of UV patterns (among others) is shaped by coevolution with pollinators, macroevolutionary traits play an important role in the evolution of colour patterns. Moreover, a number of studies suggested that UV patterns of flowers could serve as a taxonomic tool (Primack, 1982; Silberglied, 1979), while other researchers believe that UV patterns could tell us more about the importance of UV-mediated selection which alters the UV irradiance level in terrestrial systems in connection with climate change (Williamson et al., 2014; Koski & Ashman, 2015).

Plants, like animals, achieve UV reflectance in two main ways. First, they use chemical- or pigment-based colour. Pigments are compounds which absorb subsets of the visible spectrum, thus transmitting and reflecting back only that UV light which they do not absorb. The tissue which reflects UV light is then perceived as a UV pattern. Secondly, plants can produce structural colour. Structural colours are present when light of different wavelengths, in this case light in the UV range, is selectively reflected from a surface, while the remaining colours are absorbed (Glover & Whitney, 2010). Surprisingly, structural colours are far less studied in plants that in animals and they are less studied than pigments in plants. Another somewhat neglected colour phenomenon in plants is fluorescence, although it has been described that floral parts of grass, such as pollens and fruits, produce attractive blue fluorescence emissions in the UV range (366 nm) and it has been suggested that this serves as a visual cue attracting pollinators, predators, and pests (Baby et al., 2013).

Given that most studies focus only on UV patterns of blossoms of a species, it is important to note that the remaining parts of a plant usually absorb UV light. Because chlorophyll itself absorbs the UV light, most plants provide a highly contrasting background to blossoms and organismal surfaces (Hausmann et al., 2003; Andersson & Andersson, 1998).

Given the prevalence of UV patterns in plants, it is natural that UV pattern perception plays an important role in the lives of pollinators, such as insects. In the following, however, we focus on UV patterns in butterflies and UV flower patterns are only tangential concern, which is why the present discussion of this subject is not meant to be exhaustive.

2.1.2 Butterfly UV patterns

For general overview, the numbers of records, h-indexes, and sums of times cited are listed below:

UV coloration Lepidoptera: 32 records on Web of Science, *h*-index=14, Sum of Times Cited=800
UV reflectance Lepidoptera: 33 records on Web of Science, *h*-index=13, Sum of Times Cited=522
Ultraviolet Lepidoptera: 308 records on Web of Science, *h*-index=44, Sum of Times Cited=7483

The wings of butterflies feature a wide variety of UV-reflective patterns, including the most intensive reflection found in living organisms. In many species, these patterns show no or only negligible congruence with pattern visible to the human eye (Silberglied et al., 1978). These patterns can be produced by pigments, by structural means, or both. They are often species-specific and sexually dimorphic. Obviously, butterflies can use these patterns for intra- and inter-specific communication.

The first comprehensive analysis of butterfly UV patterns was undertaken by Lutz (1933), who published images of many species taken through a UV filter. His main goal was to indicate how a butterfly's pattern may appear to another insect as opposed to the human eye. Unfortunately, in his study he did not take into account fact that insects are also sensitive to the visible spectrum, although some species display a weakness in the orange and red colour range (Crane, 1954). A subsequent study by Crane analysed all possible methods of studying the UV spectrum and using UV photography, it surveyed in detail the UV patterns of 41 lepidopteran species. Crane also systematically described the colours (visible to the human eye) which correspond to the UV patterns and stated that the nature of all these colours is probably structural. One of his conclusions, namely that UV patterns are rare among lepidopteran species, was probably due to his focus on tropical species. Other scientists who importantly contributed to the study of UV patterns in Lepidoptera are the American entomologist Robert E. Silberglied, Yoshiaki Obara, Ronald R. Rutowski, Darell J. Kemp, Victor B. Meyer-Rochow, and Uwe A. Filter.

Since then, our knowledge of UV patterns on the wings of butterflies and moths had expanded to include at least ten families of Lepidoptera (Meyer-Rochow & Järvilehto, 1997; Meyer-Rochow, 1983; Lyytinen et al., 2004), such as Pieridae (Bowden & Watt, 1966; Silberglied & Taylor, 1973; Nekrutenko, 1965a; Allyn & Downey, 1977), Nymphalidae (Kemp, 2006; Kemp & Macedonia, 2006), Riodinidae (Dushkina et al., 2017), Lycaenidae (Imafuku et al., 2002; Imafuku, 2008), Lymantriidae (Lyytinen et al., 2004), and Papilionidae (Huxley, 1975; Eguchi & Meyer-Rochow, 1983; Crane, 1954). In general, UV reflectance is in Lepidoptera more often associated with a nocturnal rather than diurnal lifestyle (Lyytinen et al., 2004).

2.1.2.1 The adaptive function of UV reflectance and its perception in butterflies

2.1.2.1.1 Thermoregulation

Light reflectance plays an important role in thermoregulation, but while this holds of the visible and infrared light, it is not the case where the UV range is concerned because UV light has no thermal effect. Dark-coloured animals soak up warmth quickly, which increases their level of fitness for potential mating or nectar searching. This can improve the survival chances of ectotherms, such as butterflies (Krishna et al., 2020). Pigments, such as melanins, play a key role in the complex processes which maintain thermoregulation in ectotherms. It has been suggested that lightness is driven by the need to protect from pathogens, while melanin levels are driven by UV radiation (Caro & Mallarino, 2020). This ecogeographical rule of the thumb, known as Gloger's rule (Gloger, 1833), applies however only to endotherms while for ectotherms, it has been suggested that colour lightness in butterflies can be influenced simultaneously by thermoregulation and protection against UV radiation (Ellers & Boggs, 2004; Bishop et al., 2016; Heidrich et al., 2018). This depends on several factors such as the life history of specific species (e.g. its basking strategy) or features of its environment, such as elevation (Ellers & Boggs, 2004). The expression of pigments such as melanin and pterins could be genetically/developmentally conditioned (Ellers & Boggs, 2004; Zhang et al., 2017), which is why it is crucial to consider a link between UV reflectance and this phenomenon, although pigment expression may at first sight seem tangential to our subject. While in the following, we do touch upon the subject of thermoregulation, it is not our main subject. This work is focused more narrowly on lepidopteran communication. For further information about thermoregulation and melanisation, see e.g. Tuomaala et al. (2012), Bishop et al. (2016), Ramos and Hulshof (2019), Shanks et al. (2015).

2.1.2.1.2 Butterfly communication

Large part of recent research on UV signalling focuses on birds with well-described mating systems, social systems, and ecological niches (Endler, 1991). For most of the 20,000 species of butterflies, such information is unfortunately lacking (Kemp & Rutowski, 2011). Nevertheless, detailed empirical work in several species forms a solid platform for investigating the use of UV patterns during social and sexual interactions among butterflies. Males tend to respond to the visual stimuli produced by a female and initiate courtship by presenting visual and chemical signals to which the female responds. Since many butterfly species are sexually dimorphic with respect to UV reflectance (males and females of a species have different patterns), one potential function of these patterns could be sexual recognition. Many butterfly species have colouration that is very similar in visible light but highly distinct in the UV spectra. Extreme differences in UV patterns of males of many closely related species suggest that they might be used for species discrimination by females or even by males. On top of that, the shape and the position of UV structures are distinct enough to enable a relatively easy differentiation between species or subspecies.

The iridescent UV reflection produced by interference, with its high intensity, spectral purity, and abrupt flashing with the wingbeat, also seems to function as a long-range signal for various kinds of communication (Rutowski et al., 2007b). UV absorption may also be a signal: important is the contrast with the surrounding environment against which the wings are displayed in various interspecific ecological links.

2.1.2.1.2.1 Intraspecific communication

Petersen et al. (1951) were the first to demonstrate that the UV component of insect colour pattern might serve an intraspecific communicative function. They found that (UV-reflecting) zinc-white butterfly models were far more attractive to male *Pieris napi* and *Pieris bryoniae* than (UV-absorbing) lead-white models were.

Females of *Pieris napi* (Bowden & Watt, 1966; Stella et al., 2018), *Pieris bryoniae* (Petersen et al. 1951), as well as other species of Pieridae, such as *Eurema candina* (Obara et al., 2008; Rutowski et al., 2007a), *Pieris rapae* (Morehouse et al., 2007; Makino et al., 1952), *Pieris occidentalis* (Wiernasz, 1989), and *Belenois zochalia* (Silberglied, 1979), reflect UV light more strongly that their conspecific males: these species display different degrees of UV sexual dichromatism. For example, Stella et al. (2018) found a 25% higher level of UV reflectance in females of *Pieris napi* than in their conspecific males, while Meyer-Rochow and Järvilehto (1997) found 35–40% difference of UV reflectance in this species (for more details, see Part 2). Nevertheless, this phenomenon, i.e. females having higher UV reflectance levels than conspecific males, is in Lepidoptera rather an exception (Silberglied et al., 1978). It has been proposed that during the evolution of Pieridae, there must have been a switch in the UV pattern of the sexes (Silberglied et al., 1978).

There are several hypotheses regarding the evolution of this sexual UV dichromatism. First of all, it has been suggested that females with a higher level of UV reflectance intensity are favoured by males, which is why expression of this trait, i.e. high UV reflectance, in females could be adaptive and enhance fitness either in the context of male mate preference or competitive interactions with other females (Rutowski et al., 2007a). A second explanation could be that the expression of the UV pattern is an incidental and non-functional result of genetic correlations between sexes. This could lead to a penetration (integration) of genes which express the trait in females (Lande, 1987). But males of *Pieris napi* do not have a highly UV reflective patterns, due mainly to the presence of pterins which decrease the level of UV reflectance and increase the degree of whiteness (i.e. the level of reflectance in the visible spectrum of light) in *Pieris napi*. Males are under sexual selective pressure from females for whiter wing patterns, i.e. patterns with a higher level of whiteness (Turner, 1978; Stride, 1956; Stride, 1957), which is probably why variation in UV reflectance levels in males seems relatively low in comparison to females of the species (Stella et al., 2018; Meyer-Rochow & Järvilehto, 1997). It is expected that chemical removal of the pterin pigment on the wings of male *Pieris napi* would lead to an analogical appearance of UV reflectance as in the females. Removal of pterin pigments has been tested on *Colias eurytheme* (Rutowski et al., 2005), one of the most studied butterfly species with respect to UV reflectance. Due to the different structure of the wing (see below a chapter on Mechanism of UV pattern colouration), the removal of pterin from the wing of *Colias eurytheme* led to a largely achromatic broadband white reflectance pattern and a decline in the iridescent properties of the UV signal. It seems therefore that in this case, pterins increase colour contrast as the UV flashes on and off during wing movement.

Furthermore, the development of UV patterns in female butterflies could be explained by the hypothesis of *good genes*. During mating, males give females a costly nutrient investment in the form of spermatophore, which is used for somatic maintenance (Boggs & Gilbert, 1979; Rutowski et al., 1987). Females therefore select males according to their indicators (secondary sexual traits) such as colouring, the size of some structures (Bonduriansky, 2001), and their overall size (Rutowski & Gilchrist, 1986). This can potentially increase the survival chances or reproductive success of her offspring through, for example, a better ability to choose optimal microhabitats and nutritious plant foliage.

Sexual traits are known to be highly variable (Iwasa & Pomiankowski, 1995). In other words, individuals of high genetic quality can be in good condition regardless of their developmental environment, whereas low genetic quality specimens prosper only in favourable environments (Kemp & Rutowski, 2011; White et al., 2015). According to the Hamilton–Zuk (1982) hypothesis of parasite-mediated sexual selection, females should prefer males with the most elaborate sexual ornaments because the degree of ornament elaboration positively correlates with male's ability to resist various infections. By mating with elaborately ornamented males, females would thus acquire heritable parasite resistance for their offspring (Hamilton & Zuk, 1982). The level of UV reflectance in this case could be one such a dependent structure (Silberglied et al., 1978; Kemp, 2007a). Moreover, different signals may correlate with different aspects of bearer's condition or quality (Fitzpatrick, 1998; Brooks & Couldridge, 1999) but even with a UV pattern which involves multiple colour mechanisms. The expression of these mechanisms may thus encode a number of distinct types of information about the signaller (Grether et al., 2004).

Some theoretical models show that under certain circumstances, species could evolve multiple quality-revealing sexual ornaments (Johnstone, 1995), while other models propose that honest advertising should favour a single most revealing signal at the expense of other signals (Johnstone et al., 1996; Schluter & Price, 1993). Despite this theoretical consideration, the hypothesis on the evolution of multiple quality-indicating sexual traits finds little empirical support in existing studies on Lepidoptera. Kemp (2007b) found that in *Eurema hecabe,* male UV brightness correlates with their body size and, interestingly, with the size of their mate. This supports the hypothesis of full complexity of sexual interactions in polyandrous pierid butterflies, including e.g. potential assortative mating (Silberglied & Taylor, 1973; Lindsay et al., 2019). Furthermore, one study found multiple quality-revealing sexual ornaments (nest features, plumage quality, ecoparasite load, body size) in bowerbirds. This suggests that bowers are an extension of the male phenotype which females can use to assess male quality (Doucet & Montgomerie, 2003).

In most butterfly species, males tend to take the initiative in mating behaviour (Obara, 1970). Females may reject their advances for a number of reasons: the female may not be reproductively mature, had recently mated, or the courting male is not conspecific and lacks the appropriate signal (Silberglied et al., 1978). In some genera of Lepidoptera, such as *Pieris,* the initial visual perception plays an important role in this behaviour: previous studies showed that in *Pieris rapae,* UV reflectance is an essential and vital component for activation of male courtship behaviour (Obara, 1970). Female wing colouration (and not just UV reflectance) is undeniably a crucial feature in sexual discrimination (Ghiradella et al., 1972). This has been shown for instance in *Colias eurytheme,* where only the males display UV reflectance, which is therefore used by males for sex discrimination (Rutowski et al., 2005; Silberglied et al., 1978) and by females for mate assessment (Papke et al., 2007, Rutowski, 1985). UV colouration was in this case suggested as the best predictor of whether a male will be accepted by a female during mating. Moreover, due to having a pterin-based structure, the wings of *Colias eurytheme* strongly flash during flight and it has been suggested that the frequency of these UV flashes falls within the female's ability to discriminate the pulses of UV light (Nakagawa & Eguchi; 1994).

Sex discrimination via UV reflectance is possible and may be the case also in a number of other species, such as *Colias philodice* (Silberglied & Taylor, 1973), *Eurema candida* (Rutowski et al., 2007a), *Eurema hecabe* (Rutowski et al., 2007a), *Pieris protodice* (Meyer-Rochow & Järvilehto, 1997), *Pieris rapae* (Obara et al., 2008; Takizawa & Koyama, 1974) *Eurema lisa* (Ghiradella et al., 1972), *Hypolimnas bolina* (Kemp & Macedonia, 2006), *Pieris occidentalis* (Wiernasz, 1989), *Belenois zochalia* (Silberglied, 1979), *Chrysozephyrus sp.* (Imafuku et al., 2002), *Polyommatus icarus* (Knuttel & Fiedler, 2001), *Polyommatus andronicus* (Coutsis, 1996), *Bicyclus anynana* (Huq et al., 2019), and *Heliconius cydno* (Sweeney et al., 2003). Because the proportion of wing area devoted to UV-reflecting scales, the overall shape of the UV pattern, the level of UV reflectance, as well as perceived hue and saturation usually differ considerably between sexes, this trait can be employed in sex discrimination. Although sexual dichromatism is widespread among butterflies, it is not universal. Since UV signals function within a wider context they cannot be considered in isolation. In many species, it is likely that only the visible part of light is used in sexual recognition. This is most likely the case wing patterns of *Colias philodice* (Silberglied, 1979), *Anteos clorinde* (Rutowski et al., 2007a), *Pieris occidentalis* (Wiernasz, 1989), or of specific behavioural patterns such as those observed in *Pieris rapae* (Obara et al., 2008; Obara et al.; 2008). In some species, sexual recognition is aided by olfactory cues, for instance in *Bicyclus anynana* (Costanzo & Monteiro, 2007), and in many species it is likely that a combination of cues is used, which may depend on timing and distance. Because UV signals become quickly less prominent with distance – though their reach depends on the mechanism of UV colouration (Burkhardt, 1989) – olfactory cues can be used to signal at various distance and over time (Costanzo & Monteiro, 2007). In many cases, we find a species-specific shift from dependence on visual cues to chemical ones in intraspecific communication.

The *Pieris rapae* butterfly occurs in two subspecies: *Pieris rapae crucivora* and *Pieris rapae rapae*. UV sexual dimorphism is, however, found only in *Pieris rapae crucivora*, whereas *Pieris rapae rapae* have no UV reflective patterns on the wings of either sex (Obara et al., 2008; Takizawa & Koyama, 1974). Less than 5% of sexual difference in UV reflectance was found in the *P. rapae* subspecies and the difference that was observed consists in the morphological structure of the wing. Males of the *crucivora* subspecies have on their scales beads (sometimes called 'pigment granules'; for more, see chapter Mechanism of UV pattern colouration) which contain pterin, a UV-absorbing pigment, while females do not have it (Giraldo & Stavenga, 2007; Obara & Majerus, 2000). In the *rapae* subspecies, on the other hand, both sexes have beads on their scales and thus do not reflect UV light (Giraldo & Stavenga, 2007). Recognition of conspecific males of the *rapae* subspecies is effected by a 'flutter response', a specific behavioural feature which deters approaching males from attempting to copulate with males. In other words, it functions as a 'mechanical isolation mechanism' against maladaptive copulatory attempts between males (Obara & Majerus, 2000). The *crucivora* subspecies uses flutter response as well but only as a redundant behavioural feature (Giraldo & Stavenga, 2007). In cases where intersubspecific mating occurs in subspecies *crucivora* and *rapae*, hybrids are viable with highly variable UV pattern in females (Obara et al., 2010). Flutter response was described also in *Pieris napi* (Petersen et al., 1951; Meyer-Rochow & Järvilehto, 1997), *Hypolimnas bolina* (Kral, 2016), and in *Colias eurytheme*, where it functions as a signal of unreceptive behaviour (Silberglied et al., 1978).

The occurrence of UV reflectance in certain specific species could be explained by Amotz Zahavi's 'handicap principle' (Zahavi, 1975), which proposes that such signals are evolutionarily stable because their expression is costly. This is why they relate 'honest' information about their bearer's ability to carry such costs. The cost of sexual signals, in our case UV patterns, is disproportionately higher for low quality individuals than for high quality individuals because of their high nutritional demands (Tuomaala et al., 2012). In other words, males with a high level of UV-reflective patterning are handicapped by shouldering these costs but evidently can cope with it and do survive (Silberglied & Taylor, 1973).

In a series of experiments with *Colias eurytheme*, it was established that UV patterns play a critical role in female mating behaviour (Silberglied & Taylor, 1973; Silberglied et al., 1978). In particular, the males of this species whose UV pattern was destroyed suffered a significant decrease in the number of successful conspecific matings (Rutowski et al., 2005). Female preference for males with brighter UV patterns was also found in *Hypolimnas bolina* (White et al., 2015). A key prediction of the honest signalling theory is that the expression of directionally selected sexual traits should tightly covary with the condition of the phenotype (Kemp & Rutowski, 2011; Lindsay et al., 2019). This phenomenon is known as *condition-dependence* (Andersson, 1994). The lamellar arrays which produce a UV pattern may thus be costly to produce and may be sensu stricto condition-dependent (Morehouse, 2014), which would contribute to honesty of this signal (Rutowski et al., 2005).

Intrasexual competition between males could provide an alternative explanation of the function of UV reflectance in butterfly communication. Behaviourally, most female butterflies tend to be solitary, whereas males interact vigorously when defending their territory or fighting over potential mates. Long-distance signalling is thus necessary for males but not for females (Vane-Wright, 1984). Being able to recognise other males and clear signalling of one's own sex by UV reflectance is thus advantageous for males in the context of agonistic and territorial behaviour as well as mate location behaviour (Brunton et al., 1995; Silberglied et al., 1978).

In tropical species, the situation is different. Curiously, despite the striking colours of tropical butterflies, there exist only few studies on UV reflectance in tropical species. A handful of studies suggest that tropical butterflies have either partial or spot UV reflectance, that is, much less UV patterning than non-tropical species. Crane's study, which was the first to investigate large numbers of tropical species, concluded that UV reflectance in tropical butterflies is rare and the low percentage of UV-reflecting patches cannot have a significant effect on the insect's eye. In short, that in tropical butterflies, UV reflectance is unlikely to have adaptive value in communication (Crane, 1954; Vukusic & Sambles, 2003). Huertas et al. (unpublished data) report such patterns in genus *Eryphanis*, where the shape of small spots differs from the shape of their UV reflectance and could be used to discriminate between species or subspecies in this taxon. Another example of the adaptive function of UV reflectance in tropical butterflies are the neotropical *Heliconius,* famous for their wing pattern mimicry (Crane, 1955; Merrill et al., 2015). Their yellow aposematic spots are highly UV reflective and often viewed as a hidden channel of communication where species-specific signals are not detected by predators (Dell'Aglio, 2016).

This led to a suggestion that the visual system and wing colouration coevolved so as to facilitate communication especially among conspecifics (Bybee et al., 2011). Only two studies, however, tested specific intraspecific communication preferences in the context of variation of UV reflectance in tropical butterfly species, whereby the use of UV traits in signalling between different species has not been specifically addressed as yet. Regarding the former, Robertson et al. (2005) discovered that female African tropical *Bicyclus anynana* choose their mates based on the size and UV reflectivity of the dorsal eyespot's central white pupil. Contrary to this, a recent study shows that choosy *Bicyclus anynana* males notice these white UV-reflective pattern elements and mate with females that have them more readily than with females in whom these patterns are blocked (Huq et al., 2019). Secondly, *Heliconius* males also use UV signals to choose their mates, which indicates a trade-off between natural and sexual selection regarding visual signals, between reduction of likelihood of confusion in courtship and maintenance of the advantages of aposematic colouration (Dell'Aglio et al., 2018). Moreover, it seems that dorsal colours of some *Heliconius's* species may have evolved through selection for aposematism as a type of protection from predation, while its ventral surfaces were selected for sexual signalling.

In other tropical butterfly species, there is clear evidence of signal partitioning between dorsal and ventral wings (Rutowski et al., 2010), including UV signalling. In *Bicyclus anynana*, dorsal wing characters are involved in sexual signalling while eyespots on the ventral side of the wing play a role in predator avoidance (Robertson & Monteiro, 2005). The same trend in the function of the two wing surfaces appears in pipevine swallowtail (*Battus philenor*), where the colour pattern on ventral hindwings in both sexes acts as an aposematic signal which advertises distastefulness to potential predators (Rutowski et al., 2010). Morpho butterflies, too, have flickering flash iridescence colouration ranging from blue-green to UV on the dorsal side, which is involved in male flight patrolling (Crane, 1954; Tabata et al., 1996), while cryptic colours on the ventral side seem to serve as protection against visual predators (DeVries et al., 2010).

Quality of UV reflectance declines with age due to wing wear, scale loss, or damage. This can be used in mate assessment by females (Rutowski, 1985), as is the case for instance in *Colias eurytheme*, where structural UV colouration can serve as a valid indicator of age (Kemp, 2006). The nymphalid butterfly *Anartia fatima* is unusual in that both its UV reflection and visible colour change with age: in both sexes, their yellow UV-absorbing bands become white and UV-reflecting. Older, UV-reflecting females are then more attractive to mate-seeking males (Silberglied, 1979).

2.1.2.1.2.2 Notes on the evolution of UV pattern in Lepidoptera

Studies on the evolution of UV patterns in Lepidoptera are few and far between and only a handful of articles so far offer a discussion of UV reflectance from an evolutionary perspective. Research on molecular phylogeny (mitochondrial DNA) of Lepidoptera indicates that within the genus *Colias,* the oldest species in Europe are the Scandinavian ones and it is from the north-west of Europe that *Colias* butterflies spread south and east, eventually forming nine species. This analysis sems to indicate that UV reflectance evolved several times (a polyphyletic trait) within the genus (and each clade), which is consistent with the hypothesis that UV is a trait subjected to intra- and/or interspecific selection (Brunton, 1998). Brunton's study, however, was based only on 12 species and in the light of more recent studies (e.g. Stella et al., 2018; for more detail, see Part 2), its results cannot be considered fully reliable. UV pattern in genus *Gonepteryx* also lends itself to a phylogenetic mapping of evolution of this trait. Nekrutenko's study (1968), which was based on investigation of UV patterns, showed relationships between *Gonepteryx* species and its results were mostly congruent with later molecular studies (Brunton & Hurst, 1998; Bozano et al., 2016). Another recent study based on both mitochondrial and nuclear DNA describes the phylogeny of *Gonepteryx* butterflies in even more detail (Hanzalová et al., 2020). Moreover, this paper used UV patterns in its analyses as a covariate and showed analogous spatial trends in UV pattern as in the *Colias* genus.

2.1.2.1.2.3 Interspecific communication

Related species are often sympatrically distributed, which is why UV patterns can be used in species recognition. This is of importance especially in relation to avoidance of interspecific mating and through character displacement also in the context of sympatric speciation (Brown & Wilson, 1956). In many Pieridae species, only males exhibit UV reflectance and since male courtship harassment carries costs for females (Graham et al., 1980), UV patterns which distinguish females of related sympatric species could reduce interspecific male harassment and thereby increase time available for egg laying (Brunton & Majerus, 1995; Silberglied, 1979; Rutowski et al., 2007a).

In *Eurema, Phoebis, Colias,* and other genera, there are strong interspecific differences in male UV-reflective patterns among congeneric sympatric species. Females of *C. eurytheme*, for example, accept conspecific males who have a strong UV reflection. Behavioural observation showed that males with obliterated UV pattern mate less frequently than control males (Silberglied et al., 1978; Silberglied & Taylor, 1973). In *C. eurytheme*, it seems that UV pattern plays a role in species recognition. In contrast, females of *C. philodice* (a species where males are UV-absorbing), do not discriminate against conspecific males who were experimentally adorned with *C. eurytheme* UV-reflecting wing patches. There is no reason, therefore, to expect that all *Colias* species use UV pattern in communication and mate selection. It is quite clear that in some species, UV signalling does not play an exclusive role in communication. Moreover, Brunton and Majerus (1995) analysed intra and interspecific variation of UV patterns in a number of *Colias* species. One might expect very little variance in UV reflection between specimens of the same species. Nevertheless, a study comparing inter- with intraspecies differences in a number of *Colias* and *Gonepterix* species concluded that intraspecies variation is in several European species is so high that neither butterflies nor their predators are likely to be able to differentiate between species based on their UV patterns (Brunton et al. 1995). This is also why UV patterns are unlikely to play an important role in reproductive isolating mechanism in these butterfly species.

UV patterns thus clearly cannot be considered in isolation and with respect to communication, other channels must be taken into consideration: for instance *C. philodice* relies entirely on olfactory cues (Silberglied & Taylor, 1973; Taylor, 1973b). In other words, while UV signals can function as an isolating mechanism for some species, such as *Pieris napi* (Silberglied et al., 1978; Meyer-Rochow, 1991) or *Actinote genus* (Remington, 1973), where they reduce the risk of hybridisation, in other species, such as some *Colias* butterflies, UV reflectance probably plays a minor, if any, role in this type of interspecific communication.

Wing patterns of some butterfly species involves hidden features such as UV patterns, as described above. For this reason, UV reflectance patterns are used as taxonomic tool to distinguish between species, thus dispensing with the need of complicated morphological and genetical analyses. Pioneering taxonomical work based on UV reflectance of *Gonepteryx* species was done by Nekrutenko in number of his studies (Nekrutenko 1964, 1965a, 1965b, 1968, 1970, 1972). He hypothesised that UV patches could be a useful taxonomical trait. He described a number of characteristic features in the terminology of hidden UV wing pattern on the wings of *Gonepteryx* species and was most likely the first to describe gynandromorphy in the UV spectra.

Bozano et al. (2016) recently proposed the first molecular-based phylogeny of the genus *Gonepteryx*, which changed the taxonomic status of some traditional subspecies to a species level. More importantly, this study and number of other recent works treat *Goneptryx* UV patterns as a possible diagnostic trait of a taxonomic value (Brunton & Majerus, 1995; Pecháček et al., 2014; Bozano et al., 2016; Hanzalová et al., 2020). Further studies used UV patterns as an auxiliary taxonomical method for *Papilio* (Ferris, 1972), *Lycaena* (Schaider, 1988), and a number of *Colias* species (Stella et al., 2018). Already decades ago, Ferris believed that UV photography is a brilliant and cheap method for complicated taxonomical analysis and assigned a number of species/subspecies to various colour groups (Ferris 1973, 1975), which later genetic analysis indeed proved to be separate species or subspecies (Wheat & Watt, 2008). Furthermore, analysis of UV reflectance pattern on the wings revealed significant differences between two species of swallowtail butterfly (*Iphiclides feisthamelii* and *Iphiclides podalirius*). This study was supported by genetic (nuclear DNA) and morphological (male and female genitalia) analyses to underpin the importance of UV reflectance as a taxonomical tool (Gaunet et al., 2019). Even more recent findings support the taxonomical significance of UV reflectance in south American *Eryphanis* butterflies (Huertas et. al, unpublished data). Although UV patterning is in taxonomic studies employed relatively rarely, all of the abovementioned sources confirm the importance of this trait as a taxonomical tool that does not require complicated morphological analysis.

Butterfly UV patterns could also serve as a decoy or warning colouration (aposematism) for various species of birds, thus playing an important role in prey–predation interaction (Lyytinen et al., 1999, 2004; Silberglied, 1979). Brues (1941) was one of the first authors to hypothesise about UV patterns functioning as a Batesian or Mullerian mimicry. Without any field observation, he claimed that mimicry is the main purpose driving UV pattern formation in various butterfly species. Remington (1973) found that African butterfly models and mimics resembled one another in UV reflection pattern more than New World models and mimics did. He suggested two explanations: African predators see in the UV spectrum better than new World predators do or, alternatively, mimicry complexes in Africa had more time to develop. Selection against UV patterns may be expected because reflected UV light may attract avian predators (Viitala et al., 1995). The hypothesis of substantial negative selective pressure against UV patterns finds support in the fact that nocturnal species have UV patterns more frequently than diurnal species (Lyytinen et al., 2004) who more move around when visually hunting predators are more active. In species where there is sexual dimorphism of UV patterning, females tends to be more cryptic or mimetic than males. This should decrease the risk of predation, in other words, function as a protective pattern. Males, meanwhile, tend to preserve the ancestral pattern of species (Turner, 1978). That, however, was proven only for some species (Brunton et al., 1995; Brunton, 1998). *Pieris napi,* for example, exhibits an inverse phenomenon and females are more visible in UV light than males are.

Cryptic patterning is crucial not only for butterfly imagos but also for lepidopteran larval stages. Church et al. (1998) measured the reflectance spectra of lepidopteran larvae and their natural backgrounds. His results indicate that many caterpillars match the leaf background in both UV and visible wavelengths and are thus cryptic over the entire colour range. In this case, crypsis extends into the UV. Furthermore, resting adult butterflies fold their wings to join them into an upright position and in most species, the reverse side of wings does not reflect UV light (Meyer-Rochow, 1991; Ghiradella et al., 1972). Predators thus cannot use UV patterns on the wings of resting butterflies for prey detection (Lyytinen et al., 2004).

Studies of UV patterns include the most widely quoted example of evolution in action: the story of industrial melanism in *Biston betularia*. In light visible to humans, the speckled *typica* form appeared cryptic when seen against the background of foliose lichen, whereas the dark *carbonaria* form was conspicuous. Under UV light, the situation was reversed. Foliose lichens absorbed UV light and appeared as dark as the *carbonaria*. *Typica*, on the other hand, reflected UV and was conspicuous. Against crustose lichens, *typica* was less visible than *carbonaria* in both visible and UV light (Majerus et al., 2000). As is well known, during the Industrial Revolution in Britain, the *typica* form was in urban largely replaced with the *carbonaria* form. As pollution later decreased, the *typica* form once again became dominant. The abovementioned study by Majerus et al. (2000) somewhat complicates the story but also outlines the importance of framing the issue of UV reflectance in prey and predator should in an ecological context.

A number of studies suggested that butterfly colouration, with high frequency of orange, yellow, and while, is in fact aposematic (Kettlewell, 1965; Komárek, 1998). This finds support in the fact that a number of butterfly families include species with a wide spectrum of palatability, so their patterns may indeed have an aposematic function (Brower et al., 1968; Lyytinen et al., 1999). So far, however, the issue of aposematism and UV patterns has been addressed by only a handful of studies, whereby some research indicates that the aposematic colouration hypothesis may be wrong. For instance, a study on great tits (*Parus major*) showed that birds were incapable of learning to avoid unpalatable prey items regardless of whether the prey reflected or absorbed UV light (Lyytinen et al., 2001). In other words, birds exhibited no strong avoidance of UV-reflecting prey items. This is analogous to the finding that a removal of UV reflectance had no significant effect on foraging behaviour in avian merkwelt (Maddocks et al., 2001). Nevertheless, UV signals should not be considered in isolation and the entire spectrum of wing patterns needs to be examined (Arias et al., 2019).

In a slightly different context, however, highly reflective UV patterns could serve as effective defence against predation. Murali (2018) demonstrated that butterfly movement accompanied by striking colouration which dynamically changes over time is a functional protection against predation. It probably works by leading the prey to misinterpret the prey's location. Iridescence, too, which leads to fast colour changes, could serve as a form of dynamic disruptive camouflage based on the same principle (Kjernsmo et al., 2020). Although this was tested in context of wavelengths visible to humans, UV patterns – which can function as a strong signal in the visual merkwelt of organisms sensitive to UV light – could be included in this functional explanation of UV pattern configuration in a parallel fashion. Robertson et al. (2005) found highly reflective UV spots on the dorsal side of *Bicyclus anynana* wings but due to their central placement on the wing and their inaccurate mimicry of vertebrate eyes, such patterns are unlikely to be effective at deflecting attacks to the wing margin. Contrary to this, Prudic et al. (2015) found an association between increased butterfly survival and prominent eyespots in *Bicyclus anynana* in the context of predation by certain invertebrates, specifically praying mantids which are unable to sense UV light. Consideration of the sensory capacities of predator is therefore of crucial in such studies. Moreover, an experiment with UV eyespots in *Lopinga achine* demonstrated that natural marginal eyespots on butterfly wings can deflect predator attack to these nonvital parts of butterfly body in low intensity light with prominent UV component, that is, in light conditions which emulate conditions during dawn or dusk, the time when the butterfly's avian predators are most active (Olofsson et al., 2010).

Some kind of trade-off between the development of signals intended to conspecific and signals aimed at predators undoubtably affects UV wing patterns. This conflict between natural and sexual selection is traditionally described as follows: if predation is a relatively stronger selective force than sexual selection, colouration will be more conspicuous for aposematic species and more cryptic for camouflaged species. If, on the other hand, predation poses a relatively smaller threat, colour patterns will be closer to the optimum of mate choice (Endler, 1992). This was studied in *Heliconius* butterflies where males use UV signals for mate choice, indicating that conflicting forces of natural and sexual selection do affect visual signals: they reduce the cost of confusion in courtship and maintain the advantages of warning colouration (Dell'Aglio et al., 2018). Specifically in *Heliconius*, it seems likely that their dorsal colours may have evolved through selection for aposematism as antipredator protection, while their ventral wing surfaces were selected for sexual signalling.

The theory of sensory drive claims that signalling systems should evolve so as to optimise transmission between senders and intended receivers while, if at all possible, reducing visibility to eavesdroppers (Endler, 1992; White et al., 2015; Dong et al., 2019). High directional and iridescent UV reflectance could be an example of such a trade-off. In other words, the expression of such visual sexual signals is generally thought to represent a balance between the effects of sexual and natural selection (White et al., 2015). These signals require high conspicuousness, because to successfully compete for mates the male has to advertise certain qualities, but also maintain relatively low visibility to predators. Hastad (2005), for example, theorised that specific species attune their signalling to the visual systems of their intended addressees, i.e. their conspecifics, while reducing colour contrast to the background in spectral parts which their predators are most sensitive to. In other words, organisms use colour signals which are more readily seen by their conspecifics' (and thus also their own) visual system than by their predators, which allows for a directed communication channel for displaying male quality. Moreover, Mullen and Pohland (2008) described an association between the position of UV reflectance maxima and the possession of UV cones in almost one thousand bird species, which strongly indicates that perception system is interspecifically tuned and the prey can hide its communication within its own UV reflectance maxima. The same phenomenon of shielded communication appears, for example, in the mimic *Heliconius* butterflies (Dalbosco Dell'Aglio, 2016; Bybee et al., 2011) where UV signals are used in mate selection by females (see chapter on intraspecific communication). A similar phenomenon has been hypothesised in *Hypolimnas bolina* (White et al., 2015), where the maximally bright UV reflectance of white spots surrounded by shimmering blue scales on the black upper side of the wings of courting males can be seen only from a highly restricted angle. These angularly restricted signals have a narrow angular reflectance function: they are visible only from about 20° range of above-wing viewing angles. Specific behaviour also plays an essential role in enhancement of signal transmission to females of this species. As a consequence, the male UV pattern is clearly visible to *H. bolina* females but not to avian predators. This is clearly advantageous.

To sum up, UV patterns are subject to a dual challenge of maximisation of signal transmission to conspecifics with concurrent minimisation of possible detection by visually orienting predators. The theory of sensory drive predicts that such signals should be designed or transmitted to some degree privately (Endler, 1992). Some authors describe this phenomenon using the term private communication channel (Cummings et al., 2003; Siebeck et al., 2010).

2.1.2.2 Mechanisms of UV pattern colouration

Understanding of colour production mechanisms along with their potential nutritional, physiological, and developmental costs is important for our holistic appraisal of signal function and evolution. Given that colour is the product of interaction between light and morphological structures, eventually also chemical pigments, selection acting upon colour characteristics necessarily leads to changes in those underlying structures and chemicals. This is why we provide an overview of butterfly UV production mechanisms.

The formation of UV patterns is based on small structures in the butterfly wings. Although these structures are on a microscopic scale, they could be closely related to large-scale phenomena such as distribution of population with different level of UV reflectance pattern. For this reason, it is crucial to describe the main factors responsible for UV colouration in animals. In general, there are two main colouration mechanisms, pigmentary and structural, which often simultaneously contribute to the wing colouration (Vukusic & Sambles, 2003). Pigmentary (or chemical) colouration is due to pigments that selectively absorb light in a specific range of wavelength. Structural, or physical, colouration is due to interference of light scattered on nanostructures in the butterfly wing (Wilts et al., 2011). Interplay between these two colour mechanisms can influence the spectral composition of a signal and the information it conveys.

Wing structure is based on scales and each scale has a unique colour. A typical scale is about 200μm long and 100μm wide, but sizes in different species vary significantly. In most cases, but not always, scales form two layers, one consisting of larger cover scales, the other of smaller ground scales (Ghiradella, 2010). We could think of each scale as if it were a pixel in a digitised image, a pixel which contributes one point of colour to the overall image. A scale in most cases consists of two laminae: a lower lamina, which is usually flat and solid, and upper lamina, which is usually more complex and elaborate (Ghiradella et al., 1972). The upper lamina is structured by parallel, longitudinal folds, usually called 'ridges', which are connected by orthogonal struts, so-called 'crossribs' (Ghiradella, 2010). The ridges are composed of lamellae and feature diffraction gratings, so-called 'microribs' (Vukusic & Sambles, 2003). The lower and upper laminae are joined by pillar-like trabeculae (Ghiradella, 1989). The ridges and crossribs are often irregularly organised, so that in absence of pigments the scattering of incident light is wavelength-independent and results in a white colour of the scale (Stavenga et al., 2010).

Thin film layers are another relatively common variable feature in the structure of butterfly wings. Depending on the wavelength, these film layers can be constructive (e.g. UV iridescence in *Colias eurytheme*) or destructive (Kemp & Rutowski, 2011). Photonic crystals are specific single 3D or 2D network structures which reflect specific light band depending on the angle of illumination and configuration of the crystal itself (Ghiradella, 2010; Wilts et al., 2014). These structures, known also as 'photonic structures' (Vukusic & Sambles, 2003) are species-specific and can be modified in various ways, which results in different optical and structural characteristics and different contributions to wing appearance (Kemp & Rutowski, 2011). Moreover, if these structures are to produce maximally bright and/or achromatic UV signal, they need to be built with great precision and consistency, which is undeniably costly and hard for individuals to achieve (Kemp & Rutowski, 2007). Photonic structures are highly versatile, which allows for extravagantly varying shapes. In several cases, evolutionary experimentation led to highly aesthetic, regular landscapes which produce spectacular iridescence (Stavenga et al., 2004; Ghiradella et al., 1972). For further reading regarding on structures in butterfly wings, see e.g. Ghiradella (2010) or Nijhout (1991).

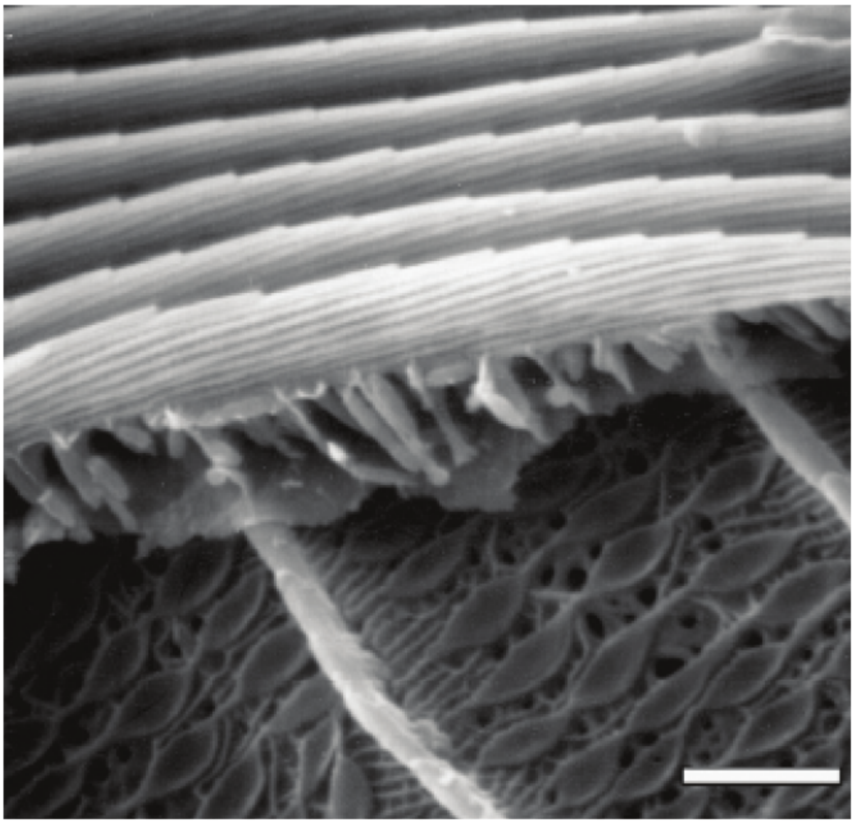


Figure 1: Colias eurytheme *male, parts of two scales. The fractured UV reflective scale (top) clearly shows lamellae of the thin film mirror on the ridges but also the interior scale trabeculae and fractured lower surface. From Ghiradella (1998).*

Pigments usually do not produce colours *per se*. They function as filters. These pigment-based colours tend to be non-directional or diffuse, changing very little with the angle of luminance or the viewing angle. Pigments responsible for colouring of butterfly wings include pterins (Pierids), melanins (Nymphalids), and flavonoids (Lycaenidae). Most studies on UV colouration focus on pterins (Kumazawa et al., 1994; Wijnen et al., 2007; Grether et al.; 2001; Rutowski et al., 2005). The main kinds of pterins are leucopterin, xanthopterin, and erythropterin (Wijnen et al. 2007; Wilts et al., 2011). Pterin pigments are deposited in small pigment granules, so-called beads, which enhance reflectance in long-wavelength range by increased scattering. In this way, they create a brighter wing colour and absorb diffuse reflectance in wavelengths below app. 550nm that is revealed when the pigments are removed (Morehouse et al., 2007; Stavenga et al., 2004). Leucopterin and xanthopterin absorb only light in the UV range. These compounds moreover amplify the iridescence properties of UV signal during wing movement

(Rutowski et al., 2005; Wijnen et al., 2007). The contrast in intensities is thus augmented in wing areas that reflect UV from the overlying lamellar thin films. These pigments also contribute to the reflection of what humans perceive as reds, oranges, and whites.

Pterins are reported to be the most nitrogen-rich pigments known. In species whose growth and development is routinely limited, pterin deposition in the scale can thus be constrained, which is why colouration could be indicative of quality of the individual (Kemp & Rutowski, 2011). An alternative view is that pteridines are the by-products of routine metabolic processes and their acquisition and expression is therefore unlikely to be particularly costly (Knuttel & Fiedler, 2001; Griffith et al., 2006).

When scale structure follows a regular periodical arrangement, it gives rise to structural colouration. In pierids, structural colouration is found in so-called cover scales, where the ridge lamellae evolved into a multi-layered structure, which creates a highly directional iridescent colouration (Ghiradella et al., 1972). Some time ago, a detailed description of four types of scales associated with physically produced UV reflectance has been proposed (Meyer-Rochow, 1983). Structural colours optically vary and can range from diffuse, broadband reflections produced by incoherent scattering, interference, or diffraction, all the way to a narrow reflection of high purity and intensity (Stavenga et al., 2004; Rutowski et al., 2005)

Illumination of the upper wings with white light can create blue light, iridescence, where the apparent colour depends on the illumination angle. This phenomenon is often discussed in the context of UV colouration and is known for instance in *Morpho* species (Glover & Whitney, 2010). This optical effect is based on taller ridges forming multilayer interference mirror, sometimes called 'multilayer reflector'. These structures are overlain by clear scales which via diffraction broaden the angle over which iridescence is visible. It has been proposed that this effect enhances the perception of the signal by intended receivers (Yoshioka & Kinoshita, 2004). Iridescence peaking in ultraviolet wavelength occurs also in Pierids, mostly in Coliadiane species (e.g. *Anteos clorinde*, *Phoebis* sp., *Eurema candida*, *E. hecabe*, *Colias eurytheme*, and *E. lisa*; Ghiradella et al., 1972; Rutowski et al., 2007a; Silberglied et al., 1978), where it is produced mainly by ridges in scales and its level is intensified via pterins which absorb short wavelengths (Wilts et al., 2011).

Distribution of iridescence in pierid wings varies among the various species. In most cases, iridescence is found only in males but in some species, females are also iridescent. Detailed spatiotemporal relationships of iridescence were studied in *Colias eurytheme* (Rutowski et al., 2007a), a butterfly which uses iridescence in its behavioural patterns mainly to communicate with conspecifics. Analysis of this behaviour also permits for some predictions regarding how signal senders and receivers need to be mutually oriented for maximal transmission and reception of this iridescent UV signal (Rutowski et al., 2007b). Angle-dependent structural colouration provides an opportunity to assess the spatiotemporal mechanisms of dynamic relations between colour, behaviour, and environment because visibility of these structurally coloured ornaments depends on both the illumination angle, i.e. an environmental feature, and observation angle, that is, on the receiver's position (Endler, 1992; Pirih et al., 2011). For example, in *Gonepteryx* species the ridge lamellae are tilted with respect to the scale plane (Ghiradella et al., 1972), whereby such tilting usually affects the visibility of the ornament in nature. This also suggests that the UV 'reflector' creates a signal which strongly contrasts with the common colours and features of the environment. The importance of inclusion of behaviour, wing pattern, and environment was highlighted a study that dealt with avian plumage (Simpson & McGraw, 2018) but the main message of that research could be generalised. Overall, however, these kinds of optical interactions between scales and their potential consequences for colour signal production, perception, and evolution remain understudied.

Several studies suggested that structural colouration serves to signal the quality of an individual. The key point of honest information regarding male mate quality is that only 'cheat-proof' signals, i.e. signals which are costly or difficult to produce and/or to maintain, can be expected to be evolutionarily stable (Smith & Harper 2003). This also implies that the expression of these signals might be condition dependent: only individuals with a good phenotype and good genetic equipment can develop and maintain an extreme version of this trait (Kemp & Rutowski, 2007).

UV colouration as secondary sexual trait is more sensitive to developmental stress than it is as a non-sexual trait. Structural colouration develops as a three-dimensional array. In most lepidopteran species, the development of colouration is restricted to a particular, restricted period of individual development: during metamorphosis, where it evolves from limited sources gathered during the larval stages. Structural UV can, therefore, function as a handicap (Zahavi, 1975) and indicate mate quality in the form of adult nutrient status (phenotypic quality), but also genetic quality due to its dependence on the ability to acquire resources during the larval stage (Kemp & Rutowski 2007). This is important because females receive fitness-enhancing nutrients during mating (Boggs & Gilbert, 1979). See also chapter on intraspecific communication.

Interestingly, to the best of our knowledge only a handful of studies so far proposed mathematical genetic models of the formation and diversification of general colour patterns in butterfly wings. Most genetic and molecular studies focus on a local patterns, such as eyespots. For example Yang et al. (2004) in their study found an abnormal specimen of *Aglais urticae*, which lacked the UV-reflective parafocal eyespots and exhibited changes of other pattern elements, which may be taken to indicate that there is a connection between proximal and distal wing colouration and the pattern in its entirety is not a single character produced along the posterior/anterior axis. On the other hand, there are only several genetical studies which investigated patterns globally, i.e. including UV patterns. Yet it is generally accepted that to understand the evolution and diversity of UV (as well as non-UV) patterns, one must investigate both genetic and molecular (developmental) traits with this in view. Such studies, however, are largely absent (Sekimura, 2014).

In general, the mechanism of UV and on-UV pattern formation varies highly among species and so does the variety of wing colours in butterflies. A unique colour signal of a species consists of a combination of structural and pigmentary elements. Angle-dependent reflectance measurements show that directional iridescence differs even among closely related species (e.g. Coliadinae *(Gonepteryx aspasia, G. Cleopatra, G. rhamni, Colias eurytheme),* Colotini (*Colotis regina, Hebomoia glaucippe*), Nymphalini (*Aglais urticae, Aglais io, Vannesa atalanta*), Papilioninae (*Parides* spp.), Theclini (*Chrysozephyrus* spp.) (Pirih et al., 2011; Stavenga et al., 2014; Wilts et al., 2014; Imafuku et al., 2002). Species-dependent scale curvature determines the spatial properties of wing iridescence. Narrow beam illumination of flat scales results in a narrow far-field iridescence pattern, while curved scales produce wider patterns. Restricted spatial visibility of iridescence probably plays a role in intraspecific signalling, as discussed above. Moreover, the position and type of pigment in the wings, the properties of the thin film (lamina), as well as scale anatomy itself, all these elements in conjunction produce a species-specific UV colouration.

When we take all of this aboard, it becomes clear that there is no simple or general mechanism of wing colouration and every species must be analysed on its own. In other words, we are far from understanding the optics of butterfly colouration in detail and we know that almost every species, and certainly those in different families, apply a variety of optical colouring methods. In many cases, pigmentary and structural colouration techniques combine in a nontrivial way (Nijhout, 1991; Wijnen et al., 2007). The mechanism of UV pattern creation is an important part of understanding other crucial topics, such as communication in UV wavelengths. As before, we must add that since this thesis focuses mainly on UV communication in the context of evolution, the present description of production of UV colour production is far from comprehensive.

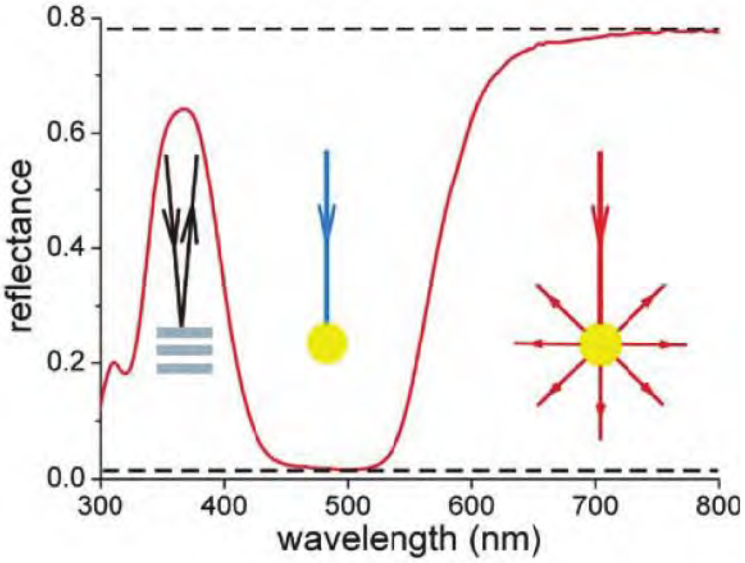


Figure 2: A summary diagram of wing reflectance in Hebomoia glaucippe *(Pieridae) as an example of the complex mechanism of UV colouration. The lamellae of cover scale ridges highly reflect UV but not all ranges of visible light. Blue light is thus absorbed.*

2.1.2.3 The effect of environment on UV reflectance

A number of spatial and temporal variables affect different functional traits in the life history of Lepidoptera, such as their body size, flight performance, wing shape, or overall fitness (Le Roy et al., 2019). These variables include temperature (Advani et al., 2019; Chen et al., 2019; Galarza, Dhaygude et al. 2019), altitude (Tuomaala et al., 2012; Sekimura & Nijhout, 2017; Brehm et al., 2019; Montejo-Kovacevich et al., 2019), latitude (Hovanitz, 1944; Dalrymple et al., 2015), net primary production (Beerli et al., 2019), but for the most part, their effect on UV patterns is studied rarely if at all.

The extent to which UV patterns are genetically fixed or changing in response to environmental pressures is not precisely understood but most insect species display a degree of phenotypic plasticity, which enables them to adapt to particular environmental conditions. Some studies did, however, investigate the purpose of UV patterns in the context of environmental variables. They worked for instance with latitude and variation across broad latitudinal space. In fact, latitude has been used as a proxy in series of macroecological studies. For instance, Meyer-Rochow and Järvilehto (1997) tested the effect of latitude on dorsal UV wing pattern and revealed significant differences between studied specimens. They, as well as a number of other researchers, found that females of *Pieris napi* from the northern part of the studied range had more prominent UV patterns, whereas males tended to remain more phenotypically constant and exhibited a smaller degree of UV pattern variability (see also Turner, 1978; Hazel, 1990; Brunton & Majerus, 1995). A similar trend was found in bumblebees: those living at higher latitudes reflect UV light more strongly than those from low latitudes (Mazokhin-Porshnyakov, 1954). One of the most likely explanations of this phenomenon is that the UV component of natural light decreases at higher latitudes due to a lower angle of the sun, which is why UV signals must be under these conditions stronger to facilitate functional communication. This is congruent with results of our study that analysed over 400 specimens of *Pieris napi* from the entire palearctic region (Stella et al., 2018; see Part 2 for further details). Generally speaking, localities characterised by extreme values of a number of variables, such as high seasonality of temperature seasonality and low overall temperatures, favour *P. napi* butterflies with stronger UV reflectance.

Large-scale environmental variables were explored in another study, which focused on *Gonepteryx rhamni* butterflies (Pecháček et al., 2014). This study found a systematic correlation between increase in the relative area of UV colouration, increased temperature and precipitation, and decreasing latitude of collected specimens (see Part 2 of this thesis). In this case, UV patches might serve as protection against UV light: such function has been proposed for flowers, where highly reflective pollen can function as UV protection after anthesis (Koski & Ashman, 2015). A follow-up on the abovementioned *Gonepteryx* study, which worked with 320 specimens of seven species, revealed that the shape of UV patch (signalling trait) is more asymmetric than the shape of wing venation (non-signalling trait). Unfortunately, the correlation between environment (latitude) and wing shape (fluctuating asymmetry) was not significant in all cases (Pecháček et al., 2019b; see Part 2 for further details). In other words, this study concluded that at least as far as *Gonepteryx* butterflies are concerned, UV patches are not a condition-dependent trait.

A study which compared two subspecies of *Pieris rape* revealed a significant gradient in wing UV reflectance along the east–west axis (Obara et al., 2008). UV reflectance of wings was higher toward the east, culminating in the Japanese subspecies (*Pieris rapae crucivora*). This variation is probably due to differences in inter-subspecies mating between UV-reflecting East Asian butterflies and ancestral UV-absorbing European butterflies (Fukano et al., 2012). Furthermore, only a weak relationship between UV pattern and habitat properties was found in 54 species of *Colias* butterflies (Stella et al., 2018). In this study, the different species tended to have different life histories, which made it difficult to associate UV reflectance with some general ecological variables. Recently, Dalrymple et al. (2018) investigated multiple dimensions of macroecological variation and their relative importance for butterfly colouring. They found that variables related to energy and resources in the environment consistently function as the most powerful predictors of butterfly colouration. This complex study, however, analysed the entire spectrum from 300nm to 700nm and paid no specific attention to UV patterns. Unfortunately, it dealt only with one relatively small region (Australia) and its results would be hard to generalise to other areas.

It is generally accepted that UV patterns correlate with UV radiation in the environment. Such association was described for example by a study on *Eurema hecabe* (Kemp, 2007a), where incident light was manipulated in such a way that mating rates of *E. hecabe* were six times higher in a UV cage (UV radiation present in the cage environment) than in a non-UV cage (UV radiation in the cage was reduced). Moreover, because the male UV signal was dulled (but not completely eliminated), these results indicate that females prefer to mate with males that have more brightly coloured wings. All in all, this study confirms the importance of configuration and level of UV irradiance in lepidopteran life history and its environment. Beckmann et al. (2014) produced a UV radiation dataset at high resolution to emphasise the importance of this factor in many ecological processes and geographical distribution of organisms. While this dataset is congruent with known patterns of global UV distribution, it also reveals further details of spatial and temporal relationships useful for ecological studies.

Although a number of direct and indirect effects of UV radiation has been described, as far as we know, UV radiation has not been systematically related to the configuration of organismal UV pattern, the only exception being one recent study on UV patterns in marsh marigold (*Caltha palustris*) (Zitko, 2019). Such link could help disentangle the complicated macroecological relationships between correlated variables, such as temperature and precipitation, and help reveal patterns in the distribution of functional response traits, such as UV pattern. Another interesting phenomenon related to ambient UV light is found in the *Anolis* lizards where cosmopolitan species are more variable in colouration. Different morphs of *Anolis conspersus* live in the various light habitats and it seems that morphs with greater colour variation tend to live in habitats with more heterogeneous light (Macedonia, 2001). UV light may the drive colouration of lepidopteran species or subspecies in the same manner but studies unfortunately have not yet been undertaken.

UV colouration is significantly affected by yet another developmental stress, namely temperature. Research in *Drosophila* (Vollmer et al., 2004) indicates that even relatively small departures from optimum developmental temperatures can result in temporary adult male sterility. In *Colias eurytheme*, the stressful effect of thermal manipulation was confirmed by a study which described a slower rate of pupal development and consequent impact on expression of UV wing pattern (Kemp & Rutowski, 2007). Congruent with these results are conclusions of research on seasonal forms in *Bicyclus anynana*, which showed that courtship, mate preference, and ornament UV reflectance in *B. anynana* change depending on developmental temperature. This suggests that seasonal temperature variation leads to a complex polyphenism in mating behaviour and morphology (Prudic et al., 2011).

Additionally, UV patterns (in fact pterins) in various butterfly species (e.g. *Colias eurytheme, Pieris rapae, Eurema hecabe*) may signal condition by being correlated with larval adaptation in host–plant quality (mostly nitrogen availability) (Slansky & Feeny, 1977; Morehouse & Rutowski, 2010; Mouchet & Vukusic, 2018). Adult *Colias eurytheme* males who are exposed to poor quality larval environment are not only smaller overall but exhibit duller and more angularly restricted UV reflectance (Kemp & Rutowski, 2007). In *Eurema hecabe*, low condition males even resemble high condition females (Kemp, 2008). Variability in the UV wing patterns of *Polyommatus icarus* can be due to differences in plants available as larval food even under otherwise identical rearing conditions among individuals from the same parents (Coutsis, 1996; Knüttel & Fiedler, 2000). In this case, flavonoids are the main driver which shifts the configuration of UV pattern in *Polyomnatus* butterflies. Previous research had shown that in *Pieris* butterflies, the degree of UV reflectance depends on the presence of pterins, UV-absorptive pigments (see chapter Mechanism of UV pattern colouration). For instance, in green-veined whites (*Pieris napi*) individuals capable of using the environment more effectively and/or those living in areas richer in nutrients reflect more UV light than those from warmer areas, which are however more suitable for breeding (Stella et al., 2018, see Part 2 for further details). In all these cases, acquisition of larval resources seems to be the main determinant of condition of particular individuals.

Bird feather pattern and structure is usually viewed as a condition-dependent secondary sexual trait (see chapter on UV reflectance in birds); in this respect, it is thus similar to butterfly wing pattern (McGraw & Hill, 2006). Like avian populations, lepidopteran populations exposed to different environmental conditions seem to face different selective pressures, which results in differences in wing structure and colouration, especially in species with large geographical distribution. In general, UV pattern is considered a condition-dependent feature and seems more sensitive to developmental stress than putatively nonsexual traits (see chapter on intrasexual communication). The precise nature of links between the development, environmental conditions, and variations in UV patterns remains, however, still unclear.

3 UV Visual System

Without an adequate understanding of perceptual cues to which an animal is responding, one cannot begin to explain its behaviour, life history, and much else. The aim of this thesis is to provide a comprehensive view of the hidden UV world. In this chapter, therefore, we shall examine the basics of UV vision with special focus on butterflies. Research into the biology of UV photosensitivity and vision had over the past decade made much progress. Researchers approach the issue from various angles including genetics, ecology, behaviour, and evolution. Understanding of UV vision of studied species is of essential importance if we are to disentangle the complex biological mechanisms involved. It is also clear that in this particular area, we cannot limit ourselves to understanding the phenomena in question, i.e. UV-sensitive visual systems, merely from our, human, perspective. That would necessarily lead to erroneous conclusions.

3.1.1 Historical records of UV light effects on organisms

Experimental investigation of the influence of UV light began in the second half of the nineteenth century. By this time, scientists learned about the existence of UV radiation, some investigations into its properties had been undertaken, and researchers started to realise that sunlight is not a single stimulus but rather a collection of stimuli of different wavelengths. The earliest studies indicated that UV light exerts some physiological and behavioural effects. In 1852, Jules-Auguste Beclard described that eggs of the common house fly, *Musca*, develop faster under violet and blue light than under green, yellow, red, or white light, and that green light even inhibits their development (Beclard 1858). Later studies (Schnetzler, 1874; Yung, 1878; Pöey, 1871) followed in his footsteps and studied the effect of UV light (as well as some other factors) on the growth and development on various animals. Unfortunately, the methods they used are not described in detail, so the effect of specifically just the UV light they observed is uncertain. Nevertheless, these studies showed certain effects of light (most likely UV light) on living organisms.

John Lubbock, 1st Baron Avebury, was the first scientist to experiment with the effect of emitted UV light on organisms (Lubbock 1883). He used a prism to emit UV light and tested ants' behavioural response to this light. He observed that ants carried their pupae away from the violet (UV) and blue parts of the spectrum and deposited them in locations with red light. This was the first experiment to demonstrate a behavioural response of an organism specifically to UV light. Other early studies focused mainly on the effects of UV light on responses of the central nervous system of e.g. *Daphnia* (Van Herwerden, 1914), Hymenoptera (Lutz, 1924; Frisch, 1924; Kühn 1924), Actinopterygii (Schiemenz, 1924), and finally also Lepidoptera (Lutz, 1924).

Lutz's famous study assumed that most lepidopteran species select the leaves to lay their eggs on based on odour rather than vision. He observed that Lepidoptera tend to lay their eggs on flowers which to human eyes look inconspicuous. Later in the twentieth century, Dora Ilse (1928) was the first person to show that butterflies can discriminate between different colours. Moreover, she demonstrated that butterflies have innate preference for specific colours and that among butterfly families, these preferences differ. Although interest in UV vision has been rapidly growing, until 1990s only a few animals were known to be sensitive to UV light, although nowadays we know that many, perhaps even most, animal species can visually detect and respond to it (Tovée, 1995).

3.1.2 Butterfly UV vision

Differences between butterfly eyes studied so far suggest that the capacity to discriminate colours varies among species and that this is driven by visual tasks determined by behaviour and habitat (Stavenga & Arikawa, 2006). The compound eyes of butterflies consist of a number of anatomical units, ommatidia, which are organised to form a more or less regular hemisphere. Ommatidia are usually present in three types characterised by their photoreceptors and pigments. In short, however, we can say that a facet lens and crystalline cone together project incident light into a photoreceptor. A butterfly ommatidium (retina) contains six or more photoreceptors (Arikawa, 2017). Their light-sensitive organelles, rhabdomeres, jointly constitute the fused rhabdom, which is a long cylinder that acts as an optical waveguide. The special feature of many butterfly eyes is the presence of a thin reflective layer, tapetum, located near the rhabdoms (Carlson & Chi, 1979). Visual perception thus starts with the absorption of a photon by visual pigments located in rhabdomere. Incident light propagates along the rhabdom and when it is not absorbed, it is reflected by the tapetum. Light then travels in a reverse direction and if not absorbed, eventually leaves the eye and it can be observed as eyeshine, also known as 'eye glow'. The eyeshine phenomenon allows for *in vivo* optical characterisation of individual ommatidia (Qiu et al., 2002).

The visual pigments, rhodopsins, are opsin proteins combined with a chromophore. Isomerisation and subsequent photochemical process form the chemical basis of photon absorption. The general organisation of butterfly colour vision is similar to that of honeybees and bumblebees, which is probably the most commonly studied subject in insect vision studies (Meyer-Rochow, 2019; Kelber & Somanathan, 2019). Butterfly vision system is in most cases based on three photoreceptor classes, with maximal sensitivity in the ultraviolet (UV), blue (B), and green (G) wavelength range (Menzel & Backhaus, 1989). Nymphalids are in this respect an exception because they have a tetrachromatic colour vision system (Briscoe et al., 2003).

The dorsal rim is a special area of compound eyes: it consists of a few omnitidia whose photoreceptors have a very high polarisation sensitivity (Stavenga & Arikawa, 2006), which is in Lepidoptera restricted to UV polarisation (Stalleicken et al., 2006). For instance the migratory monarch butterflies (*Danaus plexippus*) use this structure for flight orientation based on polarised UV light (Sauman et al., 2005). For a more detailed description of butterfly compound eye, see for example Stavenga and Arikawa (2006), Arikawa (2017), or Rutowski (2003).

The distribution of receptor types is, however, highly varied: it differs both among species and between the sexes. The reason for such diversity in butterfly colour vision is not fully understood but it is probably linked to the typical life histories of individual species. In many species, colour receptors are not uniformly distributed and patterns of expression of visual pigment across the eye can correlate with their merkwelt, or as it is sometimes called, their 'visuoecological lifestyle'. Some experts in this context proposed an 'efficient coding hypothesis', which suggests that the retina evolved so as to efficiently encode a given visual environment. In other words, it evolved to minimise redundancy of information carried by different neurons (Simoncelli & Olshausen, 2001; Baden et al., 2019).

This convergent coevolution was studied for example for flowers and their pollinators (Papiorek et al., 2016) or between three closely related sympatric species of dung beetle and their environment (Tocco et al., 2019). The small white, *Pieris rapae crucivora*, is a prominent example of a species with a heterogenous retina. In this species, we find three types of retina. The main difference between female and male photoreceptors is that the eyes of male *P. rapae crucivora* contain a fluorescent pigment which acts as a specialised UV filter and functions as a narrow-band violet receptor (Qiu et al., 2002), which probably improves the discrimination of UV contrast. In the eyes of males of another *P. rapae* subspecies, the *P. rapae rapae*, this fluorescent pigment is, however, absent (Stavenga et al., 2004). It was therefore hypothesised that the Japanese *P. rapae* subspecies *crucivora* evolved an improved discrimination of UV-visible patterns in the wings whose contrast is enhanced by scattered beads. A similar same-sex discrimination trait occurs in *Heliconius* butterflies (Dell'Aglio, 2016; Catalán et al.m 2018) or in *Asterocampa leilia* (Rutowski & Warrant, 2002). These differences in vision are due to gene duplications followed by mutations which affect amino acids in the opsin (visual protein) and lead to variation in spectral sensitivities between species (Briscoe, 2008). For example, both *Pieris rapae* and *Lycaena rubidus* have gene duplications on B opsin, but these duplications led to different extended visual spectrum ranges and sexual dimorphism in *Lycaena* eyes (Briscoe 2008). Another way in which vision can be altered is by application of spectral filtering in the ommatidia (Pirih et al., 2010).

The difference in organisation and configuration of ommatidia is often pronounced in short-wavelength photoreceptors, i.e. in UV photoreceptors (Cuthill et al., 2000). This led to suggestions that for example in the *Heliconius* complex or in the Pierids, the visual system and UV pattern co-evolved and might thus function as a unique, 'private', channel of communication (Stavenga & Arikawa, 2006; Pirih et al., 2010; Cronin & Bok, 2016; Briscoe et al., 2010). For example, it has been demonstrated that *Colias eurytheme* has a high ventral acuity and relatively larger eyes that other species. A possible adaptive explanation of this phenomenon may be that this facilitates hidden communication by male UV signals, such as was described in the previous chapter (Merry et al., 2006). In general, species which use their UV patterns in their intraspecific communication tend to exhibit some specific alteration of UV receptors (Meyer-Rochow et al., 2002), although it must be borne in mind that the importance of UV perception undoubtedly extends beyond mate recognition.

Indeed, not all communication in butterflies is based on visual signals. Silberglied (1978), for example, explored the importance of visual contact of butterflies during mating of several species and noted that no chemical stimuli are needed to elicit mating behaviour. But this is the case only in some species. For instance for *Colias eurytheme*, olfactory cues are more important than visual signals: initial mating requires visual stimuli, whereby even indistinct ones suffice, but further mating depends on chemosensory cues (Brunton & Majerus, 1995). In other words, final recognition of females by males is achieved by chemical means. For example, *Pieris napi* emits citral, a sex pheromone which positively affects female willingness to mate (Friberg et al. 2008).

Along with butterfly wings, eyes likewise are of essential importance in our quest to understand the evolution and development of various clades of butterflies (Stavenga & Arikawa 2006; Briscoe & Chittka, 2001). Although vision studies made a substantial progress in the course of the past several decades, vision is well described only in several species and there is still enormous amount of work to be done.

3.1.3 UV vision in other organisms

The fact that humans are incapable of UV vision does not imply that other clades cannot see UV light. Humans are rather an exception and since there are many other species which do use UV vision in their communication, we shall in the following present their brief description.

Humans have a trichromatic vision with three cone types that detect light in wavelength range of 400–700 nm. Birds, on the other hand, have tetrachromatic vision based on four cone types sensitive to wavelengths of 300–700nm and double cones for luminance with UV-transparent ocular media (Cuthill et al., 2000; Osorio & Vorobyev, 2008). All these cones are associated with a system of coloured oil droplets which act as filters and improve discrimination by sharpening the peaks of spectral absorbance (Cuthill et al., 2000). In addition to this, many birds species use multifocal lenses to preclude chromatic aberration, thereby improving overall vision (Cronin & Bok, 2016). The fact that birds can see in the UVA was established in 1972 in a series of pioneering experiments in hummingbirds (Huth & Burkhardt, 1972) and in the same year also in pigeons (Wright, 1972). Since that time, the ability to see UV light has been demonstrated for at least 35 diurnal bird species, mainly diurnal raptors, frugivorous, nectarivorous, and insectivorous species (Bennett & Cuthill 1994; Rajchard, 2009; Ödeen & Håstad, 2013).

Diurnal birds, which can be expected to have excellent colour vision, fall into two different classes of colour vision: a violet sensitive (VS- λ_{max} 402–426nm) and a UV sensitive (UVS- λ_{max} 355–377 nm) group (Bennett & Cuthill, 1994). Differences between these two groups are due to amino acid substitutions in specific sites of the UV/V cone opsin gene (SWS1), which led to changes in light absorption (Yokoyama et al., 2000; Osorio, 2019). In contrast to this, UV pigments in other vertebrates maintained their functions by accumulating at least one of the specific amino acid changes at a site entirely different from avian species (Yokoyama & Shi, 2000). As in Lepidoptera, there are significant interspecific variations in the relative abundance of different cone types in different parts of the retina (Bowmaker et al., 1991; Cuthill et al., 2000; Hunt et al., 1998; Baden et al., 2019). It is vital to consider UV vision in birds because it is highly likely that it functions as an important selective force on the evolution of not only birds but also insects and their traits.

The ability to perceive UV light is not unique to birds and insects. UV receptors are integrated into the visual system of most animals, including crustaceans. For instance, the Mantis shrimp with 16 photoreceptors apparently use different regions of the UV spectrum for different tasks. They can be trained to associate UV cues of wavelengths above 350nm with food rewards. In fact, this part of the spectrum often figures in various species-specific chromatic cues. This is applies for instance to fish with tetrachromatic vision, of which the zebrafish is the most commonly studied species (Leech & Johnsen, 2006; Siebeck, 2004; Wada et al., 2018; Franke et al., 2019; Nelson et al., 2019), but also amphibians, such as frogs and salamanders (Govardovskii & Zueva, 1974; Cronin & Bok, 2016; Mohun & Davies, 2019), reptiles (Tovée, 1995; Harcourt, 1996; Dong et al., 2019), and even mammals.

Most mammals have dichromatic vision: primates with their trichromatic vision are rather an exception. UV perception was found in various species of rodents (Jacobs et al., 1991; Jacobs, 1992; Jacobs et al., 2001), bats (Simões et al., 2018), and seals (Hogg et al., 2015). Until recently, it has been assumed that larger mammals were with respect to vision generally similar to humans and that they use yellow lenses to block UV radiation. Recent studies, however, revealed UV sensitivity in arctic reindeer (Hogg et al., 2011; Hogg et al., 2015). This accounts for the fact that Rudolph was so worried about entangling Santa's sleigh in power lines, which emit high amounts of UV radiation.

A comparison between various mammalian ocular media moreover shows that a fraction of environmental UVA is admitted into the eye, which counts for some UV sensitivity (Douglas & Jeffery, 2014). Even mammals without UV-specific photoreceptors are thus UV-sensitive if they have ocular medium that transmits UV wavelengths, because all visual pigments absorb significant amounts of UV if the energy level is sufficient. In general, species which are at least partially nocturnal tend to have UV-transmitting lenses, while mainly diurnal species do not, and UV radiation thus does not reach their retina (Douglas & Jeffery, 2014). Human cataract surgery is a procedure where the lens is removed and aphakic individuals, i.e. people without a lens, experience a change in their vision. For instance the painter Claude Monet (1840–1926) documented the bluish appearance of UV radiation when he pained one of his favourite scenes, water lilies, before and after his cataract surgery in 1923 (Arribas, 2012). Human eye without a lens is thus capable of perceiving a limited amount of UV light. In most mammals, the loss of UV vision is linked to either increased acuity in the remaining part of the light spectrum or with protection against potential damage to retinal tissue (Cronin & Bok, 2016). In general, however, in animals which have UV receptors those receptors tend to be incorporated into the overall colour vision of the animal.

The ecological importance of UV vision in the abovementioned organisms has been studied mostly in the context of navigation and orientation (Menzel & Backhaus, 1989; Tovée, 1995; Simpson & McGraw, 2018), management of the circadian rhythms (Mainster, 2006; Tovée, 1995), intra- and intersexual signalling (Hunt et al., 1998; Pearn et al., 2001; Cuthill et al., 1999), species signalling (Cuthill et al., 2000; Tovée, 1995), and in connection with foraging and prey and/or predator detection (Honkavaara et al., 2002; Rajchard, 2009; Hogg et al., 2011; Tedore & Nilsson, 2019). For more, see also chapter on UV patterns in organisms.

Although most animals with UV sensitivity have only one type of UV receptor incorporated in their a tri- or tetrachromatic colour vision system, there are some notable exceptions. In *Drosophila*, for example, UV vision is based on two different pigments with λ_{max} at 345nm and 375nm (Cronin & Bok, 2016). Another common feature of eye structure are UV-filtering pigments in the lens. Such pigments attenuate the shifts of UV light falling on the retina. At least one species of thrips (Thysanoptera) has a UV-absorbing pigment in certain facets of its compound eyes, which shift the likely sensitivity maximum of the visual pigment of λ_{max} 360nm down to below 330nm (Mazza et al., 2009). For a review of the history of research, phylogeny, and distribution of UV photoreceptors, see e.g. Cronin and Bok (2016).

The visual world is highly diverse. This is the case not only when we compare the natural environments inhabited by different species, but also in comparison between the different parts of the visual field of a single species, eye structure, and even the anatomy and function of the retina as such (Baden et al., 2019). Visual specialisation in eye design, large-scale inhomogeneities across the retinal surface, and local circuit motifs mean that all species' retinas are unique. To reach a better understanding of this fascinating subject, one must explore UV sensitivity in a far more comparative and complex manner.

3.1.4 Some notes on colours

Colour *per se* can never be described objectively because it is a perceptual construct (Cuthill et al., 2000) that ought to be viewed as a property of both the coloured object and sensory apparatus of the animal that perceives it (Glover & Whitney, 2010). Humans can understand hues perceived by birds about as much as knowledge of two coordinates can predict the position of a point in three-dimensional space (Bennett et al., 1994).

There are various aspects of colour and colour patterns. Human perception of colour includes brightness, chroma, and hue. Brightness is the overall intensity, chroma is saturation, that is, how much light of other wavelengths (e.g. white light) is mixed with the focal colour, and hue is the focal colour at a particular wavelength range, e.g. red, green, or blue (Kelber & Osorio, 2010). A colour pattern may result from the variation in any of these factors across the body of an organism and can often be expressed in quantitative characteristics such as a number of spots or size of a blotch (Thorpe, 2002).

Human colour vision system cannot thus be used with certainty to assess the variation of all visual colour signals. This becomes especially apparent when we consider the known differences between human vision physiology and avian visual systems. Human trichromatic vision system with its specially tuned colour receptors allows us to perceive the general colour contrast between patches in plumage. It does not, however, allow us to appreciate how birds themselves perceive their specific colour-based interactions (Håstad & Ödeen, 2008). One might wish to argue that trichromacy is ideal for colour vision: not only does it differentiate between the separate dimensions of hue, saturation, and brightness, but – as has long been known – there three components are adequate for coding most variation in the natural spectra with opsin-based pigments (Kelber & Osorio, 2010). For more on colour vision and spectral coding, see Villafuerte and Negro (1998), Vorobyev and Osorio (1998), or Osorio and Vorobyev (2008).

It seems that sensitivity to UV light is found in all major animal groups but not in humans (some researchers speak of humans being 'the odd ones out'). The aim of this overview chapter is to gain a better understanding of non-human vision to understand the world from animals' point of view. More specifically, we shall have a look at studies which try to relate the terminology of human colour vision to animal studies, look at how animals use spectral information in natural contexts, and relate this to how various animals perceive their world and live in it. Even at the outset, however, it should be noted that vast amount of work still needs to be done if we are to appreciate all aspects of UV umwelt and that this work cannot treat UV as a separate subject. When trying to understand an animal's visual umwelt, all wavelengths perceived by the animal must be taken into account.

4 Central Nervous System Processing of UV Visual Information: The Brain

Within the holistic approach of this thesis, it is vital to link the final stage of UV (and non-UV) signal processing with the visual system and thereby also with the central nervous system (CNS). Although there are some notable exceptions (Enquist & Arak 1993; Arak & Enquist, 1993), many studies on evolution and ecology of vision tended to neglect the neural networks and/or psychology of the perceiver (Lindsay et al., 2019). In other words, the neural systems which mediate colour vision are so far almost unexplored. And since, to the best of our knowledge, there is no evidence suggesting that UV signals are processed in some dedicated part of the CNS, investigation of this subject is highly challenging (Stavenga & Arikawa, 2006). The following text thus provides rather general information on the effects of light signals on the CNS in various organisms.

When sensory organs perceive a UV signal, the central nervous system receives and further processes this clue. In vision, what matters is not only the stimulation of visual receptors but also the relative stimulation of two or more receptor types and subsequently – even more importantly – their neuronal interactions. Perception is viewed as a multistage process where receptor signals are transformed by a sequence of neural mechanisms (Vorobyev & Osorio, 1998). In Lepidoptera, the CNS is composed of a double chain of ganglia joined by longitudinal connectives. The anterior ganglion is the brain. The brain is connected to the ventral chain of ganglia by two connectives which travel around the pharynx. The brain connects to the eyes, ocelli, and antennae. The suboesophageal ganglion is highly complex and innervates the sense organs and muscles associated with mouthparts, salivary glands, and the neck region. The suboesophageal ganglion exerts the primary excitatory or inhibitory influence on motor activity in all Lepidoptera (Perveen & Khan, 2017). Protocerebrum is associated mainly with compound eyes and ocelli (Swihart, 1970).

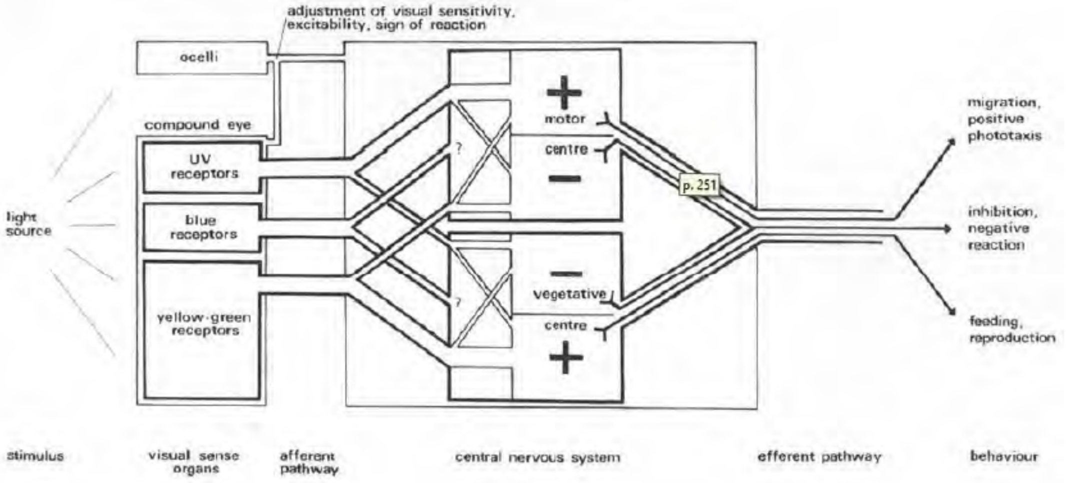


Figure 3: A functional model of perception of light, including UV spectra. CNS is represented as a tangled box with a number of question marks. Afferent pathways for sensory neurons carry nerve impulses from sensory stimuli towards the central nervous system and brain. Efferent pathways for motor neurons carry neural impulses away from the central nervous system and towards the muscles to cause movement. Originally in Mikkola (1972)

A number of authors, e.g. Brunton and Majerus (1995), theorised about the problem which arises when we measure or compare colours by human visual system, which is based on three colour-sampling elements (cone types) or colour receptors in the retina. A neural signal is caused by light of a particular wavelength: that stimulates the appropriate colour signal. But because different animal species have different composition of photoreceptors as well as different neural processing, they see colours and hues we cannot even imagine. We must therefore devise methods of colour quantification which are independent of human vision.

At this point, we ought to mention some of the main theories of colour vision in the context of neural processing. The trichromatic theory, also known as 'Young–Helmholtz theory', is based on colour matching and colour mixing studies. Trichromacy was confirmed by the discovery of three types of cones whose spectral sensitivities were predicted by psychophysical methods which account for neural activity (Vorobyev & Osorio, 1998). Young–Helmholtz theory was traditionally contrasted with the 'colour opponency model' developed by Ewald Hering. This theory claims that cone photoreceptors are linked to form three opposing colour pairs: blue and yellow, red and green, and black and white. The neural implementation for this psychophysically determined opponency mechanisms is less certain. A third model, known as 'receptor noise-limited colour opponent model', predicts the form of colour vision in animals for which physiological and anatomical data on the eye are available but direct measurements of perceptual thresholds are not known (Vorobyev & Osorio, 1998). This model has the virtue of simplicity, making minimal assumptions about post-receptoral processing.

Electroretinogram (ERG) is one of the indirect methods one can use to study the effect of signals on the CNS and explore the main pathways involved in the visual process (Jacobs et al., 2001). The nature of the insect ERG has long been a subject of controversy due because of the use of various methodological approaches, usually very invasive and cruel with respect to the experimental subject, and subsequent effects on the CNS (Ruck & Jahn, 1954; Swihart 1964). Technical stimulation of visual cues is in ERG a crucial feature. The method requires an instrument with high spatio-temporal accuracy and a UV channel. Currently, several types of ERG instruments are used, which include up to six arbitrary chromatic channels covering the UV spectrum (Franke et al., 2019).

Neuronal pathways have been investigated with the ERG in a number of lepidopteran species belonging to several families, e.g. *Papilio troilus* in Papilionidae (Swihart, 1970), *Colias eurytheme* in Pieridae (Post & Goldsmith, 1969), Lycaenidae (Eguchi et al., 1982), *Heliconius erato* in Nymphylidae (Swihart, 1964), Satyridae (Eguchi & Horikoshi, 1984), and Saturniidae (Eguchi et al. 1982). These studies have shown that efferent impulses are as important to the visual process as the afferent ones are. Moreover, they describe a variety of specific fibres with wavelength-specific patterns of activity and explore various level of responses.

Even the usually highly traditional anatomy is enjoying a renaissance thanks to a technique called serial block-face scanning electron microscopy (Denk & Horstmann, 2004) which could be used for exploring CNS pathways. This method allows us to obtain thousands of sequential images on the basis of which one can describe detailed anatomy of individual neurons and the circuits they form in a way that was simply not feasible with traditional transmission electron microscopy (Arikawa, 2017).

Behavioural tests are another way of exploring the functioning of CNS (Mazokhin-Porshnyakov, 1969; Warzecha & Egelhaaf, 1995). Different studies, however, use different methods and lack of standardisation means that their results are hard to compare. In many cases, scientists find discrepancies between behavioural and physiological experiments appear in many cases (Mikkola 1972; Telles et al., 2014). For example, Jacobs et al. (2001) found in rats a large disparity between UV sensitivity assessed based on behavioural discrimination, where results suggested a 50% contribution from UV cones, and ERG measurements, where results came up with only a 17% contribution. The authors hypothesised that somewhere beyond the outer retina, a significant enhancement in the relative strength of the UV cone signals must take place.

In any case, studies of neural events related to insect colour vision help highlight the remarkably efficient and adaptive methods which evolved in arthropods to extract vital information from their environment. This short chapter offered only a general outline of the basic concepts of neural processing of UV signals. And although studies in neurobiology of UV processing and vision as are still few and far between, in the following we shall try to explore these processes through other phenomena.

5 Notes on the methodology of UV reflected photography: UV photography as a methodological innovation

At the outset, it is crucial to differentiate between UV reflected photography and UV induced fluorescence. UV induced fluorescence is the imaging of visible or infrared light which is emitted as a result of UV hitting a surface, while in UV reflected photography the subject is illuminated directly by UV light and camera captures only UV spectra. The phenomenon of UV reflectance is, in its biological context, described by different scholars by different names, including UV reflectance (Pecháček et al., 2019a; Cuthill et al., 1999), UV phenomenon (Mullen & Pohland, 2008), or UV iridescence (Stavenga et al., 2010; Rutowski & Kemp, 2017; Kemp, 2007a). All these terms describe the same optical or biological phenomenon.

Humans cannot ordinarily detect UV light; studies of UV patterns thus necessarily rely on the assistance of technology. There are in principle two main conventional approaches: UV spectrophotometry and UV photography. The development of UV photography has been of essential importance and it is a highly varied and versatile technology because each study requires a somewhat different approach. UV photography has been evolving and used in science for almost one hundred years, ever since Lutz captured the first UV pictures using a pinhole camera (Lutz, 1924). This American entomologist was the first to use UV photography to investigate UV-reflective patterns and their function in communication among animals or plants and their pollinators. This opened the way for further studies (Lutz, 1924; Lutz, 1933). Since that time, unfortunately, UV photography received much less attention than for instance IR or standard VIS photography. Nevertheless, numerous studies from many areas of science had developed different approaches and dealt with different challenges with respect to UV photography and various companies and scientists came up with new techniques in the acquisition and standardisation of still UV photography (Eastman Kodak Company, 1972; Eastman Kodak Company, 1948; Ferris, 1975; Kevan et al., 1973; Brues, 1941) and UV cinematography (Aneshansley & Eisner, 1975; Eisner et al., 1969). They dealt with UV as such (Allman, 1973), UV photography in chemical analysis (Luner, 1968), skin analysis (Crowther, 2019), UV photography in archaeology (Conlon, 1971), forensics (Arribas, 2012), aerial UV photography (Cronin et al., 1968; Lavigne & Øritsland, 1974; Lavigne, 1976), the visual system of bees and pollination ecology (Daumer, 1958; De Bruin, 1961; Hill, 1977), and at last but not least with UV patterns in butterflies (Crane, 1954; Mazokhin-Porshnyakov, 1957; Ferris, 1972).

Figure 4: UV Photography is art ...(Jindřich Štýrský, Severní točna 1939)

Crane (1954) and Mazokhin-Porshnyakov (1957) were among the first to examine different species of butterflies with UV light by a photographic method. There are several reasons for such a long-lasting and still ongoing evolution of UV photography. First of all, this area of photography has never been commonly used for non-scientific applications. Relatively fast evolution of regular still photography over the past century provided challenging and constantly changing conditions in terms of material for the much less widely used UV photography (Nekrutenko & Didmanidze, 1975; Silberglied, 1979). Under these circumstances, it has been extremely difficult to standardise of UV photography among the widely diverse range of scientific fields, which resulted in a number of various methods in this discipline.

The invention of digital photography offered a significantly faster and more cost-effective way of producing images with the added possibility of subsequent image analyses. Visible and infrared spectra are regularly detected with conventional cameras (for butterfly wings in infrared light, see e.g. Krishna et al. 2020). Digital UV photography, however, places some specific demands on the technical equipment that must be overcome. First of all, one must have a UV-sensitive camera. For instance, FujiFilm IS Pro is a digital camera suitable for UV photography due to its broad sensitivity spanning from 330 to 900nm. Yet information regarding sensitivity is, unfortunately, rarely available. Furthermore, a camera needs to be equipped with an uncoated UV-transmitting non-distorting lens, for example a quartz lens, whereby in general, the older and simpler, the better. What one needs is a prime lens with few optical elements. A set of filters (e.g. B+W) then must be mounted on the lens to filter out the visible and infrared parts of the spectrum. Finally, one should use an artificial source of stable UV light, such as a mercury lamp, flashlight, or LED specially adapted for emitting in known UV spectra. For analysis of the emission of several commonly used UV light sources, see Rutowski & Macedonia (2008). Reflection standards for UV photography, such as Spectralon or Kodak grey card, are another required element for acquisition of UV photographs (Knüttel & Fiedler, 2000). This allows standardisation (linearization) of all photographs in a dataset (Troscianko & Stevens, 2015). Cameras are usually designed to produce images that look good, that is, they are not developed to record reality. This so-called non-linearity in capturing RGB signals is standard in most conventional cameras. Technical and high-specification cameras (sensors) usually have linear outputs.

To emphasise the nontriviality of taking UV photographic methods, we should also mention the shift of focus. Due to a different wavelength, the plane of focus is closer to the focal plane (Primack, 1982). This can be partly addressed by stopping the aperture. Some authors used the term 'harmonisation' to describe setups which bring together all these features (Crowther, 2019). A number of fairly specifically described methods of UV photography have been published (Stevens et al., 2007; Arribas, 2012; Troscianko & Stevens, 2015; Stella et al., 2018; Crowther, 2019; Dalrymple et al., 2015; Tedore & Nilsson, 2019). Data acquired by such standardised methods then can be stored in a database such as iDigBio (www.idigbio.org) or LepNet (Seltmann et al., 2017) for further high-throughput image analysis.

Unclear or erroneous methodology can result in unexpected or undesirable results of analyses. For example, Brunton and Majerus (1995) used spectrophotometry to assess inter- and intraspecific differences between various species of *Colias* and *Gonepteryx*. Since UV pattern in a species strongly depends on the angle and place of measured patches in a butterfly wing (Wilts et al., 2011), the study resulted in unclear results due to insufficiently described methodology. Standardised position of butterfly and probe for spectrophotometric analysis should be included in every study. Only when this condition is met, standardised UV photographs can provide UV pattern overview useful for further spectrophotometric measurements.

Based on described methodology, one can see that some studies used faulty methods to produce their UV pictures, although their conclusions and discussion focused mainly on analysis of UV patterns (Kodric-Brown & Johnson, 2002; Zapletalová et al., 2016; Futahashi et al., 2019). These errors originate mainly from inappropriate material used, such as unsuitable filters or cameras (CMOS sensors), ambient light and its geometry, wrong image format (JPEGs vs RAWs), or the use of non-standardised photographic methods which led to the production of photographic data that could not be properly compared for analyses (Arribas, 2012; Troscianko & Stevens, 2015). In such studies, the properties of colour images were device-dependent and images of the same scene varied depending on the use of materials and methods (Stevens et al., 2007). Finally, in many cases one can see that the image outputs were inappropriately modified.

Unsuitable methods of UV photography can lead to unfounded and/or erroneous assumptions regarding taxonomic placement, eventually ecological or evolutionary interpretation, because a UV pattern can be partially masked by visible or, more frequently, by IR reflectance. Such UV photographs are not pleasant to look at. They lack the appeal, charm, and beauty of the black and white or infrared photographs or the visible spectrum images and even so, they are rather difficult to obtain (Arribas, 2012).

The difference between UV photography and UV spectrophotometry is clear. Conventional spectrophotometers provide only point samples, while adequate characterisation of colour of a heterogenous object requires multiple samples across an appropriate sampling array, such as multiple transects (Crane, 1955; Garcia et al., 2013). This places high demands not only on sampling time but also on information about spatial relationships between colours that must be reconstructed from the geometry of the sampling array (Wilts et al., 2011). Even so, the spatial resolution is usually crude. Spectrometry also usually requires a static subject, either because of the need to sample an array or because the measuring probe needs to be close to or touch the colour patch. This is particularly challenging when working with delicate museum specimens (Stevens et al., 2007).

Spectrophotometry also does not yield data suitable for analysing the three-dimensional nature of many patterns (Troscianko & Stevens, 2015). Even digital UV photography is relatively challenging but it has many advantages over spectrophotometry, particularly in its ability to use powerful and complex image processing algorithms to analyse entire spatial patterns without the need to reconstruct topography from point samples. More obviously, the process is relatively fast and allows for rapid collection of large quantities of data, from unrestrained targets and with minimal equipment (Pike, 2011). Imag-ing programs can be used to obtain various forms of data, including colour patch size and distribu-tion measures, diverse 'brightness' and colour metrics, or broadband reflection values. Moreover, digital technology can be used in manipulation of stimuli for use in behavioural experiments.

Condition of the specimen is an important factor to consider in the context of methodology of UV imaging. A number of studies investigated various differences between old (museum) specimens and freshly killed or living specimens. They found, among other things, no significant difference in reflectance patterns between specimens freshly killed before photographing and those which had been dried, provided that specimens had been protected from light and rubbing (Stella et al., 2018; Ramos & Hulshof, 2019). It also seems that the use of chloroform or paradichlorobenzene has no observable effect on reflectance results (Crane 1954). A study using *Polyommatus icarus* as a test species showed that the blue sexual signalling colour of structural origin in males of this species exhibited stability both over the course of a year (3 broods tested) and over more than a century (Kertész et al., 2019). Another study found no significant change over decades in either visible colour or UV colouration in museum specimens of two lepidopteran species. There was just a light increase in UV reflectance, probably due to pigment degradation (Peet-Pare, 2017).

In contrast to this, the nymphalid butterfly *Anartia fatima* is unusual in that both its UV reflection and visible colour change with the specimen's age. Its yellow, UV-absorbing bands gradually become white and UV-reflective in both sexes. Older, UV-reflecting females are more attractive to mate-seeking males (Taylor, 1973a). This phenomenon was, however, later associated with another factor and given that UV patterns are not considered important communication signals in tropical butterflies, it may just coincide with some underlying genetical or physiological shifts. Also, some bird feathers, dragonflies and jumping spiders fade over time (Lim et al., 2007).

Jack J. Windig, a pioneer in digital image analysis of lepidopteran wing pattern, in his article (Windig, 1991) stated that collection of imaging data and image analysis should meet three criteria. Firstly, completeness, meaning the studied trait should be quantified with respect to all its characters. Secondly, repeatability, meaning that the procedure must be repeatable. And finally, the process should be fast relative to available methods. Although digital photography products are available, fast, and they have already transformed studies of colouration, patterns and shapes in biology, one should proceed with caution during the whole procedure and suitable calibrations before and after such investigations must be made. An English saying states that 'a picture is worth a thousand words'. In this context, we should say 'a standardised picture is worth a thousand words'.

6 Conclusion

The remarkable colours of butterflies and their possible functions have been attracting special attention of biologists ever since the time of Darwin (1909), Wallace, and Lorenz (1962). All three of these respected scientists came to basically to the same conclusion, namely that in the varied manifold of various environments, striking colours help butterflies to distinguish each other, define their territories, and attract and keep mates. UV signals are just one of the pieces of this much larger puzzle.

The aim of this dissertation is to elucidate and review the functions and relative importance of UV patterns mainly in Lepidoptera in a holistic context of UV ambient light, optical properties of various surfaces and patterns, and general knowledge of UV perception, and to briefly describe CNS as the central processing unit of UV signals. The structure of this thesis was inspired by the ground-breaking work of Silberglied (1979), who was the first to attempt a comprehensive approach to UV in biology.

The six studies enclosed to this text discuss mainly the variation in UV reflectance levels and UV pattern shapes in the context of large-scale environmental factors in several studied species. This, however, is only a small part of the UV umwelt briefly outlined above, an umwelt that encompasses many other ecological and evolutionary factors. UV patches may play a significant role in intraspecific and/or interspecific communication of Lepidoptera and we believe they are also a functional and effective taxonomical tool. The ubiquity of UV photoreceptors in the visual systems of many animals shows that UV colour is as important as other visible colours. Studies which quantify colour, or even qualitatively describe or compare colour patterns, must take UV colour into account. Descriptions of colour should not reflect merely our view of them. They should be set up with full consideration of the purpose of the colour. A relatively large proportion of studies fails to account for UV phenomena and many researchers neglect the UV spectrum as part of the integrated visual system. On the other hand, there is no reason why one should assign a higher value to UV patterns than to wing pattern visible to human eye. And there is no good reason why we should assume a special function of UV wing patterns as a signal in visual communication.

There is still much work to be done to address all aspects of UV umwelt: ambient UV, UV patterns, UV vision, and finally, UV signal processing. If we ignore these dimensions and study colour only anthropocentrically, we introduce fatal flaws into our experiments, as we do when we fail to represent all parts of the hidden UV world.

References

Advani, N.K., Parmesan, C., & Singer, M.C. (2019). Takeoff temperatures in *Melitaea cinxia* butterflies from latitudinal and elevational range limits: a potential adaptation to solar irradiance. *Ecological Entomology,* **44**(3), 389–396.

Allman, M. (1973). Photomacrography using direct ultraviolet radiation and the problem of sharp focus. *The Journal of Photographic Science,* **21**(6), 265–270.

Allyn, A.C., & Downey, J.C. (1977). Observations on male U-V reflectance and scale ultrastructure in *phoebis* (pieridae). *The Allyn Museum of Entomology,* **42**, 1–20.

Alonso-Alvarez, C., Doutrelant, C., & Sorci, G. (2004). Ultraviolet reflectance affects male–male interactions in the blue tit (*Parus Caeruleus Ultramarinus*). *Behavioral Ecology,* **15**(5), 805–809.

Altshuler, D.L. (2001). Ultraviolet reflectance in fruits, ambient light composition and fruit removal in a tropical forest. *Evolutionary Ecology Research,* **3**(7), 767–778.

Andersson, M.B. (1994). *Sexual Selection.* Princeton, USA: Princeton University Press.

Andersson, S., & Andersson, M. (1998). Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proceedings of The Royal Society of London B: Biological Sciences,* **265**(1395), 445–450.

Aneshansley, D.J., & Eisner, T. (1975). Ultraviolet Viewer. *Science,* **188**(4190), 782.

Arak, A., & Enquist, M. (1993). Hidden preferences and the evolution of signals. *Philosophical Transactions of The Royal Society of London. Series B: Biological Sciences,* **340**(1292), 207–213.

Arias, M., Mappes, J., Desbois, C., Gordon, S., Mcclure, M., Elias, M., Nokelainen, O., & Gomez, D. (2019). Transparency reduces predator detection in mimetic clearwing butterflies. *Functional Ecology,* **33**(6), 1110–1119.

Arikawa, K. (2017). The eyes and vision of butterflies. *The Journal of Physiology,* **595**(16), 5457–5464.

Arribas, O.J. (2012). The ultraviolet photography of nature: techniques, material and (especially) lacertini results. *Butlletí De La Societat Catalana D'herpetologia,* **20**(2), 72–114.

Baby, S., Johnson, A.J., Govindan, B., Lukose, S., Gopakumar, B., & Koshy, K.C. (2013). UV induced visual cues in grasses. *Scientific Reports,* **3**(2738), 1–6.

Baden, T., Euler, T., & Berens, P. (2019). Understanding the retinal basis of vision across species. *Nature Reviews Neuroscience,* **2***(340)*, 1–16.

Beckmann, M., Václavík, T., Manceur, A.M., Šprtová, L., Von Wehrden, H., Welk, E., & Cord, A.F. (2014). Gl UV: a global UV-B radiation data set for macroecological studies. *Methods in Ecology And Evolution,* **5**(4), 372–383.

Beclard, J. (1858). Note relative a l'influence de la lumiere sur les animaux. *Comptes rendus hebdomadaires des séances de l'Académie des sciences,* **46**(2), 441–443.

Beerli, N., Bärtschi, F., Ballesteros-Mejia, L., Kitching, I.J., & Beck, J. (2019). How has the environment shaped geographical patterns of insect body sizes? a test of hypotheses using sphingid moths. *Journal of Biogeography,* **46**(8), 1687–1698.

Bennett, A.T.D., & Cuthill, I.C. (1994). Ultraviolet vision in birds: What is its function? *Vision Research,* **34**(11), 1471–1478.

Bennett, A.T.D., Cuthill, I.C., & Norris, K.J. (1994). Sexual selection and the mismeasure of color. *The American Naturalist,* **144**(5), 848–860.

Biberman, L.M., Dunkelman, L., Fickett, M.L., & Finke, R.G. (1966). Levels of nocturnal illumination. *Institute for Defence Analysis,* Research Paper P-232.

Bishop, T.R., Robertson, M.P., Gibb, H., Van Rensburg, B.J., Braschler, B., Chown, S.L., Foord, S.H., Munyai, T.C., Okey, I., & Tshivhandekano, P.G. (2016). Ant assemblages have darker and larger members in cold environments. *Global Ecology and Biogeography,* **25**(12), 1489–1499.

Bleiweiss, R. (2004). Ultraviolet plumage reflectance distinguishes sibling bird species. *Proceedings of The National Academy of Sciences,* **101**(47), 16561–16564.

Boggs, C.L., & Gilbert, L.E. (1979). Male contribution to egg production in butterflies: Evidence for transfer of nutrients at mating. *Science,* **206**(4414), 83–84.

Bonduriansky, R. (2001). The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biological Reviews,* **76**(3), 305–339.

Bonnaffé, W., Martin, M., Mugabo, M., Meylan, S., & Le Galliard, J. (2018). Ontogenetic trajectories of body coloration reveal its function as a multicomponent nonsenescent signal. *Ecology and Evolution,* **24**(8), 12299–12307.

Bowden, W.B., & Watt S.R. (1966). Chemical phenotypes of pteridine colour forms in *Pieris* butterflies. *Nature,* **210**, 304–306.

Bowmaker, J.K., Thorpe, A., & Douglas, R.H. (1991). Ultraviolet-sensitive cones in the goldfish. *Vision Research,* **31**(3), 349–352.

Bozano, G.C., Coutsis, J.G., Herman, P., Allegrucci, G., Cesaroni, D., & Sbordoni, V. (2016). *Guide to the Butterflies of the Palearctic Region. Pieridae 3: Coliadinae: Rhodocerini, Euremini, Coliadini (Gonepteryx And Others) & Dismorpiinae (Leptidea).* 2nd ed. Milano: Omnes Artes.

Brehm, G., Zeuss, D., & Colwell, R.K. (2019). Moth body size increases with elevation along a complete tropical elevational gradient for two hyperdiverse clades. *Ecography,* **42**(4), 632–642.

Briscoe, A.D. (2008). Reconstructing the ancestral butterfly eye: Focus on the opsins. *Journal of Experimental Biology,* **211**(11), 1805–1813.

Briscoe, A.D., Bernard, G.D., Szeto, A.S., Nagy, L.M., & White, R.H. (2003). Not all butterfly eyes are created equal: Rhodopsin absorption spectra, molecular identification, and localization of ultraviolet-, blue-, and green-sensitive rhodopsin-encoding mRNAs in the retina of *Vanessa Cardui*. *Journal of Comparative Neurology,* **458**(4), 334–349.

Briscoe, A.D., & Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology,* **46**(1), 471–510.

Briscoe, A.D., Bybee, S.M., Bernard, G.D., Yuan, F., Sison-Mangus, M.P., Reed, R.D., Warren, A.D., Llorente-Bousquets, J., & Chiao, C.C. (2010). Positive selection of a duplicated uv-sensitive visual pigment coincides with wing pigment evolution in *Heliconius* butterflies. *Proceedings of the National Academy of Sciences of the United States of America,* **107**(8), 3628–3633.

Brooks, R., & Couldridge, V. (1999). Multiple sexual ornaments coevolve with multiple mating preferences. *American Naturalist,* **154**(1), 37–45.

Brower, L.P., Ryerson, W.N., Coppinger, L.L., & Glazier, S.C. (1968). Ecological chemistry and the palatability spectrum. *Science,* **161**(3848), 1349–1350.

Brown, W.L., & Wilson, E.O. (1956). Character displacement. *Systematic Zoology,* **5**(2), 49–64.

Brues, C.T. (1941). Photographic evidence on the visibility of color patterns in butterflies to the human and insect eye. *Proceedings of the American Academy of Arts and Sciences,* **74**(8), 281–286.

Brunton, C. (1998). The evolution of ultraviolet patterns in European *Colias* butterflies (Lepidoptera, Pieridae): A phylogeny using mitochondrial DNA. *Heredity,* **80**(5), 611–616.

Brunton, C.F., & Hurst, G.D.D. (1998). Mitochondrial DNA phylogeny of brimstone butterflies (genus *Gonepteryx*) from the Canary Islands and Madeira. *Biological Journal of The Linnean Society,* **63**(1), 69–79.

Brunton, C., & Majerus, M.N. (1995). Ultraviolet colours in butterflies: Intra-or inter-specific communication? *Proceedings of The Royal Society of London. Series B: Biological Sciences,* **260**(1358), 199–204.

Burkhardt, D. (1989). UV vision: A bird's eye view of feathers. *Journal of Comparative Physiology A,* **164**(6), 787–796.

Burkhardt, D. (1982). Birds, berries and UV. *Naturwissenschaften,* **69**(4), 153–157.

Burns, K.J., & Shultz, A.J. (2012). Widespread cryptic dichromatism and ultraviolet reflectance in the largest radiation of neotropical songbirds: Implications of accounting for avian vision in the study of plumage evolution. *Auk,* **129**(2), 211–221.

Bybee, S.M., Yuan, F., Ramstetter, M.D., Llorente-Bousquets, J., Reed, R.D., Osorio, D., & Briscoe, A.D. (2011). UV photoreceptors and UV-yellow wing pigments in *Heliconius* butterflies allow a color signal to serve both mimicry and intraspecific communication. *American Naturalist,* **179**(1), 38–51.

Carlson, S.D., & Chi, C. (1979). The Functional morphology of the insect photoreceptor. *Annual Review of Entomology,* **24**(1), 379–416.

Carney, C.L., Antonson, N., Alvarez, J., Foss, K., Kettler, K., Lloyd, A.J., Matzner, D., Mohan, D., Shroll, K., & Snyder, C. (2019). Fatal attraction: What is the role of visual cues in attracting prey to carnivorous plants? *Bios,* **90**(2), 79–86.

Caro, T., & Mallarino, R. (2020). Coloration in mammals. *Trends in Ecology and Evolution,* **35**(4), 357–366.

Catalán, A., Macias-Munoz, A., & Briscoe, A.D. (2018). Evolution of sex-biased gene expression and dosage compensation in the eye and brain of *Heliconius* butterflies. *Molecular Biology and Evolution,* **35**(9), 2120–2134.

Chadyšiene, R., & Girgždys, A. (2008). Ultraviolet radiation albedo of natural surfaces. *Journal of Environmental Engineering and Landscape Management,* **16**(2), 83–88.

Chen, Z., Xu, L., Li, L., Wu, H., & Xu, Y. (2019). Effects of constant and fluctuating temperature on the development of the oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae). *Bulletin of Entomological Research,* **109**(2), 212–220.

Church, S.C., Bennett, A.T.D., Cuthill, I.C., Hunt, S., Hart, N.S., & Partridge, J.C. (1998). Does lepidopteran larval crypsis extend into the ultraviolet? *Naturwissenschaften,* **85**(4), 189–192.

Church, S.C., Bennett, A.T.D., Cuthill, I.C., & Partridge, J.C. (1998). Ultraviolet cues affect the foraging behaviour of blue tits. *Proceedings of the Royal Society of London B: Biological Sciences,* **265**(1405), 1509–1514.

Church, S.C, Merrison, A.S.L., & Chamberlain, T.M.M. (2001). Avian ultraviolet vision and frequency-dependent seed preferences. *Journal of Experimental Biology,* **204**(14), 2491–2498.

Conlon, V.M. (1971). Some standard photographic procedures suitable for archaeological documentation. *Medical and Biological Illustration,* **21**(2), 60–65.

Costanzo, K., & Monteiro, A. (2007). The use of chemical and visual cues in female choice in the butterfly *Bicyclus anynana. Proceedings of the Royal Society B: Biological Sciences,* **274**(1611), 845–851.

Coutsis, J.G. (1996). Ultra-violet reflection pattern in *Polyommatus andronicus* Coutsis & Chávalas, 1995 and *Polyommatus icarus* (Rottenburg, 1775) (Lepidoptera: Lycaenidae). *Phegea,* **24**(99), 167–169.

Crane, J. (1954). Spectral reflectance characteristics of butterflies (Lepidoptera) from Trinidad, B.W.I.. *Zoologica,* **39**(8), 85–115.

Crane, J. (1955). Imaginal behavior of a Trinidad butterfly, *Heliconius erato hydara* Heiwitson, with special reference to the social use of color. *Zoologica,* **40**(16), 167–196.

Cronin, J.F., Rooney, T.P., Williams Jr, R.S., Molineux, C.E., & Bliamptis, E.E. (1968). *Ultraviolet Radiation and the Terrestrial Surface.* Special Reports no. 83. Bedford, Massachusetts: Airforce Cambridge Research.

Cronin, T.W., & Bok, M.J. (2016). Photoreception and vision in the ultraviolet. *Journal of Experimental Biology,* **219**(18), 2790–2801.

Crowther, J.M. (2019). *UV reflectance photography of skin: What are you imaging?* https://onlinelibrary.wiley.com/doi/abs/10.1111/ics.12591

Cummings, M.E., Rosenthal, G.G., & Ryan, M.J. (2003). A private ultraviolet channel in visual communication. *Proceedings of the Royal Society B: Biological Sciences,* **270**(1518), 897–904.

Cuthill, I.C., Bennett, A.T.D., P.Artridge, J.C., & Maier, E.J. (1999). Plumage reflectance and the objective assessment of avian sexual dichromatism. *American Naturalist,* **153**(2), 183–200.

Cuthill, I.C., Partridge, J.C., Bennett, A.T.D., Church, S.C., Hart, N.S., & Hunt, S. (2000). Ultraviolet vision in birds. *Advances in the Study of Behavior,* **29**(2), 159–214.

Dalrymple, R.L., Flores-Moreno, H., Kemp, D.J., White, T.E., Laffan, S.W., Hemmings, F.A., Hitchcock, T.D., & Moles, A.T. (2018). Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. *Ecological Monographs,* **88**(2), 204–224.

Dalrymple, R.L., Kemp, D.J., Flores-Moreno, H., Laffan, S.W., White, T.E., Hemmings, F.A., Tindall, M.L., & Moles, A.T. (2015). Birds, butterflies and flowers in the tropics are not more colourful than those at higher latitudes. *Global Ecology and Biogeography,* **24**(12), 1424–1432.

Daumer, K. (1958). Blumenfarben, wie sie die Bienen sehen. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, And Behavioral Physiology,* **41**(1), 49–110.

De Bruin, J.P. (1961). Principles of ultraviolet light and some of its applications in photography. *Journal of the Biological Photographic Association,* **29**, 53–63.

Del Giudice, E., De Ninno, A., Fleischmann, M., Mengoli, G., Milani, M., Talpo, G., & Vitiello, G. (2005). Coherent quantum electrodynamics in living matter. *Electromagnetic Biology and Medicine,* **24**(3), 199–210.

Delhey, K., Peters, A., Biedermann, P.H.W., & Kempenaers, B. (2008). Optical properties of the uropygial gland secretion: No evidence for UV cosmetics in birds. *Naturwissenschaften,* **95**(10), 939–946.

Delhey, K., Peters, A., Johnsen, A. & Kempenaers, B. (2006). Fertilization success and UV ornamentation in blue tits *Cyanistes caeruleus*: Correlational and experimental evidence. *Behavioral Ecology,* **18**(2), 399–409.

Dell'aglio, D.D. (2016). *Behavioural and Ecological Interactions Between* Heliconius *Butterflies, Their Predators And Host Plants*, Ph.D. thesis, University of Cambridge.

Dell'aglio, D.D., Troscianko, J., Mcmillan, W.O., Stevens, M. & Jiggins, C.D. (2018). The appearance of mimetic *Heliconius* butterflies to predators and conspecifics. *Evolution,* **72**(10), 2156–2166.

Denk, W., & Horstmann, H. (2004). Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *Plos Biology,* **2**(11), E329.

Devries, P.J., Penz, C.M., & Hill, R.I., (2010). Vertical distribution, flight behaviour and evolution of wing morphology in *Morpho* butterflies. *Journal of Animal Ecology,* **79**(5), 1077–1085.

Dong, C.M., Mclean, C.A., Moussalli, A., & Stuart-Fox, D. (2019). Conserved visual sensitivities across divergent lizard lineages that differ in an ultraviolet sexual signal. *Ecology and Evolution,* **9**(20): 11824–11832.

Doucet, S.M., & Montgomerie, R. (2003). Multiple sexual ornaments in satin bowerbirds: Ultraviolet plumage and bowers signal different aspects of male quality. *Behavioral Ecology,* **14**(4), 503–509.

Douglas, R.H., & Jeffery, G. (2014). The spectral transmission of ocular media suggests ultraviolet sensitivity is widespread among mammals. *Proceedings of the Royal Society B: Biological Sciences*, **281**(1780), 20132995.

Dugas, M.B. (2009). House sparrow, *Passer domesticus,* parents preferentially feed nestlings with mouth colours that appear carotenoid-rich. *Animal Behaviour,* **78**(3), 767–772.

Dushkina, N., Erten, S., & Lakhtakia, A. (2017). Coloration and structure of the wings of *Chorinea sylphina* Bates. *Journal of the Lepidopterists' Society,* **71**(1), 1–11.

Dyer, A.G. (1996). Reflection of near-ultraviolet radiation from flowers of Australian native plants. *Australian Journal of Botany,* **44**(4), 473–488.

Eastman Kodak Company (1972). *Publication M-27. Ultraviolet and Fluorescence Photography.* 1. Rochester: Eastman Kodak Company.

Eastman Kodak Company (1948). *Publication M-3. Infrared and Ultraviolet Photography.* 1. Rochester: Eastman Kodak Company.

Eaton, M.D. (2005). Human Vision fails to distinguish widespread sexual dichromatism among sexually "monochromatic" birds. *Proceedings of the National Academy of Sciences of the United States of America,* **102**(31), 10942–10946.

Eguchi, E., & Horikoshi, T. (1984). Comparison of stimulus-response (V-log I) functions in five types of lepidopteran compound eyes (46 species). *Journal of Comparative Physiology A,* **154**(1), 3–12.

Eguchi, E., & Meyer-Rochow, V.B. (1983). Ultraviolet photography of forty-three species of lepidoptera representing ten families. *Annotationes zoologicae Japonenses,* **56**(1), 10–18.

Eguchi, E., Watanabe, K., Hariyama, T., & Yamamoto, K. (1982). A comparison of electrophysiologically determined spectral responses in 35 species of lepidoptera. *Journal of Insect Physiology,* **28**(8), 675–682.

Eisner, T., Silberglied, R.E., Aneshansley, D., Carrel, J.E., & Howland, H.C. (1969). Ultraviolet video-viewing: The television camera as an insect eye. *Science,* **166**(3909), 1172–1174.

Eliseev, A.A., Ippolitov, I.I., Kabanov, M.V., Poddubnyi, V.V., Ravodina, O.V., Teodorovich, Z.S., & Shchipunov, A.A. (1998). Experimental investigation of variability of ultraviolet fluxes at the Earth's surface. *Russian Physics Journal,* **41**(9), 922–926.

Ellers, J., & Boggs, C.L. (2004). Evolutionary genetics of dorsal wing colour in *Colias* butterflies. *Journal of Evolutionary Biology,* **17**(4), 752–758.

Endler, J.A. (1992). Signals, signal conditions, and the direction of evolution. *American Naturalist,* **139,** S125–S153.

Endler, J.A. (1991). Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research,* **31**(3), 587–608.

Enquist, M., & Arak, A. (1993). Selection of exaggerated male traits by female aesthetic senses. *Nature,* **361**(6411), 446–448.

Ferris, C.D. (1975). A note on films and ultraviolet photography. *News of the Lepidopterists' Society,* **6,** 6–7.

Ferris, C.D. (1972). Ultraviolet photography as an adjunct to taxonomy. *News of the Lepidopterists' Society,* **26**(1), 210–215.

Ferris, C.D. (1973). A revision of the *Colias alexandra* complex (Pieridae) aided by ultraviolet reflectance photography with designation of a new subspecies. *Journal of the Lepidopterists' Society,* **27**(1), 57–73.

Feynman, R.P. (2006). *QED: The Strange Theory of Light and Matter.* Princeton University Press.

Fitzka, M., Simic, S., & Hadzimustafic, J. (2012). Trends in spectral UV radiation from long-term measurements at Hoher Sonnblick, Austria. *Theoretical and Applied Climatology,* **110**(4), 585–593.

Fitzpatrick, S. (1998). Colour schemes for birds: Structural coloration and signals of quality in feathers. *Annales Zoologici Fennici,* **35,** 67–77.

Fleishman, L.J., Loew, E.R., & Leal, M. (1993). Ultraviolet vision in lizards. *Nature,* **365**(6445), 397–397.

Font, E., & Molina-Borja, M. (2004). Ultraviolet reflectance of color patches in *Gallotia galloti* from Tenerife, Canary Islands. *The Biology of Lacertid Lizards. Institut Menorquí D'estudis, Menorca, Recerca* 8: 201–221.

Font, E., Pérez I De Lanuza, G., & Sampedro, C. (2009). Ultraviolet reflectance and cryptic sexual dichromatism in the ocellated lizard, Lacerta (Timon) Lepida (Squamata: Lacertidae). *Biological Journal of the Linnean Society,* **97**(4), 766–780.

Franke, K., Chagas, A.M., Zhao, Z., Zimmermann, M.J.Y., Qiu, Y., Szatko, K., Baden, T., & Euler, T. (2019). *An Arbitrary-Spectrum Spatial Visual Stimulator for Vision Research.* DOI: 10.1101/649566 (preprint).

Friberg, M., N. Vongvanich, A. Borg-Karlson, D. J. Kemp, S. Merilaita, & C. Wiklund. (2008). Female mate choice determines reproductive isolation between sympatric butterflies. *Behavioral Ecology and Sociobiology* **62**(6), 873-886.

Frisch, K.V. (1924). *Sinnesphysiologie und 'Sprache' der Bienen.* Berlin: Springer.

Fukano, Y., Satoh, T., Hirota, T., Nishide, Y., & Obara, Y. (2012). Geographic expansion of the cabbage butterfly (*Pieris rapae*) and the evolution of highly UV-reflecting females. *Insect Science,* **19**(2), 239–246.

Futahashi, R., Yamahama, Y., Kawaguchi, M., Mori, N., Ishii, D., Okude, G., Hirai, Y., Kawahara-Miki, R., Yoshitake, K., & Yajima, S. (2019). Molecular basis of wax-based color change and UV reflection in dragonflies. *Elife,* **8,** E43045.

Galarza, J.A., Dhaygude, K., Ghaedi, B., Suisto, K., Valkonen, J., & Mappes, J. (2019). Evaluating responses to temperature during pre-metamorphosis and carry-over effects at post-metamorphosis in the wood tiger moth (*Arctia plantaginis*). *Philosophical Transactions of the Royal Society B,* **374**(1783), 20190295.

Gamero, A., Senar, J.C., Hohtola, E., Nilsson, J., & Broggi, J. (2015). Population differences in the structure and coloration of great tit contour feathers. *Biological Journal of the Linnean Society,* **114**(1), 82–91.

Garcia, J.E., Rohr, D., & Dyer, A.G. (2013). Trade-off between camouflage and sexual dimorphism revealed by UV digital imaging: The case of Australian mallee dragons (*Ctenophorus fordi*). *Journal of Experimental Biology,* **216**(Pt 22), 4290–4298.

Gates, D.M., Keegan, H.J., Schleter, J.C., & Weidner, V.R. (1965). Spectral properties of plants. *Applied Optics,* **4**(1), 11–20.

Gaunet, A., Dincă, V., Dapporto, L., Montagud, S., Vodă, R., Schär, S., Badiane, A., Font, E., & Vila, R. (2019). Two consecutive Wolbachia-mediated mitochondrial introgressions obscure taxonomy in palearctic swallowtail butterflies (Lepidoptera, Papilionidae). *Zoologica Scripta*. DOI: 10.1111/zsc.12355

Ghiradella, H. (1989). Structure and development of iridescent butterfly scales: Lattices and laminae. *Journal of Morphology,* **202**(1), 69–88.

Ghiradella, H., Aneshansley, D., Eisner, T., Silberglied, R.E., & Hinton, H.E. (1972). Ultraviolet reflection of a male butterfly: Interference color caused by thin-layer elaboration of wing scales. *Science,* **178**(4066), 1214–1217.

Ghiradellia, H. (2010). Insect cuticular surface modifications: Scales and other structural formations. *Advances in Insect Physiology,* 38: 135–180.

Giraldo, M., & Stavenga, D. (2007). Sexual dichroism and pigment localization in the wing scales of *Pieris rapae* butterflies. *Proceedings of the Royal Society B: Biological Sciences,* **274**(1606), 97–102.

Gloger, C.W.L. (1833). *Das Abändern der Vögel durch Einfluss des Klima's...,* Breslau: Schulz.

Glover, B.J., & Whitney, H.M. (2010). Structural colour and iridescence in plants: The poorly studied relations of pigment colour. *Annals of Botany,* **105**(4), 505–511.

Govardovskii, V.I., & Zueva, L.V. (1974). Spectral sensitivity of the frog eye in the ultraviolet and visible region. *Vision Research,* **14**(12), 1317–1321.

Graham, S.M., Watt, W.B., & Gall, L.F. (1980). Metabolic resource allocation vs. mating attractiveness: Adaptive pressures on the "alba" polymorphism of Colias butterflies. *Proceedings of the National Academy of Sciences of the United States of America,* 77(6), 3615–3619.

Grether, G.F., Kolluru, G.R., & Nersissian, K. (2004). Individual colour patches as multicomponent signals. *Biological Reviews,* **79**(3), 583–610.

Grether, G.F., Hudon, J., & Endler, J.A. (2001). Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual coloration in guppies (*Poecilia reticulata*). *Proceedings of the Royal Society B: Biological Sciences,* **268**(1473), 1245–1253.

Griffith, S.C., Parker, T.H., & Olson, V.A. (2006). Melanin-versus carotenoid-based sexual signals: Is the difference really so black and red? *Animal Behaviour,* **71**(4), 749–763.

Häder, D.P., Kumar, H.D., Smith, R.C., & Worrest, R.C. (2007). Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical and Photobiological Sciences,* **6**(3), 267–285.

Haigh, J.D. (2007). The Sun and the Earth's climate. *Living Reviews in Solar Physics,* **4**(1), 2.

Hamilton, W.D., & Zuk, M. (1982). Heritable true fitness and bright birds: A role for parasites? *Science,* **218**(4570), 384–387.

Hankison, S.J., & Palmer, M.S. (2016). Assays to detect UV-reflecting structures and determine their importance in mate preference using the sailfin molly *Poecilia latipinna*. *Journal of Visualized Experiments,* **14**(115), 1–5.

Hanzalová, D., Pecháček, P., Bartoňová, A., Marešová, J., Rindoš, M., Kleisner, K., & Faltýnek Fric, Z. (2020). Co-evolution of UV reflectance patterns and biogeography of brimstone butterflies. Under Review

Harcourt, B.F. (1996). Ultraviolet lights for reptiles. *Veterinary Record,* **138**(21), 528–528.

Hastad, O., Victorsson, J., & Odeen, A. (2005). Differences in color vision make passerines less conspicuous in the eyes of their predators. *Proceedings of the National Academy of Sciences of the United States of America,* **102**(18), 6391–6394.

Håstad, O., & Ödeen, A. (2008). Different ranking of avian colors predicted by modeling of retinal function in humans and birds. *American Naturalist,* **171**(6), 831–838.

Hausmann, F., Arnold, K.E., Marshall, N.J., & Owens, I.P. (2003). Ultraviolet signals in birds are special. *Proceedings of the Royal Society B: Biological Sciences,* **270**(1510), 61–67.

Hawryshyn, C.W. (2003). Mechanisms of ultraviolet polarization vision in fishes. In: S.P. Collin, & N.J. Marshall, Eds., *Sensory Processing in Aquatic Environments.* Zurich: Springer, 252–265.

Hazel, W.N. (1990). Sex-limited variability mimicry in the swallowtail butterfly *Papilio polyxenes* Fabr. *Heredity,* **65**(1), 109–114.

Heidrich, L., Friess, N., Fiedler, K., Brändle, M., Hausmann, A., Brandl, R., & Zeuss, D. (2018). The dark side of Lepidoptera: Colour lightness of geometrid moths decreases with increasing latitude. *Global Ecology and Biogeography,* **27**(4), 407–416.

Heiling, A.M., Chittka, L., Cheng, K., & Herberstein, M.E. (2005). Colouration in crab spiders: Substrate choice and prey attraction. *Journal of Experimental Biology,* **208**(10), 1785–1792.

Hill, R.J. (1977). Ultraviolet reflectance-absorbance photography: An easy, inexpensive research tool. *Brittonia,* **29**(4), 382–390.

Hirota, S.K., Miki, N., Yasumoto, A.A., & Yahara, T. (2019). UV bullseye contrast of *Hemerocallis* flowers attracts hawkmoths but not swallowtail butterflies. *Ecology and Evolution,* **9**(1), 52–64.

Hobson, A. (2013). There are no particles, there are only fields. *American Journal of Physics,* **81**(3), 211–223.

Hockberger, P. (2002). a history of ultraviolet photobiology for humans, animals and microorganisms. *Photochemistry and Photobiology,* **76**(6), 561–579.

Hogg, C., Neveu, M., Folkow, L., Stokkan, K.A., Hoh Kam, J., Douglas, R.H., & Jeffery, G. (2015). The eyes of the deep diving hooded seal (*Cystophora cristata*) enhance sensitivity to ultraviolet light. *Biology Open,* **4**(7), 812–818.

Hogg, C., Neveu, M., Stokkan, K.A., Folkow, L., Cottrill, P., Douglas, R., Hunt, D.M., & Jeffery, G. (2011). Arctic reindeer extend their visual range into the ultraviolet. *Journal of Experimental Biology,* **214**(12), 2014–2019.

Honkavaara, J., Koivula, M., Korpimäki, E., Siitari, H., & Viitala, J. (2002). Ultraviolet vision and foraging in terrestrial vertebrates. *Oikos,* **98**(3), 505–511.

Hovanitz, W. (1944). The ecological significance of the color phases of *Colias chrysotheme* in North America. *Ecology,* **25**(1), 45–60.

Hsiung, B., Shawkey, M.D., & Blackledge, T.A. (2019). Color production mechanisms in spiders. *Journal of Arachnology,* **47**(2), 165–180.

Hunt, S., Cuthill, I.C., Bennett, A.T., Church, S.C., & Partridge, J.C. (2001). Is the ultraviolet waveband a special communication channel in avian mate choice? *Journal of Experimental Biology,* **204**(14), 2499–2507.

Hunt, S., Bennett, A.T.D., Cuthill, I.C., & Griffiths, R. (1998). Blue tits are ultraviolet tits. *Proceedings of the Royal Society of London B: Biological Sciences,* **265**(1395), 451–455.

Huq, M., Bhardwaj, S., & Monteiro, A. (2019). Male *Bicyclus anynana* butterflies choose females on the basis of their ventral UV-reflective eyespot centers. *Journal of Insect Science,* **19**(1), 1–25.

Huth, H., & Burkhardt, D. (1972). Der spektrale Sehbereich eines Violettohr-Kolibris. *Naturwissenschaften,* **59**(12), 650–650.

Huxley, J. (1975). The basis of structural colour variation in two species of *Papilio*. *Journal of Entomology Series A, General Entomology,* **50**(1), 9–22.

Imafuku, M. (2008). Variation in UV light reflected from the wings of *Favonius* and *Quercusia* butterflies. *Entomological Science,* **11**(1), 75–80.

Imafuku, M., Hirose, Y., & Takeuchi, T. (2002). Wing colors of *Chrysozephyrus butterflies* (Lepidoptera; Lycaenidae): Ultraviolet reflection by males. *Zoological Science,* **19**(2), 175–183.

Iwasa, Y., & Pomiankowski, A. (1995). Continual change in mate preferences. *Nature,* **377**(6548), 420–422.

Jacobs, G.H., Fenwick, J.A., & Williams, G.A. (2001). Cone-based vision of rats for ultraviolet and visible lights. *Journal of Experimental Biology,* **204**(Pt 14), 2439–2446.

Jacobs, G.H., Neitz, J., & Deegan II., J.F. (1991). Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature,* **353**(6345), 655.

Jacobs, G.H. (1992). Ultraviolet vision in vertebrates. *American Zoologist,* **32**(4), 544–554.

Johnsen, S. (2012). *The Optics of Life: A Biologist's Guide to Light in Nature.* Princeton: Princeton University Press.

Johnstone, R.A. (1995). Sexual selection, honest advertisement and the handicap principle: Reviewing the evidence. *Biological Reviews,* **70**(1), 1–65.

Johnstone, R.A., Reynolds, J.D., & Deutsch, J.C. (1996). Mutual mate choice and sex differences in choosiness. *Evolution,* **50**(4), 1382–1391.

Jouventin, P., Nolan, P.M., Örnborg, J., & Dobson, F.S. (2005). Ultraviolet beak spots in king and emperor penguins. *Condor,* **107**(1), 144–150.

Kelber, A., & Osorio, D. (2010). From spectral information to animal colour vision: Experiments and concepts. *Proceedings of The Royal Society B: Biological Sciences,* **277**(1688), 1617–1625.

Kelber, A., & Somanathan, H. (2019). Spatial vision and visually guided behavior in Apidae. *Insects,* **10**(12), 418–435.

Kemp, D.J. (2007a). Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). *Behavioral Ecology,* **19**(1), 1–8.

Kemp, D.J. (2006). Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. *Evolutionary Ecology Research,* **8**(3), 515–527.

Kemp, D.J. (2008). Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. *Evolution: International Journal of Organic Evolution,* **62**(9), 2346–2358.

Kemp, D.J., & Macedonia, J.M. (2006). Structural ultraviolet ornamentation in the butterfly *Hypolimnas bolina* l. (Nymphalidae): Visual, morphological and ecological properties. *Australian Journal of Zoology,* **54**(4), 235–244.

Kemp, D.J., & Rutowski, R.L. (2011). The role of coloration in mate choice and sexual interactions in butterflies. *Advances in the Study of Behavior,* **43**(5), 55–92.

Kemp, D.J., & Rutowski, R.L. (2007). Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. *Evolution,* **61**(1), 168–183.

Kemp, D.J. (2007b). Female butterflies prefer males bearing bright iridescent ornamentation. *Proceedings of the Royal Society B: Biological Sciences,* **274**(1613), 1043–1047.

Kertész, K., Piszter, G., Bálint, Z., & Biró, L.P. (2019). Biogeographical patterns in the structural blue of male *Polyommatus icarus* butterflies. *Scientific Reports,* **9**(1), 1–9.

Kettlewell, H.B.D. (1965). Insect survival and selection for pattern. *Science,* **148**(3675), 1290–1296.

Kevan, P.G., Grainger, N.D., Mulligan, G.A., & Robertson, A.R. (1973). A gray-scale for measuring reflectance and color in the insect and human visual spectra. *Ecology,* **54**(4), 924–926.

Kjernsmo, K., Whitney, H.M., Scott-Samuel, N.E., Hall, J.R., Knowles, H., Talas, L., & Cuthill, I.C. (2020). Iridescence as camouflage. *Current Biology,* **30**(1), 1–5.

Klomberg, Y., Kouede, R.D., Bartoš, M., Mertens, J.E.J., Tropek, R., Fokam, E.B., & Janeček, Š. (2019). The role of ultraviolet reflectance and pattern in the pollination system of *Hypoxis camerooniana* (Hypoxidaceae). *AoB Plants,* **11**(5), 1–11.

Knüttel, H., & Fiedler, K. (2000). On the use of ultraviolet photography and ultraviolet wing patterns in butterfly morphology and taxonomy. *Journal of the Lepidopterists' Society,* **54**(4), 137–144.

Knuttel, H., & Fiedler, K. (2001). Host-plant-derived variation in ultraviolet wing patterns influences mate selection by male butterflies. *Journal of Experimental Biology,* **204**(Pt 14), 2447–2459.

Kodric-Brown, A., & Johnson, S.C. (2002). Ultraviolet reflectance patterns of male guppies enhance their attractiveness to females. *Animal Behaviour,* **63**(2), 391–396.

Kohler, A.M., Olson, E.R., Martin, J.G., & Anich, P.S. (2019). Ultraviolet fluorescence discovered in New World flying squirrels (*Glaucomys*). *Journal of Mammalogy,* **100**(1), 21–30.

Koivula, M., Korpimaki, E., & Viitala, J. (1997). Do Tengmalm's owls see vole scent marks visible in ultraviolet light? *Animal Behaviour,* **54**(4), 873–877.

Koivula, M., & Viitala, J. (1999). Rough-legged buzzards use vole scent marks to assess hunting areas. *Journal of Avian Biology,* **30**(3), 329–332.

Komárek, S. (1998). *Mimicry, Aposematism and Related Phenomena*. London: Coronet Books Inc.

Koski, M.H., & Ashman, T. (2016). Macroevolutionary patterns of ultraviolet floral pigmentation explained by geography and associated bioclimatic factors. *New Phytologist,* **211**(2), 708–718.

Koski, M.H., & Ashman, T. (2015). Floral pigmentation patterns provide an example of Gloger's rule in plants. *Nature Plants,* **1**(1), 14007.

Kral, K. (2016). Implications of insect responses to supernormal visual releasing stimuli in intersexual communication and flower-visiting behaviour: A review. *European Journal of Entomology,* **113**, 429–437.

Krishna, A., Nie, X., Warren, A.D., Llorente-Bousquets, J.E., Briscoe, A.D., & Lee, J. (2020). Infrared optical and thermal properties of microstructures in butterfly wings. *Proceedings of the National Academy of Sciences of the United States Of America,* **117**(3), 1566–1572.

Kühn, A. (1924). Zum Nachweis des Farbenunterscheidungsvermögens der Bienen. *Naturwissenschaften,* **12**(6), 116–118.

Kumazawa, K., Tanaka, S., Negita, K., & Tabata, H. (1994). Fluorescence from wing of *Morpho sulkowskyi* butterfly. *Japanese Journal of Applied Physics,* **33**(4), 2119–2122.

Kurup, R., Johnson, A.J., Sankar, S., Hussain, A.A., Kumar, C.S., & Sabulal, B. (2013). Fluorescent prey traps in carnivorous plants. *Plant Biology,* **15**(3), 611–615.

Lande, R. (1987). Genetic correlations between the sexes in the evolution of sexual dimorphism and mating preferences. In: W. Bradbury J., & B. Andersson M., Eds, *Sexual Selection: Testing the Alternatives.* Chichester, UK: Wiley, 83–94.

Lavigne, D.M. (1976). Counting harp seals with ultra-violet photography. *Polar Record,* **18**(114), 269–277.

Lavigne, D.M., & Øritsland, N.A. (1974). Ultraviolet photography: A new application for remote sensing of mammals. *Canadian Journal of Zoology,* **52**(7), 939–941.

Le Roy, C., Debat, V., & Llaurens, V. (2019). Adaptive evolution of butterfly wing shape: From morphology to behaviour. *Biological Reviews,* **94**(4), 1261–1281.

Lebas, N.R., & Marshall, N.J. (2001). No evidence of female choice for a condition-dependent trait in the agamid lizard, *Ctenophorus ornatus*. *Behaviour,* **138**(8), 965–980.

Leblanc, G., Francis, C.M., Soffer, R., Kalacska, M., & De Gea, J. (2016). Spectral reflectance of polar bear and other large arctic mammal pelts: Potential applications to remote sensing surveys. *Remote Sensing,* **8**(4), 273.

Leech, D.M., & Johnsen, S. (2006). Ultraviolet vision and foraging in juvenile bluegill (*Lepomis macrochirus*). *Canadian Journal of Fisheries and Aquatic Sciences,* **63**(10), 2183–2190.

Liley, J.B., & Mckenzie, R.L. (2006). *Where on Earth Has the Highest UV.* Lauder, New Zealand: National Institute of Water and Atmospheric Research.

Lim, M.L., Land, M.F., & Li, D. (2007). Sex-specific UV and fluorescence signals in jumping spiders. *Science,* **315**(5811), 481.

Lindsay, W.R., Andersson, S., Bererhi, B., Höglund, J., Johnsen, A., Kvarnemo, C., Leder, E.H., Lifjeld, J.T., Ninnes, C.E., & Olsson, M. (2019). Endless forms of sexual selection. https://Peerj.Com/Preprints/27584/

Liu, M., Siefferman, L., & Hill, G.E. (2007). an experimental test of female choice relative to male structural coloration in eastern bluebirds. *Behavioral Ecology and Sociobiology,* **61**(4), 623–630.

Losey, G.S., Cronin, T.W., Goldsmith, T.H., Hyde, D., Marshall, N.J., & McFarland, W.N. (1999). The UV visual world of fishes: A review. *Journal of Fish Biology,* **54**(5), 921–943.

Lubbock, J.S. (1883). *Ants, Bees, And Wasps: a Record of Observations on the Habits of the Social Hymenoptera.* 6th edition. London: Kegan Paul.

Luner, S.J. (1968). Ultraviolet photography for visualization of separation patterns of zone electrophoresis and other methods. *Analytical Biochemistry,* **23**(2), 357–358.

Lutz, F.E. (1933). "Invisible" colors of flowers and butterflies. *Natural History,* **33**(1), 565–567.

Lutz, F.E. (1924). Apparently non-selective characters and combinations of characters, including a study of ultraviolet in relation to the flower-visiting habits of insects. *Annals of The New York Academy of Sciences,* **29**(1), 181–283.

Lynch, D.K., Livingston, W.C., & Livingston, W. (2001). *Color and Light in Nature.* 2nd edition. Cambridge, UK: Cambridge University Press.

Lythgoe, J.N. (1979). *Ecology of Vision.* California: Clarendon Press.

Lyytinen, A., Alatalo, R.V., Lindström, L., & Mappes, J. (2001). Can ultraviolet cues function as aposematic signals? *Behavioral Ecology,* **12**(1), 65–70.

Lyytinen, A., Alatalo, R.V., Lindström, L., & Mappes, J. (1999). Are European white butterflies aposematic? *Evolutionary Ecology,* **13**(7–8), 709–719.

Lyytinen, A., Lindström, L., & Mappes, J. (2004). Ultraviolet reflection and predation risk in diurnal and nocturnal lepidoptera. *Behavioral Ecology,* **15**(6), 982–987.

Macedonia, J.M. (2001). Habitat light, colour variation, and ultraviolet reflectance in the grand Cayman anole, *Anolis conspersus. Biological Journal of the Linnean Society,* **73**(3), 299–320.

Macedonia, J.M., Brandt, Y., & Clark, D.L. (2002). Sexual dichromatism and differential conspicuousness in two populations of the common collared lizard (*Crotaphytus collaris*) from Utah and New Mexico, USA. *Biological Journal of the Linnean Society,* **77**(1), 67–85.

Maddocks, S.A., Church, S.C., & Cuthill, I.C. (2001). The effects of the light environment on prey choice by zebra finches. *Journal of Experimental Biology,* **204**(Pt 14), 2509–2515.

Madronich, S., McKenzie, R.L., Björn, L.O., & Caldwell, M.M. (1998). Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology B: Biology,* **46**(1–3), 5–19.

Mainster, M.A. (2006). Violet and blue light blocking intraocular lenses: Photoprotection versus photoreception. *British Journal of Ophthalmology,* **90**(6), 784–792.

Majerus, M.E.N., Brunton, C.F.A., & Stalker, J. (2000). A bird's eye view of the peppered moth. *Journal of Evolutionary Biology,* **13**(2), 155–159.

Makino, K., Satoh, K., Koike, M., & Ueno, N. (1952). Sex in *Pieris rapae* L. and the pteridin content of their wings. *Nature,* **170**, 32–55.

Marshall, J., & Johnsen, S. (2017). Fluorescence as a means of colour signal enhancement. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **372**(1724), 20160335.

Marshall, N.J. (1996). Measuring colours around a coral reef. *Biophotonics International,* **3**(2), 52–56.

Martin, M., Badiane, A., & Le Galliard, J. (2019). The relative importance of body size and UV coloration in influencing male-male competition in a lacertid lizard. *Behavioral Ecology and Sociobiology,* **73**(7), 98.

Mazokhin-Porshnyakov, G.A. (1957). The reflecting qualities of the wings of butterflies and the role of ultravio-let rays in visual perception of insects. *Biofizika,* **2**, 358–368.

Mazokhin-Porshnyakov, G.A. (1954). Ultraviolet radiation of the sun as a factor in insect habitats. [in Russian]. *Zhurnal Obshchei Biologii,* **15**, 362–367.

Mazokhin-Porshnyakov, G.A., 1969. *Insect Vision.* New York: Plenum Press.

Mazza, C.A., Izaguirre, M.M., Curiale, J., & Ballaré, C.L. (2009). A Look into the invisible: Ultraviolet-B sensitivity in an insect (*Caliothrips phaseoli*) revealed through a behavioural action spectrum. *Proceedings of the Royal Society B: Biological Sciences,* **277**(1680), 367–373.

Mcgraw, K.J., & Hill, G., E. (2006). Mechanics of carotenoid-based coloration. *Bird Coloration,* **1**, 177–242.

Menzel, R., & Backhaus, W. (1989). Color vision honeybees: Phenomena and physiological mechanisms. In: D.G. Stavenga, & R.C. Hardie, Eds. *Facets of Vision.* London: Springer, 281–297.

Merrill, R.M., Dasmahapatra, K.K., Davey, J.W., Dell'aglio, D.D., Hanly, J.J., Huber, B., Jiggins, C.D., Joron, M., Kozak, K.M., & Llaurens, V. (2015). The diversification of *Heliconius* butterflies: What have we learned in 150 years? *Journal of Evolutionary Biology,* **28**(8), 1417–1438.

Merry, J.W., Morehouse, N.I., Yturralde, K., & Rutowski, R.L. (2006). The eyes of a patrolling butterfly: Visual field and eye structure in the orange sulphur, *Colias Eurytheme* (Lepdoptera, Pieridae). *Journal of Insect Physiology,* **52**(3), 240–248.

Meyer-Rochow, V.B. (1991). Differences in ultraviolet wing patterns in the New Zealand lycaenid butterflies *Lycaena salustius,* L. Rauparaha & L. Feredayi, as a likely isolating mechanism. *Journal of the Royal Society of New Zealand,* **21**(2), 169–177.

Meyer-Rochow, V.B., & Järvilehto, M. (1997). Ultraviolet colours in *Pieris napi* from northern and southern Finland: Arctic females are the brightest! *Naturwissenschaften,* **84**(4), 165–168.

Meyer-Rochow, V.B. (2019). Eyes and vision of the bumblebee: A brief review on how bumblebees detect and perceive flowers. *Journal of Apiculture,* **2**(1), 107–115.

Meyer-Rochow, V.B. (1983). Flugelfarben, wie sie die Falter sehen: A study of UV- and other colour patterns in Lepidoptera. *Annotationes zoologicae japonenses,* **56**, 85–99.

Meyer-Rochow, V.B., Kashiwagi, T., & Eguchi, E. (2002). Selective photoreceptor damage in four species of insects induced by experimental exposures to UV-irradiation. *Micron,* **33**(1), 23–31.

Meyer-Rochow, V.B., & Shimoyama, A. (2008). UV-reflecting and absorbing body regions in gentoo and king penguin: Can they really be used by the penguins as signals for conspecific recognition? *Polar Biology,* **31**(5), 557–560.

Mikkola, K. (1972). Behavioural and electrophysiological responses of night-flying insects, especially Lepidoptera, to near-ultraviolet and visible light. *Annales Zoologici Fennici,* **9**(4), 225–254.

Mohun, S.M., & Davies, W.I.L. (2019). The evolution of amphibian photoreception. *Frontiers in Ecology and Evolution,* 7(321), 1–12.

Montejo-Kovacevich, G., Smith, J.E., Meier, J.I., Bacquet, C.N., Whiltshire-Romero, E., Nadeau, N.J., & Jiggins, C.D. (2019). Altitude and life-history shape the evolution of Heliconius wings. *Evolution,* **73**(12), 2436–2450.

Morehouse, N.I. (2014). Condition-dependent ornaments, life histories, and the evolving architecture of resource-use. *Integrative and Comparative Biology,* **54**(4), 591–600.

Morehouse, N.I., & Rutowski, R.L. (2010). Developmental responses to variable diet composition in a butterfly: The role of nitrogen, carbohydrates and genotype. *Oikos,* **119**(4), 636–645.

Morehouse, N.I., Vukusic, P., & Rutowski, R. (2007). Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. *Proceedings of the Royal Society B: Biological Sciences,* **274**(1608), 359–366.

Mouchet, S.R., & Vukusic, P. (2018). Structural colours in lepidopteran scales. In: R. Constant, Ed, *Advances in Insect Physiology.* London: Elsevier, 1–53.

Mullen, P., & Pohland, G. (2008). Studies on uv reflection in feathers of some 1000 bird species: Are UV peaks in feathers correlated with violet-sensitive and ultraviolet-sensitive cones? *Ibis,* **150**(1), 59–68.

Murali, G. (2018). Now you see me, now you don't: Dynamic flash coloration as an antipredator strategy in motion. *Animal Behaviour,* **142**(2), 207–220.

Nakagawa, T., & Eguchi, E. (1994). Differences in flicker fusion frequencies of the five spectral photoreceptor types in the swallowtail butterfly' s compound eye. *Zoological Science,* **11**(5), 759–762.

Nekrutenko, Y.P. (1964). The hidden wing-pattern of some palearctic species of *Gonepteryx* and its taxonomic value. *Journal of Research on the Lepidoptera,* **3**(2), 65–68.

Nekrutenko, Y.P. (1965a). 'Gynandromorphic effect 'and the optical nature of hidden wing-pattern in *Gonepteryx rhamni* L. (Lepidoptera, Pieridae). *Nature,* **205**, 417–418.

Nekrutenko, Y.P. (1965b). Three cases of gynandromorphism in *Gonepteryx*: an observation with ultraviolet rays. *Journal of Research on the Lepidoptera,* **4**(2), 103–108.

Nekrutenko, Y.P. (1968). *Phylogeny and geographical distribution of the genus Gonepteryx (Lepidoptera, Pieridae): an attempt of study in historical zoogeography.* Kiev: Naukova Dumka.

Nekrutenko, Y.P. (1970). A new subspecies of *Gonepteryx Rhamini* from Tian-Shan Mountains, USSR. *News of the Lepidopterists' Society,* **24**(3), 218–220.

Nekrutenko, Y.P. (1972). a new subspecies of *Gonepteryx amintha* (Pieridae) from Yunnan, mainland China, with comparative notes. *Journal of Research on the Lepidoptera,* **11**(4), 235–244.

Nekrutenko, Y.P., & Didmanidze, E.A. (1975). new data on geographic variation of ultraviolet reflectance pattern in *Gonepteryx rhamni* L. (Lepidoptera, Pieridae) in the Caucasus. *Doklady Akademii Nauk Ukrainskoi Ssr* [in Russian, English summary], **4**, 370–373.

Nelson, R.F., Balraj, A., Suresh, T., Torvund, M., & Patterson, S.S. (2019). Strain variations in cone wavelength peaks in situ during zebrafish development. *Visual Neuroscience,* **36**(E10), 1–28.

Nijhout, H.F. (1991). *The Development and Evolution Of Butterfly Wing Patterns.* Smithsonian Series in Comparative Evolutionary Biology (USA). Washington: Smithsonian Institute Press.

Obara, Y., & Majerus, M.N. (2000). Initial mate recognition in the british cabbage butterfly, *Pieris rapae rapae. Zoological Science,* **17**(6), 725–730.

Obara, Y., Ozawa, G., & Fukano, Y. (2008). Geographic variation in ultraviolet reflectance of the wings of the female cabbage butterfly, *Pieris rapae. Zoological Science,* **25**(11), 1106–1110.

Obara, Y. (1970). Studies on the mating behavior of the white cabbage butterfly, *Pieris rapae crucivora* Boisduval. *Zeitschrift für Vergleichende Physiologie,* **69**(1), 99–116.

Obara, Y., Watanabe, K., & Satoh, T. (2010). UV reflectance of inter-subspecific hybrid females obtained by crossing cabbage butterflies from Japan (*Pieris rapae crucivora*) with those from New Zealand (*P. rapae rapae*). *Entomological Science,* **13**(1), 156–158.

Obara, Y., Koshitaka, H., & Arikawa, K. (2008). Better mate in the shade: Enhancement of male mating behaviour in the cabbage butterfly, *Pieris rapae crucivora,* in a UV-rich environment. *Journal of Experimental Biology,* **211**(23), 3698–3702.

Ödeen, A., & Håstad, O. (2013). The phylogenetic distribution of ultraviolet sensitivity in birds. *BMC Evolutionary Biology,* **13**(1), 36.

Olofsson, M., Vallin, A., Jakobsson, S., & Wiklund, C. (2010). Marginal eyespots on butterfly wings deflect bird attacks under low light intensities with UV wavelengths. *Plos One,* **5**(5), E10798.

Osorio, D. (2019). The evolutionary ecology of bird and reptile photoreceptor spectral sensitivities. *Current Opinion in Behavioral Sciences,* **30**, 223–227.

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research,* **48**(20), 2042–2051.

Papiorek, S., Junker, R.R., Alves-Dos-Santos, I., Melo, G.A.R., Amaral-Neto, L.P., Sazima, M., Wolowski, M., Freitas, L., & Lunau, K. (2016). Bees, birds and yellow flowers: Pollinator-dependent convergent evolution of UV patterns. *Plant Biology,* **18**(1), 46–55.

Papke, R.S., Kemp, D.J., & Rutowski, R.L. (2007). Multimodal signalling: Structural ultraviolet reflectance predicts male mating success better than pheromones in the butterfly *Colias urytheme* L. (Pieridae). *Animal Behaviour,* **73**(1), 47–54.

Parkyn, D.C., & Hawryshyn, C.W. (1993). Polarized-light sensitivity in rainbow trout (*Oncorhynchus mykiss*): Characterization from multi-unit responses in the optic nerve. *Journal of Comparative Physiology A,* **172**(4), 493–500.

Peach, D.A., Ko, E., Blake, A.J., & Gries, G. (2019). Ultraviolet inflorescence clues enhance attractiveness of inflorescence odour to *Culex pipiens* mosquitoes. *Plos One,* **14**(6), E0217484.

Pearn, S.M., Bennett, A.T., & Cuthill, I.C. (2001). Ultraviolet vision, fluorescence and mate choice in a parrot, the budgerigar *Melopsittacus undulatus. Proceedings of the Royal Society B: Biological Sciences,* **268**(1482), 2273–2279.

Pecháček, P., Stella, D., Keil, P., & Kleisner, K. (2014). Environmental effects on the shape variation of male ultraviolet patterns in the brimstone butterfly (*Gonepteryx rhamni*, Pieridae, Lepidoptera). *Naturwissenschaften,* **101**(12), 1055–1063.

Pecháček, P., Stella, D., & Kleisner, K. (2019). A morphometric analysis of environmental dependences between ultraviolet patches and wing venation patterns in *Gonepteryx* butterflies (Lepidoptera, Pieridae). *Evolutionary Ecology,* **33**(1), 89–110.

Peet-Pare, C.A. (2017). *Mimicry in the Ultraviolet: a Predator Perspective*. MS thesis. Carleton University.

Peichl, L., Chavez, A.E., Ocampo, A., Mena, W., Bozinovic, F., & Palacios, A.G. (2005). Eye and vision in the subterranean rodent cururo (*Spalacopus cyanus*, Octodontidae). *Journal of Comparative Neurology,* **486**(3), 197–208.

Perveen, F.K., & Khan, A. (2017). *Introductory Chapter: Lepidoptera.* https://www.intechopen.com/books/lepidoptera/introductory-chapter-lepidoptera Edn.

Petersen, B., Toernblom, O., & Bodin, N. (1951). Verhaltensstudien am Rapsweissling und Bergweissling (*Pieris napi* L. und *Pieris bryoniae* Ochs.). *Behaviour,* **4**(1), 67–84.

Pike, T.W. (2011). Using digital cameras to investigate animal colouration: Estimating sensor sensitivity functions. *Behavioral Ecology and Sociobiology,* **65**(4), 849–858.

Pirih, P., Arikawa, K., & Stavenga, D.G. (2010). An expanded set of photoreceptors in the eastern pale clouded yellow butterfly, *Colias erate*. *Journal of Comparative Physiology A,* **196**(7), 501–517.

Pirih, P., Wilts, B.D., & Stavenga, D.G. (2011). Spatial reflection patterns of iridescent wings of male pierid butterflies: Curved scales reflect at a wider angle than flat scales. *Journal of Comparative Physiology A,* **197**(10), 987–997.

Pöey, A. (1871). Influence e la lumiere violette sur la croissance de la vigne, des cochons et des taureaux. *Comptes rendus hebdomadaires des séances de l'Académie des sciences,* **73**, 1236–1238.

Post, C.T., & Goldsmith, T.H. (1969). Physiological evidence for color receptors in the eye of a butterfly. *Annals of the Entomological Society of America,* **62**(6), 1497–1498.

Primack, R.B. (1982). Ultraviolet patterns in flowers, or flowers as viewed by insects. *Arnoldia,* **42**(3), 139–146.

Prudic, K.L., Jeon, C., Cao, H., & Monteiro, A. (2011). Developmental plasticity in sexual roles of butterfly species drives mutual sexual ornamentation. *Science,* **331**(6013), 73–75.

Prudic, K.L., Stoehr, A.M., Wasik, B.R., & Monteiro, A. (2015). Eyespots deflect predator attack increasing fitness and promoting the evolution of phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences,* **282**(1798), 20141531.

Qiu, X., Vanhoutte, K.A.J., Stavenga, D.G., & Arikawa, K. (2002). Ommatidial heterogeneity in the compound eye of the male small white butterfly, *Pieris rapae crucivora*. *Cell and Tissue Research,* **307**(3), 371–379.

Rajchard, J. (2009). Ultraviolet (UV) light perception by birds: A review. *Veterinární Medicína,* **54**(8), 351–359.

Ramos, M.E., & Hulshof, C.M. (2019). Using digitized museum collections to understand the effects of habitat on wing coloration in the Puerto Rican monarch. *Biotropica,* **51**(4), 477–483.

Remington, C.L. (1973). Ultraviolet reflectance in mimicry and sexual signals in the lepidoptera. *Journal of the New York Entomological Society,* **81**(2), 124.

Ries, C., Spaethe, J., Sztatecsny, M., Strondl, C., & Hödl, W. (2008). Turning blue and ultraviolet: Sex-specific colour change during the mating season in the Balkan moor frog. *Journal of Zoology,* **276**(3), 229–236.

Robertson, K.A., & Monteiro, A. (2005). Female Bicyclus Anynana butterflies choose males on the basis of their dorsal UV-reflective eyespot pupils. *Proceedings of the Royal Society B: Biological Sciences,* 272(1572), 1541–1546.

Romero-Diaz, C., Rivera, J.A., Ossip-Drahos, A.G., Zúñiga-Vega, J.J., Vital-García, C., Hews, D.K., & Martins, E.P. (2019). Losing the trait without losing the signal: Evolutionary shifts in communicative colour signalling. *Journal of Evolutionary Biology,* **32**(4), 320–330.

Rosenthal, G.G., Wagner Jr, W.E., & Ryan, M.J. (2002). Secondary reduction of preference for the sword ornament in the pygmy swordtail *Xiphophorus nigrensis* (Pisces: Poeciliidae). *Animal Behaviour,* **63**(1), 37–45.

Rowe, J.W., Gradel, J.R., Bunce, C.F., & Clark, D.L. (2012). Sexual dimorphism in size and shell shape, and dichromatism of spotted turtles (*Clemmys guttata*) in southwestern Michigan. *Amphibia–Reptilia,* **33**(3–4), 443–450.

Ruck, P., & Jahn, T.L. (1954). Electrical studies on the compound eye of *Ligia occidentalis* Dana (Crustacea: Isopoda). *Journal of General Physiology,* **37**(6), 825–849.

Rutowski, R.L. (1985). Evidence for mate choice in a sulphur butterfly (*Colias eurytheme*). *Zeitschrift für Tierpsychologie,* **70**(2), 103–114.

Rutowski, R.L., & Kemp, D.J. (2017). Female iridescent colour ornamentation in a butterfly that displays mutual ornamentation: Is it a sexual signal? *Animal Behaviour,* **126**, 301–307.

Rutowski, R.L., Macedonia, J.M., Kemp, D.J., & Taylor-Taft, L. (2007a). Diversity in structural ultraviolet coloration among female sulphur butterflies (Coliadinae, Pieridae). *Arthropod Structure and Development,* **36**(3), 280–290.

Rutowski, R.L., Macedonia, J.M., Morehouse, N., & Taylor-Taft, L. (2005). Pterin pigments amplify iridescent ultraviolet signal in males of the orange sulphur butterfly, *Colias eurytheme*. *Proceedings of the Royal Society B: Biological Sciences,* **272**(1578), 2329–2335.

Rutowski, R.L. (2003). Visual ecology of adult butterflies. In: C.L. Boggs, W.B. Watt, & P.R. Ehrlich, Eds, *Butterflies: Ecology and Evolution Taking Flight.* Chicago: University of Chicago Press, 9–25.

Rutowski, R.L., & Gilchrist, G.W. (1986). Copulation in *Colias eurytheme* (Lepidoptera: Pieridae): Patterns and frequency. *Journal of Zoology,* **209**(1), 115–124.

Rutowski, R.L., Gilchrist, G.W., & Terkanian, B. (1987). Female butterflies mated with recently mated males show reduced reproductive output. *Behavioral Ecology and Sociobiology,* **20**(5), 319–322.

Rutowski, R.L., & Macedonia, J.M. (2008). Limits on the wavelengths represented in ultraviolet images of lepidoptera. *Journal of the Lepidopterists' Society,* **62**(3), 133–137.

Rutowski, R.L., Nahm, A.C., & Macedonia, J.M. (2010). Iridescent hindwing patches in the pipevine swallowtail: Differences in dorsal and ventral surfaces relate to signal function and context. *Functional Ecology,* **24**(4), 767–775.

Rutowski, R.L., & Warrant, E.J. (2002). Visual field structure in the *Empress leilia, Asterocampa leilia* (Lepidoptera, Nymphalidae): Dimensions and regional variation in acuity. *Journal of Comparative Physiology A,* **188**(1), 1–12.

Rutowski, R.L., Macedonia, J.M., Merry, J.W., Morehouse, N.I., Yturralde, K., Taylor-Taft, L., Gaalema, D., Kemp, D.J., & Papke, R.S. (2007b). Iridescent ultraviolet signal in the orange sulphur butterfly (*Colias eurytheme*): Spatial, temporal and spectral properties. *Biological Journal of the Linnean Society,* **90**(2), 349–364.

Sabol, A.C., Hellmann, J.K., Gray, S.M., & Hamilton, I.M. (2017). The role of ultraviolet coloration in intrasexual interactions in a colonial fish. *Animal Behaviour,* **131**, 99–106.

Sauman, I., Briscoe, A.D., Zhu, H., Shi, D., Froy, O., Stalleicken, J., Yuan, Q., Casselman, A., & Reppert, S.M. (2005). Connecting the navigational clock to sun compass input in monarch butterfly brain. *Neuron,* **46**(3), 457–467.

Schaider, P. (1988). Unterschiede von *Lycaena hippothoe* und *candens* im UV-Licht (Lep., Lycaenidae). *Atalanta,* **18**, 415–425.

Schiemenz, F. (1924). Über den Farbensinn der Fische. *Zeitschrift Für Vergleichende Physiologie,* **1**(1–2), 175–220.

Schluter, D., & Price, T. (1993). Honesty, perception and population divergence in sexually selected traits. *Proceedings of the Royal Society B: Biological Sciences,* **253**(1336), 117–122.

Schnetzler, J. (1874). *De l'influence de la lumiere sur le dévelopement des larves de grenouilles.* Paris: Archiv Des Sciences Physiques Et Naturelles.

Sekimura, T. (2014). Pattern formation and diversity in butterfly wings: Experiments and models. In: O. Jenkins P., Ed, *Advances in Zoology Research.* New York: Nova Science Publishers, 1–26.

Sekimura, T., & Nijhout, H.F. (2017). *Diversity and Evolution of Butterfly Wing patterns.* Singapore: Springer.

Seltmann, K.C., Cobb, N.S., Gall, L.F., Bartlett, C.R., Basham, M.A., Betancourt, I., Bills, C., Brandt, B., Brown, R.L., & Bundy, C. (2017). Lepnet: The Lepidoptera of North America Network. *Zootaxa,* **1**(4247), 73–77.

Shanks, K., Senthilarasu, S., & Mallick, T.K. (2015). White butterflies as solar photovoltaic concentrators. *Scientific Reports,* **5**, 12267.

Shashar, N. (1994). *UV Vision by marine animals: Mainly questions.* In: D. Gulko, & P.L. Jokiel, Eds, *Ultraviolet Radiation and Coral Reefs.* HIMB Technical Report no. 41, 201–206.

Siebeck, U.E. (2004). Communication in coral reef fish: The role of ultraviolet colour patterns in damselfish territorial behaviour. *Animal Behaviour,* **68**(2), 273–282.

Siebeck, U.E., Parker, A.N., Sprenger, D., Mäthger, L.M., & Wallis, G. (2010). A species of reef fish that uses ultraviolet patterns for covert face recognition. *Current Biology,* **20**(5), 407–410.

Siitari, H., Honkavaara, J., & Viitala, J. (1999). Ultraviolet reflection of berries attracts foraging birds. A laboratory study with redwings (*Turdus iliacus*) and bilberries (*Vaccinium myrtillus*). *Proceedings of The Royal Society B: Biological Sciences,* **266**(1433), 2125–2129.

Silberglied, R.E., Taylor, J., & Orley, R. (1978). Ultraviolet reflection and its behavioral role in the courtship of the sulfur butterflies *Colias eurytheme* and *C. philodice* (Lepidoptera, Pieridae). *Behavioral Ecology and Sociobiology,* **3**(3), 203–243.

Silberglied, R.E., & Taylor, O.R. (1973). Ultraviolet differences between the sulphur butterflies, *Colias eurytheme* and *C. philodice,* and a possible isolating mechanism. *Nature,* **241**, 406–408.

Silberglied, R.E. (1979). Communication in the ultraviolet. *Annual Review of Ecology and Systematics,* **10**, 373–398.

Simões, B.F., Foley, N.M., Hughes, G.M., Zhao, H., Zhang, S., Rossiter, S.J., & Teeling, E.C. (2018). As blind as a bat? Opsin phylogenetics illuminates the evolution of color vision in bats. *Molecular Biology and Evolution,* **36**(1), 54–68.

Simoncelli, E.P., & Olshausen, B.A. (2001). Natural image statistics and neural representation. *Annual Review of Neuroscience,* **24**(1), 1193–1216.

Simpson, R.K., & Mcgraw, K.J. (2018). It's not just what you have, but how you use it: Solar-positional and behavioural effects on hummingbird colour appearance during courtship. *Ecology Letters,* **21**(9), 1413–1422.

Slansky, F., & Feeny, P. (1977). Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological Monographs,* **47**(2), 209–228.

Smith, J.M., & Harper, D. (2003). *Animal Signals.* Oxford: Oxford University Press.

Stalleicken, J., Labhart, T., & Mouritsen, H. (2006). Physiological characterization of the compound eye in monarch butterflies with focus on the dorsal rim area. *Journal of Comparative Physiology A,* **192**(3), 321–331.

Stapley, J., & Whiting, M.J. (2006). Ultraviolet signals fighting ability in a lizard. *Biology Letters,* **2**(2), 169–172.

Stavenga, D.G., & Arikawa, K. (2006). Evolution of color and vision of butterflies. *Arthropod Structure and Development,* **35**(4), 307–318.

Stavenga, D.G., Giraldo, M.A., & Leertouwer, H.L. (2010). Butterfly wing colors: Glass scales of *Graphium sarpedon* cause polarized iridescence and enhance blue/green pigment coloration of the wing membrane. *Journal of Experimental Biology,* **213**(10), 1731–1739.

Stavenga, D.G., Leertouwer, H.L., & Wilts, B.D. (2014). Coloration principles of nymphaline butterflies: Thin films, melanin, ommochromes and wing scale stacking. *Journal of Experimental Biology,* **217**(12), 2171–2180.

Stavenga, D.G., Stowe, S., Siebke, K., Zeil, J., & Arikawa, K. (2004). Butterfly wing colours: Scale beads make white pierid wings brighter. *Proceedings of the Royal Society B: Biological Sciences,* **271**(1548), 1577–1584.

Steffen, J.E., Hultberg, J., & Drozda, S. (2019). The effect of dietary carotenoid increase on painted turtle spot and stripe color. *Comparative Biochemistry and Physiology Part B: Biochemistry And Molecular Biology,* **229**, 10–17.

Stella, D., Faltýnek, Z., Rindoš, M., Kleisner, K., & Pecháček, P. (2018). Distribution of ultraviolet ornaments in *Colias* butterflies (Lepidoptera: Pieridae). *Environmental Entomology,* **47**, 1344–1354.

Stella, D., Pecháček, P., Meyer-Rochow, V.B., & Kleisner, K. (2018). UV reflectance is associated with environmental conditions in Palaearctic *Pieris napi* (Lepidoptera: Pieridae). *Insect Science,* **25**(3), 508–518.

Stevens, M., & Cuthill, I.C. (2007). Hidden messages: Are ultraviolet signals a special channel in avian communication? *AIBS Bulletin,* **57**(6), 501–507.

Stevens, M., Parraga, C.A., Cuthill, I.C., Partridge, J.C., & Troscianko, T.S. (2007). Using digital photography to study animal coloration. *Biological Journal of the Linnean Society,* **90**(2), 211–237.

Stoehr, A.M., & Mcgraw, K.J. (2001). Ultraviolet reflectance of color patches in male *Sceloporus undulatus* and *Anolis carolinensis*. *Journal of Herpetology,* **35**(1), 168–171.

Stride, G.O. (1957). Investigations into the courtship behaviour of the male of *Hypolimnas misippus* L. (Lepidoptera, Nymphalidae), with special reference to the role of visual stimuli. *British Journal of Animal Behaviour,* **5**(4), 153–167.

Stride, G.O. (1956). On the courtship behaviour of *hypolimnas nisippus* l. (Lepidoptera, Nymphalidae), with notes on the mimetic association with *Danaus chrysippus* L. (Lepidoptera, Danaidae). *British Journal of Animal Behaviour,* **4**(2), 52–68.

Sweeney, A., Jiggins, C., & Johnsen, S. (2003). Insect communication: Polarized light as a butterfly mating signal. *Nature,* **423**(6935), 31–2.

Swihart, S.L. (1964). The nature of the electroretinogram of a tropical butterfly. *Journal of Insect Physiology,* **10**(4), 547–562.

Swihart, S.L. (1970). The neural basis of colour vision in the butterfly *Papilio troilus*. *Journal of Insect Physiology,* **16**(8), 1623–1636.

Tabata, H., Hasegawa, T., Nakagoshi, M., Takikawa, S., & Tsusue, M. (1996). Occurrence of biopterin in the wings of *Morpho* butterflies. *Experientia,* **52**(1), 85–87.

Takizawa, T., & Koyama, N. (1974). Reflection of ultraviolet light from the wing surface of the cabbage butterfly, *Pieris rapae crucivora* Boisduval (Lepidoptera: Pieridae). *Journal of the Japanese Academy of Sciences, Series A: Biology,* **61**(17), 1–12.

Taylor, O.R. (1973a). A non-genetic "polymorphism" in *Anartia fatima* (Lepidoptera: Nymphalidae). *Evolution,* **27**(1), 161–164.

Taylor, O.R. (1973b). Reproductive isolation in *Colias eurytheme* and *C. philodice* (Lepidoptera: Pieridae): Use of olfaction in mate selection. *Annals of the Entomological Society of America,* **66**(3), 621–626.

Tedore, C., & Nilsson, D. (2019). Avian UV vision enhances leaf surface contrasts in forest environments. *Nature Communications,* **10**(238), 1–12.

Telles, F.J., Lind, O., Henze, M.J., Rodríguez-Gironés, M.A., Goyret, J., & Kelber, A. (2014). Out of the blue: The spectral sensitivity of hummingbird hawkmoths. *Journal of Comparative Physiology A,* **200**(6), 537–546.

Thorpe, R.S., & Richard, M. (2001). Evidence that ultraviolet markings are associated with patterns of molecular gene flow. *Proceedings of the National Academy of Sciences of the United States of America,* **98**(7), 3929–3934.

Thorpe, R.S. (2002). Analysis of color spectra in comparative evolutionary studies: Molecular phylogeny and habitat adaptation in the St. Vincent anole (*Anolis trinitatis*). *Systematic Biology,* **51**(4), 554–569.

Tobiska, W., & Nusinov, A. (2007). *Iso 21348-Process for determining solar irradiances.* Iso/Tc 20/Sc 14th edition. USA: 49.140.

Tocco, C., Dacke, M., & Byrne, M. (2019). Eye and wing structure closely reflects the visual ecology of dung beetles. *Journal of Comparative Physiology A,* **205**(2), 211–221.

Tovée, M.J. (1995). Ultra-violet photoreceptors in the animal kingdom: Their distribution and function. *Trends in Ecology and Evolution,* **10**(11), 455–460.

Troscianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox. a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution,* **6**(11), 1320–1331.

Tuomaala, M., Kaitala, A., & Rutowski, R.L. (2012). Females show greater changes in wing colour with latitude than males in the green-veined white butterfly, *Pieris napi* (Lepidoptera: Pieridae). *Biological Journal of the Linnean Society,* **107**(4), 899–909.

Turner, J., & Parisi, A. (2018). Ultraviolet radiation albedo and reflectance in review: The influence to ultraviolet exposure in occupational settings. *International Journal of Environmental Research and Public Health,* **15**(7), 1507.

Turner, J.R.G. (1978). Why male butterflies are non-mimetic: Natural selection, sexual selection, group selection, modification and sieving. *Biological Journal of the Linnean Society,* **10**(4), 385–432.

Van Herwerden van, M.A. (1914). Ueber Die Perzeptionsfahigkeit des Daphnienauges fur ultraviolette Strahlen. *Biologisches Zentralblatt,* **34**, 213–216.

Vane-Wright, R.I. (1984). The role of pseudosexual selection in the evolution of butterfly colour pattern. In: R.I. Vane-Wright, & P.R. Ackery, Eds, *The Biology of Butterflies.* New Jersey: Princeton University Press, 251–253.

Viitala, J., Korplmäki, E., Palokangas, P., & Koivula, M. (1995). Attraction of kestrels to vole scent marks visible in ultraviolet light. *Nature,* **373**(6513), 425.

Villafuerte, R., & Negro, J.J. (1998). Digital imaging for colour measurement in ecological research. *Ecology Letters,* **1**, 151–154.

Vorobyev, M., Osorio, D., Bennett, A.T.D., Marshall, N.J., & Cuthill, I.C. (1998). Tetrachromacy, oil droplets and bird plumage Colours. *Journal of Comparative Physiology A,* **183**(5), 621–633.

Vorobyev, M., & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society of London B: Biological Sciences,* **265**(1394), 351–358.

Vukusic, P., & Sambles, J.R. (2003). Photonic structures in biology. *Nature,* **424**(6950), 852.

Wada, S., Shen, B., Kawano-Yamashita, E., Nagata, T., Hibi, M., Tamotsu, S., Koyanagi, M., & Terakita, A. (2018). Color opponency with a single kind of bistable opsin in the zebrafish pineal organ. *Proceedings of the National Academy of Sciences of the United States of America,* **115**(44), 11310–11315.

Wallace, A.R. (1865). On the phenomena of variation and geographical distribution as illustrated by the Papilionidæ of the Malayan region. *Transactions of the Linnean Society of London,* **25**(1), 1–71.

Warzecha, A., & Egelhaaf, M. (1995). Visual pattern discrimination in a butterfly: A behavioral study on the Australian lurcher, *Yoma sabina. Naturwissenschaften,* **82**(12), 567–570.

Wheat, C.W., & Watt, W.B., 2008. A mitochondrial-DNA-based phylogeny for some evolutionary-genetic model species of *Colias* butterflies (Lepidoptera, Pieridae). *Molecular Phylogenetics and Evolution,* **47**(3), 893–902.

White, T.E., Zeil, J., & Kemp, D.J. (2015). Signal design and courtship presentation coincide for highly biased delivery of an iridescent butterfly mating signal. *Evolution,* **69**(1), 14–25.

Whiting, M.J., Stuart-Fox, D.M., O'Connor, D., Firth, D., Bennett, N.C., & Blomberg, S.P. (2006). Ultraviolet signals ultra-aggression in a lizard. *Animal Behaviour,* **72**(2), 353–363.

Wiernasz, D.C. (1989). Female choice and sexual selection of male wing melanin pattern in *Pieris occidentalis* (Lepidoptera). *Evolution,* **43**(8), 1672–1682.

Wijnen, B., Leertouwer, H., & Stavenga, D. (2007). Colors and pterin pigmentation of pierid butterfly wings. *Journal of Insect Physiology,* **53**(12), 1206–1217.

Williamson, C.E., Zepp, R.G., Lucas, R.M., Madronich, S., Austin, A.T., Ballaré, C.L., Norval, M., Sulzberger, B., Bais, A.F., & Mckenzie, R.L., (2014). Solar ultraviolet radiation in a changing climate. *Nature Climate Change,* **4**(6), 434.

Wilts, B.D., Ijbema, N., & Stavenga, D.G. (2014). Pigmentary and photonic coloration mechanisms reveal taxonomic relationships of the cattlehearts (Lepidoptera: Papilionidae: Parides). *BMC Evolutionary Biology,* **14**(1), 160.

Wilts, B.D., Pirih, P., & Stavenga, D.G. (2011). Spectral reflectance properties of iridescent pierid butterfly wings. *Journal of Comparative Physiology A,* **197**(6), 693–702.

Windig, J.J. (1991). Quantification of lepidoptera wing patterns using an image analyzer. *Journal of Research on the Lepidoptera,* **30**(1–2), 82–94.

Winter, Y., López, J., & Von Helversen, O. (2003). Ultraviolet vision in a bat. *Nature,* **425**(6958), 612.

Wright, A.A. (1972). The Influence of ultraviolet radiation on the pigeons color discrimination. *Journal of the Experimental Analysis of Behavior,* **17**(3), 325–337.

Yang, M., Pyornila, A., & Meyer-Rochow, V.B. (2004). UV-Reflectivity of parafocal eyespot elements on butterfly wings in normal and abnormal specimens. *Entomologica Fennica,* **15**(1), 34–40.

Yokoyama, S., Radlwimmer, F.B., & Blow, N.S. (2000). Ultraviolet pigments in birds evolved from violet pigments by a single amino acid change. *Proceedings of the National Academy of Sciences of the United States of America,* **97**(13), 7366–7371.

Yokoyama, S., & Shi, Y. (2000). Genetics and evolution of ultraviolet vision in vertebrates. *FEBS Letters,* **486**(2), 167–172.

Yoshioka, S., & Kinoshita, S. (2004). Wavelength-selective and anisotropic light-diffusing scale on the wing of the *Morpho* butterfly. *Proceedings of the Royal Society B: Biological Sciences,* **271**(1539), 581–587.

Yung, E. (1878). De l'influence des differentes couleurs du spectre sur le developpement des animaux. *Comptes rendus de l'Académie des Sciences,* **87**, 998–1000.

Zahavi, A. (1975). Mate selection: a selection for a handicap. *Journal of Theoretical Biology,* **53**(1), 205–214.

Zapletalová, L., Zapletal, M., & Konvička, M. (2016). Habitat impact on ultraviolet reflectance in moths. *Environmental Entomology,* **45**(5), 1300–1305.

Zhang, L., Martin, A., Perry, M.W., Van Der Burg, K.R., Matsuoka, Y., Monteiro, A., & Reed, R.D. (2017). Genetic basis of melanin pigmentation in butterfly wings. *Genetics,* **205**(4), 1537–1550.

Zitko, M. (2019). *Ekologické faktory ovlivňující variabilitu ultrafialového zbarvení květů*. BS thesis. Faculty of Science, Charles University, Czech Republic.

Part 2

Stella, D., Pecháček, P., Meyer-Rochow, V.B. and Kleisner, K. (2018) UV reflectance is associated with environmental conditions in Palaearctic *Pieris napi* (Lepidoptera: Pieridae). *Insect Science*, 25, 508–518.

CHARLES UNIVERSITY
Faculty of science

Declaration of publication co-authorship

This is to confirm that PhD candidate David Stella significantly contributed to the following publication: *Stella, D., Pecháček, P., Meyer-Rochow, V.B. and Kleisner, K. (2018) UV reflectance is associated with environmental conditions in Palaearctic* Pieris napi *(Lepidoptera: Pieridae). Insect science, 25, 508-518.*

He reviewed the literature, acquired the data, drafted the manuscript and worked on the manuscript revision.

The co-authors agree with submitting this publication as part of his PhD.

Doc. Mgr. Karel Kleisner PhD

Department of Philosophy and History of Science, Faculty of Science, Charles University

Insect Science

Insect Science (2018) 25, 508–518, DOI 10.1111/1744-7917.12429

ORIGINAL ARTICLE

UV reflectance is associated with environmental conditions in Palaearctic *Pieris napi* (Lepidoptera: Pieridae)

David Stella[1], Pavel Pecháček[1], Victor Benno Meyer-Rochow[2,3] and Karel Kleisner[1]

[1]Faculty of Science, Department of Philosophy and History of Science, Charles University, Prague, Czech Republic; [2]Department of Biology, Research Institute of Luminescent Organisms, Tokyo, Japan and [3]Department of Genetics and Physiology, Oulu University, Oulu, Finland

Abstract The subject of our investigation was the visual features of wing color with special focus on the UV reflectance in the green-veined white butterfly (*Pieris napi*). Previous studies had concluded that UV reflectance on dorsal wing surfaces is found only in the female *P. napi*. Based on UV sensitive photography, we analyzed a correlation between 12 geographic and environmental factors and UV reflectance patterns on 3 patches on the forewings of 407 *P. napi* specimens from the Palaearctic region. Results had shown that females significantly differ from males: they exhibit a 25% higher UV reflectance. To investigate whether and how UV reflectance levels on the forewings and hindwings of both sexes are influenced by the environment, we performed a principal component analysis (PCA) with several environmental variables. For several variables (in particular, latitude and longitude, mean annual temperature and precipitation, and temperature annual range and altitude), the generalized linear model (GLM) model revealed a significant correlation in both sexes. This suggests a link between UV reflectance levels and the environment and distribution of *P. napi.* We found that stronger UV reflectance is associated with generally more hostile environments and concluded that large-scale environmental factors influence the UV reflectance on the forewings of both male and female green-veined white butterflies.

Key words geographical variation; green-veined white; Palaearctic; UV photography; UV reflectance

Introduction

Darwin (1871) encouraged zoologists and ecologists of his era to study animal coloration (Poulton, 1890; Beddard, 1892). Ever since then, issues related to coloration have been investigated in a wide range of evolutionary, taxonomic, ecological, and ethological contexts (Cott, 1940). Although generally less well researched than other forms of visual phenomenon, UV reflectance (Lutz, 1924; DeVoe *et al.*, 1969; Huth & Burkhardt, 1972) has been documented especially in numerous species of butterflies from Europe (Meyer-Rochow & Järvilehto, 1997; Pecháček *et al.*, 2014), Asia (Eguchi & Meyer-Rochow, 1983), New Zealand (Meyer-Rochow, 1991), and North America (Silberglied & Taylor, 1973).

An essential part of the biological role of UV reflection is its sensory detection. Most insects, including butterflies, sense UV-A with their photoreceptors (Briscoe & Chittka, 2001). This ability helps them in intraspecific communication (Silberglied, 1984; Rutowski, 1991; Meyer-Rochow & Järvilehto, 1997; Kemp & Rutowski, 2011) and sexual recognition (Kemp *et al.*, 2005; Morehouse *et al.*, 2007), and can assist them in locating nectar-bearing flowers and open spaces (Mazokhin-Porshniakov, 1957; Shkurikhin & Oslina, 2015).

Correspondence: David Stella, Faculty of Science, Department of Philosophy and History of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic. Tel: +420 603 421 814; Email: David.Stella@natur.cuni.cz

For our study, we chose the green-veined white butterfly (*Pieris napi*, Linnaeus 1758, Lepidoptera: Pieridae). Due to its wide distribution from Western Europe throughout the entire Palaearctic region to Eastern Asia and Japan (Eitschberger, 1984), it is an excellent subject for studying relations between ecological variables and UV reflectance. Previous studies had shown that *P. napi* is a polymorphic species whose morphology and life history change with altitude and latitude (Espeland *et al.*, 2007; Välimäki & Kaitala, 2007; Shkurikhin & Oslina, 2015). This trend, however, varies both within and between the sexes (Rutowski *et al.*, 1981; Tuomaala *et al.*, 2012). Furthermore, in comparison with other butterfly species such as *Colias eurytheme* (Silberglied *et al.*, 1978), the age of *P. napi* individuals has little effect on their UV reflectance (Meyer-Rochow & Järvilehto, 1997). In the multivoltine *P. napi*, females follow various heritable strategies associated with mating tactics, which range from strict monandry (i.e., females mate only once) to a high degree of polyandry (Meyer-Rochow, 1999; Wedell *et al.*, 2002; Välimäki & Kaitala, 2006; Kivelä & Välimäki, 2008).

Another important aspect of the life history of *P. napi* is related to its mating behavior. Males actively search for females (Obara, 1970; Fukano *et al.*, 2012), but since they are subsequently unable to enforce copulation, females decide whether to copulate with a male (Silberglied *et al.*, 1978; Kaitala & Wiklund, 1994). This important feature of *P. napi* life history is addressed in the discussion.

Color polymorphism in butterflies has been extensively studied along geographic and environmental gradients, such as variation across latitude (Hovanitz, 1944), longitude (Obara *et al.*, 2008a), altitude (Tuomaala *et al.*, 2012), level of ultraviolet radiation (Meyer-Rochow & Järvilehto, 1997), temperature, and/or photoperiod (Hazel & West, 1983). Furthermore, ultraviolet coloration of butterflies may also be influenced by the quality of food ingested during their larval development (Knuttel & Fiedler, 2001; Kemp, 2006; Rutowski *et al.*, 2007). Almost all existing studies, however, deal with specimens from a relatively small region: large regions such as the entire Palaearctic have rarely been investigated. Most studies, moreover, measure UV reflectance using conventional spectrophotometry, whereas in our investigation, we measured and visualized UV reflectance using digital photography.

In our study, we search for associations between the level of UV reflectance and ecological variables (geographic and environmental) on a large geographical scale. Further, based on hypotheses proposed in earlier works (Rutowski, 1981; Tuomaala *et al.*, 2012), we test for differences in UV reflectance between males and females *P. napi*. We demonstrate that environmental conditions correlate with the amount of UV reflectance of *P. napi* wings and discuss the possible evolutionary and ecological implications of these results.

Materials and methods

Variables

Our analysis included a total of 12 geographic and environmental variables: latitude (specimen museum label), longitude (specimen museum label), altitude (Google Earth 1.6.2015), mean annual temperature, mean annual precipitation, isothermality, temperature seasonality, precipitation seasonality, temperature annual range (Hijmans *et al.*, 2005), mean annual Net Primary Production (NPP) (Running *et al.*, 2004; Zhao *et al.*, 2005; Zhao & Running, 2010), Normalized Difference Vegetation Index (NDVI) (Holben, 1986; Eklundh, 1995; Huete *et al.* 2002), and Actual Evapotranspiration (AET) (Mu *et al.*, 2011). Since no data on the UV irradiation in the particular localities where our specimens were collected were available, we used latitude as a proxy for the missing UV irradiation GIS layer (cf., UV levels at different latitudes; Munakata *et al.*, 2006). These variables can be divided in 2 groups: geographical and environmental. The geographical group includes latitude, longitude, and altitude. Annual precipitation and mean annual temperature are considered major, the remaining variables minor environmental variables. These 2 groups of variables were used as independent variables since they influence insect development, distribution, and body size. All variables were resolved at 30 arcsec (approximately 1 km at the equator). The spatial position of each specimen was subsequently used to extract values of the variables listed above (ESRI, 2011).

To avoid co-linearity, we performed principal component analysis (PCA) on all environmental and geographical variables. PCA was centered to zero mean and standardized to a variance of one. Only 2 PCA axes were used for further analysis.

Acquisition of photographs in the ultraviolet (UV-A)

Reports often fail to include precise and detailed descriptions of how images they work with were obtained (Stevens *et al.*, 2007). We decided to include the details of the entire process. All specimens were photographed using a digital camera (for details, see below). The advantages of a photographic method (digital or celluloid film photography) are substantial: it is a nondestructive method of recording UV reflectance, which enables the recording of UV reflectance of the whole wing area with a potential spatial pattern of UV reflectance (Knüttel & Fiedler, 2000). The main difference of a traditional film photography in the acquisition of UV patterns is related to image standardization, which is hard to obtain due to the developing and processing of the film material. The spectrophotometric method, on the other hand, unlike photography requires the use of a part of a butterfly wing, making it impossible to obtain and visualize UV reflectance across an entire specimen (Brunton & Majerus, 1995; Obara & Majerus, 2000).

We used a FujiFilm IS Pro digital camera suitable for UV photography due to its broad sensitivity spectrum, which spans from 330 to 900 nm. The camera was equipped with uncoated UV transmitting nondistorting lenses. We used 2 photographic filters: B+W 403 and B+W BG 38. The black filter B+W 403 prevented light in the visible spectrum, that is, 400–700 nm, from passing through the lens. This filter, however, does not stop infrared light (IR), which is a stronger component than the UV in both daylight and artificial light. The second filter B+W BG 38 was then used to filter out the IR light ($\lambda >$ 700 nm).

To illuminate the objects, which were to be photographed, we used the artificial light source UVP MRL-58 multiple-Ray-Lamp (8-watt, 230V-50Hz, 0.16A) equipped with a mercury fluorescent lamp 8w F8T5 longwave 365 nm or 8w F8T5BLB 352 nm. All objects were illuminated under the same angle (45°) and photographed in a standardized position from a dorsal view (Fig. 5). To follow the same method for all specimens, several standardized procedures were implemented. The FujiFilm IS Pro digital camera was set to ISO 400, shutter time 15 min, and maximum aperture value. All objects were standardized using an 18% gray card, Kodak color separation guide, and a 15 cm length scale. All images were developed in Photoshop Lightroom 3.4.1. And finally, no exposure or chromatic changes were applied (i.e., output settings were not optimized for each image).

Specimens were examined from 4 collections: Natural History Museum in Tartu, the personal collection of Prof. Stanislav Komárek (Prague), Natural History Museum in Prague, and Natural History Museum in Vienna. A total of 407 specimens (155 males/252 females) of *P. napi* were acquired. Information about the location and date of collection was recorded from tags to obtain accurate information about spatial position. See Table S1 and Figure 1.

Measurement of UV reflectance from images

The 3 patches our analysis focused on were defined by morphologically corresponding (homologous) elements on the dorsal side of the butterfly wing. These were wing

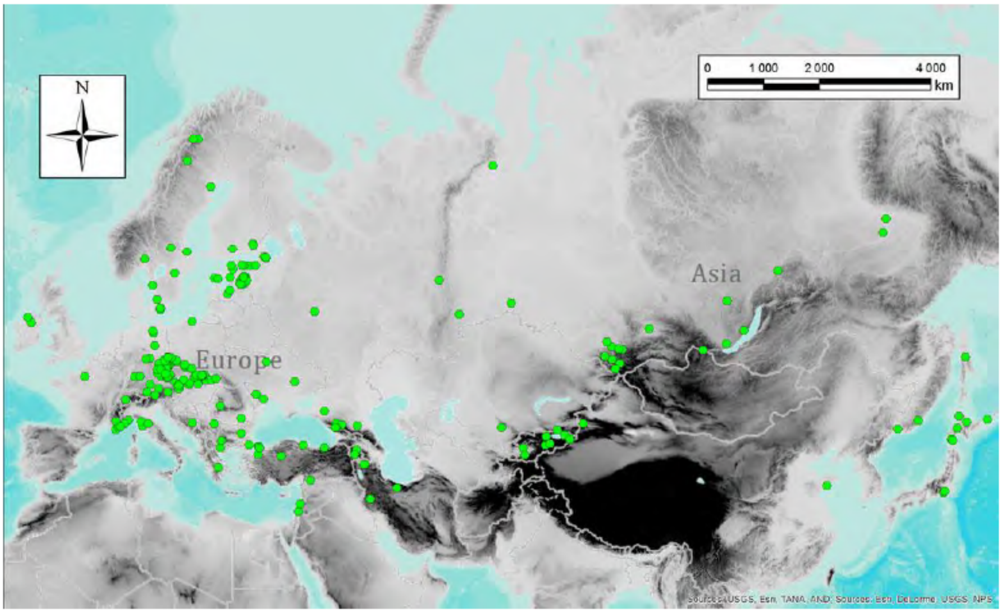


Fig. 1 Locations of the 407 specimens of the green-veined white (Pieris napi) from the Palaearctic region. All data are available in Table S1.

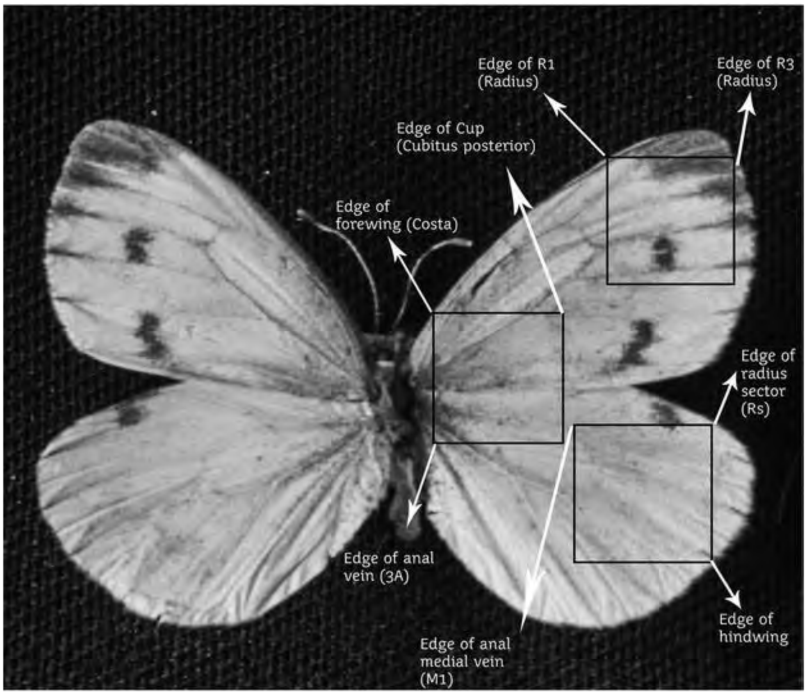


Fig. 2 Patches of measured UV reflectance on butterfly wing delimited by morphologically homologous elements.

veins, specifically Radius (R1 and R3), Cubitus posterior veins and edge of forewing (Costa) on the forewing and Radius (Rs), Medial (M1), Anal (3A) veins on the hindwing (see Fig. 2). The average perimeter was 650 pixels, that is, the *XY* length was 150 pixels. Calibrated UV images of the patches were analyzed using software Image-J ver. 1.46 (Abràmoff et al., 2004). The following values were calculated for the chosen patches: (a) an area (sum of pixels), (b) the mean (the average gray value being the sum of the gray values of all pixels divided by the number of pixels, and for RGB images each pixel converted to grayscale using the formula gray = red + green + blue), (c) standard deviation (i.e., standard deviation of the gray values), (d) modal gray value, (e) minimum and maximum values within the selection, (f) the perimeter (i.e., length of outside boundary of the selection), and (g) the median (i.e., the median value of the selected pixels). The means were used for further analyses of UV reflectance.

Statistical analysis

To investigate the influence of the environment on the UV reflectance, we employed 2 statistical techniques: First we used linear modeling (*lm* function in R) based on the idea that UV reflectance depends on the main environmental factors, that is, annual precipitation, annual temperature, and on latitude. Previous studies had shown that these variables are the main correlates of UV reflectance (Obara *et al.*, 2008a,b). The dependent variable was set to UV reflectance and independent ones were set to the 2 main environmental variables and latitude. We used the function *Calc.relimp* (*lmg metrics*) in R to measure the relative importance metrics for this linear model (Grömping, 2007). Furthermore, we employed a generalized linear model (*glm*) to investigate the relationship between UV reflectance and the first 2 PCs on all environmental and geographical variables. All statistics were calculated in R software ver. 3.2.3 (R Development Core Team, 2015) using packages "stat" and "ggplot2." All effect sizes were reported as adjusted R^2.

Results

Sexual dichromatism

We analyzed differences in UV reflectance between males and females using a method of digital photography expressed as an RGB signal of an image. The results of Shapiro–Wilk confirmed nonnormally distributed data (males: $N = 155$, $W = 0.962$, $P < 0.001$; females: $N = 252$, $W = 0.954$, $P < 0.001$), which prompted us to use

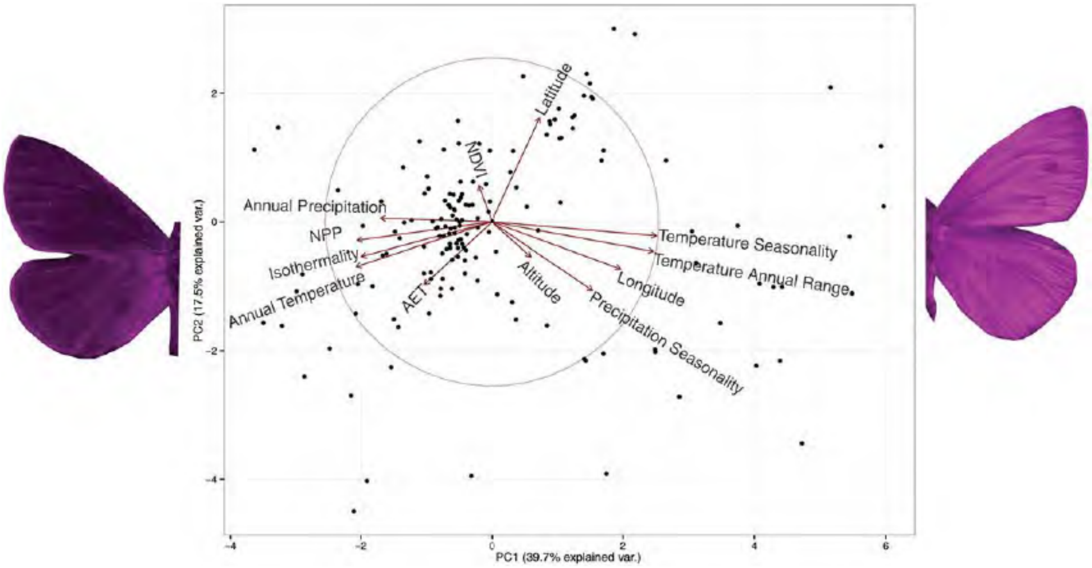


Fig. 3 PCA of geographical and environmental variables of female specimens for UV reflectance. The first axis explains 39.7% and the second axis 17.5% of all variability. Red vector stands for geographical and environmental variables.

a nonparametric method of analysis. The nonparametric Mann–Whitney U test revealed significant differences ($P < 0.001$) in UV reflectance signals between males and females, whereby females exhibited a 25% greater reflectance than the males (Fig. 5).

The effect of variables on UV reflectance

We tested whether the time elapsed since the specimen was caught had any effect on UV reflectance, but did not find any significant correlation between the date of collection and UV reflectance ($N = 322$, $t = 1.453$, $P = 0.147$). Since the date of capture was not indicated in all specimens, we analyzed only the 322 specimens for which the data were available.

Females

For females, UV reflectance was statistically significantly associated with latitude ($t_{(3,247)} = 3.24$, $P < 0.001$, $R^2 = 0.076$) and mean annual temperature ($t_{(3,247)} = -2.351$, $P = 0.016$, $R^2 = 0.058$). Mean annual precipitation ($t_{(3,247)} = -1.730$, $P = 0.085$, $R^2 = 0.017$) does not significantly correlate with UV reflectance. The proportion of variance explained by this model was 15%. Females are on average brighter in UV in northern areas with lower mean annual temperature.

The principal component analysis of the 12 geographic and environmental variables resulted in 2 PCA axes for female specimens (Fig. 3). The first PCA axis explained 39.7% and the second 17.5% of all variability. The first axis represents mainly variations in temperature seasonality, annual temperature range, annual mean precipitation, annual mean temperature, Net Primary Production (NPP), and isothermality. This axis, therefore, represents mainly *environmental* variables. The second axis accounts for latitude, altitude, and Actual Evapotranspiration (AET). This axis represents mainly *geographic* variables.

Further, we used GLM to test the relationship between UV reflectance (dependent variable) and both environmental PCs. Variation in UV reflectance turned out to significantly correlate with ecological factors associated with PC1 ($t_{(1,249)} = 4.325$, $P = <0.001$) as well as with those associated with PC2 ($t_{(1,249)} = -4.415$, $P = <0.001$). The GLM model explained 12.6% of overall variability. Female butterflies possess lower levels of UV reflectance in south-westerly regions of the Palaearctic and in localities with lower altitude. Moreover, a high level of temperature seasonality (variability of temperature during the year) and annual temperature range (range of temperature between the warmest and the coldest month of the year) is associated with higher levels of UV reflectance. We also found that the rate of precipitation, NPP, isothermality, annual temperature, and AET negatively correlate with the level of UV reflectance.

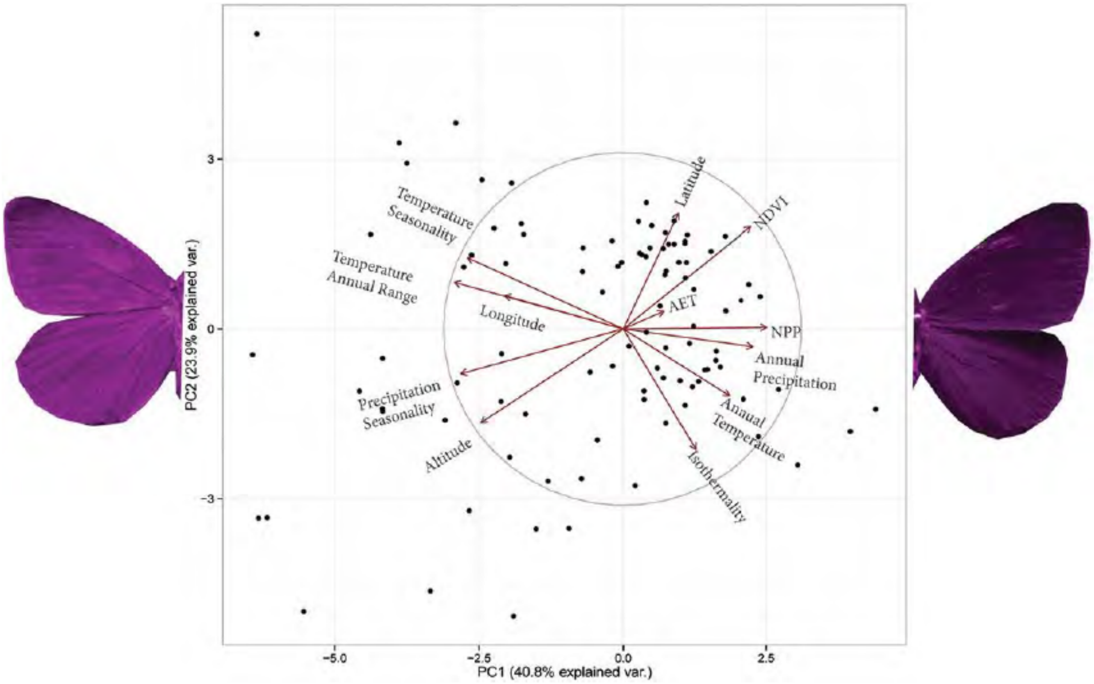


Fig. 4 Biplot showing first 2 principal components for male specimens. The first component axis explains 40.8% and the second axis 23.9% of all variability from the dataset. Red vectors stand for geographical and environmental variables.

Males

In males, UV reflectance was significantly correlated with latitude ($t_{(3,151)} = 2.862$, $P < 0.01$, $R^2 = 0.072$), annual temperature ($t_{(3,151)} = -1.942$, $P = 0.05$ $R^2 = 0.035$), and mean annual precipitation ($t_{(3,151)} = 3.024$, $P < 0.01$, $R^2 = 0.047$). The model explained 15.4% of variance. Northern areas characterized by low temperature and low rates of precipitation are inhabited by butterflies with a higher level of UV reflectance.

When we ran PCA on male data, the first axis accounted for 40.8% and the second axis for 19% of all variation (Fig. 4). The first axis stands for temperature seasonality, temperature annual range, precipitation seasonality, NPP, precipitation annual mean, and temperature annual mean. The second PC axis represents latitude, NDVI, and isothermality.

GLM revealed no statistically significant relationship between UV reflectance and the first PCA axis ($t_{(1,153)} = -1.539$, $P = 0.126$). The second PCA axis, on the other hand, did significantly predict a variation in UV reflectance ($t_{(1,153)} = -3.724$, $P < 0.001$). *P. napi* with stronger UV reflectance inhabit northern areas and lower altitudes. *P. napi* butterflies generally show stronger UV reflectance in areas with high values of NDVI and low values of isothermality. This model explains 9.5% of overall variability.

Discussion

Our results confirmed that there are significant differences in UV reflectance between the males and the females of *Pieris napi* and showed that UV reflectance in this species is also correlated with geographic and environmental variables. This latter result might suggest a causal link between the environment and observed variation in UV reflectance.

Sexual dimorphism in UV reflectance

UV reflectance in female *P. napi* was 25% more intense than that in male individuals. Previous studies had revealed that in 2 related *Pieris* species, sexual dimorphism in ultraviolet reflectance is employed in the recognition of sex of an individual in preference to chemical signals or wing patterns in the visible (VIS) spectrum (Rutowski, 1981; Obara *et al.*, 2008a). As shown by Makino *et al.* (1952) and Petersen and Luxton (1982), females prefer males with a higher degree of reflectance in the VIS spectrum (i.e., whiter specimens). The relatively low variation in the of UV reflectance in male *P. napi* could therefore be the result of a consistent female preference for white males. These males thus possess high fitness due to sexual selection

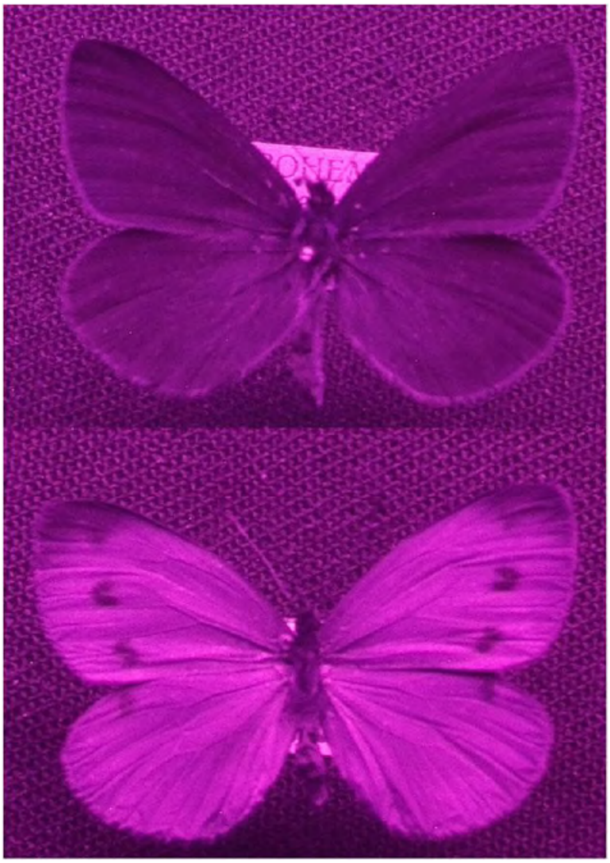


Fig. 5 The photograph demonstrates difference in UV reflectance between male (above) and female (beneath) specimen of *Pieris napi*.

even if their dorsal wing surface is constrained from evolving the dark color, which is a thermoregulatory adaptation for heat absorption (Tuomaala *et al.*, 2012; Shanks *et al.*, 2015).

A greater degree of "whiteness" can be explained by higher amounts of pterins, which decrease the level of diffuse UV reflectance and, at the same time, increase the reflectance in mid- to long-wavelength radiation in the VIS spectrum (Watt & Bowden, 1966; Morehouse *et al.*, 2007; Wijnen *et al.*, 2007). Males had been under sexual selective pressure from females (Turner, 1978), which is probably why the variation of UV reflectance in males is relatively low in comparison with the females.

The sex-specific photoreceptors discovered in *P. rapae crucivora*, whose sexual wing dichromatism is similar to that of *P. napi*, offer an alternative explanation. Males of this species possess a double-peaked sensitivity maximum in the violet region of the spectrum (around 425 nm), which differs strongly from what is found in females (Arikawa *et al.*, 2005). The sex-specific pigmentation of the males (pterins) has presumably evolved to improve a spectral discrimination of colors of conspecifics at around 400 nm, where wing reflectance between sexes abruptly changes (Obara & Majerus, 2000; Arikawa *et al.*, 2005; Stavenga & Arikawa, 2011).

UV reflectance and environmental conditions

Female *P. napi* generally exhibit a less intense UV reflectance in western and southern regions of the Palaearctic. We found that a high level of temperature seasonality and annual temperature range is associated with a more intense UV reflectance. Furthermore, the rate of precipitation, NPP, isothermality, annual temperature, and the AET negatively correlate with UV reflectance. Generally speaking, localities characterized by extreme values of a number of variables (such as high temperature seasonality and low temperatures) favor female *P. napi* butterflies with stronger UV reflectance. Males with stronger UV reflectance occur in areas with high values of NDVI and low values of isothermality. Similarly as in females, variables associated with a higher UV reflectance in males generally indicate that they live in an environment less suitable for breeding (especially in the peripheral populations of *P. napi*).

Based on these results, we suggest 2 possible but not necessarily exclusive explanations: First, higher levels of UV reflectance of butterfly wings, which help facilitate mate recognition, may be a way of compensating for low levels of environmental UV radiation in northern areas and lower altitude. At higher latitudes, the sun is lower in the horizon so the UV rays travel a greater distance through UV-absorbing layers of the atmosphere (Meyer-Rochow & Järvilehto 1997, Madronich *et al.*, 1998). We also suggest that in females, UV reflectance is negatively correlated with environmental UV irradiation.

Second, the more unfit for breeding environment (northern localities, low annual temperature, high levels of temperature variability during the year, etc.), the smaller the numbers of butterflies (Shreeve, 1981). Under these conditions, an encounter of 2 butterflies is thus less likely. As reported for *Pieris rapae crucivora* and *Pieris protodice*, the initial and final mate recognition by the male is based entirely on visual cues. The female UV pattern is therefore an essential factor (Obara, 1970; Rutowski, 1981) of mate recognition. Assuming that sensitivity to UV radiation in the eyes of the males is constant in all localities, high levels of UV reflectance observed in females that inhabit a hostile environment may thus be an adaptation to facilitate mate recognition (Meyer-Rochow & Järvilehto 1997).

Obara (2008a,b) suggests that a higher temperature and a long day photoperiod predict a higher level of UV reflectance on the butterfly wing. Although Obara's conclusions are based on the study of a different species (*P. rapae*), we are of the opinion that our model species is subjected to similar environmental pressures as *P. rapae* and our conclusions therefore contradict Obara (Obara *et al.*, 2008a). In our dataset, the negative correlation between temperature and levels of wing UV reflectance is statistically highly significant. It has been suggested that pterin granules in the wing scale, which are responsible for UV absorption, are the most nitrogen-rich pigments known (Morehouse *et al.*, 2007). Specimens surviving in low-nitrogen environments would thus find the synthesis and deposition of these compounds more difficult than individuals living in more favorable conditions (Giraldo & Stavenga, 2007; Kemp & Rutowski, 2011). One could thus expect that butterflies in habitats less suitable for breeding have lower levels of pterins and the level of UV reflectance in these specimens is higher (Morehouse & Rutowski, 2010a,b).

Moreover, the deposition of pterins in wing scales and UV coloration could be indicative of the quality (health status) of an individual. Males capable of obtaining sufficient amounts of nitrogen from plants have strongly UV-absorbing wing patterns. Such males, which appear whitest to the female butterfly eye, could therefore be favored by females. A similar scenario is known in *Polyomnatus icarus*, where the ultraviolet wing pattern is determined by the amount of flavonoids in the diet (Knuttel & Fiedler, 2001).

Numerous earlier studies have shown that seasonal generations of various insect species can differ in color, body size, behavior, and fertility (Shapiro, 1977; Stjernholm & Karlsson, 2008). The subject of our study, *P. napi*, is a multivoltine species (i.e., a species, which produces more than one generation during a year). This characteristic, however, depends to a large extent on environmental conditions. In other words, in hostile environmental conditions only 1 or 2 generations may be present, while warmer and more fertile localities may produce up to 4 generations a year (Cizek *et al.*, 2006). Phenotypic expressions differ between the generations. For example, spring specimens of *P. napi* can be easily distinguished from the second generation by a higher level of melanism in their wing pattern in the VIS spectrum (Shkurikhin & Oslina, 2015). We therefore suggest that environmental conditions are the primary factor, which determines both wing UV reflectance and the number of generations.

For further research, it would be interesting to measure the reflectance in the VIS spectrum (or at least to obtain standardized photographs in the VIS spectrum and thus measure the level of melanization) and correlate it with UV reflectance. Tuomaala *et al.* (2012) carried out such a research on a regional scale and demonstrated a negative correlation between the UV and VIS reflectance in females of *P. napi*. Her study (reported in the abovementioned paper) was based on specimens from across the Palaearctic region. If we consider smaller scale analyses, such as analyses by geographical regions, they too could provide valuable results. Such studies could focus on just 1 or 2 species, which would exclude potential differences in life histories and behaviors.

Conclusions

We have investigated the effects of environmental conditions on the level of UV reflectance in *P. napi*. Museum specimens proved to be a suitable source for quantitative analyses of butterfly wing patterns, since their UV patterns are stable. Males and females differ significantly in the level of UV reflectance. Environmental conditions significantly affect UV reflectance on the forewings of both sexes. In particular, less suitable conditions predict stronger UV reflectance. Two possible explanations were suggested: one based on pterin deposition, the other based on the notion of adaptation, which facilitates mate location. And while intraspecific communication cannot be based only on UV patterns, our finding of different levels of UV reflectance in the 2 sexes suggests that UV wing patterns could play a role in communication within the genus *Pieris*.

Acknowledgments

We wish to thank Jaan Luig, Pavel Chvojka, Stanislav Komárek, Gaal Sabine, Uwe Filter, and Blanca Huertas for providing butterfly specimens. This research was supported by the Charles University Grant project GAUK 764313.

Authors' contributions

DS collected museum data, designed the study, analyzed data and drafted the manuscript. PP helped with collecting of the data and participated in data analysis. VBMR helped draft the manuscript. KK conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

Disclosure

The authors declare no conflict of interest.

References

Abràmoff, M.D., Magalhães, P.J. and Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International*, 11, 36–42.

Arikawa, K., Wakakuwa, M., Qiu, X., Kurasawa, M. and Stavenga, D.G. (2005) Sexual dimorphism of short-wavelength photoreceptors in the small white butterfly, *Pieris rapae crucivora. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 25, 5935–5942.

Beddard, F.E. (1892) *Animal Coloration: An Account of the Principal Facts and Theories Relating to the Colours and Markings of Animals*. S. Sonnenschein, New York.

Briscoe, A.D. and Chittka, L. (2001) The evolution of color vision in insects. *Annual Review of Entomology*, 46, 471–510.

Brunton, C. and Majerus, M.N. (1995) Ultraviolet colours in butterflies: intra-or inter-specific communication? *Proceedings of the Royal Society of London Series B: Biological Sciences*, 260, 199–204.

Cizek, L., Fric, Z. and Konvicka, M. (2006) Host plant defences and voltinism in European butterflies. *Ecological Entomology*, 31, 337–344.

Cott, H.B. (1940) *Adaptive Coloration in Animals*. Methuen, London.

Darwin, C. (1871) *The Descent of Man*. John Murray, London.

DeVoe, R.D., Small, R.J. and Zvargulis, J.E. (1969) Spectral sensitivities of wolf spider eyes. *The Journal of General Physiology*, 54, 1–32.

Eguchi, E. and Meyer-Rochow, V.B. (1983) Trophospongium-like structures in an insect eye: response of retinula cells of *Papilio xuthus* (Lepidoptera) to irradiation with ultraviolet light. *Cell and Tissue Research*, 231, 519–526.

Eitschberger, U. (1984) Systematische Untersuchungen am *Pieris napi-bryoniae* Komplex (s.l.) (Lepidoptera, Pieridae). *Herbipoliana*, 1, 1–504.

Eklundh, L. (1995) Noise estimation in NOAA AVHRR maximum-value composite NDVI images. *Remote Sensing*, 16, 2955–2962.

Espeland, M., Aagaard, K., Balstad, T. and Hindar, K. (2007) Ecomorphological and genetic divergence between lowland and montane forms of the *Pieris napi* species complex (Pieridae, Lepidoptera). *Biological Journal of the Linnean Society*, 92, 727–745.

ESRI (2011) *ArcGIS Desktop: Release 10.1*, 1st edn, Redlands, California.

Fukano, Y., Satoh, T., Hirota, T., Nishide, Y. and Obara, Y. (2012) Geographic expansion of the cabbage butterfly (*Pieris rapae*) and the evolution of highly UV-reflecting females. *Insect Science*, 19, 239–246.

Giraldo, M. and Stavenga, D. (2007) Sexual dichroism and pigment localization in the wing scales of *Pieris rapae* butterflies. *Proceedings of the Royal Society B: Biological Sciences*, 274, 97–102.

Google Earth (2015) Version Earth 7.1.5.1557. Available: http://www.google.cz/intl/cs/earth/.

Grömping, U. (2007) Estimators of relative importance in linear regression based on variance decomposition. *The American Statistician*, 61, 139–147.

Hazel, W.N. and West, D.A. (1983) The effect of larval photoperiod on pupal colour and diapause in swallowtail butterflies. *Ecological Entomology*, 8, 37–42.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. and Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978.

Holben, B.N. (1986) Characteristics of maximum-value composite images from temporal AVHRR data. *International Journal of Remote Sensing*, 7, 1417–1434.

Hovanitz, W. (1944) The ecological significance of the color phases of *Colias chrysotheme* in North America. *Ecology*, 25, 45–60.

Huete, A., Didan, K., Miura, T., Rodriguez, E., Gao, X. and Ferreira, L. (2002) Overview of the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing of Environment*, 83, 195–213.

Huth, H. and Burkhardt, D. (1972) Der spektrale sehbereich eines violettohr-kolibris. *Naturwissenschaften*, 59, 650–650.

Kaitala, A. and Wiklund, C. (1994) Female mate choice and mating costs in the polyandrous butterfly *Pieris napi* (Lepidoptera: Pieridae). *Journal of Insect Behavior*, 8, 355–363.

Kemp, D.J. (2006) Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. *Evolutionary Ecology Research*, 8, 515–527.

Kemp, D.J. and Rutowski, R.L. (2011) The role of coloration in mate choice and sexual interactions in butterflies. *Advances in the Study of Behavior*, 43, 55–92.

Kemp, D.J., Rutowski, R.L. and Mendoza, M. (2005) Colour pattern evolution in butterflies: a phylogenetic analysis of structural ultraviolet and melanic markings in North American sulphurs. *Evolutionary Ecology Research*, 7, 133–141.

Kivelä, S.M. and Välimäki, P. (2008) Competition between larvae in a butterfly *Pieris napi* and maintenance of different life-history strategies. *Journal of Animal Ecology*, 77, 529–539.

Knüttel, H. and Fiedler, K. (2000) On the use of ultraviolet photography and ultraviolet wing patterns in butterfly morphology and taxonomy. *Journal of the Lepidopterists' Society*, 54, 137–144.

Knuttel, H. and Fiedler, K. (2001) Host-plant-derived variation in ultraviolet wing patterns influences mate selection by male butterflies. *The Journal of Experimental Biology*, 204, 2447–2459.

Lutz, F.E. (1924) Apparently non-selective characters and combinations of characters, including a study of ultraviolet in relation to the flower-visiting habits of insects. *Annals of the New York Academy of Sciences*, 29, 181–283.

Madronich, S., McKenzie, R.L., Björn, L.O. and Caldwell, M.M. (1998) Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology B: Biology*, 46, 5–19.

Makino, K., Satoh, K., Koike, M. and Ueno, N. (1952) Sex in *Pieris rapae* L. and the pteridin content of their wings. *Nature*, 170, 32–55.

Mazokhin-Porshniakov, G. (1957) Reflecting properties of butterfly wings and role of ultra-violet rays in the vision of insects. *Biophysics*, 2, 352–362.

Meyer-Rochow, V.B. (1991) Differences in ultraviolet wing patterns in the New Zealand lycaenid butterflies *Lycaena salustius*, *L. rauparaha*, and *L. feredayi* as a likely isolating mechanism. *Journal of the Royal Society of New Zealand*, 21, 169–177.

Meyer-Rochow, V.B. (1999) Compound eye: circadian rhythmicity, illumination, and obscurity. *Atlas of Arthropod Sensory Receptors—Dynamic Morphology in Relation to Function* (eds. E. Eguchi & Y. Tominaga), pp. 97–124. Springer Verlag, Tokyo.

Meyer-Rochow, V. and Järvilehto, M. (1997) Ultraviolet colours in *Pieris napi* from northern and southern Finland: Arctic females are the brightest! *Naturwissenschaften*, 84, 165–168.

Morehouse, N.I. and Rutowski, R.L. (2010a) Developmental responses to variable diet composition in a butterfly: the role of nitrogen, carbohydrates and genotype. *Oikos*, 119, 636–645.

Morehouse, N.I. and Rutowski, R.L. (2010b) In the eyes of the beholders: female choice and avian predation risk associated with an exaggerated male butterfly color. *The American Naturalist*, 176, 768–784.

Morehouse, N.I., Vukusic, P. and Rutowski, R. (2007) Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. *Proceedings of the Royal Society B: Biological Sciences*, 274, 359–366.

Mu, Q., Zhao, M. and Running, S.W. (2011) Improvements to a MODIS global terrestrial evapotranspiration algorithm. *Remote Sensing of Environment*, 115, 1781–1800.

Munakata, N., Cornain, S., Kanoko, M., Mulyadi, K., Lestari, S., Wirohadidjojo, W., Bolseé, D., Kazadzis, S., Meyer-Rochow, V. and Schuch, N. et al. Schuch N, Casiccia C, Kaneko M, Liu C-M, Jimbow K, Saida T, Nishigori C, Ogata K, IOnafuku K, Hieda K, and Ichihashi M (2006) Biological monitoring of solar UV radiation at 17 sites in Asia, Europe and South America from 1999 to 2004. *Photochemistry and Photobiology*, 82, 689–694.

Obara, Y. (1970) Studies on the mating behavior of the white cabbage butterfly, *Pieris rapae crucivora* Boisduval. *Zeitschrift für vergleichende Physiologie*, 69, 99–116.

Obara, Y. and Majerus, M.N. (2000) Initial mate recognition in the British cabbage butterfly, *Pieris rapae rapae*. *Zoological Science*, 17, 725–730.

Obara, Y., Ozawa, G. and Fukano, Y. (2008a) Geographic variation in ultraviolet reflectance of the wings of the female cabbage butterfly, *Pieris rapae*. *Zoological Science*, 25, 1106–1110.

Obara, Y., Koshitaka, H. and Arikawa, K. (2008b) Better mate in the shade: enhancement of male mating behaviour in the cabbage butterfly, *Pieris rapae* crucivora, in a UV-rich environment. *The Journal of Experimental Biology*, 211, 3698–3702.

Pecháček, P., Stella, D., Keil, P. and Kleisner, K. (2014) Environmental effects on the shape variation of male ultraviolet patterns in the Brimstone butterfly (*Gonepteryx rhamni*, Pieridae, Lepidoptera). *Naturwissenschaften*, 101, 1055–1063.

Petersen, H. and Luxton, M. (1982) A comparative-analysis of soil fauna populations and their role in decomposition processes. *Oikos*, 39, 287–388.

Poulton, E.B. (1890) *The Colours of Animals: Their Meaning and Use, Especially Considered in the Case of Insects*. D. Appleton, New York.

R Development Core Team (2015) *R: A Language and Environment for Statistical Computing*, 3.2.3 edn, R Foundation for Statistical Computing, Vienna.

Running, S.W., Nemani, R.R., Heinsch, F.A., Zhao, M., Reeves, M. and Hashimoto, H. (2004) A continuous satellite-derived measure of global terrestrial primary production. *Bioscience*, 54, 547–560.

Rutowski, R.L. (1991) The evolution of male mate-locating behavior in butterflies. *American Naturalist*, 138, 1121–1139.

Rutowski, R.L. (1981) Sexual discrimination using visual cues in the checkered white butterfly (*Pieris protodice*). *Zeitschrift für Tierpsychologie*, 55, 325–334.

Rutowski, R.L., Long, C.E., Marshall, L.D. and Vetter, R.S. (1981) Courtship solicitation by *Colias* females

(Lepidoptera: Pieridae). *American Midland Naturalist*, 105, 334–340.

Rutowski, R.L., Macedonia, J.M., Kemp, D.J. and Taylor-Taft, L. (2007) Diversity in structural ultraviolet coloration among female sulphur butterflies (Coliadinae, Pieridae). *Arthropod Structure & Development*, 36, 280–290.

Shanks, K., Senthilarasu, S. and Mallick, T.K. (2015) White butterflies as solar photovoltaic concentrators. *Scientific Reports*, 5. Available: http://www.nature.com/articles/srep12267.

Shapiro, A.M. (1977) Phenotypic induction in *Pieris napi* L.: role of temperature and photoperiod in a coastal California population. *Ecological Entomology*, 2, 217–224.

Shkurikhin, A. and Oslina, T. (2015) Seasonal phenotypic plasticity of the polyvoltine white butterfly *Pieris napi* L. (Lepidoptera: Pieridae) in the southern Urals. *Russian Journal of Ecology*, 46, 96–102.

Shreeve, T.G. (1981) Flight patterns of butterfly species in woodlands. *Oecologia*, 51, 289–293.

Silberglied, R.E., Taylor, J. and Orley, R. (1978) Ultraviolet reflection and its behavioral role in the courtship of the sulfur butterflies *Colias eurytheme* and *C. philodice* (Lepidoptera, Pieridae). *Behavioral Ecology and Sociobiology*, 3, 203–243.

Silberglied, R.E. (1984) Visual communication and sexual selection among butterflies, *The biology of butterflies. Symposium of the Royal Entomological Society of London Number* 11., eds. R.I. Vane-Wright & P.R. Ackery, Academic Press, London, pp. 207–223.

Silberglied, R. and Taylor, O. (1973) Ultraviolet differences between the sulphur butterflies, *Colias eurytheme* and *C. philodice*, and a possible isolating mechanism. *Nature*, 241, 406–408.

Stavenga, D.G. and Arikawa, K. (2011) Photoreceptor spectral sensitivities of the small white butterfly *Pieris rapae* crucivora interpreted with optical modeling. *Journal of Comparative Physiology A*, 197, 373–385.

Stevens, M., Parraga, C.A., Cuthill, I.C., Partridge, J.C. and Troscianko, T.S. (2007) Using digital photography to study animal coloration. *Biological Journal of the Linnean Society*, 90, 211–237.

Stjernholm, F. and Karlsson, B. (2008) Flight muscle breakdown in the green-veined white butterfly, *Pieris napi* (Lepidoptera: Pieridae). *European Journal of Entomology*, 105, 87–91.

Tuomaala, M., Kaitala, A. and Rutowski, R.L. (2012) Females show greater changes in wing colour with latitude than males in the green-veined white butterfly, *Pieris napi* (Lepidoptera: Pieridae). *Biological Journal of the Linnean Society*, 107, 899–909.

Turner, J.R.G. (1978) Why male butterflies are non-mimetic: natural selection, sexual selection, group selection, modification and sieving. *Biological Journal of the Linnean Society*, 10, 385–432.

Välimäki, P. and Kaitala, A. (2007) Life history tradeoffs in relation to the degree of polyandry and developmental pathway in *Pieris napi* (Lepidoptera, Pieridae). *Oikos*, 116, 1569–1580.

Välimäki, P. and Kaitala, A. (2006) Does a lack of mating opportunities explain monandry in the green-veined white butterfly (*Pieris napi*)? *Oikos*, 115, 110–116.

Watt, W.B. and Bowden, S.R. (1966) Chemical phenotypes of pteridine colour forms in *Pieris* butterflies. *Nature*, 210, 304–306.

Wedell, N., Wiklund, C. and Cook, P.A. (2002) Monandry and polyandry as alternative lifestyles in a butterfly. *Behavioral Ecology*, 13, 450–455.

Wijnen, B., Leertouwer, H. and Stavenga, D. (2007) Colors and pterin pigmentation of pierid butterfly wings. *Journal of Insect Physiology*, 53, 1206–1217.

Zhao, M., Heinsch, F.A., Nemani, R.R. and Running, S.W. (2005) Improvements of the MODIS terrestrial gross and net primary production global data set. *Remote Sensing of Environment*, 95, 164–176.

Zhao, M. and Running, S.W. (2010) Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. *Science*, 329, 940–943.

Manuscript received July 14, 2016
Final version received October 17, 2016
Accepted November 22, 2016

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Specimens with all variables.

Pecháček, P., Stella, D., Keil, P. and Kleisner, K. (2014) Environmental effects on the shape variation of male ultraviolet patterns in the Brimstone butterfly (*Gonepteryx rhamni*, Pieridae, Lepidoptera). *Naturwissenschaften*, 101, 1055–1063.

CHARLES UNIVERSITY
Faculty of science

Declaration of publication co-authorship

This is to confirm that PhD candidate David Stella significantly contributed to the following publication: *Pecháček, P., Stella, D., Keil, P. and Kleisner, K. (2014) Environmental effects on the shape variation of male ultraviolet patterns in the Brimstone butterfly (*Gonepteryx rhamni*, Pieridae, Lepidoptera). Naturwissenschaften, 101, 1055-1063.*

He reviewed the literature, acquired the data and worked on the manuscript revision.

The co-authors agree with submitting this publication as part of his PhD.

Doc. Mgr. Karel Kleisner PhD

Department of Philosophy and History of Science, Faculty of Science, Charles University

Naturwissenschaften (2014) 101:1055–1063
DOI 10.1007/s00114-014-1244-5

ORIGINAL PAPER

Environmental effects on the shape variation of male ultraviolet patterns in the Brimstone butterfly (*Gonepteryx rhamni*, Pieridae, Lepidoptera)

Pavel Pecháček · David Stella · Petr Keil · Karel Kleisner

Received: 16 May 2014 / Revised: 18 September 2014 / Accepted: 19 September 2014 / Published online: 4 October 2014

Abstract The males of the Brimstone butterfly (*Gonepteryx rhamni*) have ultraviolet pattern on the dorsal surfaces of their wings. Using geometric morphometrics, we have analysed correlations between environmental variables (climate, productivity) and shape variability of the ultraviolet pattern and the forewing in 110 male specimens of *G. rhamni* collected in the Palaearctic zone. To start with, we subjected the environmental variables to principal component analysis (PCA). The first PCA axis (precipitation, temperature, latitude) significantly correlated with shape variation of the ultraviolet patterns across the Palaearctic. Additionally, we have performed two-block partial least squares (PLS) analysis to assess covariation between intraspecific shape variation and the variation of 11 environmental variables. The first PLS axis explained 93 % of variability and represented the effect of precipitation, temperature and latitude. Along this axis, we observed a systematic increase in the relative area of ultraviolet colouration with increasing temperature and precipitation and decreasing latitude. We conclude that the shape variation of ultraviolet patterns on the forewings of male Brimstones is correlated with large-scale environmental factors.

Communicated by: Sven Thatje

P. Pecháček · D. Stella · K. Kleisner (✉)
Department of Philosophy and History of Science, Faculty of Science, Charles University in Prague, Viničná 7, 128 44 Praha 2, Czech Republic
e-mail: karel.kleisner@natur.cuni.cz

P. Keil
Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect Street, New Haven, CT 06520, USA

P. Keil
Center for Theoretical Study, Charles University and the Academy of Sciences of the Czech Republic, Jilská 1, 110 00 Praha 1, Czech Republic

Keywords Ultraviolet reflectance · Geographic variation · Evolution · Sexual selection · Geometric morphometrics · UV ornaments

Introduction

The discovery that some animals perceive ultraviolet radiation goes back to Darwin's contemporary John Lubbock, the 1st Baron Avebury (Lubbock 1882). Sensitivity to ultraviolet light has been observed in many invertebrates (Lutz and Richtmyer 1922; Lutz 1924, 1933b; DeVoe et al. 1969) and later also in vertebrates (Huth and Burkhardt 1972; Wright 1972; Silberglied 1979; Tovee 1995). Around the same time, it was also found that there exists a relationship between ultraviolet patterns in flowers and some of their pollinators (Lutz 1924). Moreover, UV-reflective surfaces with signalling and communicative functions have been found in many clades of invertebrates including butterflies (Lutz 1933a; Mazokhin-Porshnyakov 1957; Nekrutenko 1965a; Silberglied and Taylor 1978; Silberglied 1979, 1984; Eguchi and Meyer-Rochow 1983; Brunton and Majerus 1995; Brunton 1998), spiders (Heiling et al. 2003, 2005) and beetles (Pope and Hinton 1977).

As a model species for our study, we chose the Common Brimstone (*Gonepteryx rhamni*, Linnaeus, 1758), a Palaearctic species widely distributed from Western Europe to Eastern Asia (see Fig. 1). The genus *Gonepteryx* has been studied by zoologists since the early days of UV reflectance research (Mazokhin-Porshnyakov 1957). The ultraviolet wing pattern of *G. rhamni* was repeatedly under consideration as a trait of potential taxonomic value (Nekrutenko 1965b, 1968, 1970; Kudrna 1975). Later, the Brimstone became a popular model in studies focussing on the structural basis and physical nature of UV colouration in butterflies (Wijnen et al. 2007; Pirih et al. 2011; Wilts et al. 2011).

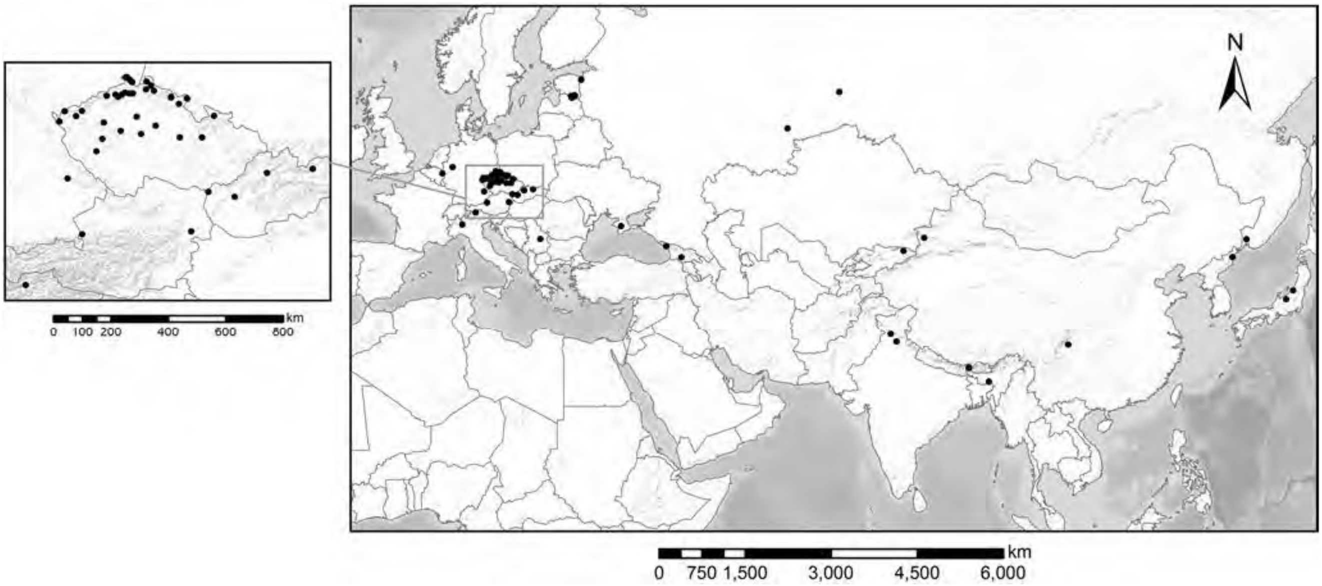


Fig. 1 Locations (*black dots*) of the 110 specimens of the Brimstone butterfly (*Gonepteryx rhamni*) from the Palaearctic region

Previous studies of butterflies have shown an environmental dependence of different aspects of wing colouration such as the variation in colour across the latitude (Hovanitz 1944), intensity of ultraviolet reflectance (Meyer-Rochow and Järvilehto 1997), degree of melanization (Ellers and Boggs 2002; Karl et al. 2009; Fischer and Karl 2010), and seasonal polyphenism together with variability in the expression of size and composition of the eyespots (Brakefield 1987; Brakefield and French 1999; Beldade and Brakefield 2002; Brakefield et al. 2007; De Jong et al. 2010). The ultraviolet colouration in butterflies may also be influenced by temperature and the quality of food ingested during development (Kemp et al. 2006; Kemp and Rutowski 2007; Kemp 2008b). These studies, however, focussed mainly on the intensity and hue of the UV pattern without taking into account the shape and relative size of ultraviolet patches on butterfly wings.

Although a role of the size of UV patterns in sexual selection has not yet been demonstrated in the Brimstone butterfly, it has been shown that females of butterfly *Bicyclus anynana* select males on the basis of the size and brightness of the ultraviolet-reflecting pupils of dorsal eyespots (Robertson and Monteiro 2005). Moreover, the absence or presence of ultraviolet wing pattern may serve as isolating mechanism in New Zealand lycaenid butterflies (Meyer-Rochow 1991). The importance of UV signals during mate choice was directly evidenced in *Eurema* and *Colias* species (Papke et al. 2007; Kemp 2008a). Based on an analogy with related pierid butterflies, we suppose that male dorsal UV patterns in *G. rhamni* may play a role in sexual selection and that this may influence the observed variation of this trait in the natural populations of the Brimstone.

Selection pressures acting upon the formation of animal patterns are not necessarily limited to sexual selection. Morphology can also be influenced by environmental selection, as demonstrated by classical examples such as the geographic patterns of body size (Bergmann 1847) and the length of extremities in ectotherms (Allen 1877). Recent studies of animal surfaces brought further evidence pertaining to geographic patterns of plumage colours in hummingbirds (Schmitz-Ornés 2006), the elytral patterns in carabid beetles (Kleisner et al. 2012) and colour patterns in bumblebees (Williams 2007). The variation of some traits, however, cannot be fully explained by an adaptive evolutionary hypothesis (Gould and Lewontin 1979; Kleisner et al. 2012). Although particular traits may later be co-opted for various functions such as sexual signalling, their variation cannot be sufficiently explained by these new selection pressures alone (Gould and Vrba 1982; Kleisner 2008, 2011; Maran and Kleisner 2010).

Our main goal was to find possible associations between the shape of UV patterns in *G. rhamni* and broad-scale environmental conditions (climate, productivity). We demonstrate that environmental conditions indeed correlate with the relative size and shape of the patterns and conclude with a discussion of possible evolutionary and ecological causes of these correlations.

Material and methods

Specimens

We have used a set of 110 individual male specimens: 59 observations were made in the Czech Republic and 51 observations come from the Palaearctic outside the Czech Republic. Photographs of all specimens were deposited in the entomological collections of the Natural History Museum of the University of Tartu (Estonia) and the National Museum in Prague (Czech Republic). We have recorded the geographic coordinates describing where each specimen was caught (Fig. 1).

Acquisition of photographs in the ultraviolet spectra (UV-A)

We have used a FujiFilm IS Pro digital camera which, thanks to its broad sensitivity to 330–900-nm spectrum, is suitable for UV photography (Pike 2011). The camera was equipped with an uncoated UV-transmitting lens. We used photographical filters B+W 403 and B+W BG 53. Ultraviolet band-pass filter B+W 403 (transmission range 290–410 nm with peak at 355 nm) filtered out the visible spectrum (400–700 nm) and the B+W BG 38 filter (transmission range 290–750 nm with peak at 500 nm) blocked the IR light ($\lambda > 700$ nm). To illuminate the photographed objects, we have used a UVP MRL-58 Multiple-Ray Lamp (8 W, 230 V-50 Hz, 0.16 A) equipped with mercury fluorescent lamp 8 W F8T5 long-wave 365 nm. All objects were illuminated under the angle of 45° and photographed in a standardized position (dorsal view). Based on our previous experience with the model species, the shape and size of ultraviolet pattern of *G. rhamni* remain the same even after a considerable change of the angle, which was also shown by Pirih et al. (2011). For all specimens, we used the following setting of the FujiFilm IS Pro camera: ISO 400, shutter time 15′ and aperture of 3.5. All images were standardized using 18 % grey card, Kodak colour separation guide and a 15-cm length scale.

Environmental and geographic correlates

As potential correlates of the UV patterns, we chose longitude, latitude and altitude. These broad-scale variables describe the spatial position of each specimen; we obtained them from locality labels on the pinned specimens. Then, we used these coordinates to assign (in ArcGIS 10.0; ESRI Inc.) to each specimen the mean annual temperature and precipitation, mean temperature in the warmest and coldest month, mean precipitation in the wettest and driest month, net primary productivity (NPP) and normalized difference vegetation index (NDVI) of the locality of the specimen. We have used these variables as predictors as they were previously demonstrated to affect insect distributions (Hawkins and Porter 2003; Battisti et al. 2005), development (Dixon et al. 2009), body size (Chown and Gaston 2010) and insect personality (Tremmel and Müller 2013). The data on temperature, precipitation and altitude came from 10 arc-min WorldClim layers (Hijmans et al. 2005). Our NPP layer came from the Postdam Institute for Climate Impact Research (Cramer et al. 1999): It represents an averaged (over 1961–1990) net production of organic compounds from atmospheric CO_2. NDVI represents the amount of green vegetation cover and was downloaded from NASA Goddard DAAC. To mitigate the effect of potential geo-referencing errors, we used climatic data of a relatively coarse resolution (note that finer, 2.5 and 5 arc-min, data were also available). We made this decision in order to minimize the risk of assigning an inappropriate (spatially mismatched) climate to the specimens.

To avoid co-linearity between predictors, we performed principal component analysis (PCA) on all climatic and geographic variables, centred to zero mean and standardized to variance of 1 (package 'stats' in R software; R Development Core Team 2012). We used only the first two axes of this PCA for further analyses.

Landmark definitions and procrustes analysis

Ultraviolet digital photographs of the left forewing of each of the 110 male specimens of *G. rhamni* were analysed using geometric morphometrics. At each forewing, we placed 32 landmarks (including 12 semi-landmarks) using tpsDig2 software ver. 2.14 (Rohlf 2009a). Landmarks are corresponding points which can be placed on the forewing of each specimen in the set, while semi-landmarks denote curves and outlines of the forewing where proper landmarks cannot be defined (for definitions of landmark and semi-landmark locations on the butterfly forewing, see Fig. 2).

All configurations of landmarks and semi-landmarks were superimposed by a generalized Procrustes analysis (GPA) performed in tpsRelw ver. 1.49 (Rohlf 2008). This

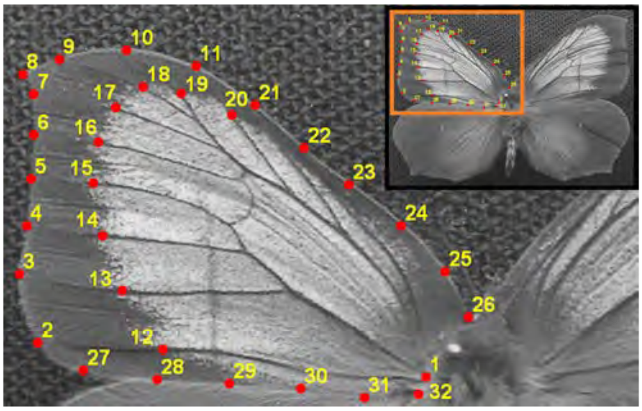


Fig. 2 A definition of landmarks on the left forewing. *Points 1–20* represent homologous anatomical landmarks found in all analysed subjects: landmark *1*, wing base (the connection of anal and cubital vein); landmark *8*, wing apex. The other landmarks are located at vein endings at the edge of the wing and the edge of the UV-reflecting pattern: landmarks *2* and *12*, first anal vein; landmarks *3* and *13*, cubitus 3; landmarks *4* and *14*, cubitus 2; landmarks *5* and *15*, cubitus 1; landmarks *6* and *16*, media 2; landmarks *7* and *17*, media 1; landmarks *9* and *18*, radius 4; landmarks *10* and *19*, radius 3; landmarks *11* and *20*, radius 2. *Points 21–32* represent semi-landmarks which serve to denote curves

procedure standardizes the size of objects and optimizes their rotation and translation until the coordinates of corresponding landmarks align as closely as possible. To reduce the dimensionality of procrustes residuals, we carried out a principal component analysis (PCA) in tpsRelw, ver. 1.49. The scores on the 10 PC axes carrying information about the wing shape were then saved and used for further analyses.

Correlations between environment and shape

To estimate the relationship between wing morphology and environmental (and geographic) variables, we have used two complementary analytical methods.

First, we applied the Permutational Multivariate Analysis of Variance using Distance Matrices (Adonis) function in the Vegan package in R (Oksanen et al. 2011) with Euclidean distance measure. We fitted a multivariate multiple regression model using Adonis, where the responses were the 10 shape PC axes which explain 90 % of shape variability of forewing and the predictors were the first two environmental PC axes. To control for shape variation due to allometry, we added wing size (computed as centroid size for each landmark configuration) as a covariate in the model. Shape changes associated with explanatory variables were visualized by thin-plate spline deformation grids available in tpsRegr, ver. 1.36 (Rohlf 2009b).

Second, we applied the two-block partial least squares (2B-PLS) method (Rohlf and Corti 2000) in order to explore covariation between the shape variables representing wing morphology and 11 environmental/geographic variables (unlike in the previous analysis, we used the original environmental/geographic variables, not PCs). Landmark configurations were transformed into partial warp scores (Rohlf et al. 1996) and analysed in tpsPLS software, ver. 1.18 (Rohlf 2006). In particular, the 2B-PLS created a pair of new variables which were linear combinations of variables within both of the original data sets (blocks). These new variables were produced so as to maximize covariation between the two original sets of variables (Rohlf and Corti 2000). Thin-plane splines were used to display the results in form of deformation grids of observed variation along the first PLS axis. These visualizations were made in tpsPLS 1.18 software (Rohlf 2006).

Geographic and environmental bias in specimen locations

There is a bias in the geographic distribution of our specimens (Fig. 1), since about half of them (59 out of 110) come from the small area of the Czech Republic, which contrasts with the rest coming from all over the Palaearctic. Such bias is commonly found in many large-scale data sets extracted from museum collections (Diniz-Filho et al. 2010). To assess whether the geographic bias affects our shape-environment correlations, we reran the 2B-PLS analysis using only specimens from outside the Czech Republic and compared them with results obtained from the complete data set.

Results

The principal component analysis of 11 environmental and geographical variables resulted in two interpretable PCA axes (Fig. 3). The first PC axis explained 46 % and the second 16 % of all variability. The first PC axis accounted mainly for the mean annual temperature, mean annual precipitation, mean precipitation in the wettest month, and latitudinal and longitudinal variation. The second axis represented variation in the NDVI and the mean precipitation in the driest month. Relative loadings of geographic and environmental variables for the first and second PC axes are presented in Table 1.

Multivariate multiple regressions (‘Adonis’ function in ‘vegan’ R package) of shape data on the two PCs extracted from the predictors (with wing centroid size as a covariate) showed that the first environmental PC axis significantly affects the shape space of the forewing ($F_{1,106}=15.49$, $p=0.0001$, $R^2=0.12$). The effect of the second PC axis was significant but it explained only a small part of variance ($F_{1,106}=3.48$, $p=0.02$, $R^2=0.027$). The effect of the centroid size was also significant ($F_{1,106}=3.05$, $p=0.029$, $R^2=0.023$).

All p values were based on 9,999 permutations. Specimens inhabiting environments with higher precipitation and temperature tend to have larger UV patches which cover most of the forewing surface.

The two-block partial least squares (2B-PLS) analysis (Rohlf and Corti 2000) focussed on covariation between the shape variables and the ecological variables. The first PLS axis accounted for 93 % of squared covariance (permutation test for 9,999 iterations, $p=0.0003$), while the second PLS axis accounted only for approximately 3 % and was not statistically significant ($p=0.999$). Correlations between variables and shape vectors were 0.57 ($p=0.0001$) for the first PLS axis and 0.46 ($p=0.0031$) for the second PLS axis. We have observed shape differences linked to the first PLS axis (Fig. 4): The association was principally with precipitation, temperature and latitude. Shape variation along the first PLS axis revealed constriction/dilation along the anterior-posterior axis of the wing. With an increase in temperature and precipitation and a decrease in latitude, the relative size of the ultraviolet pattern markedly increases at the expense of the UV non-reflective wing area (Fig. 4).

To examine the potential confounding effect of the remaining environmental bias, we reran the 2B-PLS analysis only on specimens from outside the Czech Republic. The relationship between shape and ecological variables remained clearly significant, $p<0.001$(permutation test for 9999 iterations); the first PLS axis accounted for 94 % of squared covariance. The correlation between variables and shape vectors was 0.65 ($p<0.001$).

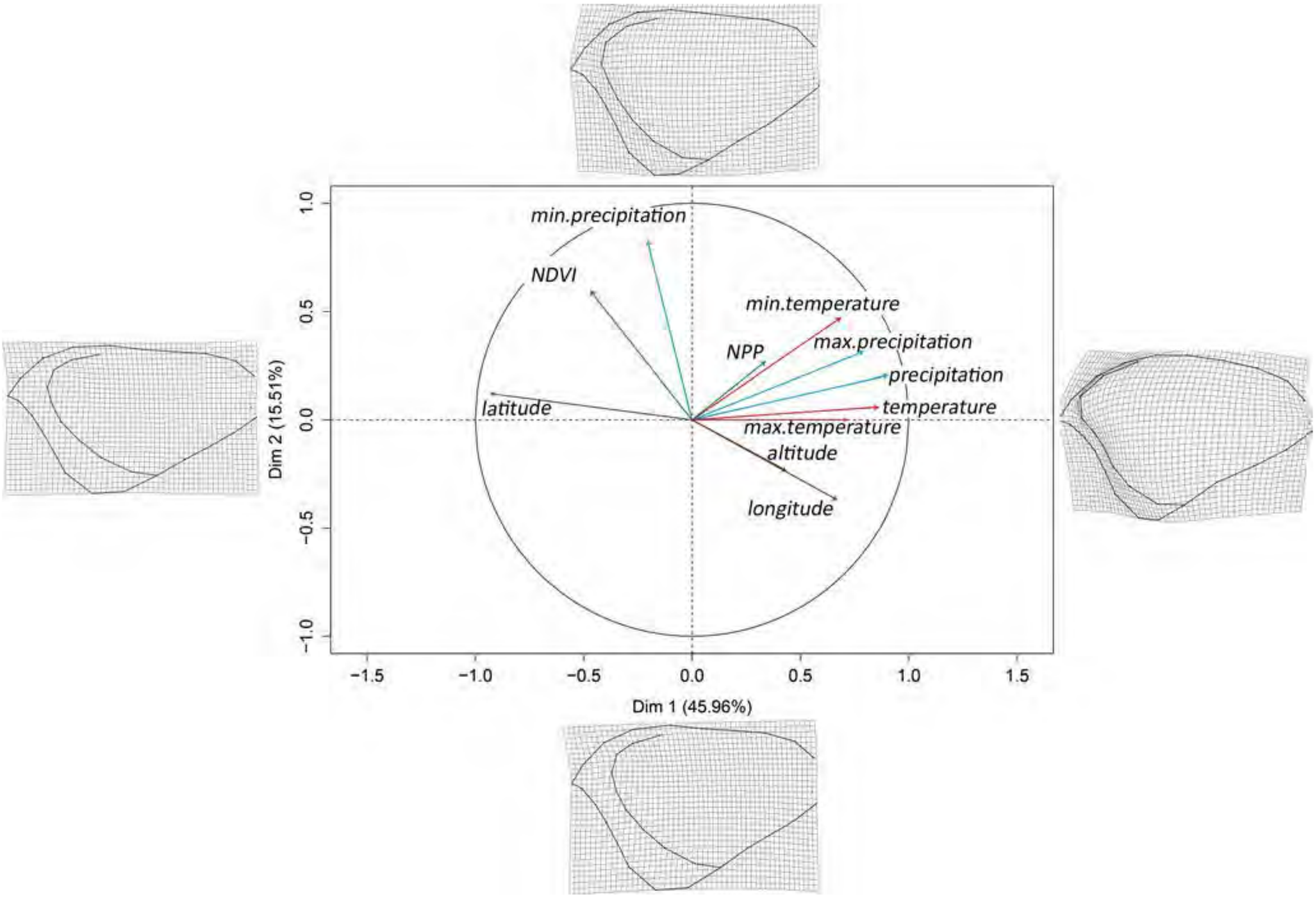


Fig. 3 Principal component analysis of environmental and topographical variables (standardized, centred). The first and second axes explain 46 and 15.5 % of variability in environmental data respectively. The first principal component accounts mainly for annual mean temperature and precipitation, mean temperature in the warmest month (*max.temperature*), and the latitudinal effect (*latitude*), while the second axis can be interpreted as a joined effect of the normalized difference vegetation index (NDVI) and the mean precipitation in the driest month (*min.precipitation*). *NPP* net primary production. Thin-plate spline visualizations are not part of the environmental principle component analysis. They were added manually (based on the regressions of the shape data to environmental principle components) to illustrate how the wing shape changes across a range of environmental conditions

Table 1 Relative loadings of geographic and environmental variables for first (PC1) and second (PC2) principal component

Ecological variable	PC1	PC2
Latitude	0.409	−0.092
Longitude	−0.296	0.282
Temperature	−0.408	−0.159
Precipitation	−0.352	−0.241
Altitude	−0.188	0.174
NDVI	0.210	−0.459
NPP	−0.151	−0.208
Precipitation minimum	0.093	−0.641
Precipitation maximum	−0.386	−0.046
Temperature minimum	−0.306	−0.362
Temperature maximum	−0.319	−0.001

NPP net primary production, *NDVI* normalized difference vegetation index

Discussion

We have demonstrated that the shape variability of ultraviolet patterns and the overall shape of the forewing correlate with the environmental conditions in which the specimens were collected, which may suggest a causal link between the environment and the observed variability. The area covered by the UV patterns on the wings decreases towards cooler and drier locations in higher latitudes, while specimens inhabiting warmer and more humid environment tend to have broader wings with UV patterns covering most of the forewing surface.

The wing area covered by the UV pattern covaries with forewing dimensions, and both are affected by the environment. The shape variation of the UV pattern and the shape variation of the whole forewing are not, however, isomorphic (see the deformation grids in Fig. 4), meaning that the area of

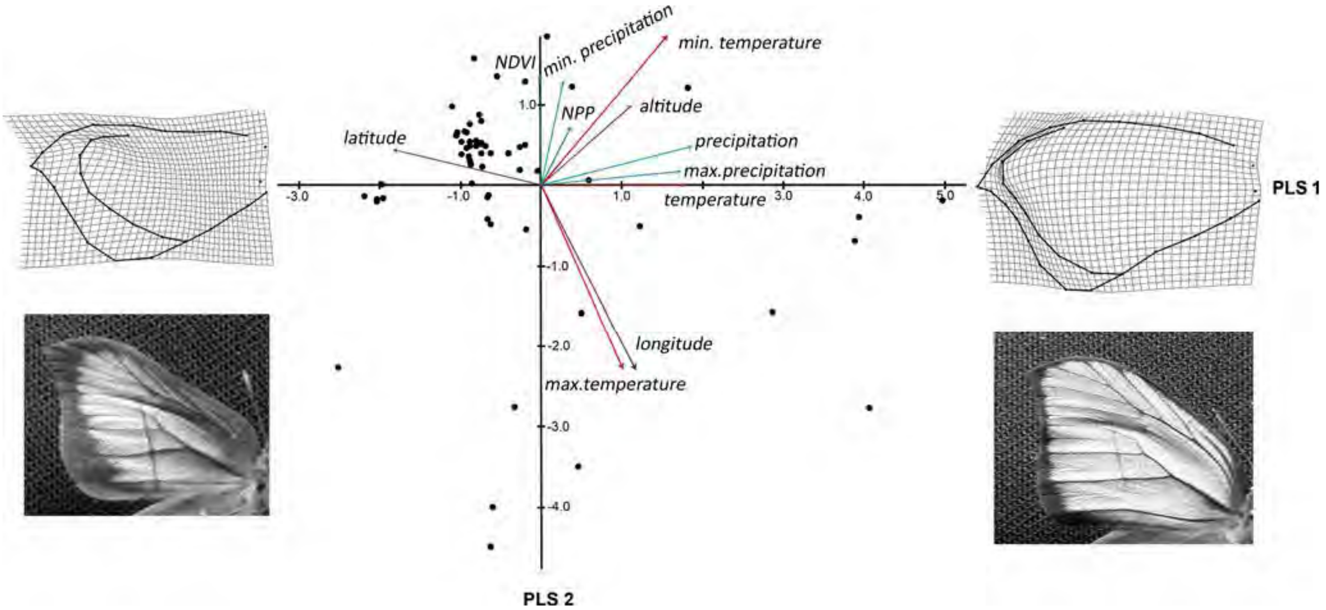


Fig. 4 Two-block partial least squares plot for projections of *Gonepteryx rhamni* specimens onto ordination vectors. Deformation grids on both extremes of the *x*-axis show changes in shape associated with the first axis. Vectors designate estimates of standardized scores for the mean annual temperature, mean temperature in the warmest (*max.temperature*) and the coldest month (*min.temperature*), mean annual precipitation, mean precipitation in the wettest (*max.precipitation*) and driest month (*min.precipitation*), altitude, net primary production (*NPP*), normalized difference vegetation index (*NDVI*), latitude and longitude

the wing covered by a UV pattern increases at a higher rate towards lower latitudes and especially towards higher temperatures and amounts of precipitation than the UV non-reflective wing area. This suggests that the UV pattern is more environment dependent than the overall forewing shape and that in addition to the hypothesized sexual selection (Silberglied and Taylor 1978; Silberglied 1984; Brunton and Majerus 1995; Kemp 2006; Kemp and Rutowski 2007, 2011), the environmental selection, too, may contribute to the variation of the UV patterns. The role of sexual selection in formation of the UV pattern in *G. rhamni*, however, has not been explicitly evidenced yet.

Temperature, which has the highest correlation with the first PLS axis, seems to be an especially significant environmental factor. In ectotherms, thermoregulatory costs of maintaining the main body temperature are rather high. It has been shown, for instance, that temperature—but not the energy supply—crucially affects the expression of sexually selected UV colouration in male European green lizards (Bajer et al. 2012). Ambient temperature during larval and early pupal development affects the metabolic rate of developing butterflies (Stevens 2004), which may further affect the expression of ultraviolet patterns (Kemp et al. 2006; Kemp and Rutowski 2007; Kemp 2008b; Prudic et al. 2011). Moreover, cold treatment of pupae in *Aglais urticae* led to a non-expression of UV spots on the wings of the adults (Anonymous 1910). It is thus possible that other ecological variables associated with the shape of UV patterns, such as precipitation and latitude, may affect the UV patterns indirectly.

The role of ecology and nutrition of larvae in the formation of wing scale morphology responsible for the reflectance and potential signalling was thoroughly studied in *Pieris* butterflies. It was shown that differences in the density of pterin granules deposited within the nanostructure of scales are responsible for sexual dichromatism of many pierid species (Morehouse et al. 2007). Although different dietary regimes influence the development and phenotype of *Pieris rapae*, the effect seems to be restricted to larvae (Morehouse and Rutowski 2010). Feeding experiments using other butterfly species have shown that composition of larval food (both the plant species and the plant parts) significantly affects the variation in UV patterns in the European Common Blue butterfly, *Polyommatus icarus* (Knuttell and Fiedler 2000). In *Colias eurytheme* and *Eurema hecabe*, the quality of larval food resources also affects some properties of the ultraviolet patterns, such as brightness (Kemp et al. 2006; Kemp and Rutowski 2007; Kemp 2008b).

Another explanation is that the male UV pattern of *G. rhamni* indicates a whole range of mate qualities (Kemp and Rutowski 2011) including the accessibility of larval resources, the ability to acquire and assimilate these resources, thermal stability of pupal development, developmental stability and resistance to environmental perturbations, or the effectiveness of morphogenetic mechanisms responsible for the partitioning of developmental resources to UV signalling structures (Kemp 2006, 2008b; Kemp et al. 2006; Kemp and Rutowski 2007). It seems that southern specimens have access to relatively richer resources compared to the northern specimens. The costly UV pattern could be an indicator of male's ability to assimilate and utilize these resources and can be subject to female sexual selection. Subsequently, the trade-off between the availability of environmental resources and the ability to assimilate these resources may be responsible for the latitudinal variation in the UV patterns.

Alternatively, the UV patches could be a protective adaptation against high UV exposure at high altitudes and low latitudes (Herman et al. 1999). If that were the case, UV-reflective patterns would cover larger wing areas in specimens from lower latitudes and higher altitudes (as in the case of *G. rhamni*), while in fact the opposite occurs in *Pieris napi* whose northernmost females possess brighter UV reflectivity than their southern sisters (Meyer-Rochow and Järvilehto 1997). Although our results may be seen as partially supporting this conjecture, there is a serious problem. In particular, one would need to find a good reason why such protection against the high levels of UV occurs only in males (as is the case of *G. rhamni*). Another possibility is that the dorsal UV patterns in males are primarily sexual patterns which were only later co-opted for a protective function. Females thus lack the adaptation simply because they have no dorsal pattern which could be used for such secondary protective function.

In order to disentangle the complex links between the development, environmental conditions, and the variation in the UV pattern, one would have to carry out breeding experiments with controlled exposure of the developing larvae to varying environmental factors. We also note that despite our efforts to account for it, our results could be affected by the uneven spread of our specimens in the environmental space and future research might benefit from a more systematic stratified random sampling. Further research could also investigate whether the comparatively smaller size of UV patterns in colder and drier environments is in any way compensated, for example, by higher intensity (or brightness) of UV reflectance. For the moment being, we tentatively conclude that the shape variation of UV patterns in male Brimstone butterflies may be due to a combination of both sexual and environmental selection.

Acknowledgments We wish to thank Jaan Luig and Pavel Chvojka for their help with providing the butterflies and David Hořák, Victor Benno Meyer-Rochow, Nathan Morehouse and two anonymous referees for their useful comments. This research was supported by the Czech Grant Agency project GACR P505/11/1459. PK was supported by EU FP7 People Programme (Marie Curie Actions; REA agreement no. 302868; project WORLDIVERSITY). PP and DS were supported by the Charles University Grant Agency project GAUK 764313.

References

Allen JA (1877) The influence of physical conditions in the genesis of species. Radic Rev 1:108–140

Anonymous (1910) Schmetterlinge. In: Meyers Grosses Konversations-Lexikon (6th edition, vol. 21). Bibliographisches Institut, Leipzig and Wien, pp 803–807

Bajer K, Molnar O, Torok J, Herczeg G (2012) Temperature, but not available energy, affects the expression of a sexually selected ultraviolet (UV) colour trait in male European green lizards. PLoS One 7: e34359. doi:10.1371/journal.pone.0034359

Battisti A, Stastny M, Netherer S, Robinet C, Schopf A, Roques A, Larsson S (2005) Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. Ecol Appl 15:2084–2096

Beldade P, Brakefield PM (2002) The genetics and evo-devo of butterfly wing patterns. Nat Rev Genet 3:442–452. doi:10.1038/nrg818

Bergmann C (1847) Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Göttinger Stud 3:595–708

Brakefield PM (1987) Tropical dry and wet season polyphenism in the butterfly *Melanitis leda* (Satyrinae): phenotypic plasticity and climatic correlates. Biol J Linn Soc 31:175–191. doi:10.1111/j.1095-8312.1987.tb01988.x

Brakefield PM, French V (1999) Butterfly wings: the evolution of development of colour patterns. BioEssays 21:391–401. doi:10.1002/(sici)1521-1878(199905)21:5<391::aid-bies6>3.0.co;2-q

Brakefield P, Pijpe J, Zwaan B (2007) Developmental plasticity and acclimation both contribute to adaptive responses to alternating seasons of plenty and of stress in *Bicyclus* butterflies. J Biosci 32: 465–475. doi:10.1007/s12038-007-0046-8

Brunton CFA (1998) The evolution of ultraviolet patterns in European *Colias* butterflies (Lepidoptera, Pieridae): a phylogeny using mitochondrial DNA. Heredity 80:611–616. doi:10.1046/j.1365-2540.1998.00336.x

Brunton CFA, Majerus MEN (1995) Ultraviolet colors in butterflies—intraspecific or inter-specific communication. Proc R Soc B Biol Sci 260:199–204. doi:10.1098/rspb.1995.0080

Chown SL, Gaston KJ (2010) Body size variation in insects: a macroecological perspective. Biol Rev 85:139–169

Cramer W et al (1999) Comparing global models of terrestrial net primary productivity (NPP): overview and key results. Glob Chang Biol 5:1–15. doi:10.1046/j.1365-2486.1999.00009.x

De Jong MA, Kesbeke FMNH, Brakefield PM, Zwaan BJ (2010) Geographic variation in thermal plasticity of life history and wing pattern in *Bicyclus anynana*. Clim Res 43:91–102. doi:10.3354/cr00881

DeVoe RD, Small RJ, Zvargulis JE (1969) Spectral sensitivities of wolf spider eyes. J Gen Physiol 54:1–32. doi:10.1085/jgp.54.1.1

Diniz-Filho JAF, De Marco JP, Hawkins BA (2010) Defying the curse of ignorance: perspectives in insect macroecology and conservation biogeography. Insect Conserv Divers 3:172–179. doi:10.1111/j.1752-4598.2010.00091.x

Dixon AFG, Honěk A, Keil P, Kotela MAA, Šizling AL, Jarošík V (2009) Relationship between the minimum and maximum temperature thresholds for development in insects. Funct Ecol 23:257–264. doi:10.1111/j.1365-2435.2008.01489.x

Eguchi E, Meyer-Rochow VB (1983) Ultraviolet photography of forty-three species of lepidoptera representing ten families. Annot Zool Jpn 56:10–18

Ellers J, Boggs CL (2002) The evolution of wing color in *Colias* butterflies: heritability, sex linkage, and population divergence. Evolution 56:836–840

Fischer K, Karl I (2010) Exploring plastic and genetic responses to temperature variation using copper butterflies. Clim Res 43:17–30. doi:10.3354/cr00892

Giese AC (1946) Comparative sensitivity of sperm and eggs to ultraviolet radiations. Biol Bull 91:81–87

Gould SJ, Lewontin RC (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc R Soc B Biol Sci 205:581–598. doi:10.1098/rspb.1979.0086

Gould SJ, Vrba ES (1982) Exaptation; a missing term in the science of form. Paleobiology 8:4–15

Hawkins BA, Porter EE (2003) Water–energy balance and the geographic pattern of species richness of western Palearctic butterflies. Ecol Entomol 28:678–686. doi:10.1111/j.1365-2311.2003.00551.x

Heiling AM, Herberstein ME, Chittka L (2003) Pollinator attraction: crab-spiders manipulate flower signals. Nature 421:334. doi:10.1038/421334a

Heiling AM, Chittka L, Cheng K, Herberstein ME (2005) Colouration in crab spiders: substrate choice and prey attraction. J Exp Biol 208: 1785–1792. doi:10.1242/jeb.01585

Herman JR, Krotkov N, Celarier E, Larko D, Labow G (1999) Distribution of UV radiation at the Earth's surface from TOMS-measured UV-backscattered radiances. J Geophys Res Atmos 104: 12059–12076. doi:10.1029/1999jd900062

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978. doi:10.1002/joc.1276

Hovanitz W (1944) The Ecological significance of the color phases of *Colias chrysotheme* in North America. Ecology 25:45–60

Huth HH, Burkhardt D (1972) Der spektrale Sehbereich eines Violettohr-Kolibris. Die Naturwissenschaften 59:650

Karl I, Geister TL, Fischer K (2009) Intraspecific variation in wing and pupal melanization in copper butterflies (Lepidoptera: Lycaenidae). Biol J Linn Soc 98:301–312. doi:10.1111/j.1095-8312.2009.01284.x

Kemp DJ (2006) Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. Evol Ecol Res 8:515–527

Kemp DJ (2008a) Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). Behav Ecol 19:1–8. doi:10.1093/beheco/arm094

Kemp DJ (2008b) Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. Evolution 62:2346–2358. doi:10.1111/j.1558-5646.2008.00461.x

Kemp DJ, Rutowski RL (2007) Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. Evolution 61:168–183. doi:10.1111/j.1558-5646.2007.00014.x

Kemp DJ, Rutowski RL (2011) The role of coloration in mate choice and sexual interactions in butterflies. In: Brockmann HJ, Roper T, Naguib M, Wynne-Edwards K, Barnard C, Mitani J (eds) Advances in the study of behavior, vol 43. Elsevier, Amsterdam, pp 55–92. doi:10.1016/B978-0-12-380896-7.00002-2

Kemp DJ, Vukusic P, Rutowski RL (2006) Stress-mediated covariance between nano-structural architecture and ultraviolet butterfly coloration. Funct Ecol 20:282–289. doi:10.1111/j.1365-2435.2006.01100.x

Kleisner K (2008) Homosemiosis, mimicry and superficial similarity: notes on the conceptualization of independent emergence of similarity in biology. Theory Biosci 127:15–21. doi:10.1007/s12064-007-0019-3

Kleisner K (2011) Perceive, Co-opt, modify, and live! Organism as a centre of experience. Biosemiotics 4:223–241

Kleisner K, Keil P, Jaroš F (2012) Biogeography of elytral ornaments in Palearctic genus *Carabus*: disentangling the effects of space, evolution and environment at a continental scale. Evol Ecol 26:1025–1040. doi:10.1007/s10682-011-9537-z

Knuttell H, Fiedler K (2000) On the use of ultraviolet photography and ultraviolet wing patterns in butterfly morphology and taxonomy. J Lepidopterol Soc 54:137–144

Kudrna O (1975) A revision of the genus Gonepteryx Leach (Lep., Pieridae). Entomol Gaz 26:3–37

Lubbock J (1882) Ants, bees, and wasps. A record of observations on the habits of the social Hymenoptera. D. Appleton and Co., New York

Lutz FE (1924) Apparently non-selective characters and combinations of characters, including a study of ultraviolet in relation to the flower-visiting habits of insects. Ann N Y Acad Sci 29:181–283

Lutz FE (1933a) Experiments with "stingless bees" (*Trigona cressoni parastigma*) concerning their ability to distinguish ultraviolet patterns. Am Mus Novit 641:1–26

Lutz FE (1933b) Invisible colors of flowers and butterflies. Nat Hist 33: 565–567

Lutz FE, Richtmyer FK (1922) The reaction of *Drosophila* to ultraviolet. Science 55:519–519

Maran T, Kleisner K (2010) Towards an evolutionary biosemiotics: semiotic selection and semiotic co-option. Biosemiotics 3:189–200

Mazokhin-Porshnyakov GA (1957) Reflecting properties of butterfly wings and the role of ultra-violet rays in the vision of insects. Biophysics 2:285–296

Meyer-Rochow VB (1991) Differences in ultraviolet wing patterns in the New Zealand lycaenid butterflies *Lycaena salustius*, *L. rauparaha*, and *L. feredayi* as a likely isolating mechanism. J R Soc N Z 21:169–177. doi:10.1080/03036758.1991.10431405

Meyer-Rochow VB, Järvilehto M (1997) Ultraviolet colours in *Pieris napi* from northern and southern Finland: arctic females are the brightest! Naturwissenschaften 84:165–168. doi:10.1007/s001140050373

Morehouse NI, Rutowski RL (2010) Developmental responses to variable diet composition in the cabbage white butterfly, *Pieris rapae*: the role of nitrogen, carbohydrates and genotype. Oikos 119:636–645. doi:10.1111/j.1600-0706.2009.17866.x

Morehouse NI, Vukusic P, Rutowski R (2007) Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. Proc R Soc B Biol Sci 274:359–366

Nekrutenko YP (1965a) Gynandromorphic effect and the optical nature of hidden wing-pattern in *Gonepteryx rhamni* L. (Lepidoptera, Pieridae). Nature 205:417–418

Nekrutenko YP (1965b) Three cases of gynandromorphism in *Gonepteryx*. J Res Lepidoptera 4:103–108

Nekrutenko YP (1968) Phylogeny and geographical distribution of the genus *Gonepteryx* (Lepidoptera, Pieridae): An attempt of study in historical zoogeography. Naukova dumka, Kiev

Nekrutenko YP (1970) A new subspecies of *Gonepteryx rhamni* from Tian-shan Mountains, U.S.S.R. J Lepid Soc 34:218–220

Oksanen J et al. (2011) vegan: Community ecology package. R package version 2.0–2

Papke R, Kemp D, Rutowski R (2007) Multimodal signalling: structural ultraviolet reflectance predicts male mating success better than pheromones in the butterfly *Colias eurytheme* L. (Pieridae). Anim Behav 73:47–54. doi:10.1016/j.anbehav.2006.07.004

Pike T (2011) Using digital cameras to investigate animal colouration: estimating sensor sensitivity functions. Behav Ecol Sociobiol 65: 849–858. doi:10.1007/s00265-010-1097-7

Pirih P, Wilts BD, Stavenga DG (2011) Spatial reflection patterns of iridescent wings of male pierid butterflies: curved scales reflect at a wider angle than flat scales. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197:987–997. doi:10.1007/s00359-011-0661-6

Pope RD, Hinton HE (1977) A preliminary survey of ultraviolet reflectance in beetles. Biol J Linn Soc 9:331–348. doi:10.1111/j.1095-8312.1977.tb00275.x

Prudic KL, Jeon C, Cao H, Monteiro A (2011) Developmental plasticity in sexual roles of butterfly species drives mutual sexual ornamentation. Science 331:73–75. doi:10.1126/science.1197114

R Development Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Robertson KA, Monteiro A (2005) Female *Bicyclus anynana* butterflies choose males on the basis of their dorsal UV-reflective eyespot pupils. Proc R Soc B Biol Sci 272:1541–1546. doi:10.1098/rspb.2005.3142

Rohlf JF (2006) TpsPLS (version 1.18). Department of Ecology and Evolution, State University of New York at Stony Brook, New York

Rohlf JF (2008) tpsRelw version 1.46. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook

Rohlf JF (2009a) TpsDig2 (version 2.14). New York: Department of Ecology and Evolution, State University of New York at Stony Brook

Rohlf JF (2009b) TpsRegr (version 1.36). New York: Department of Ecology and Evolution, State University of New York at Stony Brook

Rohlf FJ, Corti M (2000) Use of two-block partial least-squares to study covariation in shape. Syst Biol 49:740–753. doi:10.1080/106351500750049806

Rohlf FJ, Loy A, Corti M (1996) Morphometric analysis of old world talpidae (Mammalia, Insectivora) using partial-warp scores. Syst Biol 45:344–362. doi:10.1093/sysbio/45.3.344

Schmitz-Ornés A (2006) Using colour spectral data in studies of geographic variation and taxonomy of birds: examples with two hummingbird genera, *Anthracothorax* and *Eulampis*. J Ornithol 147: 495–503. doi:10.1007/s10336-006-0053-9

Silberglied RE (1979) Communication in the ultraviolet. Annu Rev Ecol Syst 10:373–398. doi:10.1146/annurev.es.10.110179.002105

Silberglied RE (1984) Visual communication and sexual selection among butterflies. In: Vane-Wright RI, Ackery PR (eds) The biology of butterflies. Academic, London, pp 207–223

Silberglied RE, Taylor OR (1978) Ultraviolet reflection and its behavioral role in courtship of sulfur butterflies *Colias eurytheme* and *Colias philodice* (Lepidoptera, Pieridae). Behav Ecol Sociobiol 3:203–243. doi:10.1007/Bf00296311

Stevens DJ (2004) Pupal development temperature alters adult phenotype in the speckled wood butterfly, *Pararge aegeria*. J Therm Biol 29: 205–210. doi:10.1016/j.jtherbio.2004.02.005

Tovee MJ (1995) Ultra-violet photoreceptors in the animal kingdom: their distribution and function. Trends Ecol Evol 10:455–460. doi:10.1016/S0169-5347(00)89179-x

Tremmel M, Müller C (2013) Insect personality depends on environmental conditions. Behav Ecol 24:386–392

Wijnen B, Leertouwer HL, Stavenga DG (2007) Colors and pterin pigmentation of pierid butterfly wings. J Insect Physiol 53:1206–1217. doi:10.1016/j.jinsphys.2007.06.016

Williams P (2007) The distribution of bumblebee colour patterns worldwide: possible significance for thermoregulation, crypsis, and warning mimicry. Biol J Linn Soc 92:97–118. doi:10.1111/j.1095-8312.2007.00878.x

Wilts BD, Pirih P, Stavenga DG (2011) Spectral reflectance properties of iridescent pierid butterfly wings. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197:693–702. doi:10.1007/s00359-011-0632-y

Wright AA (1972) The influence of ultraviolet radiation on the pigeon's color discrimination. J Exp Anal Behav 17:325–337. doi:10.1901/jeab.1972.17-325

Stella, D., Z. Faltýnek Fric, M. Rindoš, Kleisner, K. and Pecháček P. (2018) Distribution of Ultraviolet Ornaments in *Colias* Butterflies (Lepidoptera: Pieridae). *Environmental Entomology,* 47, 1344–1354.

CHARLES UNIVERSITY
Faculty of science

Declaration of publication co-authorship

This is to confirm that PhD candidate David Stella significantly contributed to the following publication: *Stella, D., Z. Faltýnek Fric, M. Rindoš, K. Kleisner, and P. Pecháček. 2018. Distribution of Ultraviolet Ornaments in* Colias *Butterflies (Lepidoptera: Pieridae). Environ. Entomol. 47,1344-1354.*

He reviewed the literature, acquired the data, drafted the manuscript and worked on the manuscript revision.

The co-authors agree with submitting this publication as part of his PhD.

Doc. Mgr. Karel Kleisner PhD

Department of Philosophy and History of Science, Faculty of Science, Charles University

Environmental Entomology, XX(X), year, 1–11
doi: 10.1093/ee/nvy111
Research

Molecular Ecology and Evolution

Distribution of Ultraviolet Ornaments in *Colias* Butterflies (Lepidoptera: Pieridae)

David Stella,[1,4] Zdeněk Faltýnek Fric,[2] Michal Rindoš,[2,3] Karel Kleisner,[1] and Pavel Pecháček[1]

[1]Department of Philosophy and History of Science, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 44 Praha 2, Czech Republic, [2]Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic, [3]University of South Bohemia, Faculty of Science, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic, and [4]Corresponding author, e-mail: david.stella@natur.cuni.cz

Subject Editor: Andy Michel

Received 4 April 2018; Editorial decision 5 July 2018

Abstract

Ultraviolet patterns in butterflies have been recognized and studied for many years. They are frequently involved in both intraspecific and interspecific interactions. Only a handful of studies, however, have investigated possible links between ultraviolet (UV) reflectance and ecological properties in some genera of the Lepidoptera as a whole. This study examines the impact of habitat and distribution on UV reflectance patterns on the wings of 106 species and subspecies of *Colias* butterflies. Based on standardized digital photographs, we performed a multivariate analysis of relations between UV reflectance, preferred habitat (alpine, arctic, dry grasslands, humid, forest, and ubiquitous), and distribution area (Afrotropical, Nearctic, Neotropical, European, Caucaso–Anatolian, boreal Eurasian, Central Asian mountains, northern China and Japan, and northern Oriental region). UV patterns occur more frequently in the male (60 taxa) than in female (25 taxa) Coliads. This difference in presence of UV patterns is used for differentiating between the males and females of a given species or subspecies. Further possible explanations of this phenomenon are also discussed. This study also shows that particular configurations of UV patterns are significantly associated with particular distribution areas. This relation is relatively strong but overall trends remain unclear. Based on the results of this study, it can be concluded that there exists a significant difference in the configuration of UV reflectance between the sexes, and that the configuration of UV reflectance significantly interacts with the geographical distribution of *Colias* species and subspecies.

Key words: Lepidoptera, UV reflectance, *Colias* butterflies, distribution, habitat

Ultraviolet (UV) patterns have been extensively studied since the 1930s (Lutz 1924, Lutz 1933). Various groups of butterflies have received different amount of attention, probably due to the presence or absence of conspicuous coloration in their species. UV patterns are known to exist in at least ten families of Lepidoptera (Meyer-Rochow 1991, Lyytinen et al. 2004, Obara et al. 2008a) and not only diurnal, but even nocturnal species of Lepidoptera exhibit wing patterns perceptible only in the UV part of the spectrum (Allyn and Downey 1977, Silberglied 1979, Brunton and Majerus 1995, Meyer-Rochow and Järvilehto 1997). In some species, UV patterns serve as a protection against predators (Lyytinen et al. 2004, Olofsson et al. 2010). It is also possible that they function as part of reproductive isolation mechanisms between sympatric species (Silberglied and Taylor 1973, Meyer-Rochow 1991, Imafuku 2008), and they seem to be involved in sexual interactions (Silberglied 1984, Stella et al. 2018). Diurnal butterflies in particular tend to exhibit sexual dichromatism that is often more pronounced in the UV range (Brunton and Majerus 1995). As important as the ability to produce UV patterns, however, it is the butterflies' ability to perceive UV radiation. Similarly to numerous other organisms, butterflies are capable of distinguishing colors (Arikawa et al. 2005, Stavenga and Arikawa 2006), and many species have UV-sensitive photoreceptors (Briscoe and Chittka 2001, Osorio and Vorobyev 2008). All in all, UV sensitivity plays an important role in butterflies' lives (Kemp and Rutowski 2011).

UV patterns on butterfly wings are formed by two distinct mechanisms. They can be determined by the presence of pigments (pigment coloration), which enhance the scattering of incident light in longer wavelengths and cause selective absorption of particular wavelengths (Wijnen et al. 2007, Wilts et al. 2011). This is typical of, for instance, the pierid butterflies (Morehouse et al. 2007). The second way in which UV patterns are formed is by special microscopic structures on wing surfaces that reflect the light of a small part of the spectrum (structural coloration). Patterns formed by structural coloration tend to be more conspicuous and 'purer' in terms of colors than pigment-based coloration (Kemp and Rutowski 2011). The resulting color of a pattern is, however, often the result of combination of both of these mechanisms (Rutowski et al. 2005).

Structural UV coloration is found in various butterfly taxa (Ghiradella et al. 1972, Kemp and Macedonia 2006, Wijnen et al. 2007, Rutowski et al. 2007b, Wilts et al. 2011), but its best description was produced for the genus *Colias* Fabricius, 1807. In *Colias* butterflies, UV patterns seem to serve a number of functions. In some cases, they seem to help individuals to recognize suitable potential sexual partners, thus preventing interspecific breeding (Silberglied et al. 1978). They also indicate the age and condition of their bearers (Kemp 2006). Kemp (2007) had demonstrated that UV pattern is sensitive to food quality and transient heat/cold shocks during metamorphosis. Moreover, UV patterns vary in relation to environmental conditions and can therefore signal some properties of the environment where the individual had developed. Temperature, rate of precipitation, and possibly also the level of UV radiation are the most important factors that influence the quality and presence of UV pattern, especially in Pieridae (Meyer-Rochow and Järvilehto 1997, Obara et al. 2008b, Pecháček et al. 2014). In the Coliads, the quality of the pattern also influences the females' choice of sexual partners (Papke et al. 2007). So far, most studies that dealt with the properties and importance of UV ornaments in the Coliads focused on the North American *Colias eurytheme* Boisduval, 1852 (Pieridae:Lepidoptera) (Silberglied et al. 1978, Papke et al. 2007, Rutowski et al. 2007b).

Two studies then surveyed the distribution of UV patterns in another 12 (Brunton 1998) and 26 (Kemp et al. 2005) European and American species of the genus. These studies aimed at ascertaining whether UV patterns indeed play a role in sexual selection. This research was based on a theoretical assumption that secondary sexual traits are subject to rapid evolution, i.e., that they evolve faster than traits that play no role in sexual selection (Iwasa and Pomiankowski 1995). Secondary sexual traits also frequently display a polyphyletic distribution within a taxon (Brunton 1998, Kemp et al. 2005). Both of the abovementioned studies had concluded that UV-reflective patterns in the Coliads exhibit both a pronounced polyphyletic distribution and a variability that are higher than that found in traits not subjected to sexual selection. Furthermore, UV patterns also serve as an important taxonomic trait; this has already been shown in *Colias alexandra* Edwards, 1863 (Pieridae:Lepidoptera) and related species (Nekrutenko 1965, Nekrutenko 1968, Ferris 1972, Ferris 1973, Knüttel and Fiedler 2000).

Variation in the UV patterns of Coliatid butterflies may be influenced by two kinds of selection regimes. The first kind of selection amounts to a claim that the emergence of UV patterns is constrained by environmental suitability (Obara et al. 2008a, Pecháček et al. 2014, Stella et al. 2018). In environments with high productivity, butterflies may be able to generate a UV pattern more easily so that occurrences of UV patches in less productive environments will be lower. The second kind of selection that should be considered is sexual selection, including the ability of UV signaling in open and high-latitude environments. We may thus expect the presence of species with highly developed UV patches in high productivity and/or environments characterized by high exposure to UV radiation.

Although UV ornaments do seem to play an important role in the lives of the Coliads, relatively few species have been investigated from this perspective. Moreover, existing comparative studies focus only on the males of some European and North American species, with females being completely neglected (Brunton 1998, Kemp et al. 2005). The aim of the present study was to carry out a comprehensive investigation of UV patterns within the genus *Colias*, with its entire geographical range and both sexes being taken into consideration. Apart from unique photographic data, this study also offers a thorough overview of the presence of UV patterns in *Colias* butterflies in relation to properties of the environment and ecological aspects of studied taxa. It is hoped that this overview could serve as a helpful instrument for other researchers looking for a suitable model species for their studies.

Materials and Methods

Genus *Colias*

The genus *Colias* contains 84 species and almost 120 subspecies (Grieshuber 2014), which inhabit almost all parts of the world. The only regions where Coliads are not found are Australian region and Southeast Asia. Most *Colias* species inhabit the Palearctic region: 20 species live in North America, another 5 species are present in South America all the way to Tierra del Fuego, and 2 species are known from the Afrotropics (Verhulst 2000, Grieshuber 2014). No comprehensive molecular phylogeny of the genus *Colias* has been proposed as yet. Only partial phylogenetic trees are available (Brunton 1998, Pollock et al. 1998, Wheat and Watt 2008, Laiho and Stahls 2013), which is also why the specific taxonomic structure of the genus varies between publications and many taxa are treated either as species or as subspecies (Verhulst 2000, Grieshuber 2014). It is thus likely that in future, we will witness some changes to the taxonomy of the genus.

The various species and subspecies of *Colias* butterflies are adapted to different habitats (alpine rocky slopes, wetlands, forest, cultivated habitats, or steppes). Generally speaking, they can be divided into mountain species, lowland species, and species inhabiting medium elevations. Most Coliads feed on plants belonging to the pea family (Fabaceae), with only a handful exceptions, such as *Colias behrii* Edwards, 1866 (Pieridae:Lepidoptera), *Colias gigantea* Verity, 1911 (Pieridae:Lepidoptera), *Colias interior* Scudder, 1862 (Pieridae:Lepidoptera), *Colias palaeno* Linnaeus, 1761 (Pieridae:Lepidoptera), *Colias pelidne* Boisduval & Le Conte, 1830 (Pieridae:Lepidoptera), and *Colias scudderi* Reakirt, 1865 (Pieridae:Lepidoptera) (Grieshuber 2014). Their host plants range from dwarf shrubs, such as *Vaccinium*, all the way to willows (Layberry et al. 1998). Lowland species, such as *Colias croceus* Geoffroy, 1785 (Pieridae:Lepidoptera), *Colias erate* Esper, 1805 (Pieridae:Lepidoptera), *C. eurytheme*, and *Colias hyale* Linnaeus, 1758 (Pieridae:Lepidoptera), usually have a wide range of distribution and tend to be polyvoltine (Tolman and Lewington 1997, Grieshuber 2014). In the mountains and in northern latitudes, Coliads (such as *Colias heos* Herbst, 1792 (Pieridae:Lepidoptera), *Colias christina* Edwards, 1863 (Pieridae:Lepidoptera), and *Colias nastes* Boisduval, 1832 (Pieridae:Lepidoptera)) usually feed on vetches, such as *Astragalus* sp. and *Hedysarium*. Several species of mainly New World Coliads (e.g., *Colias lesbia* Fabricius, 1775 (Pieridae:Lepidoptera) and *Colias philodice* Godart, 1819 (Pieridae:Lepidoptera)) are due to their effect on alfalfa fields viewed as agricultural pests (Tabashnik 1980, Serra et al. 2013).

Specimens

Photographs of all specimens were acquired from entomological collections of the Natural History Museum in London (BMNH, United Kingdom), Natural History Museum in Vienna (NHMW, Austria), Moravian Museum in Brno (MMBC, Czech Republic), and the National Museum in Prague (NMPC, Czech Republic). We have recorded information from the label of each specimen (such as place and date of collection) and in some cases reidentified the individuals. In total, we had at our disposal 106 taxa of *Colias* butterflies.

Photography Acquisition and UV Patches Recognition

We have used a FujiFilm IS Pro digital camera, which was well-suited for UV photography thanks to its broad sensitivity to the 330–900 nm (Pecháček et al. 2014). The camera is equipped with uncoated, UV-transmitting lenses. We used photographic filters B+W 403 and B+W BG 53. The black filter B+W 403 blocked the visible spectrum (400–700 nm), whereas the B+W BG 38 filter blocked the IR light ($\lambda > 700$ nm). To illuminate the photographed objects, we used a UVP MRL-58 multiple-Ray-Lamp (8-watt, 230 V–50 Hz, 0.16 A) equipped with a mercury fluorescent lamp 8w F8T5 with peak emission at 365 nm. All objects were illuminated at a 45° angle and photographed in a standardized position (dorsal view). For all specimens, we used the following setting of the FujiFilm IS Pro camera: ISO 400, shutter time 15′, and aperture of 22. All images were standardized using an 18% gray card, Kodak color separation guide, and a 15-cm photographic measuring tape.

A rotation of sample wings by 5° towards the chip plane would have resulted in a distinct decrease in the level of UV reflectance (±365 nm). Accurate position of the sample was thus of crucial importance. This also indicates that UV patterns on the wings of *Colias* butterflies have a prominent directional component which cannot be appreciated within the spectrum visible to human eye (Kemp and Macedonia 2006, Rutowski et al. 2007a, Wilts et al. 2011).

The two patches we focused on in order to reveal the configuration of UV pattern in our analysis were defined by morphologically corresponding (homologous) elements on the dorsal side of the butterfly wing. These were wing veins, specifically radius and cubitus posterior veins on the forewing (FW) and radius and medial veins on the hindwing (HW). The average perimeter was 650 pixels, that is, the XY length was 150 pixels. Calibrated UV images of the patches were analyzed using software Image-J ver. 1.46 (Abràmoff et al. 2004). The means of grayscale values were then used for further analyses of UV intensity (for more details, see Stella et al. (2018). Patches with a grayscale value of <180 were rated as nonreflective, whereas patches with a value of ≥180 were evaluated as UV reflective wings.

Statistical Analyses

We have distinguished two positions of a UV-reflecting pattern, on a FW and on a HW. The presence or absence of these patterns differs also between the sexes. This results in four different UV pattern configurations: FW for males/females and HW for males/females. We have traced a relation between the presence of these four UV pattern states (response variables) and the distribution and habitat of a species in question (explanatory variables). Based on the actual distribution of *Colias* species, we defined nine distribution areas (Grieshuber 2014): Afrotropical, Nearctic, Neotropical, European, Caucaso–Anatolian (including also the high Balkan Mountains), boreal Eurasian, Central Asian mountains, northern China and Japan, and a northern Oriental region excluding the alpine zone. With respect to habitats, we distinguished between alpine, arctic, dry grasslands, humid, forest, and ubiquitous species. Based on accessible information (Pena and Ugarte 1996, Tuzov et al. 1997, Layberry et al. 1998, Verhulst 2000, Grieshuber et al. 2012, Grieshuber 2014) as well as own experience, we have also included information about voltinism (uni- or polyvoltine). For details, see Supplementary Table S1.

We have analyzed the effect of distribution and habitat on the presence or absence of the four configurations of UV pattern using multivariate Canonical Correspondence analysis (CCA) implemented in the program Canoco 5, version 5.10 (Smilauer and Leps 2014). We have tested the significance of measured effects using Monte Carlo permutation test with 999 iterations.

Results

Out of the 106 taxa representing 54 species of *Colias* (Grieshuber 2014) we followed, we found a UV reflectance pattern in 62 taxa. The pattern is found significantly more frequently in males (60 taxa) than in females (25 taxa). In over one half of *Colias* taxa, UV pattern is present only in males. Moreover, even where a UV pattern is found in the females is not as bright as in the males of the same taxon (Fig. 1).

The patterns of UV on females are highly nested in the male UV patterns. If females have a UV pattern on their FWs, then UV pattern is almost always present on the FWs of the males of that taxon (92%, missing in *Colias dimera f. meridensis* Neuburger, 1905 (Pieridae:Lepidoptera) and *Colias christophi* Grum-Grshimailo, 1885 (Pieridae:Lepidoptera)). If females have a UV pattern on HWs, then UV pattern is present always on both FWs and HWs in the males of that species. The presence of UV patterns on FWs and HWs is closely correlated, but there are some exceptions. Ninety-six per cent of males that have a UV pattern on FWs also have a UV pattern on HWs, and 93% of taxa that have a UV pattern on HWs also have a UV pattern on FWs. When females have UV pattern on HWs, they always have also UV pattern on FWs, but when they have a UV pattern on FWs, UV pattern is present on HWs only in 68% of taxa.

It has also turned out that there exist certain regional tendencies. In particular, within a given region, the various species and subspecies of *Colias* butterflies tend to share the same configuration of UV pattern on their wings, and this holds even in cases where in other regions, these taxa display a different UV pattern configuration (Fig. 2). Moreover, in different regions, the proportion of *Colias* species and subspecies that possess particular UV pattern configurations distinctly varies. Coliads inhabiting the Afrotropical region and northern Oriental region show a highly consistent frequency of UV patterning within each sex (Africa 50%, northern Orient 60% in both sexes), whereas a highly erratic ratio in the configuration of UV pattern displays was found, for example, in taxa in South America and Caucaso–Anatolian region.

Regional distribution on its own predicted the presence of the four configurations of UV pattern extremely well: the model explained 26.7% of data variability ($F = 1.6$, $P = 0.016$ on the first axis; $F = 2.0$, $P = 0.16$ on all axes). The first axis divided the plot by sexes (Fig. 3). Taxa originating from northern China and North America have a UV pattern on their FW, but only in males. The second axis divided areas with a high proportion of UV on hind wings on males and FWs of females (especially Europe).

The effect of habitat was not significant ($F = 1.2$, $P = 0.086$) on the first axis, but some trends could be observed. The other axes did not significantly explain the pattern ($F = 1.4$, $P = 0.132$), and they jointly account for 24.65% of data variability. The first axis again divided the sexes (Fig. 4). For example, we found that a higher frequency of UV patterns in females was associated with polyvoltine or alpine species, whereas in females of univoltine, arctic, and forest species, a lower frequency of UV patterns was found. The situation

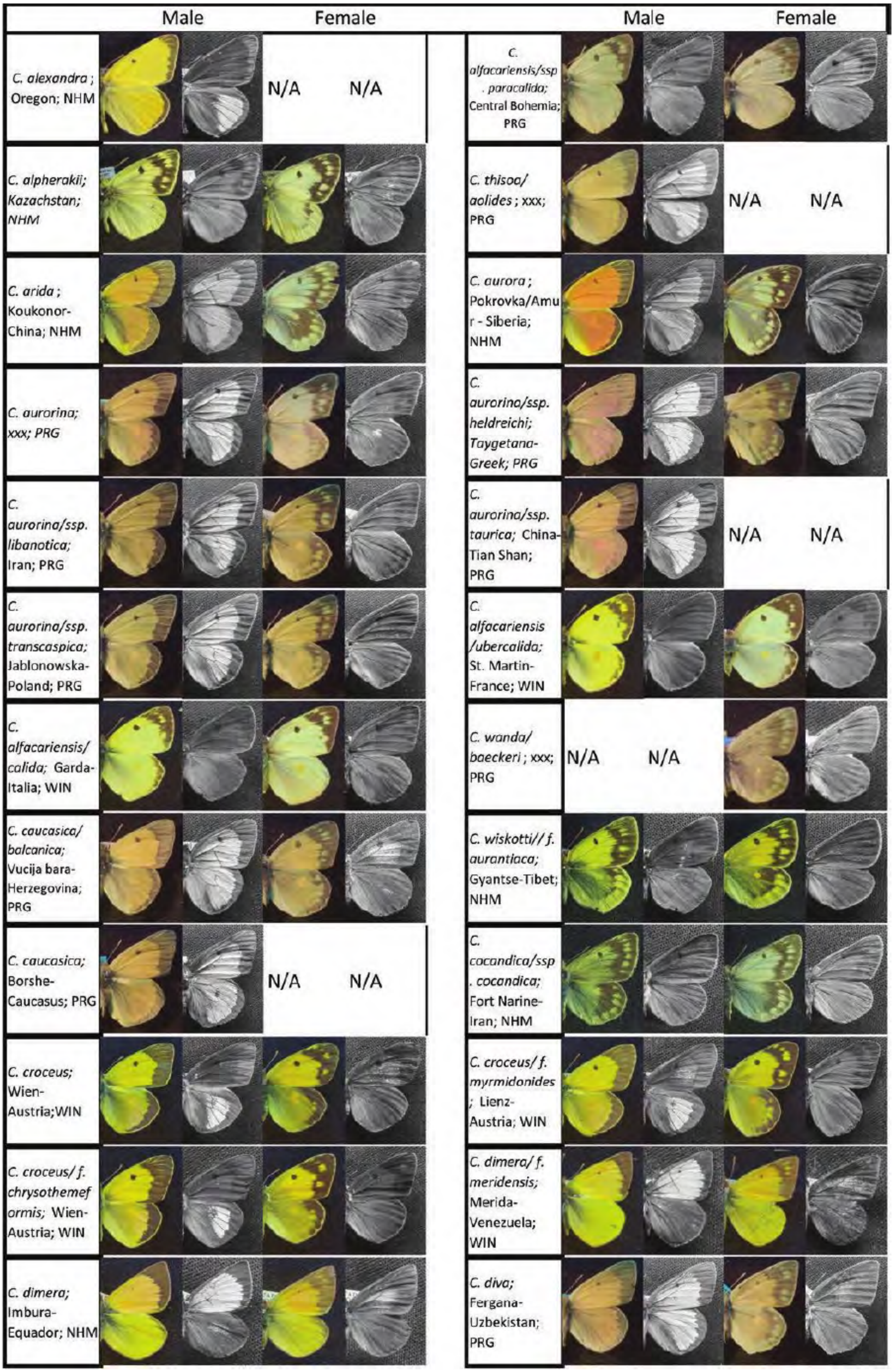


Fig. 1. A list of 106 taxa of *Colias* butterflies. Each taxon is presented in 4 photographs: 1. Male in a VIS light; 2. Male in UV light; 3. Female in VIS light; 4. Female in UV light.

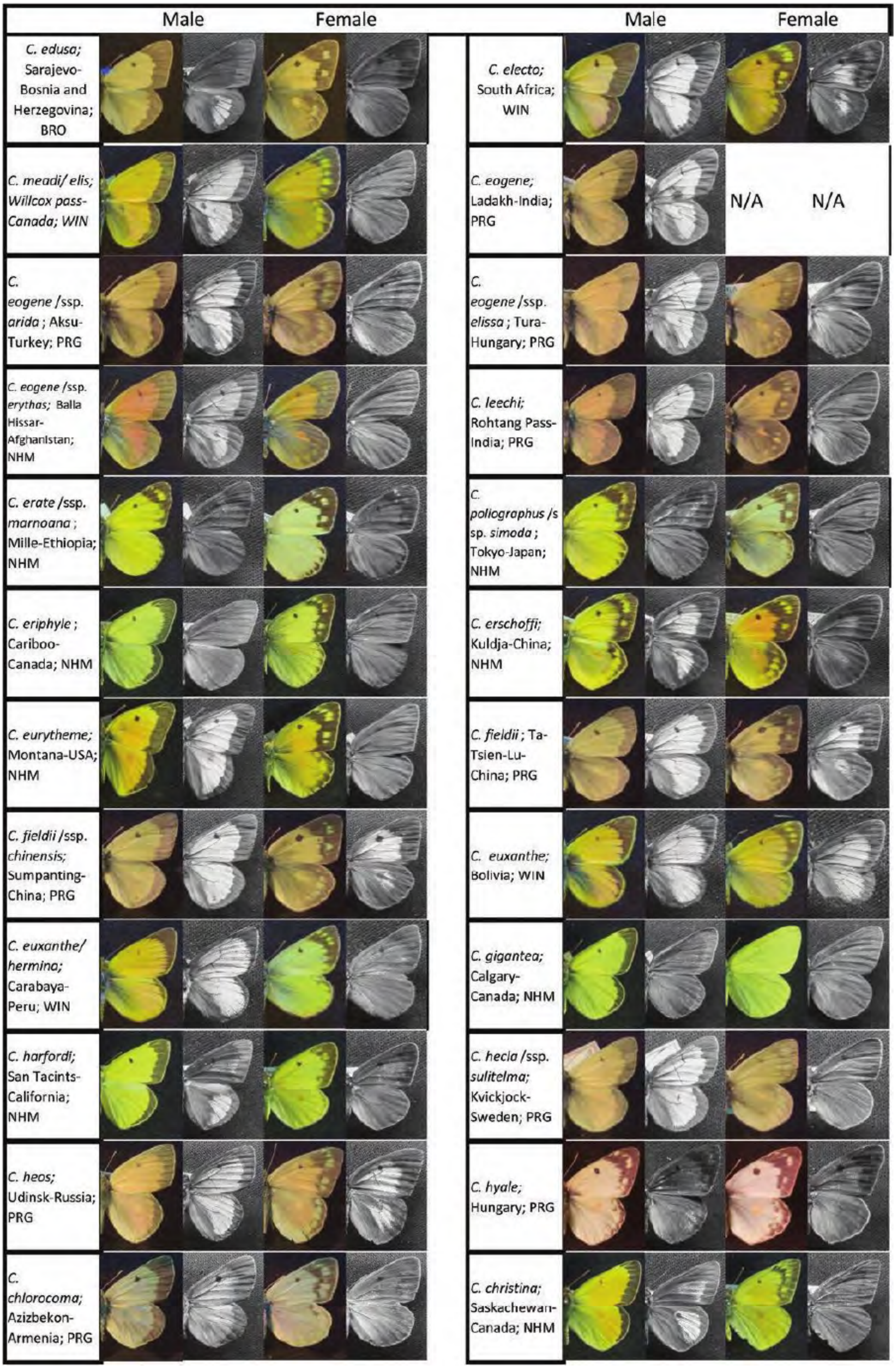


Fig. 1. Continued

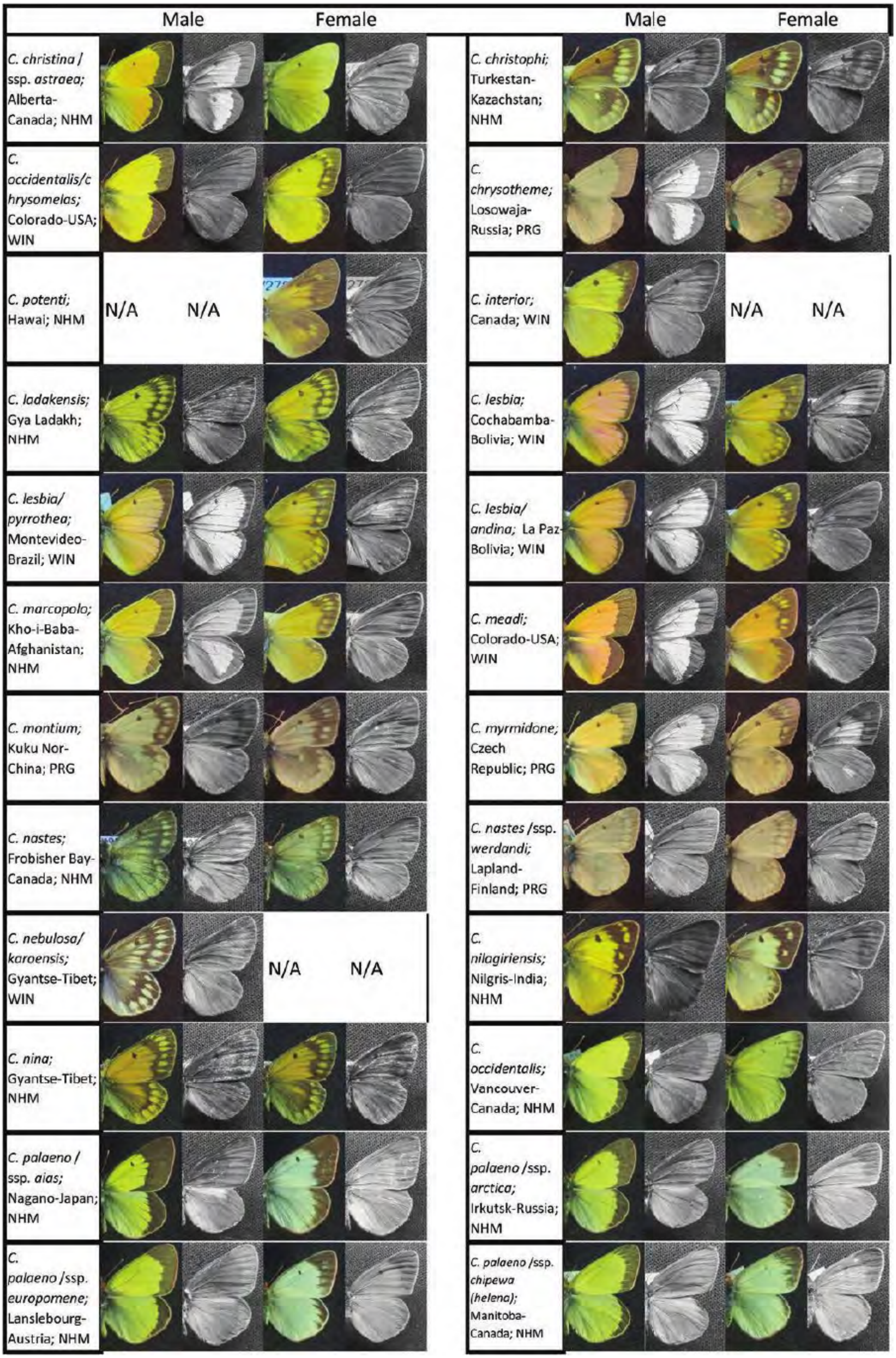


Fig. 1. Continued

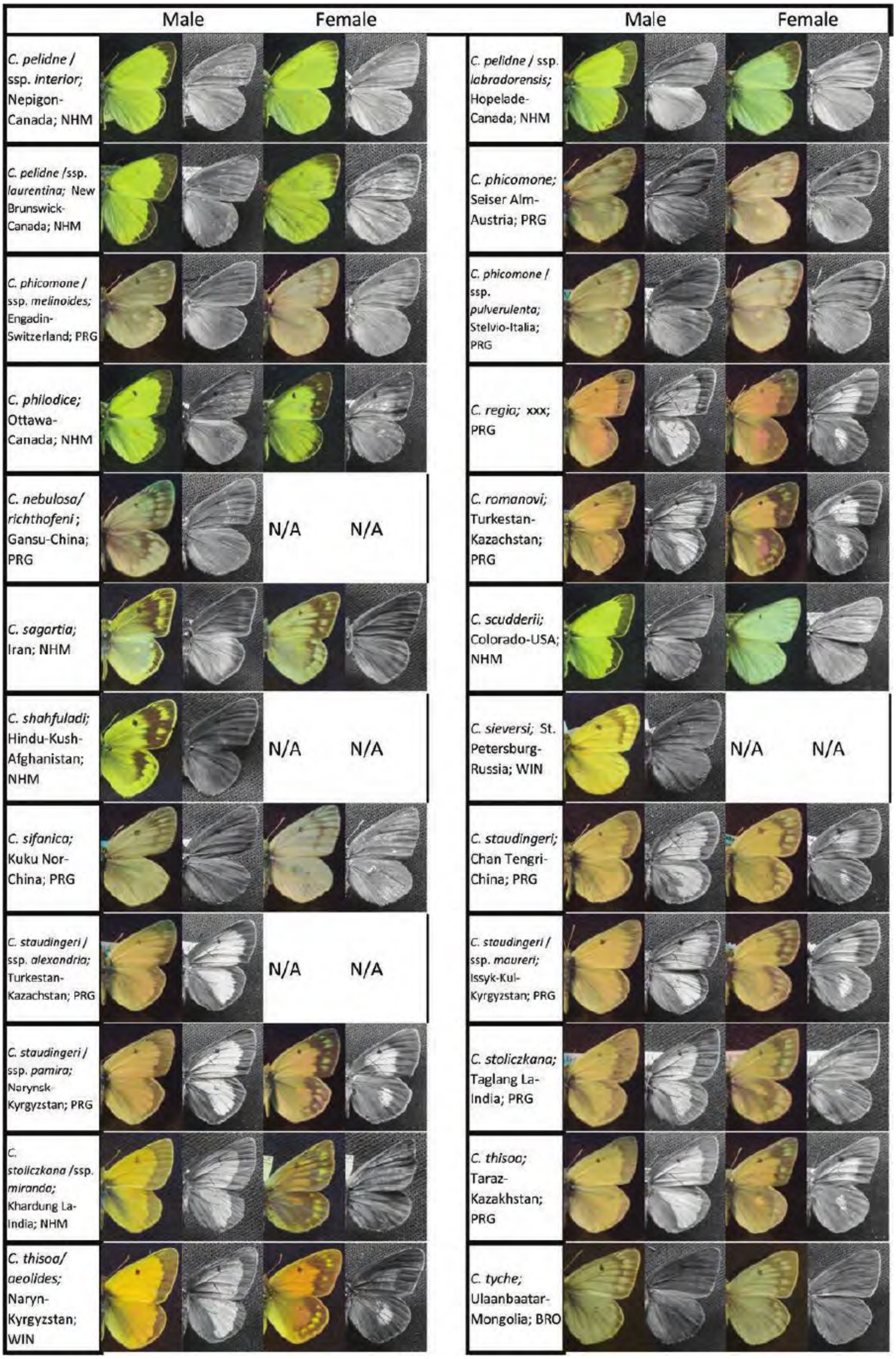


Fig. 1. Continued

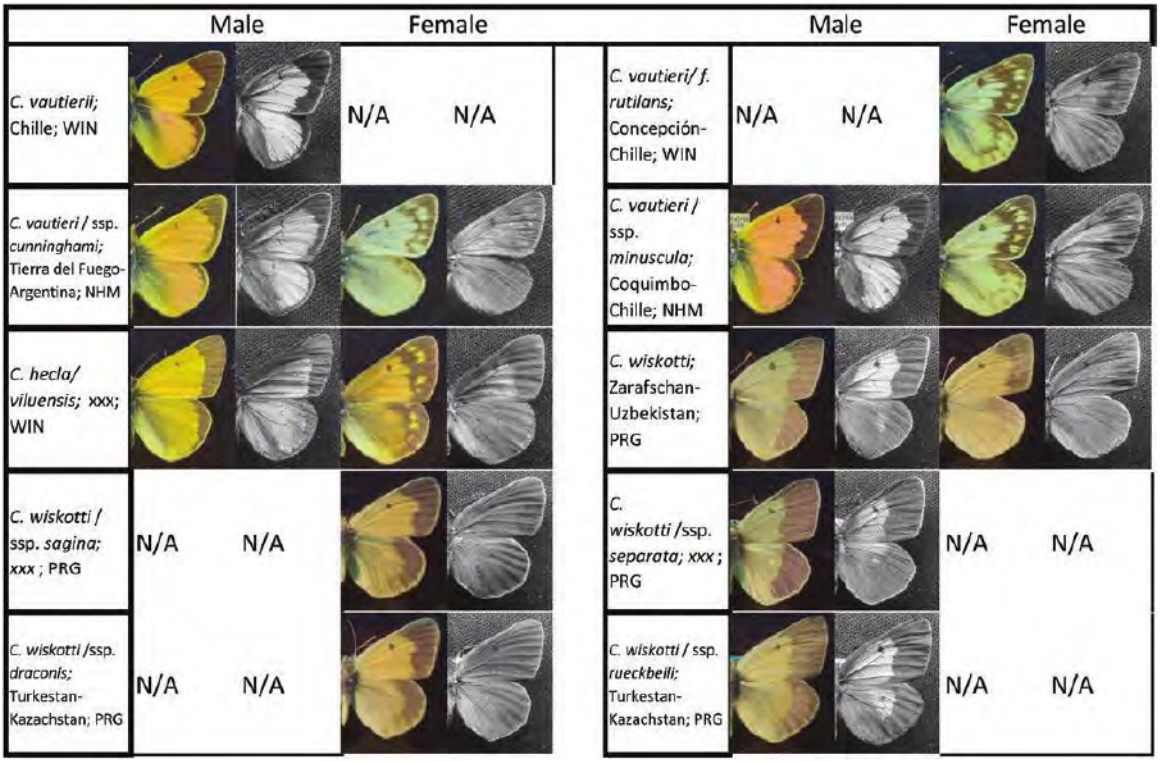


Fig. 1. Continued

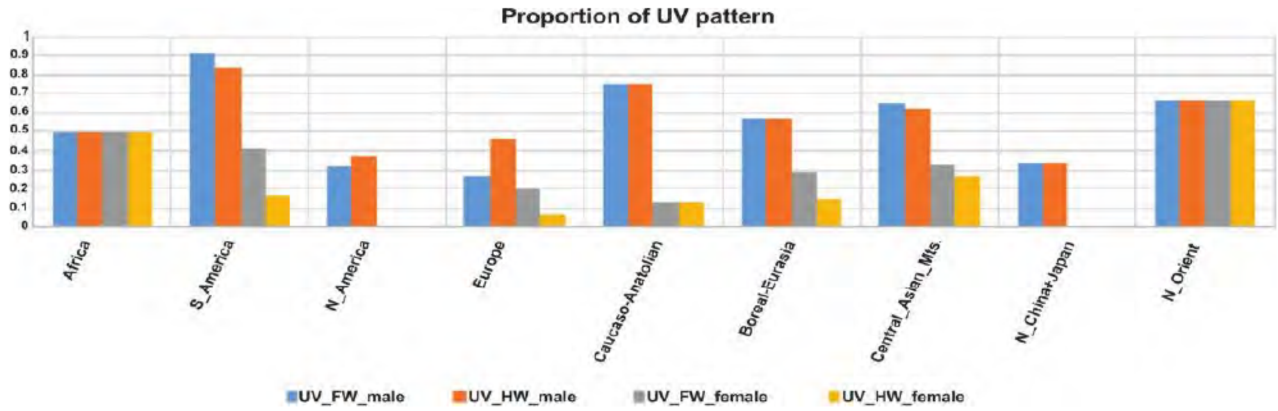


Fig. 2. The proportion of *Colias* taxa with a UV pattern within a particular region. UV = presence of the UV pattern in a taxon; FW/HW = forewing/hindwing.

is different in males, where a higher frequency of UV pattern was found in forest or arctic species. A higher frequency of UV pattern on hind wings was present in both sexes of ubiquitous species. Inclusion of distribution data in the habitat model as covariables did not improve the explanatory power of the model ($F = 1.1$, $P = 0.129$ on the first axis; $F = 1.4$, $P = 0.185$ on all axes).

Discussion

We have shown that over half of studied taxa (62%) of Coliads exhibit some UV reflectance pattern. UV patterning is present predominantly in males, but in some species also in females (60/25 taxa). This observation, namely, that UV patterns are found more frequently in males than in females, led many researchers to conclude that this patterning is the product of intra- and/or inter-sexual interactions (Silberglied and Taylor 1973, Rutowski 1985, Kemp et al. 2005). In species where males do display a UV pattern, females often also express this trait in either a full or partial form. The presence of UV pattern in both sexes remains poorly understood: of the proposed explanations, neither the antiharassment hypothesis nor the male mate choice hypothesis is broadly accepted (Kraaijeveld et al. 2007, Rutowski and Kemp 2017). A prior study had suggested that a specific UV-reflecting patch on female wings serves as a sex recognition signal (Rutowski and Kemp 2017). This could help explain the relatively high proportion of UV-reflecting females in our study. If this hypothesis is correct, a UV pattern functions as a signal that helps the males distinguish a female from a male (functional role), making it a crucial part of each individual taxon's system of visual communication. Properties of the pattern, such as its brightness or hue (as the main properties of a color), are then taxon-specific, and this role is further assisted by the fact that butterflies are capable of very fine color discrimination across both the UV and visible parts of the spectrum (Silberglied et al. 1978).

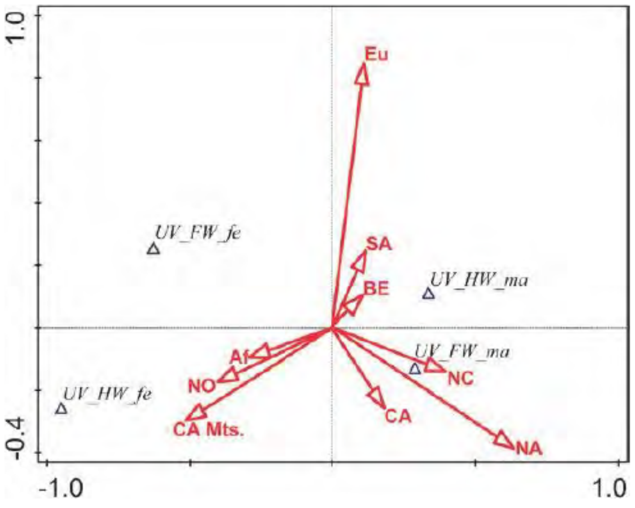


Fig. 3. A biplot diagram summarizing the effect of the region of distribution on the configuration of UV pattern. The first axis separates males (right) from females (left). The second axis separates UV patterns on FW and HW, opposite in males and females. *Eu* = Europe; *SA* = South America; *BE* = Boreal Eurasian; *NC* = northern China and Japan; *NA* = North America; *Ca* = Caucaso–Anatolian; *CA Mts.* = Central Asian mountains; *NO* = Neotropic; *Af* = Afrotropical; a blue triangle refers to centroid of treatments.

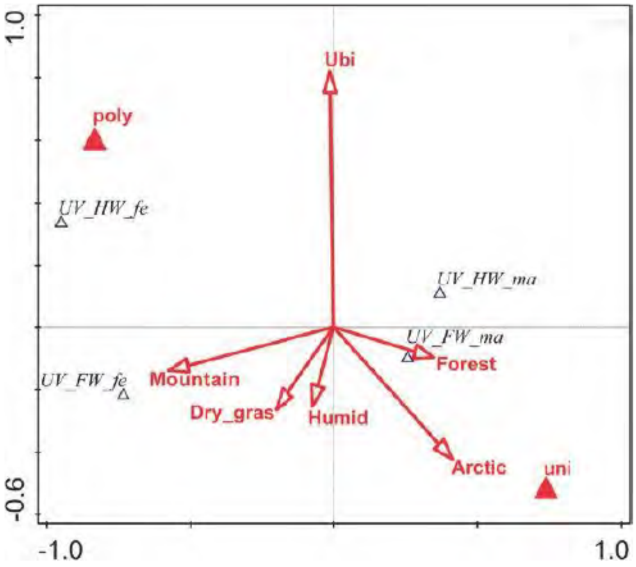


Fig. 4. Biplot diagram from the CCA with habitat variables, status of voltinism, and configurations of UV patches. The first axis separates males (right) and females (left) and also UV patterns on FW (bottom) from HW (top). *Ubi* = ubiquitous species; *Dry grass* = dry grasslands species; *poly* = polyvoltine species; *uni* = univoltine species. Other abbreviations as in Fig. 3.

On the other hand, behavioral experiments have revealed that females preferentially mate with males with a higher level of UV reflectance, i.e., with brighter patterns (Rutowski 1981, Kemp 2007a, Kemp 2007b). It has been suggested that there is strong selection for this trait. The rate of occurrence expressed among individuals that carry the same gene is therefore high in males, whereas in females it is either a result of genetic correlation between the sexes with no benefit to the females (Lande 1987, Andersson 1994), or just a rudimentary expression of the male UV pattern with no functional role (Swierk and Langkilde 2013). This seems supported by the fact that polymorphism with respect to UV patterning is common in females, whereas in males, the pattern remains relatively uniform within a taxon (Brunton and Majerus 1995). Moreover, this study confirmed that with respect to UV patterns, there is a great deal of interspecies variation among the Coliad taxons. A previous study had suggested that UV patterns may serve as part of a reproductive isolation between two sympatric taxa (Silberglied and Taylor 1973, Silberglied et al. 1978), and although this hypothesis was based only on North American species of the Coliads, our study suggest that it may apply to other *Colias* species as well, i.e., that it could be the case even with respect to other species with sympatric distribution. For this reason, it would be beneficial to compare the appearance of ultraviolet patterns in all other sympatric species and subspecies of *Colias* butterflies. These hypotheses would need to be confirmed by some robust in situ experiments. Several studies had worked with color manipulation models, but in many cases, this setup does not enable the testing of a specific hypothesis (Rutowski and Kemp 2017). Moreover, some *Colias* species cannot adapt to cage environments, which makes the implementation of such experiments rather challenging (Grieshuber 2014).

We have observed a relation between presence of a UV pattern in a species and its geographical distribution, but only a week relation between UV patterning and habitat. Although the relation presence of UV patterning and geographic regions is quite pronounced, it is hard to interpret, and no clear trends emerge. A factor that contributes to this difficulty is that the configuration of UV pattern is most probably polyphyletic within the studied taxa, and therefore consistent with the hypothesis that UV pattern is subject to a relatively fast sexual selection (Mazokhin-Porshniakov 1957, Iwasa and Pomiankowski 1995, Brunton 1998, Kemp et al. 2005). One species in a closely related pair thus may possess a UV pattern, whereas in its sister species it has disappeared (e.g., *Colias crocea/C. erate*) (Brunton 1998).

We must therefore conclude that in the *Colias* butterflies, UV patterning has no clear and obvious intra- or inter-specific role within the ecosystem. In this respect, the Coliads are quite unlike, for instance, some moths (Zapletalová et al. 2016). Brunton et al. (1998) show that UV patterns may have strong phylogenetic signal, but a comprehensive phylogenetic study of the genus *Colias* is still not available, probably because the most frequently used mitochondrial marker, namely, the Cytochrome Oxidase Subunit I, displays a low or no differentiation between species of the genus (Kramp et al. 2016). Although previous studies have revealed that UV reflectance is associated with some large-scale variables, such as temperature or precipitation, these analyses tended to focus on just one species (Meyer-Rochow and Järvilehto 1997, Pecháček et al. 2014, Stella et al. 2018). This was not the case in our study, since we targeted ecological properties relevant to 106 taxa of the *Colias* butterflies. Different species tend to have different life histories, which makes it difficult to associate UV reflectance in the Coliads with some general ecological variables. It must be concluded that more comprehensive conceptual framework is still needed if UV pattern configuration in the *Colias* butterflies is to be explained. Such a framework would most likely significantly contribute to a more thorough understanding of the genetic and ecological background of UV patterns in the *Colias* butterflies.

Generally speaking, UV patterns play a crucial role in butterflies' life history. Although many studies focus on particular species, this study mapped UV patterns in most *Colias* species and subspecies, while taking into account both sexes and the entire geographical range of the taxa under consideration. The configuration of UV patterns, captured in the unique photographic data presented here, is related to the ecological context of *Colias* taxa.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

This research was supported by the Charles University Grant project GAUK 964216. The work would be impossible without the support of the large collections of Blanca Huertas from the Natural History Museum in London, Sabine Gaal-Haszler from the Natural History Museum in Vienna, Petr Baňař from the Moravian Museum in Brno, and January Šumpich and Pavel Chvojka from the National Museum in Prague. Payam Zehzad for a help with checking of species identifications.

Conflict of interest statement

The authors declare no conflict of interest.

References Cited

Abràmoff, M. D., P. J. Magalhães, and S. J. Ram. 2004. Image processing with ImageJ. Biophoton Int. 11: 36–42.

Allyn, A. C., and J. C. Downey. 1977. Observations on male U-V reflectance and scale ultrastructure in *Phoebis* (Pieridae). Allyn Mus. Entomol. 42: 1–20.

Andersson, M. B. 1994. Sexual selection. Princeton University Press, Princeton, USA.

Arikawa, K., M. Wakakuwa, X. Qiu, M. Kurasawa, and D. G. Stavenga. 2005. Sexual dimorphism of short-wavelength photoreceptors in the small white butterfly, Pieris rapae crucivora. J. Neurosci. 25: 5935–5942.

Briscoe, A. D., and L. Chittka. 2001. The evolution of color vision in insects. Annu. Rev. Entomol. 46: 471–510.

Brunton, C. 1998. The evolution of ultraviolet patterns in European *Colias* butterflies (Lepidoptera, Pieridae): a phylogeny using mitochondrial DNA. Heredity 80: 611–616.

Brunton, C., and M. N. Majerus. 1995. Ultraviolet colours in butterflies: intra-or inter-specific communication? Proceedings of the Royal Society of London.Series B: Biological Sciences 260: 199–204.

Ferris, C. D. 1972. Ultraviolet photography as an adjunct to taxonomy. Lepidopterists Soc. J. 26: 210–215.

Ferris, C. D. 1973. A revision of the *Colias alexandra* complex (Pieridae) aided by ultraviolet reflectance photography with designation of a new subspecies. J. Lepidopterists' Soc. 27: 57–73.

Ghiradella, H., D. Aneshansley, T. Eisner, R. E. Silberglied, and H. E. Hinton. 1972. Ultraviolet reflection of a male butterfly: interference color caused by thin-layer elaboration of wing scales. Science. 178: 1214–1217.

Grieshuber, J. 2014. Guide to the butterflies of the palaearctic region. Pieridae part II.Subfamily Coliadinae. Omnes Artes, Tribe Coliadini.Milano.

Grieshuber, J., B. Worthy, and G. Lamas. 2012. The genus *Colias fabricus*, 1807: January Haugum's Annotated catalogue of the Old world *Colias* (Lepidoptera, Pieridae). Tshikolovets Publications, Pardubice, Czech Republic.

Imafuku, M. 2008. Variation in UV light reflected from the wings of *Favonius* and *Quercusia* butterflies. Entomol. Sci. 11: 75–80.

Iwasa, Y., and A. Pomiankowski. 1995. Continual change in mate preferences. Nature. 377: 420–422.

Kemp, D. J. 2006. Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. Evol. Ecol. Res. 8: 515–527.

Kemp, D. J. 2007a. Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). Behav. Ecol. 19: 1–8.

Kemp, D. J. 2007b. Female butterflies prefer males bearing bright iridescent ornamentation. Proc. Biol. Sci. 274: 1043–1047.

Kemp, D. J., and J. M. Macedonia. 2006. Structural ultraviolet ornamentation in the butterfly *Hypolimnas bolina* L. (Nymphalidae): visual, morphological and ecological properties. Aust. J. Zool. 54: 235–244.

Kemp, D. J., and R. L. Rutowski. 2007. Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. Evolution. 61: 168–183.

Kemp, D. J., and R. L. Rutowski. 2011. The role of coloration in mate choice and sexual interactions in butterflies. Adv. Study Behav. 43: 55–92.

Kemp, D. J., R. L. Rutowski, and M. Mendoza. 2005. Colour pattern evolution in butterflies: a phylogenetic analysis of structural ultraviolet and melanic markings in North American sulphurs. Evol. Ecol. Res. 7: 133–141.

Knüttel, H., and K. Fiedler. 2000. On the use of ultraviolet photography and ultraviolet wing patterns in butterfly morphology and taxonomy. J. Lepidopterists' Soc. 54: 137–144.

Kramp, K., O. Cizek, P. M. Madeira, A. A. Ramos, M. Konvicka, R. Castilho, and T. Schmitt. 2016. Genetic implications of phylogeographical patterns in the conservation of the boreal wetland butterfly *Colias palaeno* (Pieridae). Biol. J. Linn. Soc. 119: 1068–1081.

Kraaijeveld, K., F. J. L. Kraaijeveld-Smit, and J. Komdeur. 2007. The evolution of mutual ornamentation. Anim. Behav. 74: 657–677.

Laiho, J., and G. Stahls. 2013. DNA barcodes identify Central Asian *Colias* butterflies (Lepidoptera, Pieridae). Zookeys. 365: 175–196.

Lande, R. 1987. Genetic correlations between the sexes in the evolution of sexual dimorphism and mating preferences, pp. 83-94. *In* J. W. Bradbury and M. B. Andersson (eds.), Sexual selection: testing the alternatives. Wiley, Chichester, UK.

Layberry, R. A., P. W. Hall, and J. D. Lafontaine. 1998. The butterflies of Canada. University of Toronto Press, Toronto, Canada.

Lutz, F. E. 1924. Apparently non-selective characters and combinations of characters, including a study of ultraviolet in relation to the flower-visiting habits of insects. Ann. N. Y. Acad. Sci. 29: 181–283.

Lutz, F. E. 1933. Experiments with "stingless bees" (*Trigona cressoni parastigma*) concerning their ability to distinguish ultraviolet patterns. Am. Mus. Novit. 641: 1–26.

Lyytinen, A., L. Lindström, and J. Mappes. 2004. Ultraviolet reflection and predation risk in diurnal and nocturnal Lepidoptera. Behav. Ecol. 15: 982–987.

Mazokhin-Porshniakov, G. 1957. Reflecting properties of butterfly wings and role of ultra-violet rays in the vision of insects. Biophysics 2: 352–362.

Meyer-Rochow, V. B. 1991. Differences in ultraviolet wing patterns in the New Zealand lycaenid butterflies *Lycaena salustius*, *L. rauparaha*, and *L. feredayi* as a likely isolating mechanism. J. R. Soc. N. Z. 21: 169–177.

Meyer-Rochow, V. B., and M. Järvilehto. 1997. Ultraviolet colours in *Pieris napi* from northern and southern Finland: arctic females are the brightest! Naturwissenschaften 84: 165–168.

Morehouse, N. I., P. Vukusic, and R. Rutowski. 2007. Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. Proc. Biol. Sci. 274: 359–366.

Nekrutenko, Y. P. 1965. 'Gynandromorphic Effect' and the optical nature of hidden wing-pattern in *Gonepteryx rhamni* L. (Lepidoptera, Pieridae). Nature 205: 417.

Nekrutenko, Y. P. 1968. Phylogeny and geographical distribution of the genus *Gonepteryx* (Lepidoptera, Pieridae): an attempt of study in historical zoogeography. Naukova dumka, Kiev.

Obara, Y., H. Koshitaka, and K. Arikawa. 2008. Better mate in the shade: enhancement of male mating behaviour in the cabbage butterfly, Pieris rapae crucivora, in a UV-rich environment. J. Exp. Biol. 211: 3698–3702.

Obara, Y., G. Ozawa, and Y. Fukano. 2008. Geographic variation in ultraviolet reflectance of the wings of the female cabbage butterfly, *Pieris rapae*. Zoolog. Sci. 25: 1106–1110.

Olofsson M, Vallin A, Jakobsson S, and Wiklund C. 2010. Marginal eyespots on butterfly wings deflect bird attacks under low light intensities with UV wavelengths. PLoS ONE. 5: e10798. doi:10.1371/journal.pone.0010798

Osorio, D., and M. Vorobyev. 2008. A review of the evolution of animal colour vision and visual communication signals. Vision Res. 48: 2042–2051.

Papke, R. S., D. J. Kemp, and R. L. Rutowski. 2007. Multimodal signalling: structural ultraviolet reflectance predicts male mating success better than pheromones in the butterfly *Colias eurytheme* L.(Pieridae). Anim. Behav. 73: 47–54.

Pecháček, P., D. Stella, P. Keil, and K. Kleisner. 2014. Environmental effects on the shape variation of male ultraviolet patterns in the Brimstone butterfly (Gonepteryx rhamni, Pieridae, Lepidoptera). Naturwissenschaften 101: 1055–1063.

Pena, L. E., and A. J. Ugarte. 1996. The butterflies of Chile – Las Mariposas de Chile. Editorial Universitaria, Chile.

Pollock, D. D., W. B. Watt, V. K. Rashbrook, and E. V. Iyengar. 1998. Molecular phylogeny for *Colias* butterflies and their relatives (Lepidoptera: Pieridae). Ann. Entomol. Soc. Am. 91: 524–531.

Rutowski, R. L. 1981. Sexual discrimination using visual cues in the checkered white butterfly (*Pieris protodice*). Z. Tierpsychol. 55: 325–334.

Rutowski, R. L. 1985. Evidence for mate choice in a sulphur butterfly (*Colias eurytheme*). Z. Tierpsychol. 70: 103–114.

Rutowski, R. L., and D. J. Kemp. 2017. Female iridescent colour ornamentation in a butterfly that displays mutual ornamentation: is it a sexual signal? Anim. Behav. 126: 301–307.

Rutowski, R. L., J. M. Macedonia, N. Morehouse, and L. Taylor-Taft. 2005. Pterin pigments amplify iridescent ultraviolet signal in males of the orange sulphur butterfly, *Colias eurytheme*. Proceedings of the Royal Society B: Biological Sciences 272: 2329–2335.

Rutowski, R. L., J. M. Macedonia, J. W. Merry, N. I. Morehouse, K. Yturralde, L. Taylor-Taft, D. Gaalema, D. J. Kemp, and R. S. Papke. 2007a. Iridescent ultraviolet signal in the orange sulphur butterfly (*Colias eurytheme*): spatial, temporal and spectral properties. Biol. J. Linn. Soc. 90: 349–364.

Rutowski, R. L., J. M. Macedonia, D. J. Kemp, and L. Taylor-Taft. 2007b. Diversity in structural ultraviolet coloration among female sulphur butterflies (Coliadinae, Pieridae). Arthropod Struct. Dev. 36: 280–290.

Serra, G. V., N. C. Porta, S. Avalos, and V. Mazzuferi. 2013. Fixed-precision sequential sampling plans for estimating alfalfa caterpillar, *Colias lesbia*, egg density in alfalfa, *Medicago sativa*, fields in Córdoba, Argentina. J. Insect Sci. 13: 41.

Silberglied, R. E. 1979. Communication in the ultraviolet. Annu. Rev. Ecol. Syst. 10: 373–398.

Silberglied, R. E. 1984. Visual communication and sexual selection among butterflies, pp. 207–223. *In* R. I. Vane-Wright and P. R. Ackery (eds.), The biology of butterflies. Symposium of the Royal Entomological Society of London Number 11. Academic Press, London.

Silberglied, R. E., and O. R. Taylor. 1973. Ultraviolet differences between the Sulphur Butterflies, *Colias eurytheme* and *C. philodice*, and a possible isolating mechanism. Nature 241: 406–408.

Silberglied, R. E., J. Taylor, and R. Orley. 1978. Ultraviolet reflection and its behavioral role in the courtship of the sulfur butterflies *Colias eurytheme* and *C. philodice* (Lepidoptera, Pieridae). Behav. Ecol. Sociobiol. 3: 203–243.

Šmilauer, P., and J. Lepš. 2014. Multivariate analysis of ecological data using CANOCO 5. Second Edition. Cambridge University Press, Cambridge, 374 pp.

Stavenga, D. G., and K. Arikawa. 2006. Evolution of color and vision of butterflies. Arthropod Struct. Dev. 35: 307–318.

Stella, D., P. Pecháček, V. B. Meyer-Rochow, and K. Kleisner. 2018. UV reflectance is associated with environmental conditions in Palaearctic Pieris napi (Lepidoptera: Pieridae). Insect Sci. 25: 508–518.

Swierk, L., and T. Langkilde. 2013. Bearded ladies: females suffer fitness consequences when bearing male traits. Biol. Lett. 9: 1–4.

Tabashnik, B. E. 1980. Population structure of pierid butterflies: III. Pest populations of Colias philodice eriphyle. Oecologia 47: 175–183.

Tolman, T., and R. Lewington. 1997. Collins field guide butterflies Britain and Europe. Harper Collins, London.

Tuzov, V., P. Bogdanov, A. Devyatkin, L. Kaabak, V. Korolev, V. Murzin, G. Samodurov, and E. Tarasov. 1997. A guide to the butterflies of Russia and adjacent territories (Lepidoptera, Rhopalocera). Vol. 1: Hesperiidae, Papilionidae, Pieridae, Satyridae. Pensoft: Sofia. p. 480.

Verhulst, J. T. 2000. Les *Colias* Du globe: Texte. Antiquariat Geock & Evers, Keltern, Germany.

Wheat, C. W., and W. B. Watt. 2008. A mitochondrial-DNA-based phylogeny for some evolutionary-genetic model species of Colias butterflies (Lepidoptera, Pieridae). Mol. Phylogenet. Evol. 47: 893–902.

Wijnen, B., H. L. Leertouwer, and D. G. Stavenga. 2007. Colors and pterin pigmentation of pierid butterfly wings. J. Insect Physiol. 53: 1206–1217.

Wilts, B. D., P. Pirih, and D. G. Stavenga. 2011. Spectral reflectance properties of iridescent pierid butterfly wings. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 197: 693–702.

Zapletalová, L., M. Zapletal, and M. Konvička. 2016. Habitat impact on ultraviolet reflectance in moths. Environ. Entomol. 45: 1300–1305.

Stella, D., Pecháček, P. and Kleisner, K. (2016) Nondestructive, fast, ultraviolet: Applications of UV photography in ecology, taxonomy and evolutionary biology. *Klapalekiana,* 52, 111–118.

CHARLES UNIVERSITY
Faculty of science

Declaration of publication co-authorship

This is to confirm that PhD candidate David Stella significantly contributed to the following publication: *Stella, D., Pecháček, P. and Kleisner, K. (2016) Nondestructive, fast, ultraviolet: Applications of UV photography in ecology, taxonomy and evolutionary biology. Klapalekiana, 52, 111-118.*

He reviewed the literature, acquired the data, drafted the manuscript and worked on the manuscript revision.

The co-authors agree with submitting this publication as part of his PhD.

Doc. Mgr. Karel Kleisner PhD

Department of Philosophy and History of Science, Faculty of Science, Charles University

Published September 26, 2016

Klapalekiana, **52**: 111–118, 2016
ISSN 1210-6100

Nejlepší přednáška / Best oral presentation

Příbuznost hostitelských rostlin určuje strukturu potravních sítí mezi rostlinami a herbivorním hmyzem

Host-plant phylogenetic diversity drives insect-plant food web structure

Martin VOLF (1, 2), Petr PYSZKO (3), Tomokazu ABE(4), Martin LIBRA (1, 2),
Nela KOTÁSKOVÁ (3), Rajesh KUMAR (5), Martin ŠIGUT (3), Ondřej KAMAN (1),
Phillip BUTTERILL (1), Jan ŠIPOŠ (3), Haruka ABE (6), Hiroaki FUKUSHIMA (6),
Pavel DROZD (3), Naoto KAMATA (4), Masashi MURAKAMI (6)
& Vojtěch NOVOTNÝ (1, 2)

Současné odhady počtu druhů hmyzu se pohybují v řádu několika milionů. Konzervativní odhady počítají s přibližně 6 miliony popsaných i nepopsaných druhů členovců, mezi kterými zaujímá čelní postavení herbivorní, neboli býložravý, hmyz. Linie herbivorního hmyzu jsou často druhově výrazně bohatší než jejich draví a jinak se živící příbuzní. Za obrovskou rozmanitost vděčí herbivorní hmyz zejména koevoluci s rostlinami a dlouhé historii vzájemných interakcí se svými hostiteli. Během tohoto procesu se herbivoři postupně specializovali, což vedlo ke vzniku nových druhů a celkové diverzifikaci hmyzu. Místa s vysokou diverzitou rostlin, jako třeba tropický deštný les, proto hostí i nejbohatší společenstva herbivorního hmyzu. Některé teorie předpokládají, že k vysoké diverzitě hmyzu v takovýchto podmínkách přispívá i jeho vysoká specializace na několik málo hostitelů v prostředí s vysokou diverzitou živných rostlin a jejich vývojových linií.

V této studii jsme provedli sběry společenstev herbivorního hmyzu na třech plochách o rozloze 0.1 hektaru v lesích mírného pásu s různou diverzitou hostitelských rostlin. Sběry proběhly v Japonsku a České republice. Drtivá většina hmyzu se v lesních biotopech vyskytuje v korunovém patře. Ke sběru hmyzu jsme proto využili korunový jeřáb, vysokozdvižnou plošinu, případně jsme provedli sběr z korun pokácených stromů. Zaměřili jsme se především na tři skupiny herbivorů – listy okusující larvy, členovce tvořící hálky a hmyz minující v listech a prostřednictvím potravních sítí jsme rekonstruovali vztahy s jejich hostiteli. Poté jsme analyzovali vliv fylogeneze (příbuznosti) hostitelských rostlin na strukturu potravních sítí mezi hmyzem a rostlinami. Na základě několika lokusů DNA (ITS, matK, trnL-trnF a rbcL) jsme rekonstruovali časově kalibrovanou fylogenzi hostitelů, zachycující příbuzenské vztahy mezi rostlinami na jednotlivých plochách. Ukázalo se, že členovci tvořící hálky a hmyz minující v listech byli v podstatě výhradně monofágní a ani velmi příbuzné druhy rostlin nesdílely téměř žádné druhy těchto herbivorů. Jejich diverzita tak byla korelována především s počtem druhů hostitelských rostlin. Oproti tomu druhy herbivorů patřících mezi listy okusující larvy vykazovaly poměrně vysokou míru polyfagie a ve většině případů se živily několika druhy hostitelů. Jejich výběr hostitelů byl ovlivněn fylogenezí hostitelských rostlin a jejich příbuzenskými vztahy. Provedli jsme proto několik analýz abychom zjistili, jaký konkrétní význam má fylogeneze hostitelských rostlin pro utváření potravních sítí mezi listy okusujícími larvami a jejich hostiteli. V první analýze jsme analyzovali potravní sítě pro datové soubory, v nichž byly údaje o společenstvech hmyzu pro některé druhy hostitelských rostlin spojené. V rámci této analýzy jsme postupovali podle fylogenetického stáří jednotlivých linií hostitelských rostlin a vždy spojovali data pro linie rostlin, které byly mladší než specifikované stáří. V druhé analýze jsme analyzovali potravní sítě mezi hmyzem a monofyletickými skupinami hostitelských rostlin a výsledky porovnali s analýzou, do níž byly zahrnuty náhodně vybrané skupiny rostlin (které proto nebyly ve většině případů monofyletické) a s nimi asociovaný hmyz.

Analýzy ukázaly prudký nárůst specializace potravních sítí pro datové soubory, v rámci nichž byly spojeny linie rostlin mladší než 20–50 milionů let, což odpovídá stáří řady rostlinných čeledí. To ukazuje, že velká míra generalizovaných potravních vztahů byla vázána na blízce příbuzné rostliny. Další analýzy potvrdily, že v rámci potravních sítí vázaných na monofyletické skupiny hostitelských rostlin je ve srovnání s náhodně vybranými druhy hostitelů vyšší počet realizovaných potravních vztahů a sítě jsou celkově více propojené. Tyto výsledky ukazují, že hostitelská spektra listy okusujících larev často zahrnují příbuzné druhy hostitelských rostlin. Důležitou se zdá být zejména příslušnost hostitelských rostlin do čeledí. Zatímco rostliny ze stejné čeledi sdílí vysoký počet listy okusujících larev, vzdáleněji příbuzné druhy rostlin pocházející z různých čeledí sdílí výrazně nižší počet těchto herbivorů. To následně podporuje jeho specializaci a diverzifikaci v místech s vysokou diverzitou rostlin a jejich čeledí.

Naše závěry naznačují, že zatímco pro diverzitu listy okusujících herbivorů je patrně rozhodující fylogenetická diverzita hostitelských rostlin a počet jejich čeledí, je diverzita členovců tvořící hálky a hmyzu minující v listech ovlivněna především prostým počtem hostitelských rostlin. Zdá se proto, že ačkoliv je diverzita hostitelských rostlin jedním z klíčových mechanismů ovlivňujících druhové bohatství hmyzu, konkrétní mechanismy jejího působní se mohou mezi různými skupinami hmyzu lišit. Tato práce vznikla za podpory GAČR 14-04258S a ERC GA669609.

With recent estimates of around 6.1 million species, arthropods are one of the most species-rich groups of known organisms. Arthropods and insects in particular owe their enormous diversity, to large extent, to coevolution with plants. Insect-plant coevolution has led to the emergence of many specialized insect groups, and diversification of the whole class. Insect taxa feeding on plants are generally more species-rich than their counterparts exploiting other food sources. In turn, areas with a large number of plant species support high diversity of insects. It has been suggested that high diversity of insects in such areas may be a result not only of a large numbers of host-plants, but also of different insect specializations in conditions of increased host-plant diversity.

To investigate the effects of host-plant diversity on insect communities, we sampled herbivore assemblages in three 0.1 ha plots located in temperate forests with different host-plant diversity in the Czech Republic and Japan. Insects were sampled from a canopy crane, a cherry picker and felled trees, allowing complete sampling of canopy assemblages of leaf-chewing larvae, miners and gall formers. We reconstructed insect-plant food webs and time-calibrated host-plant phylogenies for individual guilds and sites. Gall formers and miners were almost exclusively monophagous, whereas leaf-chewers showed substantial levels of polyphagy at all three sites. While communities of gall formers and miners were affected by the species of host-plant communities, the specialization of leaf-chewers was affected by host-plant phylogenetic distance. To investigate the role of host-plant phylogeny in structuring insect plant-food webs we ran two separate analyses. First, we collated various clades of the host-plant phylogeny to identify what levels are the most crucial for herbivore food-choice. Second, we analyzed characteristics of herbivore food-webs associated with monophyletic subsets of host-plant community and subsets of the communities sampled in random to reveal relationships between insect specialization and plant phylogeny.

Food web reconstruction for communities revealed a steep decrease in food web generality when the insect data from plant taxa younger than 20–50 myr were collated. Further, the connectance within monophyletic plant groups was higher than between plants sampled at random. This shows that a large proportion of insect generality was generated through feeding on closely related and especially confamiliar hosts, with fewer insects being shared between older plant families and higher taxa. This suggests that high phylogenetic diversity of host-plants supports insect specialization, contributing to insect herbivore diversity. In summary, our results suggest that whereas the diversity of monophagous guilds seems to be directly correlated with the number of host-plant species, the diversity of polyphagous taxa is correlated with host-plant phylogenetic diversity at individual sites. Although high plant diversity thus always seems to significantly contribute to insect diversity, the exact mechanisms are guild-specific.

This project was supported by GACR 14-04258S and ERC GA669609.

Adresa autorů / Authors' address
(1) Biology Center, Czech Academy of Sciences, České Budějovice, Czech Republic;
e-mail: volf@entu.cas.cz;
(2) University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic;
(3) University of Ostrava, Faculty of Science, Ostrava, Czech Republic;
(4) The University of Tokyo, Faculty and Graduate School of Agriculture and Life Sciences, Tokyo, Japan;
(5) Central Muga Eri Research & Training Institute, Jorhat, India;
(6) Chiba University, Faculty of Science, Chiba, Japan

Nejlepší poster / Best poster

Nedestruktivně, rychle, ultrafialově: O využití UV fotografie v ekologii, taxonomii a evoluční biologii

Nondestructive, fast, ultraviolet: Applications of UV photography in ecology, taxonomy and evolutionary biology

David STELLA, Pavel PECHÁČEK & Karel KLEISNER

První ultrafialové obrázky několika rostlin a motýlů byly s využitím tzv. dírkové komory pořízeny už ve dvacátých a třicátých letech 20. století. Od padesátých let byla pro získávání UV snímků využívána klasická fotografie, ale jednalo se o postup technicky i časově poměrně náročný. Rozvoj digitálních metod záznamu znamenal ve srovnání s běžnou fotografií významné zjednodušení co do rychlosti, potřebných nákladů i možností, jak získané snímky zpracovávat. V případě UV fotografie však zůstává několik překážek, jejichž překonání vyžaduje použití specializované fotografické výbavy. V první řadě se jedná o fotoaparát citlivý

Obr. 1. *Gonepteryx rhamni* (Linnaeus, 1758). Fotografie samce žluťáska řešetlákového v ultrafialovém světle. Na fotografii je velmi dobře patrná plocha s UV reflektantní kresbou.
Fig. 1. *Gonepteryx rhamni* (Linnaeus, 1758). Photography of male common brimstone in UV spectra. UV patches are very apparent and visible.

k UV záření. Pro tyto účely je vhodná například širokospektrální digitální zrcadlovka Fuji IS pro, jejíž čip je senzitivní v oblasti od 330 nm do 900 nm. Přístroj je nutné doplnit objektivem propustným pro ultrafialové paprsky (čočky běžných objektivů jsou běžně pokryty vrstvou pro UV nepropustnou). Pro získání ultrafialové podoby zkoumaného objektu je vhodné fotoaparát osadit sérii filtrů (B+W), které zamezí průchodu viditelného a infračerveného záření. Vhodné je také použití umělého zdroje UV záření, tím může být například rtuťová zářivka, zábleskové světlo, případně LED osvětlení uzpůsobené na svícení v UV spektru. Tato metoda je rychlou a nedestruktivní cestou k zachycení komplexní podoby UV reflektantních struktur na nejrůznějších organizmálních površích a byla použita ve všech zmíněných případových studiích.

Vznik UV-reflektantních vzorů je u některých motýlů spojen s přítomností speciálních mikrostruktur na povrchu křídla. Předpokládá se, že strukturální zbarvení by mohla vzhledem ke své nákladnosti fungovat v pohlavním výběru jako znaky indikující kvalitu nositele. Jednou z možností jak tuto hypotézu testovat je sledovat velikost a tvar takových znaků v závislosti na prostředí, ve kterém se jedinec vyvíjel. V tomto ohledu je velmi přínosné využití metod geometrické morfometriky (GM). Tradiční morfometrické metody vycházely z přeměřování několika vybraných vlastností sledovaného znaku. GM nám naopak s využitím tvarových proměnných (landmarků) umožňuje zachytit tvar jako celek a následně analyzovat jeho proměnlivost v rámci celého souboru pozorování. Propojením metody GM a UV fotografie jsme zkoumali ultrafialové vzory na křídlech samců žluťáska řešetlákového (*Gonepteryx rhamni* (Linnaeus, 1758), Pieridae, obr. 1). Zjistili jsme, že samci, kteří pocházeli z teplejších a vlhčích oblastí, nesli na svých křídlech větší kresby než ti z vyšších zeměpisných šířek a z oblastí s nedostatkem srážek. To napovídá, že exprese UV-reflektantních kreseb u tohoto žluťáska je závislá na podmínkách, v kterých se daný jedinec vyvíjel.

UV-reflektance u motýlů ale nemusí být nutně spojena s přítomností zvláštních mikroskopických struktur jako v případě žluťásků. Zdrojem odražených UV paprsků může být i pouhá difuze světla dopadajícího na povrch křídla. V takovém případě nenacházíme pravidelné vzory, ale ultrafialové světlo je reflektováno celým povrchem křídla, přičemž intenzita odrazu je regulována pigmenty z rodiny pterinů, které paprsky z této části spektra pohlcují. Tyto pigmenty jsou považovány za nákladné a jejich množství by opět mohlo ukazovat na kvalitu jedince a jeho schopnost v juvenilním stádiu získat dostatek zdrojů pro maximální produkci těchto látek. Tuto hypotézu jsme testovali na příkladu běláska řepkového (*Pieris napi* (Linnaeus, 1758), Pieridae). Analýza více než 400 jedinců z celé Palearktické oblasti odhalila signifikantní vliv prostředí na intenzitu UV reflektance, přičemž křídla jedinců pocházející z nehostinnějších oblastí opravdu odrážela vyšší porce UV paprsků (křídla obsahovala méně UV absorpčních pigmentů). V mezipohlavním srovnání vykazovali samci mnohem nižší hodnoty UV-reflektance než samice, což napovídá, že tento znak zřejmě slouží coby indikátor kvality především v případě samců. To pravděpodobně souvisí s epigamním chováním daného druhu.

U některých druhů motýlů, kteří jsou si ve viditelném světle velmi podobní, nacházíme v UV spektru velmi nápadné rozdíly. Ultrafialové znaky mohou fungovat jako prekopulační reprodukčně izolační mechanismy (RIM), na což bylo v minulosti poukázáno u dvou velmi podobných žluťásků rodu *Colias* Fabricius, 1807 (Pieridae). Tyto vzory často vykazují nápadnou druhovou či poddruhovou stálost, což umožňuje jejich využití coby pomocného taxonomického znaku. V tomto smyslu jsme studovali charakter UV-reflektantních znaků u několika poddruhů jihoamerického motýla *Eryphanis zolvizora* (Hewitson, 1877) (Nymphalidae, obr. 2). Tyto poddruhy byly dříve rozpoznávány pouze na základě drobných odlišností v morfologii pohlavních orgánů. S využitím ultrafialové fotografie se nám podařilo ukázat, že jednotlivé poddruhy mohou být velmi dobře a jednoduše rozeznatelné pouze na základě tvaru a rozložení svých UV vzorů.

The first ultraviolet photographs of plants and butterflies were taken, using a pinhole camera, in the 1920s and 1930s. Only later, around the year 1950, the classical film photography method was employed to produce UV photographs, but this procedure was technically complicated and time consuming. In comparison, the development of digital photography offered a much quicker and more cost-effective way of producing images with the added possibility of subsequent image analyses. However, in case of UV photography, there are specific challenges regarding the necessary technical equipment used that need to be overcome. First is the requirement of UV sensitive camera; FujiFilm IS Pro is a digital camera suitable for UV photography due to its broad sensitivity spectrum spanning from 330 to 900 nm. Furthermore, the camera needs to be equipped with an uncoated UV transmitting non-distorting lens. A set of filters (B+W) needs to be mounted on the lens to filter out the visible and infrared parts of the spectrum. Finally, the use of an artificial source of UV light, e.g. a mercury lamp, flashlight, or LED light specially adapted for emitting in UV spectra, is highly advised. Notwithstanding the aforementioned technological requirements, the UV

Obr. 2. *Eryphanis zolvizora* (Hewitson, 1877). UV struktury lze u poddruhů tohoto jihoamerického motýla použít jako pomocné taxonomické znaky.
Fig. 2. *Eryphanis zolvizora* (Hewitson, 1877). UV structures at subspieces on wing of South American butterfly could act as auxiliary taxonomic characters.

photography technique is relatively fast and provides a nondestructive way of capturing the complex shape of UV pattern on various organisms and organismal structures. Therefore, this technique was employed in all case studies described below.

Formation of the UV pattern is mainly based on the occurrence of microstructures on a wing surface. Moreover, it has been suggested that, due to its complexity, the structural coloration may indicates a whole range of mate qualities. One of the possibilities of how to test this hypothesis is to correlate the shape variability of the ultraviolet pattern with the observed environmental variables of studied specimens. Using UV photography and methods of geometrical morphometry, we have studied the UV pattern on a dorsal wing of a Brimstone butterfly (*Gonepteryx rhamni* (Linnaeus, 1758), Pieridae, Fig. 1). Thus, we observed a systematic increase in the relative area of ultraviolet coloration with increasing temperature and precipitation and decreasing latitude. These findings are pointing to a correlation between the shape variations of ultraviolet patterns on the forewings of male Brimstones and environmental factors.

UV pattern is not necessarily associated with microstructures on a butterfly wing. Another mechanism is pigmentary based reflection of UV light. In this case, the whole wing surface is predominantly reflecting UV light with some pigments acting as its absorbers. These pigments are usually from the pterin group, and they are thought to entail high energetic demands during development. The deposition of pterins in wing scales and UV coloration could be therefore indicative of the quality (health status) of an individual. We tested this hypothesis on green-veined white (*Pieris napi* (Linnaeus, 1758), Pieridae). Analysis of more than 400 specimens from the Palaearctic Region revealed that higher UV reflectance intensities are associated with generally more hostile environments. Furthermore, males and females differ significantly in the intensity of UV reflectance. This could be related to epigamic behavior in certain species.

The coloration of some species of butterflies looks very similar in visible light, but proves to be very distinct in UV spectra. UV pattern could serve as reproduction isolation mechanism (RIM) as was suggested previously in the genus *Colias* Fabricius, 1807 (Pieridae). These structures are frequently highly phylogenetically conserved, both in in species and subspecies. Therefore, they could act as auxiliary taxonomic characters, a possibility of which was the aim in our study of the South American butterfly *Eryphanis zolvizora* (Hewitson, 1877) (Nymphalidae, obr. 2), subspecies of which were previously distinguished only based on small differences in morphology of the genital organs. Using UV photography, we demonstrated that the shape and position of UV structures are distinct enough to enable a relatively easy differentiation of these subspecies.

Adresa autorů / Authors' addresses
Charles University in Prague, Faculty of Science, Department of Philosophy and History of Science, Albertov 6, CZ-128 43 Praha 2, Czech Republic;
e-mails: David.Stella@natur.cuni.cz; pavel.pechacek@gmail.com;
karel.kleisner@natur.cuni.cz

Blažek, R. and Stella, D. (2020) Polyphenism and ultraviolet reflectance in *Araschnia levana* (Lepidoptera: Nymphalidae). *Acta rerum naturalium,* 24, 1–5.

CHARLES UNIVERSITY
Faculty of science

Declaration of publication co-authorship

This is to confirm that PhD candidate David Stella significantly contributed to the following publication: *Blažek, R. and Stella, D. (2018) Polyphenism and ultraviolet reflectance in* Araschnia levana *(Lepidoptera: Nymphalidae). Acta rerum naturalium. 24, 1-5..*

He reviewed the literature, acquired the data, drafted the manuscript and worked on the manuscript revision.

The co-authors agree with submitting this publication as part of his PhD.

Radomír

Radomír Blažek

Hviezdoslavova 1070, SK-015 01 Rajec

Acta rerum naturalium, 24: 1–5, 2020 ISSN 2336-7113 (Online), ISSN 1801-5972 (Print)

Polyfenismus a ultrafialová reflektance křídel *Araschnia levana* (*Lepidoptera: Nymphalidae*)

Polyphenism and ultraviolet reflectance in *Araschnia levana* (*Lepidoptera: Nymphalidae*)

RADOMÍR BLAŽEK[1], DAVID STELLA[2]

[1] Hviezdoslavova 1070, SK-015 01 Rajec; e-mail: radom.blazek@gmail.com, [2] Přírodovědecká fakulta, Univerzita Karlova, Katedra filozofie a dějin přírodních věd, Viničná 7, CZ-120 00 Praha; e-mail: david.stella@natur.cuni.cz

Abstract: This study presents a brief summary of knowledge of ultraviolet (UV) reflectance in butterflies and moths (Lepidoptera), focusing mainly on the distribution of this phenomenon in various forms of the European Map Butterfly - *Araschnia levana* (Linnaeus, 1758). Furthermore, we provide available information regarding the reproduction and heredity of some forms of butterflies. The genus *Araschnia* was not studied for UV reflectance phenomenon, therefore representative patterns were selected from 45 forms of *A. levana* and photographed under UV light with peak emission at 365 nm. Comparison of UV and visible light photographs revealed that white colour has wing pattern the highest level of UV reflectance. Mid grey colours were found to relate to rusty red and orange colours. The lowest reflectance was recorded in various shades of brown. Iridescent bright purple patches possess a high level of UV reflectance. The configuration of UV patterns in the studied species forms was not consistent. For that reason, we conclude that the importance of UV reflectance in *A. levana* in its inter- and/or intraspecific traits remains unclear.

Keywords: *Araschnia levana*, UV reflectance, reproductive process, Lepidoptera, phenotypic plasticity

ÚVOD

Motýli jsou jedna z nejbarevnějších živočišných skupin na zemi. Na svých křídlech disponují celou škálou barev a odlesků pozorovatelných nejen ve viditelném světle (dále VIS), ale i v ultrafialové části spektra (dále UV). Barva křídel může být dána přítomností organických pigmentů, z velké části patřících do skupin melaninů, ommochromů, pterinů, méně často flavonidů, případně jiných barviv. Barevnost může být způsobena též interferencí světla na povrchových útvarech dutých poloprůsvitných šupinek nebo i kombinací pigmentů a interference (Obenberger 1964, Wilts et al. 2011). Nutno dodat, že plochy odrážející UV jsou na křídlech někdy patrné z různého úhlu s různou intenzitou odrazu, což souvisí převážně s tvarem a polohou šupinek na křídlech (Wilts et al. 2011, Kemp et Rutowski 2011). Výskyt UV reflektancí byl v minulosti sledován především u druhů motýlů čeledí Pieridae, méně u Lycaenidae a ostatních.

Přestože jsou feromony považovány za nejdůležitější složku při páření, vizuální signály jsou u motýlů též důležitým prvkem iniciace vzájemné komunikace. Imafuku (2013) uvádí, že největší citlivost motýlů k světelnému záření leží v oblasti 440–460 nm. Brunton et al. (1995) konstatují, že denní motýli vnímají v celém barevném spektru a jejich schopnost rozlišovat pohybující se objekt závisí více na kontrastu barev než na jejich intenzitě. Na druhé straně jsou schopni vnímat i světlo v UV části spektra dopadajícího na zemský povrch. Barevné vidění hmyzu je zajištěno přítomností nejméně tří různých opsinů citlivých na různé vlnové délky, které umožňují vnímat světlo už od 300 nm, toto je však velmi často druhově specifické (Stavenga et Arikawa 2006). Barevné vidění někdy souvisí též s ekologickými podmínkami prostředí nebo i s požadavkem inter- a/nebo intraspecifických komunikačních prostředků (Lebhardt et Desplan 2017).

Víceré práce se zabývají přítomností, významem a změnou UV reflektančních obrazců na těle živočichů. O těchto vzorech (patternech) se předpokládalo, že mohou například u denních motýlů sloužit k předání informace o celkové fyzické kondici (Pecháček et al. 2014). Při výběru vhodných jedinců pro páření je důležité, aby samice z různých důvodů byly schopny určit kvalitu samců ještě před pářením. Důležitými faktory úspěšnosti páření jsou stáří (mechanické poškození) a kondice samce (množství ejakulátu a v něm obsažených výživných látek důležitých pro vývoj vajíček). S tím pochopitelně taktéž souvisí i předpokládaná délka kopulace a tím pádem třeba i zvýšení rizika možné predace (Pecháček 2010). UV reflektanční obrazce, jakožto důležité sekundární pohlavní znaky mohou být ovlivněny i různými zeměpisně-přírodními podmínkami, jako např. zeměpisná šířka i délka, nadmořská výška, průměrná roční teplota, množství dešťových srážek nebo produktivita prostředí. Tyto vnější podmínky mění nejen úroveň UV reflektance, např. u druhu *Colias eurytheme* Boisduval, 1852, viz Kemp et Rutowski (2007), ale i tvar reflektančních plošek, např. u druhu *Gonepteryx rhamni* (Linnaeus, 1758), viz Pecháček (2013). Druhá studie poukazuje na korelaci vícerých parametrů, přičemž výsledky některých vlivů jsou podobné, ne-li totožné. UV reflektance u motýlů hraje roli nejen při vnitrodruhové interakci (Obara et Majerus 2000), ale má také celou řadu mezidruhových interakcí (Brunton et Majerus 1995) a v neposlední řadě může tento jev posloužit taktéž k determinaci druhů (Pecháček et al. 2012). Potěšující je též zjištění, že výsledky studií nejsou postaveny jen na pozorování exemplářů z přírody, ale jsou sledovány i na experimentální bázi. Například přídavek flavonoidů k larvální potravě *Polyommatus icarus* (Rottemburg, 1775) zvyšuje absorpci UV světla, čímž zásadně mění vzory na křídlech motýlů (Knuttel et Fiedler 2001).

Naším cílem nebylo exaktně změřit UV plošky u všech forem druhu *A. levana*, ale předpokládáli jsme, že bychom touto prací mohli přispět k objasnění jevu u druhu, u kterého dosud nebyl studován, a pochopit ho v širším obecném ekologickém kontextu.

UV REFLEKTANCE U *ARASCHNIA LEVANA*

Araschnia levana (Linnaeus, 1758) - babočka síťkovaná je denní motýl, vyznačující se výraznou sezónní dvojtvárností (polyfenizmem) – jarní rezavěčervená forma *levana* (Linnaeus, 1758) a černo-bílá letní forma *prorsa* (Linnaeus, 1758). Při vhodných klimatických podmínkách se v přírodě vyskytuje i třetí generace – forma *porima* (Ochsenheimer, 1807), která nese znaky kresby obou předcházejících generací. Přechodné formy se znaky druhé i třetí generace se vyskytují příležitostně. Změnou vnějších podmínek (teplotní šoky, změna fotoperiody, endokrinní zásahy) lze experimentálně vyvolat vznik celé řady forem, jež jsou totožné s formami, které lze nalézt ve volné přírodě (Windig et Lammar 1999, Morehouse et al. 2013). Kresby jsou tvořeny pigmenty ommochromů na základě kombinací v genomu uložených informací. To může přispět k výskytu nových vzorů, pro jejichž vznik doposud nenastaly vhodné podmínky (Povolný 1996, Blažek 2016). Otaki et Yamamoto (2004) uvádějí, že změna barev a vzorů na křídlech motýlů může být způsobena řadou proměnných, to znamená, nejen teplotními šoky, ale i působením iontů těžkých kovů, například wolframu a molybdenu, viz též Zaćwilichowski (1936) a Dąbrowski (1966). Mechanismus vždy probíhá na bázi reakcí protein-tyrosin fosfatázy a je společný pro všechny druhy.

Blažek (2016) sumarizoval informace o formách *A. levana*, patřících zbarvením k všeobecně velmi variabilním druhům. Seznam dosud známých forem tohoto druhu doplnil na základě experimentální činnosti na celkový počet 45 (obr. viz Blažek 2016, str. 60). V tomto příspěvku jsme v souvislosti s množstvím odlišně zbarvených forem studovali, zda existují nějaké společné znaky UV reflektančních obrazců u různých forem uvedeného druhu. Dalo se předpokládat, že úroveň UV reflektance u světlejší f. *levana* je vyšší než u tmavší f. *prorsa*.

Změny kreseb (tj. fenotypový projev) exemplářů (rodičů) ovlivněné podmínkami jejich vývoje ve stádiu housenek nebo kukel jsou do určité míry a v určitém poměru (do 10 %) děděny v následující jedné až dvou generacích potomků žijících za obvyklých (normálních) podmínek (Prochnow 1927, van't Hof et al. 2011). Prochnow popisuje pokusy s druhy *Aglais urticae* (*Linnaeus*, 1758), viz Standfuss (1898), *Arctia caja* (Linnaeus, 1758), viz Fischer (1901) a *Abraxas grossulariata* (Linnaeus, 1758), viz Schröder (1903). Pro předloženou studii je nyní důležitá skutečnost, že barevné formy se mohou pářit a poskytují životaschopné potomstvo.

METODY

Pro snímkování v UVA oblasti od 315 do 400 nm byl v našem případě použit zdroj osvětlení UVP MRL-58 multiple-Ray-Lamp s rtuťovou zářivkou 8 W F8T5 emitující záření s vlnovou délkou λ-max = 365 nm s úhlem nasvícení 45°. UV obraz byl snímán fotoaparátem FujiFilm IS Pro senzitivním v oblasti od 330–900 nm s fotografickým objektivem Helios 44 58/2, černým filtrem B+W 403 zamezující vstupu VIS 400–700 nm a filtrem B+W BG 38, jenž odfiltruje záření nad 700 nm. Délka expozice s clonou f22 byla 15 s (Stella et al. 2018).

Pro snímkování byli z celé škály popsaných forem vybráni příslušníci obou pohlaví bez ohledu na jejich další rozlišení, to znamená jen část dostupných exemplářů s výrazně rozdílnou kresbou. Obrazy osmi jedinců nasnímaných pod UV světlem byly vizuálně porovnány s fotografiemi pořízenými při VIS (obr. 1). Standardizovaná UV fotografie poskytla možnost jedince posuzovat dle zjednodušené metodiky, viz Stella et al. (2018).

VÝSLEDKY A DISKUSE

Vzhledem k tomu, že tento druh motýla nebyl dle našich informací fotografován v UV světle, jedná se o unikátní data. Po vyhodnocení snímků jsme dospěli k závěru, že naše výsledky jsou v souladu s fundamentální myšlenkou o větší odrazivosti světlých barev na křídlech různých forem motýlů. U námi studovaných exemplářů měla v UV světle největší odrazivost pigmentová barva bílá, která i u jiných druhů bílých barviv, mívá vysokou úroveň reflektance i v UV oblasti (Wilts et al. 2011). Šedě se zobrazují barvy rezavěčervené a oranžové, což je možné považovat za zvláštnost, neboť leží od UV na opačné straně spektra. To je zřejmě dáno charakteristikami jejich remisních křivek a také mírně zvýšenou schopností použitého fotoaparátu zachytit červenou barvu v UV oblasti do 400 nm (viz Stevens et al. 2007). Nejmenší reflektance byla zaznamenána u odstínů barvy hnědé a černé. Všechny uvedené barevné odstíny vyskytující se na vrchní i spodní straně křídel všech forem a vztahy mezi nimi ve VIS a UV jsou shodné. Světlé šupinky s iridiscenčním nafialovělým odstínem na spodní straně křídel, nejlépe pozorovatelné u forem f. *levana*, f. *rubigilatofasciata* a f. *expanda,* se jeví v UV světle jako bílé.

Předchozí studie potvrdily, že variabilita prostředí mění úroveň a tvar UV reflektance křídel některých motýlů (Meyer-Rochow 1991, Pecháček et al. 2014, Stella et al. 2018). U různých forem druhu *A. levana*, vzniklých právě změnou ekologických podmínek v ontogenezi, tomu tak evidentně není. Na základě porovnání více forem *A. levana* (též u základních forem *prorsa* a *levana*) ve VIS a UV lze konstatovat, že příspěvek UV reflektance k remisním křivkám pro celou škálu pigmentů je minimální a při bohatosti vzorů na křídlech ve VIS je celkem zanedbatelný. Z toho důvodu zůstává sporné, zda je tato konfigurace UV reflektance významným prvkem inter- a/nebo intraspecifickým v ekologii tohoto druhu. Diskutabilní zatím u tohoto druhu také zůstává, jakou mají tak vizuálně (UV i VIS pattern) rozdílné formy reprodukční úspěšnost.

Je také možné, že UV vzorům je připisován větší význam, než ve skutečnosti mají. Podle některých autorů (Silberglied 1979, Rutowski et al. 2007) zůstává stále nejasné, zda během poměrně krátké doby a z různých úhlů mohou být tyto nepatrné změny velikosti, tvaru a úrovně reflektance obrazců vyhodnoceny, a hlavně, zda jsou jejich vypovídací informace při rozhodování se pro páření dostatečné.

U čeledi Nymphalidae je evoluce křídla nejčastěji spojována s mírou a efektivitou predace. Z tohoto pohledu efekt UV reflektance u studovaného druhu zatím zůstává nejasný. Černo-bílá kresba letních forem *prorsa* je odvozený stav, a tak je možné, že z pohledu ekologie druhu ve vztahu k UV reflektanci se u jarní formy *levana* jedná o aposematický vzor, kvůli němuž je tato červená forma méně lovena. Celá řada červených příslušníků čeledi Nymphalidae je nepoživatelná. U ptáků (např. *Parus major* Linnaeus, 1758) byla pozorována snížená predace vůči exemplářům f. *levana* oproti f. *prorsa* (Joiris et al. 2010). K tomu mohou přispívat i UV reflektance bleděfialových šupinek na spodní straně předních i zadních křídel f. *levana*, které u f. *prorsa* téměř úplně chybí. Naproti tomu černá barva f. prorsa je považována za účinné kryptické zbarvení, a tak tomu může být i v případě UV reflektance této formy (Fric et al. 2004).

U polyfenizmu je ještě nutné zmínit též genetické pozadí. Sezónní dvojtvárnost vznikla v rodu *Araschnia* jen jednou, a to na bázi celého rodu a nezávisle na výskytu dvojtvárnosti v sesterské větvi *Symbrenthia-Mynes* (Windig et Lammar 1999). Ukázalo se, že fylogeneticky primitivnějším stavem bylo zbarvení červené, viz též Fric et Konvička (2004), Fric et al. (2004).

Pozoruhodným jevem zůstává, že pleziomorfním znakem regresních forem některých druhů čeledi Nymphalidae je zbarvení hnědé typu f. *terminalia* (Blažek 2012).

ZÁVĚR

Tato práce jako první předkládá a současně popisuje unikátní obrazovou dokumentaci v podobě snímků UV reflektance různých forem *A. levana*. Lze říci, že byla pozorována určitá pravidla u UV reflektance křídel. Světlejší křídla (VIS) mají vyšší UV reflektanci nežli tmavší více pigmentovaná křídla, která spíše UV pohlcují. Pokud *A. levana* iniciuje intraspecifickou komunikaci na základě vizuálních vjemů, UV reflektance je pravděpodobně jen doplňkovou, zřejmě ne vždy přítomnou funkční informací. UV reflektance je pak možná pouze fyzikálním derivátem struktury povrchu křídla bez hlubších inter- a/nebo intraspecifických ekologic-

Obr. 1. Formy *Araschnia levana* ve viditelném a UV světle 365 nm. Foto: Stella, Kšiňan, Blažek

Fig. 2. Forms *Araschnia levana* in VIS and UV light 365 nm.

1. f. *levana*, 2. f. *prorsa*, 3. f. *reducta*, 4. f. *terminalia*, 5. f. *rubigilatofasciata*, 6. f. *rubigilatogerardini*, 7. f. *gerardini*, 8. f. *expanda*. Photo: Stella, Kšiňan, Blažek

kých vztahů. Pro novost poznatků není možné porovnání s poznatky publikovanými v literatuře a tyto informace naměřené exaktními metodami mají pouze informativní charakter. Plnou platnost informací o zapojení se forem do reprodukčního procesu a funkce UV reflektance křídel u *A. levana* bude potřebné dokázat dalšími experimentálními studiemi.

LITERATURA

Blažek R. (2012): Variabilita hmyzu II. [Insect variability II.]. – Entomofauna Carpathica, 24: 31–35.

Blažek R. (2016): Variability of insects V. New forms of *Araschnia levana*. – Entomofauna carpathica, 28: 45–68.

Brunton C. et Majerus M. N. (1995): Ultraviolet colours in butterflies: intra-or inter-specific communication? – Proceedings of the Royal Society of London Series B: Biological Sciences, 260: 199–204.

Dąbrowski J. S. (1966): Changes of the wing pattern in the moths of the genus Zygaena FABR.(Lepidoptera: Zygaenidae) obtained by intrapupae injections. – Acta entomologica bohemoslovaca, 63: 411–419.

Fischer E. (1901): Experimentelle Untersuchungen über d. Vererbung erworbener Eigenschaften. – Allgemeine Zeitschrift für Entomologie, 6: 363–381.

Fric Z. et Konvička M. (2004): Sezonní dvojtvárnost babočky síťkované. – Vesmír, 83: 442–446.

Fric Z., Konvička M. et Zrzavý J. (2004): Red & black or black & white? Phylogeny of the Araschnia butterflies (Lepidoptera: Nymphalidae) and evolution of seasonal polyphenism. – J. Evol. Biol., 17: 265–278.

Imafuku M. (2013): Sexual differences in spectral sensitivity and wing colouration of 13 species of Japanese Thecline butterflies (Lepidoptera: Lycaenidae). – Eur. J. Entomol., 110: 435–442.

Joiris A., Korati S. et Van Dyck H. (2010): Differential bird predator attack rate on seasonal forms of the map butterfly (*Araschnia levana* L.): does the substrate matter? – Ethology, 116: 1020–1026.

Kemp D. J. et Rutowski R. L. (2007): Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. – Evolution, 61: 168–183.

Kemp D. J. et Rutowski R. L. (2011): The role of coloration in mate choice and sexual interactions in butterflies. – Advances in the Study of Behavior, 43: 55–92.

Knuttel H. et Fiedler K. (2001): Host-plant-derived variation in ultraviolet wing patterns influences mate selection by male butterflies. – J. Exp. Biol., 204: 2447–2459.

Lebhardt F. et Desplan C. (2017): Retinal perception and ecological significance of color vision in insects. – Current opinion in insect science, 24: 75–83.

Meyer-Rochow V. B. (1991): Differences in ultraviolet wing patterns in the New Zealand lycaenid butterflies Lycaena salustius, L. rauparaha, and L. feredayi as a likely isolating mechanism. – J. R. Soc. N. Z., 21: 169–177.

Morehouse N., Mandon N., Christides J., Body M., Bimbard G. et Casas J.: (2013): Seasonal selection and resource dynamics in a seasonally polyphenic butterfly. – J. Evol. Biol., 26: 175–185.

Obara Y. et Majerus M. N. (2000): Initial mate recognition in the British cabbage butterfly, *Pieris rapae rapae*. – Zool. Sci., 17: 725–730.

Obenberger J. (1964): Entomologie, vol. 5, 1st ed. Přírodovědecké vydavatelství, Praha.

Otaki J. M. et Yamamoto H. (2004): Species-specific color-pattern modifications of butterfly wings. – Dev. Growth Differ., 46: 1–14.

Pecháček P. (2010): Biologický výzkum v ultrafialové oblasti: evoluční a eto-ekologické implikace UV reflektance organismálních povrchů. Praha, 2010. – Ms. [Bakalářská práce, depon. in: Univerzita Karlova, Přírodovědecká fakulta, Katedra filosofie a dějin přírodních věd].

Pecháček P. (2013): Žluťásek řešetlákový a plasticita ultrafialových kreseb motýlů v závislosti na prostředí. – Živa, 2: 79–81.

Pecháček P., Stella D. et Kleisner K. (2012): Ultrafialový svět bezobratlých. – Živa, 159: 25–28.

Pecháček P., Stella D., Keil P. et Kleisner K. (2014). Environmental effects on the shape variation of male ultraviolet patterns in the Brimstone butterfly (*Gonepteryx rhamni*, Pieridae, Lepidoptera). – Naturwissenschaften, 101: 1055–1063.

Povolný D. (1996): Sezonní dvojtvárnost babočky síťkované. – Vesmír, 75: 217–222.

Prochnow O. (1927): Die Färbung der Insekten (nach Beobachtungen und Versuchsergebnissen), pp. 430-572. – In: C. Schröder (ed.), Handbuch der Entomologie, vol. 2. G. Fischer, Jena.

Rutowski R. L., Macedonia J. M., Kemp D. J. et Taylor-Taft L. (2007): Diversity in structural ultraviolet coloration among female sulphur butterflies (Coliadinae, Pieridae). – Arthropod Structure & Development, 36: 280–290.

Schrőder CH. (1903): Die Zeichnungsvariabilität von *Abraxas grossulariata L.* (Lep.), gleichzeitig ein Beitrag zur Descendenztheorie. – Allgemeine Zeitschrift für Entomologie. Bd, 8: 105–119.

Silberglied R. E. (1979). Communication in the ultraviolet. Annu. – Rev. Ecol. Syst., 10: 373–398.

Standfuss M. (1898): Experimentelle zoologische Studien mit Lepidopteren, vol. 36, 1st ed. Zürcher & Furrer, Zürich.

Stavenga D. G. et Arikawa K. (2006): Evolution of color and vision of butterflies. –Arthropod Structure & Development, 35: 307–318.

Stella D., Pecháček P., Meyer-Rochow V. B. et Kleisner K. (2018): UV reflectance is associated with environmental conditions in Palaearctic *Pieris napi* (Lepidoptera: Pieridae). – Insect Science, 25: 508–518.

Stevens M., Parraga C. A., Cuthill I. C., Partridge J. C. et Troscianko T. S. (2007): Using digital photography to study animal coloration. – Biol. J. Linn. Soc., 90: 211–237.

Van't Hof A. E., Edmonds N., Dalíková M., Marec F.

et Saccheri I. J. (2011): Industrial melanism in British peppered moths has a singular and recent mutational origin. – Science (New York, N. Y.), 332: 958–960.

Wilts B. D., Pirih P. et Stavenga D. G. (2011): Spectral reflectance properties of iridescent pierid butterfly wings. – Journal of Comparative Physiology (A), 197: 693–702.

Windig J. J. et Lammar P. (1999): Evolutionary genetics of seasonal polyphenism in the map butterfly Araschnia levana (Nymphalidae: Lepidoptera). – Evol. Ecol. Res., 1: 875–894.

Zaćwilichowski J. (1936): Eine neue Methode, aberrative Formen von Schmetterlingen durch chemische Einwirkung zu erzielen. – Bulletin international de l'Académie Polonaise des Sciences et des Lettres, Classe des Sciences Mathématiques et Naturelles, Série B, Sciences Naturelles, 8: 481–497.

Pecháček, P., Stella, D. and Kleisner, K. (2019) a Morphometric Analysis of Environmental Dependences Between Ultraviolet Patches and Wing Venation Patterns in *Gonepteryx* Butterflies (Lepidoptera, Pieridae). *Evolutionary Ecology,* 33, 89–110.

CHARLES UNIVERSITY
Faculty of science

Declaration of publication co-authorship

This is to confirm that PhD candidate David Stella significantly contributed to the following publication: *Pecháček, P., Stella, D. and Kleisner, K. (2018) A Morphometric Analysis of Environmental Dependences Between Ultraviolet Patches and Wing Venation Patterns in* Gonepteryx *Butterflies (Lepidoptera, Pieridae). Ecological Entomology, Under Review.*

He acquired the data and worked on the manuscript revision.

The co-authors agree with submitting this publication as part of his PhD.

Doc. Mgr. Karel Kleisner PhD

Department of Philosophy and History of Science, Faculty of Science, Charles University

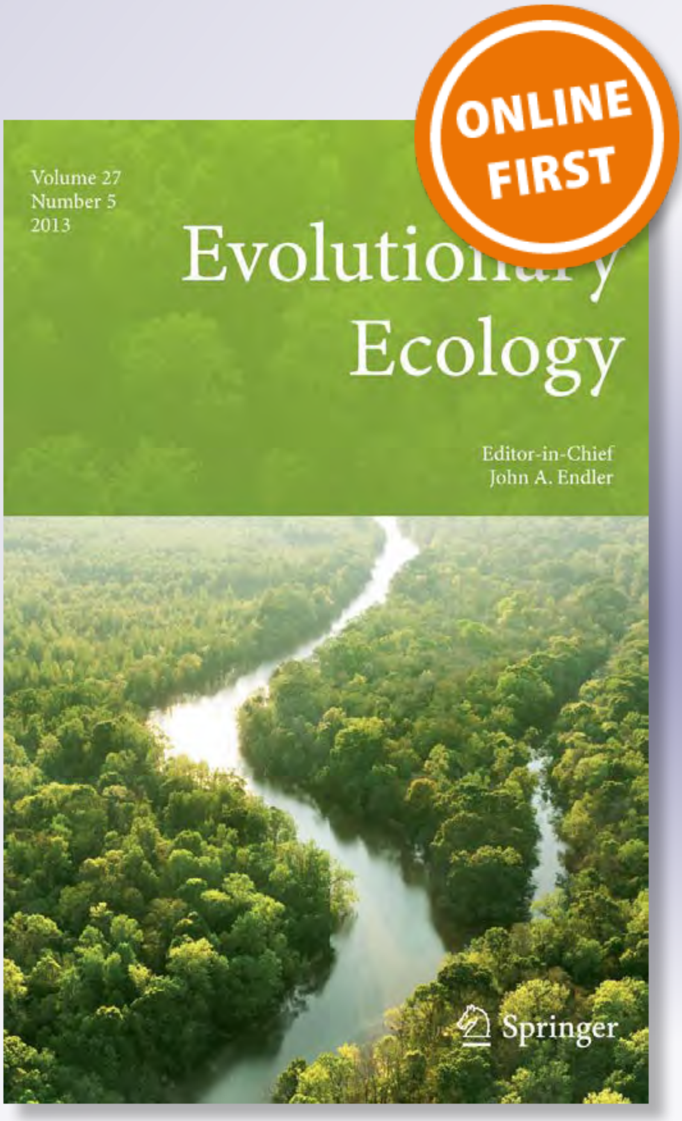
A morphometric analysis of environmental dependences between ultraviolet patches and wing venation patterns in Gonepteryx *butterflies (Lepidoptera, Pieridae)*

Pavel Pecháček, David Stella & Karel Kleisner

Evolutionary Ecology

ISSN 0269-7653

Evol Ecol
DOI 10.1007/s10682-019-09969-0



Springer

Evolutionary Ecology
https://doi.org/10.1007/s10682-019-09969-0

ORIGINAL PAPER

A morphometric analysis of environmental dependences between ultraviolet patches and wing venation patterns in *Gonepteryx* butterflies (Lepidoptera, Pieridae)

Pavel Pecháček[1] · David Stella[1] · Karel Kleisner[1]

Received: 28 September 2018 / Accepted: 9 January 2019

Abstract

It has been suggested that structural ultraviolet (UV) patches on the wings of butterflies play a role in sexual selection. UV patches may be condition-dependent signals of mate quality. In the current study, we investigated associations between the morphological properties of two male wing patterns (one signalling and one non-signalling trait) and between these patterns and various environmental variables in seven species of the genus *Gonepteryx* (*G. rhamni*, *G. nepalensis*, *G. maxima*, *G. amintha*, *G. aspasia*, *G. niphonica* and *G. cleopatra*). We collected UV photographs of a total of 320 male specimens and analysed them using geometric morphometrics. Our results show that the shape of UV patches (a signalling trait) is more asymmetric than the wing venation (a non-signalling trait). In both examined traits, however, relationship between the environment and fluctuating asymmetry is significant only in a minority of species. Our results thus do not support the hypothesis that fluctuating asymmetry is a reliable indicator of an individual's quality, in other words, that UV patches are condition-dependent trait. Examination of correlations between the two investigated shapes and the environment yielded similar results, and while the shape of UV patterns tended to be more strongly associated with the environment than the venation patterns, the correlation reached a level of significance only in a minority of cases. Due to the ambiguity of our findings, we cannot corroborate the hypothesis that UV patches act as biological signals of male quality in *Gonepteryx* butterflies, which is the case in various other related butterfly species. Finally, we found that UV patches discriminate among various *Gonepteryx* species better than the venation patterns do, which indicates that UV patches play a role in species recognition. It also suggests that UV patterns could be a useful taxonomic trait.

Keywords *Gonepteryx* · Lepidoptera · Sexual selection · Geometric morphometrics · UV reflectance

---

✉ Pavel Pecháček
pavel.pechacek@natur.cuni.cz

[1] Department of Philosophy and History of Science, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic

Introduction

Butterflies are among the most colourful animal taxa, which is also why they are the subject of many studies on animal colouration (Poulton 1890; Shawkey et al. 2009; Hiyama et al. 2012; Dalrymple et al. 2015). Butterflies often display conspicuous sexual dichromatism (Eguchi and Meyer-Rochow 1983; Allen et al. 2011; Oliver and Monteiro 2011) noticeable especially in the ultraviolet (UV) part of the electromagnetic spectrum. Sensitivity to UV light is widespread among various vertebrate and invertebrate taxa (Silberglied 1979; Tovée 1995; Losey et al. 1999; Briscoe and Chittka 2001; Hunt et al. 2001; Osorio and Vorobyev 2008; Fleishman et al. 2011; Painting et al. 2016) and butterflies are no exception (Bernard and Remington 1991; Stavenga and Arikawa 2006; Bybee et al. 2012; Chen et al. 2016). UV signals are involved in both intra- and intersexual communication (Kemp and Rutowski 2011) and often play a crucial role in identifying a potential mate of the same species (Silberglied and Taylor 1973; Obara et al. 2010). In some instances, UV patterns also have a protective or mimetic function (Remington 1973; Olofsson et al. 2010; Zapletalová et al. 2016).

It has been suggested for several species of the genus *Pieris* (Tuomaala et al. 2012) that ultraviolet patches are function as indicators of mate quality. Wing scales of these butterflies contain special pigments, usually from the pterin family, which are responsible for the scattering of light and selective absorption of specific wavelengths (Morehouse et al. 2007; Wijnen et al. 2007). Pterins are rich in nitrogen. As such, they are sensitive to the availability of nitrogen in the environment and consequently indicate the individual's ability to acquire nitrogen-rich nutrition during its larval stage (Morehouse and Rutowski 2010). An alternative way of producing colour is by optically active nanostructures on the wing surface which reflect the light of particular wavelengths (Stavenga et al. 2006; Kemp et al. 2012). This form of colour production, which often results in iridescent and highly limited-view UV reflection, is found in members of the Coliadinae subfamily (Ghiradella et al. 1972; Rutowski et al. 2007; Pirih et al. 2011) as well as in other groups of diurnal butterflies (Kemp and Macedonia 2006; Imafuku 2008). It is frequently the case that UV colouration is achieved by a combination of pigmentary and structural mechanisms (Rutowski et al. 2005; Stavenga 2014). The production of structural UV colours may be costly to its bearer and pattern's properties, such as the size of such patches or levels of UV reflectance, may signal the bearer's quality (Kemp and Rutowski 2007; Kemp 2008b). Structural UV colours can provide information about an individual's age (Kemp 2006a; Kemp and Macedonia 2006), which may have various evolutionary-ecological consequences, for instance for the size of nutritious ejaculate provided by the male (Rutowski et al. 1987; Kemp and Rutowski 2011; Meslin et al. 2017). Due to the precision required of wing scale nanostructures if they are to produce constructive interference effectively (Mouchet and Vukusic 2018), structural colours are, during an individual's development, influenced by external stress factors more than the pigment-based colours are (Kemp and Rutowski 2007). Nutrition may also influence structural colouration through its effects on wing scale architecture (Kemp et al. 2006; Kemp 2008b).

One possible approach to studying the effect of the environment on UV ornament expression is by searching for covariation between the properties of these ornaments (shape, amount of reflected light) and selected geographical and environmental variables. Previous research had shown that if the degree of UV reflectance in a butterfly species depends on the presence of UV-absorptive pigments, higher-quality individuals—i.e. those capable of using the environmental resources more effectively or simply those that live in areas richer in nutrients—should have wings with a lower degree of UV reflectance because their wings contain more UV-absorptive pigments. For instance in green-veined whites (*Pieris napi*), individuals that inhabit harsher environments reflect more UV light than those from warmer areas, which are more suitable for breeding. Furthermore, the wings of males are less UV-reflective than the wings of females (Meyer-Rochow and Järvilehto 1997; Tuomaala et al. 2012; Stella et al. 2018b). This trend, however, seems to be reversed in species with structural UV colouration. In male common brimstones (*Gonepteryx rhamni*), the relative size of structural UV wing patches positively correlates with habitat temperature and humidity (Pecháček et al. 2014). Structurally produced male UV patches could indicate a range of mate-quality predictors including access to larval resources, developmental stability, and resistance to environmental perturbations (Kemp and Rutowski 2007; Kemp 2008b). These findings support the hypothesis that UV-reflective patches are condition-dependent and could serve as indicators of an individual's quality.

Fluctuating asymmetry (FA) is a widely used measure for studying phenotypic response to environmental stress in many animal taxa, including butterflies (Tsubaki and Matsumoto 1998; Windig and Nylin 1999; Gibbs and Breuker 2006; Breuker et al. 2010) and it has been suggested that it is an indicator of an individual's quality (Leamy and Klingenberg 2005). Fluctuating asymmetry takes the form of small, random deviations from bilateral symmetry, which arise as a consequence of imprecisions in the development of individual organisms (Palmer and Strobeck 2003; Klingenberg 2015). Earlier studies revealed that sexually selected signals of quality show a greater variation in fluctuating asymmetry than non-signalling traits (Møller and Pomiankowski 1993; Sheehan and Tibbetts 2011). This phenomenon has several possible explanations: first of all, increased fluctuating asymmetry in sexually selected traits could be due to the fact that the production of these signals is costly and stressful for the organism (Møller and Pomiankowski 1993) and/or secondly, these signals of quality are more influenced by environmental stress (Polak and Starmer 2005). Fluctuating asymmetry has been measured in studies of a wide variety of organisms, from plants to humans (Graham et al. 2010; Beasley et al. 2013; Graham and Özener 2016; Kleisner et al. 2017). The link between fluctuating asymmetry and UV reflectance has not yet been studied in butterflies, but there is some evidence from birds. For example, Galván (2011) found a negative correlation between the ultraviolet-blue reflectance of the crown patch and fluctuating asymmetry of feathers in blue tits (*Cyanistes caeruleus*).

Iwasa and Pomiankowski (1995) have shown that Fisherian runaway process of sexual selection is unstable and leads to a continuous change of sexual traits, which is also why it can be supposed that secondary sexual characteristics ought to be more variable than non-sexual traits. Moreover, due to the need for effective differentiation between potential mating partners belonging to one's own species and individuals of a related species, sexually selected traits in closely related species should have a different appearance. *Gonepteryx* butterflies look very similar in the visible spectrum but when observed in the UV spectrum, differences between the various species are quite striking (see Fig. 1). One can thus assume that UV patterns are subjected to sexual selection for better mate recognition and this mechanism is responsible for the phenotypic differentiation of particular species within the UV spectrum. A similar situation can be found among the closely related *Colias* species, where sexually selected traits can have a very different appearance in allied species, whereas nonsexual traits are much more similar (Brunton 1998; Kemp et al. 2005). It can therefore be supposed that UV-reflective characteristics should be relatively stable within a species but quite distinct among closely related species, especially among species that live sympatrically. In such cases, UV-reflective patches may function as a mechanism of reproductive isolation.

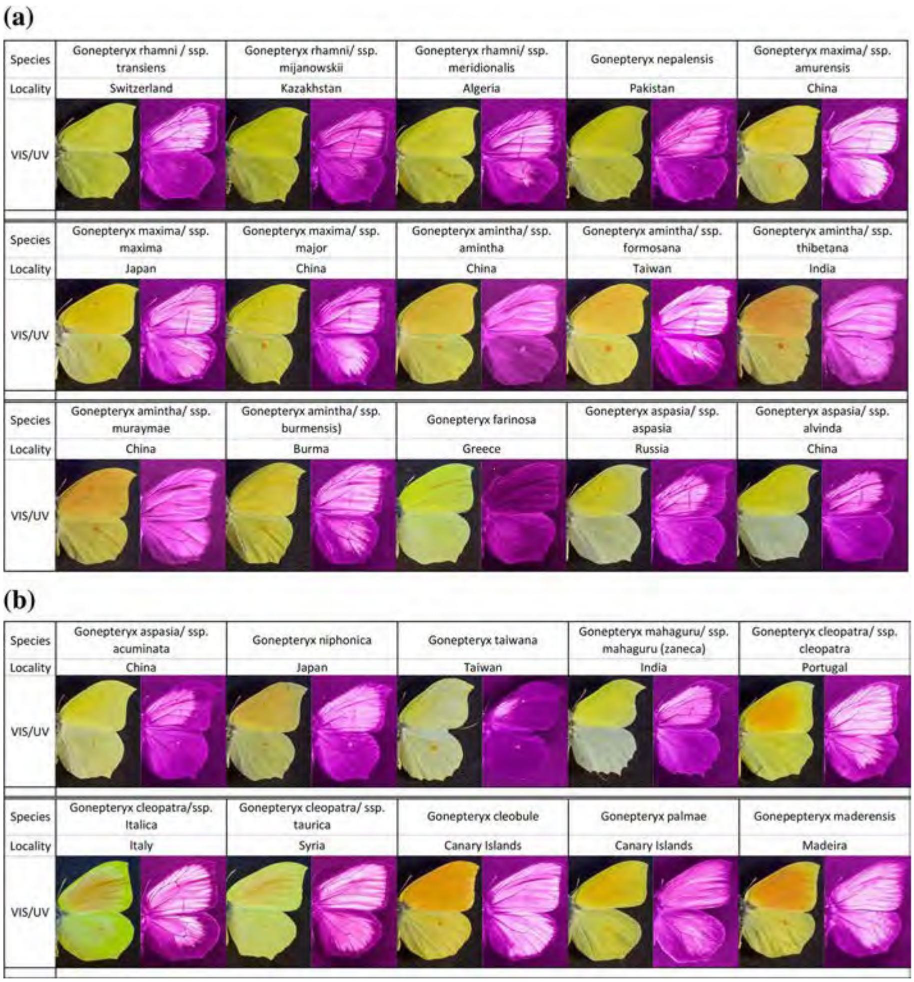


Fig. 1 Dorsal wing surfaces of *Gonepteryx* males in visible (left images) and ultraviolet (right images) light. Pictures are provided for all species of this genus, with the exception of *G. chinensis*, *G. chitralensis*, and *G. eversii*, which should, however, strongly resemble the *G. cleobule* in both parts of the spectrum. The subspecies included are not meant to be representative of the entire *Gonepteryx* genus. Their function is merely to demonstrate the diversity of UV-reflecting patches on fore- and hindwings of this genus

This hypothesis was tested on representatives of the butterfly subfamily Coliadinae, specifically on the *Colias* and *Gonepteryx* genera (Brunton and Majerus 1995). The study revealed a highly significant intraspecific variability in the levels of UV reflectance intensity. Brunton and Majerus (1995) assumed that the UV patterns of coliadinid butterflies play a role on an intraspecific level by mediating sex recognition. Nevertheless, spectrophotometric measurements are necessarily limited because they do not deliver any information about possible variations in the shape of UV patches. The presence or absence, shape and size of UV patches may play an equally important role during courtship (Rutowski 1977; Silberglied and Taylor 1978; Kemp 2007, 2008a). It has also been shown that UV signals prevent interspecific crossbreeding in *Colias* butterflies

(Silberglied and Taylor 1973, 1978), and similar phenomena have been reported in several lycaenid butterflies (Meyer-Rochow 1991; Lukhtanov et al. 2005; Imafuku 2008). In some coliadinid butterflies, even females have UV patches on their wing surfaces, but their function in courtship is unclear (Rutowski and Kemp 2017; Stella et al. 2018a).

In this study, we focus on investigating possible associations between the morphological properties of male UV patches and several ecological (both environmental and geographical) variables in seven species of genus *Gonepteryx* butterflies. We expect UV patches, which are hypothetically signals of quality, to be condition-dependent, i.e. more influenced by environmental conditions than non-signalling traits (Badyaev 2004; Cotton et al. 2004). Specifically, we hypothesise that (1) UV patches, which are a signalling trait, should show higher levels of fluctuating asymmetry than wing venation, which is a non-signalling trait; (2) UV patches of butterflies living in more hostile environments should show higher levels of fluctuating asymmetry; (3) variation in the shape of UV patches should be more influenced by environmental conditions (such as altitude, mean annual temperature, and mean annual precipitation) than the shape variation of wing venation. And finally, (4) we compare interspecific morphological differences in two selected configurations of wing shapes to examine UV patch stability within a taxon and their possible role in mate recognition. If a UV patch is indeed a sexually selected trait, it should differentiate between the taxa better than a trait that is not subject to sexual selection, in our case a wing venation pattern.

Materials and methods

Genus Gonepteryx

Species belonging to the genus *Gonepteryx*, commonly known as brimstones, are found throughout almost the entire Palaearctic, from the British Isles to Japan. In the past, this genus has undergone two major revisions (Nekrutenko 1968; Kudrna 1975). While Nekrutenko (1968) placed emphasis especially on the ultraviolet patches of particular species and subspecies as a diagnostic trait, Kudrna (1975) focused on analysing some traditional taxonomic characters such as the morphology of male genitalia. Bozano et al. (2016) have recently proposed the first molecular-based phylogeny of the genus, which changed the taxonomic status of some traditional subspecies to a species level. There are now 15 recognised species within the genus *Gonepteryx* (*G. rhamni*, *G. nepalensis*, *G. chinensis*, *G. maxima*, *G. amintha*, *G. farinosa*, *G. chitralensis*, *G. niphonica*, *G. aspasia*, *G. mahaguru G. taiwana*, *G. cleopatra*, *G. maderensis*, *G. palmae*, and *G. cleobule*) and over 30 subspecies, but the taxonomic status of some brimstones remains unclear (Kudrna 1975; Bozano et al. 2016). A vast majority of *Gonepteryx* males have iridescent ultraviolet patches on the dorsal surfaces of the wings, especially on the forewings. The presence and size of UV patches does, however, vary among *Gonepteryx* species and for instance some species and subspecies have UV patches on their hindwings as well (Fig. 1). In females, UV patches are mostly absent, the only exception being the females of *G. cleobule* from the Canary Islands, which have UV patches on their forewings, albeit much smaller than those present in conspecific males. UV patches of *Gonepteryx* butterflies have also been viewed as a possible diagnostic trait of a taxonomic value (Mazokhin-Porshnyakov 1957; Nekrutenko 1964, 1968, 1972; Matsuno 1988; Brunton and Majerus 1995; Brunton et al. 1996; Pecháček et al. 2014; Bozano et al. 2016).

Specimens

We photographed of 522 males of all *Gonepteryx* species. Photographs of the specimens were taken in the entomological collections of the Natural History Museum in London (Great Britain), Natural History Museum in Vienna (Austria), Natural History Museum of the University of Tartu (Estonia), Moravian Museum in Brno, and the National Museum in Prague (both Czech Republic). We recorded the geographic coordinates where each specimen was captured (Fig. 2). All damaged butterflies were removed from the dataset to avoid inaccurate application of shape variables (landmarks and semi-landmarks). In the next step, we discarded specimens with missing, misspelled, or untraceable localities, which is a common problem with older museum specimens. For morphometric analysis, we avoided using species with less than 15 specimens or species with UV reflecting patches which are either too small or missing (*G. taiwana* and *G. farinosa*). After removing unsuitable specimens, our dataset included 320 individual male specimens of seven species (*G. rhamni*—75 observations, *G. nepalensis*—26, *G. maxima*—18, *G. amintha*—64, *G. aspasia*—49, *G. niphonica*—16 and *G. cleopatra*—72).

Photographing in *UV wavelength range*

We used a FujiFilm IS Pro digital camera suitable for UV photography thanks to its broad sensitivity spectrum, which spans from 330 to 900 nm. The camera was equipped with an uncoated UV-transmitting lens (Helios 44-2 58 mm f/2 lens). We used photographical filters B + W 403 and B + W BG 53. The black filter B + V 403 blocks the visible spectrum (400–700 nm), while the B + W BG 38 filter blocks the IR light ($\lambda > 700$ nm). As a result, only UV light ($\lambda < 400$ nm) is transmitted through the lens. To illuminate the photographed

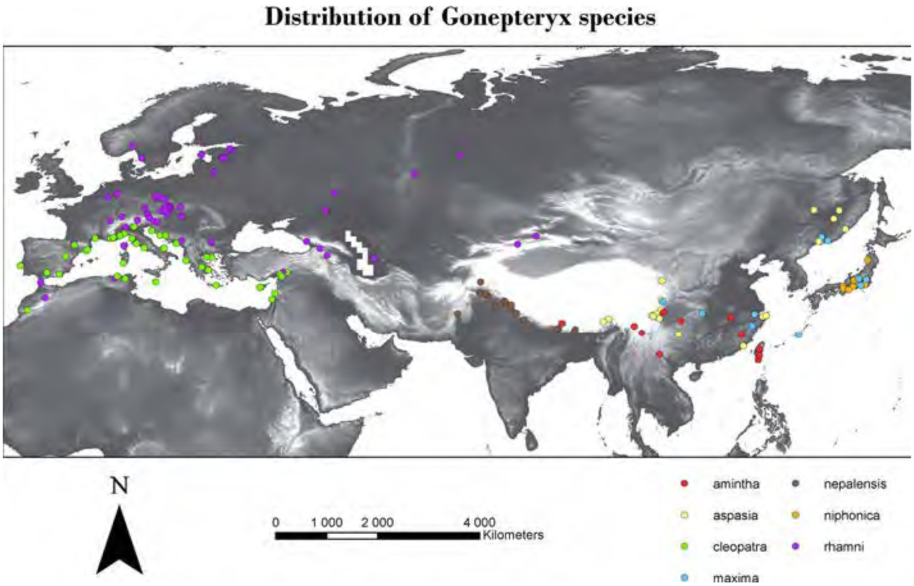


Fig. 2 Distribution of specimens of seven *Gonepteryx* species from the Palaearctic region used in the shape analysis

objects, we used a UVP MRL-58 multiple-Ray-Lamp (8-watt, 230 V-50 Hz, 0.16A) equipped with a mercury fluorescent lamp 8w F8T5 long-wave 365 nm. All objects were illuminated *at a 45 degree angle* and photographed in a standardised position, in this case dorsal view which is most appropriate way to photograph iridescent UV patches, at least in the *Gonepteryx* genus (Pirih et al. 2011; Pecháček et al. 2014). For all specimens, we used the following setting of the FujiFilm IS Pro camera: ISO 400, shutter time 15´, aperture of 3.5. All images were standardised using 18% grey card, Kodak colour separation guide, and a 15 cm length scale.

Environmental and geographical variables

We used longitude and latitude obtained from the specimen labels as potential geographical correlates of UV ornamentation. Then we used these coordinates to assign (in ArcGIS 10.0; ESRI Inc.) to each specimen seven environmental variables: altitude, mean annual temperature, mean annual precipitation, isothermality, temperature seasonality, precipitation seasonality, and temperature annual range (Hijmans et al. 2005). These variables were chosen because it has been demonstrated that they have an impact on insect development, size, and colouration (Dixon et al. 2009; Fischer and Karl 2010; Chown and Gaston 2010; Pecháček et al. 2014; Stella et al. 2018b). All variables were resolved at 30 arcsec (approximately 1 km at the equator).

To avoid co-linearity between environmental and geographical variables, we performed a Principal Components Analysis [PCA, centred, standardised; package 'stats' in R software, version 3.2.3 (R Development Core Team R 2015)] on all climatic and geographic variables and used the first principal component (PC1) axe, which explained 40.92% of all environmental variability, for further analyses. For relative loadings of geographic and environmental variables for first principal component, see Table 1.

Table 1 Relative loadings of nine geographic and environmental variables for the first (PC1) principal component and the percent of variability explained by PC1

	All	*rhamni*	*nepalensis*	*maxima*	*amintha*	*aspasia*	*niphonica*	*cleopatra*
Latitude	0.448	−0.294	0.422	−0.428	0.382	−0.408	−0.273	0.476
Longitude	−0.166	−0.383	−0.359	−0.237	−0.377	−0.428	−0.100	−0.344
Altitude	−0.255	0.176	−0.058	0.196	0.309	0.356	−0.461	−0.310
Isothermality	−0.409	0.394	−0.321	0.307	−0.180	0.384	0.105	−0.133
Precipitation annual mean	−0.290	0.335	−0.382	0.334	−0.409	0.024	−0.031	0.247
Precipitation seasonality	−0.310	−0.090	−0.290	−0.221	0.143	0.113	−0.091	−0.427
Temperature annual mean	−0.242	0.193	0.011	0.317	−0.376	0.115	0.508	−0.134
Temperature annual range	0.356	−0.426	0.410	−0.430	0.394	−0.398	−0.424	−0.387
Temperature seasonality	0.416	−0.491	0.433	−0.427	0.314	−0.437	−0.496	−0.362
Var. explained by PC1 (%)	40.92	41.99	55.03	57.1	57.43	54.55	38.98	35.46

Landmark definitions and Procrustes analysis

Ultraviolet digital photographs of the left and right forewing of each of the 320 male specimens of the *Gonepteryx* genus were analysed using geometric morphometrics. On each forewing, we placed 41 landmarks, including 12 semi-landmarks (see Fig. 3), using tpsDig2 software ver. 2.17 (Rohlf 2013a). Landmarks are homologous points which can be placed on the forewing of each specimen in the set, whereas semi-landmarks denote the curves and outlines of the forewing where fixed landmarks cannot be defined. We defined two spatial regions on the wing: the first landmark configuration is labelled '*Venation*' and defined by the outline of the wing (1–11, 30–41) and vein bifurcation points (21–29). The second landmark configuration, which we call '*UV patch*', was in this case defined as the outline of the wing supplemented by the shape of the ultraviolet patch, i.e. the UV-reflective part of the wing (12–20). Each specimen was landmarked twice (by the same person) to estimate measurement errors stemming from random inaccuracy of manual landmark application.

All configurations of landmarks and semi-landmarks were superimposed by a generalised Procrustes analysis (GPA) performed in tpsRelw ver. 1.53 (Rohlf 2013b). This procedure standardises the size of the objects and optimises their rotation and translation so that distances between the corresponding landmarks are minimised.

Fluctuating asymmetry (FA)

Fluctuating asymmetry was computed by Procrustes ANOVA implemented in MorphoJ, version 1.05f (Klingenberg 2011). Measurement of fluctuating asymmetry using geometric morphometrics provides a more sensitive and comprehensive approach than either linear or meristic methods (Beasley et al. 2013; Klingenberg 2015). Shape coordinates on the left

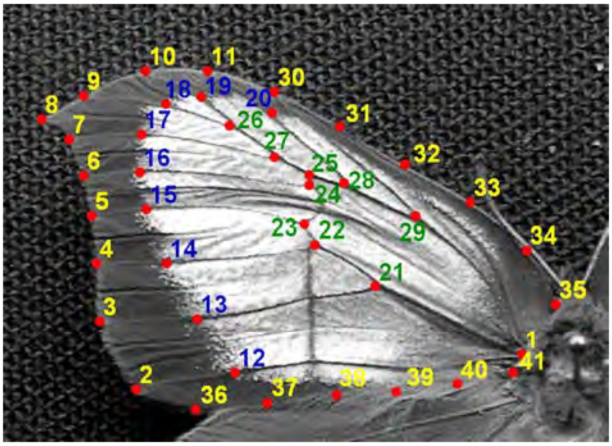


Fig. 3 Locations of landmarks on the left forewing. Points 1–31 represent homologous anatomical landmarks found in all analysed subjects. Points 30–41 represent semi-landmarks which denote curves. Yellow numbers mark the shape of the wing, blue numbers show the shape of the UV patch, and green numbers indicate bifurcation of the veins. Landmark 1 is placed at the wing base (the connection of anal and cubital vein), landmark 8 on the wing apex. Landmarks 2–20 are located at vein endings at the edge of the wing and the edge of the UV-reflecting patch. Landmarks 21–29 represent the bifurcation points of the veins

Table 2 Procrustes ANOVA for *UV patch* (defined by the outline of the wing and UV patch shape) [landmarks 1–11, 30–41 + 12–20; see Fig. 3]

Effect	SS	MS	df	F	*p* value
Individual	10.13149943	0.0005293364	19,140	30.52	<.0001
Side	0.21996082	0.0036660137	60	211.35	<.0001
Ind * side	0.3319895	0.0000173453	19,140	2.64	<.0001
Error 1	0.25250793	0.0000065757	38,400		

Columns indicate procrustes sums of squares (SS), procrustes mean squares (MS), degrees of freedom (df), Goodall's F statistic (F), and the associated *p* value. The effect of interaction between individuals and sides represents a fluctuating asymmetry

Table 3 Procrustes ANOVA for *Venation* (shape pattern defined by the outline of the wing and vein bifurcation points) [lm 1–11, 30–41 + 21–29]

Effect	SS	MS	df	F	*p* value
Individual	3.35482045	0.000175278	19140	12.27	<.0001
Side	0.30864103	0.0051440172	60	359.98	<.0001
Ind * side	0.27350254	0.0000142896	19,140	2.65	<.0001
Error 1	0.20708975	0.000005393	38,400		

and the right wing (wing on one side was reflected to its mirror image along the axis of symmetry in order to align the corresponding landmarks on both sides) were then used as independent variables (Klingenberg and McIntyre 1998; Mardia 2005).

In the ANOVA design, the main effect of 'individuals' is a variation among individuals corrected for any effect of asymmetry, while the main effect of sides corresponds to the average difference between the left and right side of the wing (directional symmetry). Fluctuating asymmetry was quantified as interaction between the main effects of 'individuals' and 'sides'. Measurement error was computed from variations between replicate measurements (Klingenberg et al. 2002). In the current study, Procrustes ANOVA revealed a significant effect of directional (sides) and fluctuating asymmetry (interaction of sides × individuals) for the wings of *Gonepteryx* individuals. These results are displayed in Tables 2 and 3. Values of fluctuating asymmetry, where higher values indicate higher wing fluctuating asymmetry, were then used for a further analysis.

Correlations between environmental and geographical variables, fluctuating asymmetry, and shape of the signalling and non-signalling trait

To test whether the levels of fluctuating asymmetry of the signalling trait (*UV patch*) and the non-signalling trait (*Venation*) statistically differ, we performed the Wilcoxon Rank Sum Test (in R 'stats' package) for all 320 specimens. We used this nonparametric test because the scores of fluctuating asymmetry were not normally distributed. To quantify a correlation between fluctuating asymmetry and the environment represented by the PC1, we performed Linear Mixed-Effects Models [function 'lmer' within R package 'lme4' (Bates et al. 2015)]. Centroid size (CS) of the wing was added to the model to control for the trait size effect on fluctuating asymmetry.

To estimate the relationship between wing morphology, represented by the left forewing, and environmental (and geographic) variables, we used Phylogenetic regression for Procrustes shape variables with permutation procedures (function 'procD.pgls') implemented in the 'geomorph' package in R (Adams and Otarola-Castillo 2013). To control for shape variation due to allometry, we added wing size (computed as the centroid size for each landmark configuration) as a covariate in the model. To control for variation due to the presence of multiple species in the dataset, we used a phylogenetic tree of genus *Gonepteryx* based on Bozano et al. (2016) and our own unpublished data. To analyze all species separately, we used Procrustes ANOVA with permutation procedures (function 'procD.lm' within R package 'geomorph'). All *p* values were based on 9999 permutations. We generated 1000 bootstrap replicates of estimated R-squared values in the case of a model with *UV shape* and *Venation*, respectively, as a response variable. Subsequently, we evaluated the overlap of corresponding confidence intervals of estimated R-squared values with a two-tailed *t* test, which corresponded to the significance test of difference between the observed R-squared values for the explanatory variables of *UV patch* and *Venation*.

Canonical variate analysis

We used a canonical variate analysis (CVA) to compare two previously defined shapes (*Venation* and *UV patch*) in the seven selected species. We performed a regression of shape on size and the residuals were used to carry out the CVA in order to control for the trait size effect (Klingenberg 2016). To evaluate the effect of UV ornaments, we compared the results of CVA computed using the two different sets of morphometric variables, namely *UV patch* and *Venation*. The analysis was performed in MorphoJ, version 1.05f (Klingenberg 2011).

Results

Fluctuating asymmetry

Wilcoxon Rank Sum Test was used to compare the values of fluctuating asymmetry in the two sets of shape measurements (*UV patch* and *Venation*). These two measurements were significantly different (N = 320, W = 61,586, $p < 0.001$). The shape of the wing area with UV patterns turned out to be more asymmetric (FA mean = 0.031) than the shape defined by the wing margin and venation (FA mean = 0.028).

Further, we used all 320 males to test the relationship between the fluctuating asymmetry of *UV patch*/*Venation* and the environment (PC1). We found a significant relationship between size (represented by the centroid size of the left forewing) and both *Venation* and *UV patch* (*UV patch*: ß = −0.119, *SE* = 0.047, t = −2.503, $p = 0.013$; *Venation*: ß = −0.131, *SE* = 0.045, t = −2.905, $p = 0.004$). The relationship between the environment and shapes (FA) was significant for the *UV patch*, whereas for *Venation* it approached significance (*UV patch*: ß = 0.031, *SE* = 0.012, t = 2.595, $p = 0.011$; *Venation*: ß = 0.022, *SE* = 0.012, t = 1.862, $p = 0.065$). We analysed all species separately in the same manner and the effects of the environment on fluctuating asymmetry were significant only in a minority of cases, in particular in *G. maxima* for both shapes and in *G. nepalensis* and *G. niphonica* for *Venation* only (see Table 4).

Table 4 Fluctuating asymmetry (FA)

Species	PC1—*UV patch*				PC1—*Venation*			
	ß	*SE*	t	*p*	ß	*SE*	t	*p*
All species	0.031	0.012	2.595	0.011*	0.022	0.012	1.862	0.065
rhamni	−0.020	0.018	−1.082	0.283	−0.005	0.015	−0.300	0.765
nepalensis	0.028	0.024	1.197	0.244	0.058	0.028	2.082	0.049*
maxima	−0.161	0.055	−2.918	0.011*	−0.117	0.051	−2.291	0.046*
amintha	0.003	0.024	0.107	0.917	−0.004	0.023	−0.195	0.849
aspasia	−0.015	0.015	−0.970	0.337	−0.026	0.016	−1.672	0.122
niphonica	−0.033	0.035	−0.953	0.358	−0.087	0.031	−2.794	0.015*
cleopatra	−0.001	0.020	−0.076	0.940	0.002	0.018	0.130	0.897

This table demonstrates relationships between the FA scores of the two measured traits, namely *UV* patch (the outline of the wing and shape of the UV patch) [landmarks 1–11, 30–41 + 12–20; see Fig. 3] and *Venation* (the outline of the wing and vein bifurcation points) [lm 1–11, 30–41 + 21–29], and the environment (represented by PC1). The species were first analysed together (controlled for species identity) and then separately. Controls for trait size effect on fluctuating asymmetry were performed in all cases

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Relationship between the shape and the environment

Using a dataset of all 320 males, we investigated the relationship between the two defined shapes (*Venation* and *UV patch*) and environment. Environment was represented by the first principal axis (PC1). The environment (PC1) significantly influenced the shape of the *UV patch* ($F = 33.852$, $p < 0.001$, $R^2 = 0.082$). Statistically significant was also the environment's effect on the wing size ($F = 64.306$, $p < 0.001$, $R^2 = 0.155$). Relationships between *Venation* and PC1, and size: PC1 ($F = 7.458$, $p = 0.022$, $R^2 = 0.02$), size ($F = 46.058$, $p \leq 0.001$, $R^2 = 0.124$).

Further, we ran a separate analysis to test for the effect of the environment on shape for all species separately (Table 5). The effect of the environment on both *Venation* and *UV patch* was significant in *G. amintha* and *G. cleopatra*, whereby variation explained by the environment was significantly higher in the *UV patch* than in *Venation*. *Gonepteryx rhamni* and *G. aspasia* also displayed a non-random relationship between both shapes and the environment but the difference between *Venation* and *UV patch* with respect to variation explained by the environment was not significant. In general, the explained variance was higher for the *UV patch* in all cases, with the most distinct effect found in *G. aspasia* (see Table 5 for a comparison of environmental effects in both *Venation* and *UV patch*).

Shape differences between species

We found that *UV patch* and *Venation* shape patterns in the seven *Gonepteryx* species were significantly different. Procrustes distances represent the value of shape differentiation between the compared groups (species), whereby—as shown in the Table 6 where higher values indicate a greater morphological distance between species—*UV patch* is a better discriminating character than *Venation* in a vast majority of cases (with the exception of

Table 5 Relationship between the shape of two measured traits (*UV patch/Venation*) and the environment represented by the first principal axis (PC1) (controlled for size)

Species	PC1—*UV patch*			PC1—*Venation*			R^2 comp.
	F	*p*	R^2	F	*p*	R^2	*p*
All species	33.852	<0.001***	0.082	7.458	0.022*	0.020	<0.001***
rhamni	2.647	0.036*	0.032	1.928	0.036*	0.023	0.265
nepalensis	1.115	0.320	0.045	0.697	0.841	0.028	0.039*
maxima	0.716	0.669	0.041	0.627	0.796	0.035	0.478
amintha	6.993	<0.001***	0.099	4.701	<0.001***	0.067	<0.001***
aspasia	21.555	<0.001***	0.281	3.418	<0.001***	0.061	0.341
niphonica	1.814	0.115	0.101	0.835	0.639	0.055	<0.001***
cleopatra	2.101	0.027*	0.029	1.974	0.023*	0.027	0.001**

R^2 comp., the significance of statistical comparison between the $R_{UV patch}^2$ and $R_{Venation}^2$

$^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$

niphonica/*nepalensis* and *cleopatra*/*maxima*). This finding suggests a strong taxon stability of UV patches. Figure 4a, b show the scatter plots of PC1 and PC2 for the two defined shapes for all 320 males. Specimens are more densely clustered within species along the first PC axis and differences between the species are more prominent. It is evident that *UV patch* discriminates between the species better than *Venation* does.

Discussion

We found that the shape of the *UV patch*, defined by the outline of the wing and by the margins of the ultraviolet ornament, is more asymmetric than the shape marked as *Venation*, defined by the outline of the wing and vein bifurcation points. This result indicates that the production of sexually selected UV patches may be costlier to the butterfly than the development of a non-signalling trait (Møller and Pomiankowski 1993). On the other hand, we revealed a statistically significant relationship between the environmental variables and fluctuating asymmetry only in a minority of observations (both in all species together and in *G. maxima* for *UV patch* and *G. nepalensis*, *G. maxina*, and *G. niphonoca* in the case of *Venation*), and even in those cases, no distinct trend emerged. We therefore conclude that our results do not corroborate the hypothesis that fluctuating asymmetry is a reliable indicator of an individual's quality or a general indicator of environmental stress (Bjorksten and Pomiankowski 2000; Bjorksten et al. 2000; Lens et al. 2002; Talloen et al. 2004).

Furthermore, we showed that overall shape variation (for all 320 specimens) of UV patches is dependent on environmental conditions. This relationship is stronger than that between the environment and wing venation. UV patches thus seem to be more condition-dependent, i.e. more influenced by the environment, than the non-signalling trait (*Venation*).

When the species were analysed separately, the amount of variance in the shape of the *UV patch* explained by environmental factors was again higher than in the case of *Venation*, and this was especially apparent in *G. aspasia*. This trend was not, however, significant in all of the studied species. In comparison to our previous study of *G. rhamni* (Pecháček et al. 2014), results of our current work revealed a significant but not

Table 6 Procrustes distances between groups for the two defined shape patterns: *UV patch* and *Venation* (higher values indicate a greater morphological distance)

	rhamni		*nepalensis*		*maxima*		*amintha*		*aspasia*		*niphonica*	
	UV patch	*Venation*	*UV patch*	*Venation*	*UV patch*	*Venation*	*UV patch*	*Venation*	*UV patch*	*Venation*	*UV patch*	*Venation*
nepalensis	0.0686 (0.0341)	0.0345										
maxima	0.0924 (0.0582)	0.0342	0.059 (0.0157)	0.0433								
amintha	0.1353 (0.0908)	0.0445	0.0765 (0.049)	0.0275	0.0626 (0.0143)	0.0483						
aspasia	0.1018 (0.0321)	0.0697	0.1493 (0.0714)	0.0779	0.1786 (0.1126)	0.066	0.2091 (0.1424)	0.0667				
niphonica	0.0666 (0.0004)	0.0662	0.0714 (−0.0001)	0.0715	0.0986 (0.0312)	0.0674	0.1166 (0.0552)	0.0614	0.0977 (0.0728)	0.0249		
cleopatra	0.0967 (0.0562)	0.0405	0.0688 (0.0171)	0.0517	0.0399 (−0.0043)	0.0442	0.0728 (0.0139)	0.0589	0.1845 (0.0968)	0.0877	0.107 (0.0204)	0.0866

The number in parentheses represents a difference between *UV patch* Procrustes distances and *Venation* Procrustes distances. A positive number means the Procrustes distance between the UV patches of two species is greater than the distance between the same species measured by the venation pattern. Positive numbers in parentheses thus indicate that *UV patch* discriminates between two species better than *Venation* does. The higher number, the better discrimination

p value was < 0.001 for all observations

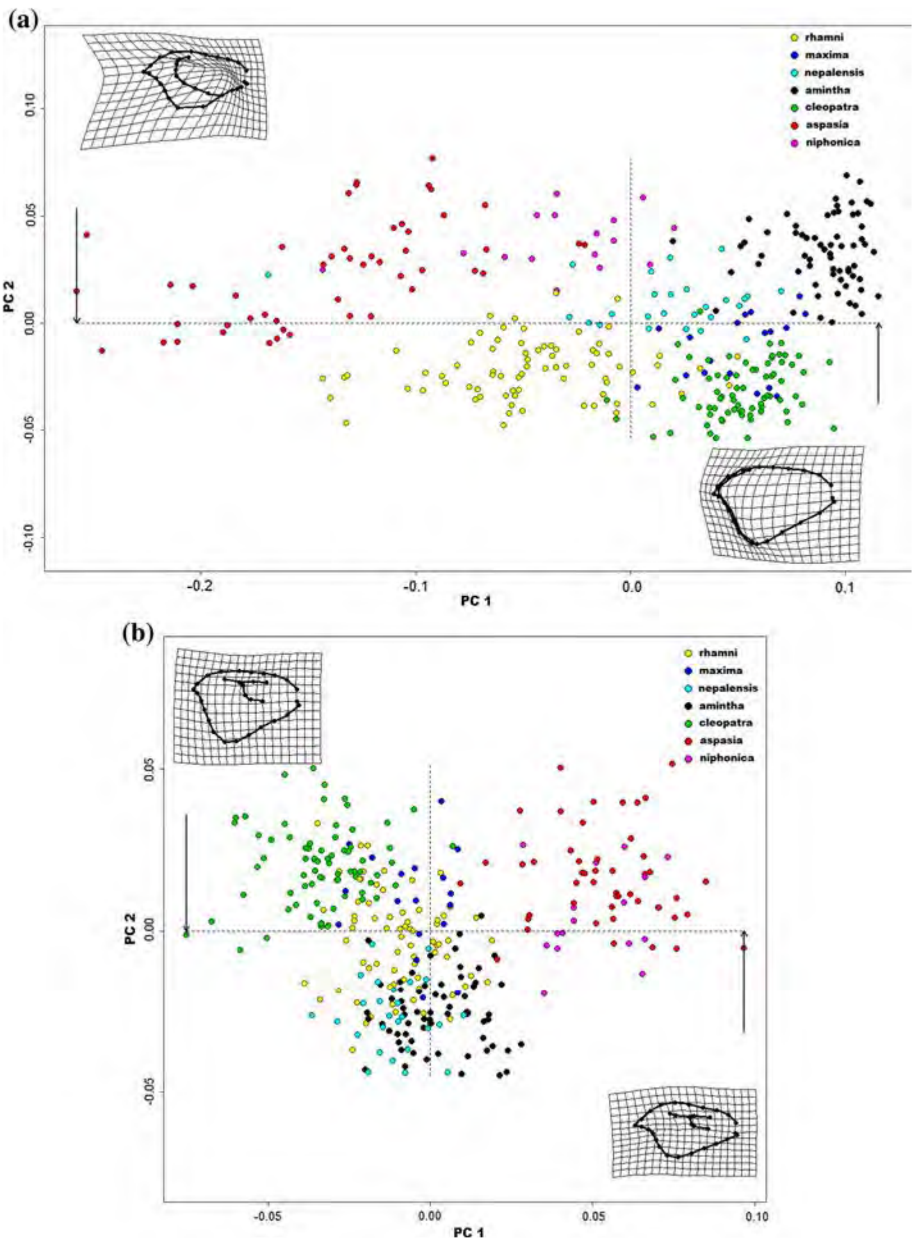


Fig. 4 The plots show shape differences between the seven analysed species along the first two principal component axes (PC1 and PC2) according the UV patches (**a**) and the venation pattern (**b**)

strong condition dependency of UV patches in this species. This difference in results might arise from a decrease in the range of variation within specimens of *G. rhamni* caused by a new taxonomy of the genus *Gonepteryx*, according to which *G. nepalensis* is a separate species (Bozano et al. 2016). Formerly, *G. nepalensis* was regarded as a subspecies of *G. rhamni* and it was as such that it was included in the abovementioned study of Pecháček et al. (2014).

Canonical variate analysis revealed that *UV patch* discriminates among species better than *Venation* does. This outcome supports the hypothesis that UV signals, being a possible secondary sexual characteristic, are more variable than non-sexual traits even in closely related species (Iwasa and Pomiankowski 1995). In the context of UV colouration, this hypothesis was investigated—with similar results—already in the *Colias* butterflies (Brunton 1998; Kemp et al. 2005), which are relatively closely related to the genus *Gonepteryx* (Wahlberg et al. 2014). Furthermore, we have shown that the analysed species statistically differ in the shape of UV patches on their forewing. This finding supports the classical claim of the Ukrainian entomologist Yuri Nekrutenko, who viewed UV patches as an important taxonomic characteristic within the genus *Gonepteryx* (Nekrutenko 1968). Usefulness of UV traits for taxonomy has been discussed also for other genera (Ferris 1972; Knuttell and Fiedler 2000). Furthermore, in the case of *Gonepteryx* butterflies, dorsal UV patches on both fore- and hindwings appears to be more variable than their colouration in the visible spectrum. For instance, males of *Gonepteryx farinosa* and *G. taiwana* have either none or very small UV patches on their wings. In many localities, *G. farinosa* is sympatric with *G. rhamni* so that absence of UV colouration in *G. farinosa* may have evolved as an adaptation that facilitates mate recognition. Similarly, *G. taiwana* is sympatric with *G. amintha formosana* in Taiwan. This fact suggests that different UV patterns may play a role in discriminating among possible mates (Fordyce et al. 2002). Mate recognition based on the absence or presence of UV signals has been hypothesised for *Colias* butterflies (Silberglied and Taylor 1973, 1978) and to a certain extent also for *Pieris rapae* (Obara et al. 2008; Fukano et al. 2012). Further support for the mate recognition function of UV patches may be found in the fact that very different UV patches can be found on the hindwings of all *amintha* subspecies (forewings are nearly completely covered by iridescent UV patch in all subspecies): no pattern (just a UV spot) in *G. amintha amintha*, larger UV patches in the *G. amintha formosana*, and UV patches covering almost the entire area of the hindwings in *G. amintha thibetana* and *G. amintha murayamae* (syn. *G. amintha yunnanensis*). Note, however, that *thibetana* and *murayamae* occur very far from each other (they are allopatric). Their identical UV appearance therefore cannot lead to any problems in mate recognition because they are not present in the same locality (Bozano et al. 2016). Both subspecies, however, may use the hindwing pattern to distinguish themselves from the *G. amitha amintha*, which has a much larger area of distribution and in parts of its distribution area is sympatric with one or the other subspecies.

All in all, our comparison of morphological properties (shape and fluctuating asymmetry) of the two chosen wing patterns (*UV patch* and *Venation*) indicates a tendency towards a larger condition-dependency in UV patches, which are hypothetical signalling traits, than in the non-signalling venation pattern. Statistically, however, the tendency was significant only in a minority of cases.

With exception of *G. aspasia*, the amount of shape variation explained by the environment was rather small in all the analyses. This outcome could be partly due to the fact that we controlled for the effect of wing size, which explained more than 1/4 of the variation in the *UV patch* and approximately 5 percent in *Venation*. By controlling for wing size, we eliminated a significant amount of variance, which left only a small part of variance to be accounted for by environmental effects. Without controlled experiments, however, we cannot estimate the proportion of size variation that may be due to environmental conditions and genetic background. By controlling for size, we may have thus dismissed some part of variation that is, to some extent, due to the environment. In this study, we were unable to differentiate between different sources of allometry (Klingenberg 2016).

Another limitation of our study may be due to selection of a non-signalling trait, i.e., wing venation. One always ought to be cautious when comparing different classes of traits, in this case structural and colour traits (Sheehan and Tibbetts 2011). It is for instance possible that the size and shape of the butterflies' wings are also influenced by sexual selection (Wickman 1992). Nevertheless, we used a functionally and developmentally constrained venation pattern, not the wing shape per se as a non-signalling trait.

Alternatively, the lower responsiveness of *Venation* (and thus the higher response of *UV patch*) to environmental fluctuations may be due to the greater evolutionary burden of the venation pattern (Schoch 2010). Venation underlies the functional and developmental structures of the wing and its variation during evolution may be therefore restricted (Shimmi et al. 2014).

For reasons discussed above, we cannot conclude that UV patterns in the seven studied brimstones are biological signals of mate quality in the same way in which it has been shown for UV colouration in other related species of butterflies (Kemp 2006b; Papke et al. 2007; Kemp and Rutowski 2011). Still, we have shown that there are significant interspecific differences in the shape of forewing UV patterns in the genus *Gonepteryx* and this indicates that UV patches may play a role in mate recognition.

Considering the connection between the UV reflective patterns and sexual selection, we need to ask in which stage of courtship could these patterns be actually significant. Like many other butterflies (Silberglied 1984), brimstone males actively search for females and, at least in *G. rhamni*, mating takes place soon after the females emerge from hibernation (Wiklund et al. 1996). The males emerge a few weeks earlier and start patrolling for mates. If a male brimstone finds a suitable female sitting somewhere, he circles around her and tries to convince her to copulate (Glaeser et al. 2017). If the female had already coupled with another male, she displays a distinctive mate-refusal posture (Wiklund et al. 1996; Friberg et al. 2008). If she has not, the male can be accepted. Then there follows a relatively complicated courtship flight, where the couple often flies high into the air. This ritual can be repeated several times. Using controlled manipulation of the level of UV reflectance or the shape size of the UV patches, as previously done in *Eureme hecabe* (Rutowski and Kemp 2017) and in *Hypolimnas bolina* (Kemp 2007), it could be investigated whether ultraviolet patterns indeed play a role in sexual selection of brimstones. Furthermore, using a similar set-up, we should be able to learn in which courtship phase these patches are relevant. If UV colouration is important during the initial contact with the female, a male with altered or removed UV patches should be rejected or ignored not only by females that have already mated but also by virgin females, such as in *Colias* butterflies (Silberglied and Taylor 1978) or jumping spiders (Lim et al. 2007). In potential experiments, however, one ought to take into account the role of pheromones in butterfly courtships (Vane-Wright and Boppré 1993). In *G. rhamni*, for instance, pheromones probably do play a role in rejecting the male (Scott 1972).

In context of our results, which suggests that there is a relationship between the environment and a UV pattern expression at least in some of the investigated species, it can be hypothesised that species living in areas where there is more shadow or dense, strongly UV-absorptive vegetation (Silberglied 1979), should have larger UV patterns or that these patterns should cover both fore- and hindwings to fulfil their signalling function. This hypothesis is linked to a proposal by Meyer-Rochow and Järvilehto (1997) who claim that the wings of *Pieris rapae* individuals from northern regions reflect the UV light more strongly because the percentage of UV irradiation on Earth's surface decreases from equator towards the poles (Herman et al. 1999). Extrapolating from this proposal, however, we should expect butterflies from arid or alpine areas to have larger UV patterns as well because in such areas, there is less grass with a low UV albedo, and sand or other materials with a relatively high UV albedo prevail (Chadyšiene and Girgždys 2008), with the consequence that as the UV albedo increases, contrast between UV iridescent wings and the background declines. Against a highly UV-absorptive background (e.g. grass), even smaller patterns should thus be large enough to fulfil a signalling function. On the other hand, if the UV pattern size positively depends on availability of accessible nutrients, the patches should be smaller in arid regions. It would be thus a good idea to focus on those attributes of UV reflective patterns which may depend on the type of habitat in which a species, subspecies, or population commonly occur.

Potentially significant but utterly neglected is the fact that UV reflectance appears not only in brimstone males but also in the females. It is usually stated that the wings of females are, with the exception of occasional UV spots, fully UV absorptive. That is true, but only for the dorsal side of the wings. Females of many brimstone species have a distinct UV iridescent pattern on the ventral side of their wings: UV light is reflected by the entire surface except for the area where the wings overlap (own unpublished data): in this way, the so-called Oudemans' phenomenon (i.e. the pattern covers only the exposed parts of overlapping morphological structures) comes into play (Oudemans 1903; Komárek 2003). It is therefore possible that female UV reflectance on ventral sides of their wings plays a role in the courtship of brimstone butterflies. Earlier studies suggest that female UV colouration may be used by males to find a female or to recognise that she is a suitable mating partner, as is the case of *Pieris rapae cruccivora* (Obara 1970; Obara and Majerus 2000).

The fact that in females, the ventral side of the wings often features a UV pattern has interesting ecological implications for brimstones. It is generally assumed that their leaf-like wing shape serves as a camouflage, that it allows the owner to blend in with the vegetation, thus protecting the specimen from a predator (Brakefield et al. 1992). This camouflage is supplemented by the colouring of the underside of their pale yellow-green wings. This is the case especially in females and it is apparent at least to human observers (when sitting on vegetation, these butterflies often close their wings). On the other hand, however, such females should be quite distinctive to birds, which are usually sensitive to UV light (Hart and Vorobyev 2005; Ödeen and Håstad 2013). We also should ask whether, given that males have a UV iridescent upper wing side, UV reflectance could play a role in visual communication with predators. Since brimstones hibernate as adults, they are among the first butterflies to emerge in spring (Wiklund et al. 1996). At that time, they are highly conspicuous and although they are edible, birds do not usually prey on them. Their colouration is thought to be aposematic, because it is reminiscent of colouration of some unpalatable pierids (Endler 1981; Rothschild 1981). We can also hypothesise that UV iridescent surfaces can be used to startle predators, as is the case of large and conspicuous eyespots of some other butterflies (Stevens 2005). Nonetheless, when attacked by a bird, butterflies stiffen and pretend to be dead (thanatosis) instead of actively defending themselves (Rothschild 1981).

Although our study suggests that UV patterns are involved in the sexual selection of *Gonepteryx* butterflies and comparisons with other butterflies make this suggestion very likely, further thorough manipulative and behavioural mating experiments are needed to confirm these conclusions. Such studies might confirm the abovementioned hypotheses, although long-lived brimstones are likely to make this process somewhat more difficult than the more traditional model species would.

Acknowledgements The authors would like to thank Blanca Huertas, Sabine Gaal-Haszler, Pavel Chvojka, Jan Šumpich, Petr Baňař, and Jaan Luig for providing butterfly collections, Zdeněk Faltýnek Fric for his help with preparing a phylogenetic tree used in the analyses, and Anna Pilátová for English proofreading. We would also like to thank T. White and two anonymous reviewers for making constructive comments to an earlier version of this manuscript. This research was supported by the Charles University Grant project GAUK 964216.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

Adams DC, Otarola-Castillo E (2013) Geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods Ecol Evol 4:393–399

Allen CE, Zwaan BJ, Brakefield PM (2011) Evolution of sexual dimorphism in the Lepidoptera. Annu Rev Entomol 56:445–464

Badyaev A (2004) Integration and modularity in the evolution of sexual ornaments. In: Pigliucci M, Preston K (eds) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Oxford University Press, Oxford, pp 50–79

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Beasley DAE, Bonisoli-Alquati A, Mousseau TA (2013) The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. Ecol Indic 30:218–226

Bernard GD, Remington CL (1991) Color vision in *Lycaena* butterflies: spectral tuning of receptor arrays in relation to behavioral ecology. Proc Natl Acad Sci USA 88:2783–2787

Bjorksten D, Pomiankowski F (2000) Fluctuating asymmetry of sexual and nonsexual traits in stalk-eyed flies: a poor indicator of developmental stress and genetic quality. J Evol Biol 13:89–97

Bjorksten TA, Fowler K, Pomiankowski A (2000) What does sexual trait FA tell us about stress? Trends Ecol Evol 15:163–166

Bozano GC, Coutsis JG, Herman P, Allegrucci G, Cesaroni D, Sbordoni V (2016) Pieridae Part 3 (Guide to the butterflies of the Palearctic region). Omnes Artes, Milano

Brakefield PM, Shreeve TG, Thomas JA (1992) Avoidance, concealment and defence. In: Dennis RLH (ed) The ecology of butterflies in Britain. Oxford University Press, Oxford, pp 93–119

Breuker CJ, Gibbs M, Van Dongen S, Merckx T, Van Dyck H (2010) The use of geometric morphometrics in studying butterfly wings in an evolutionary ecological context. In: Elewa AMT (ed) Morphometrics for nonmorphometricians. Springer, Berlin, pp 271–287

Briscoe AD, Chittka L (2001) The evolution of color vision in insects. Annu Rev Entomol 46:471–510

Brunton CFA (1998) The evolution of ultraviolet patterns in European *Colias* butterflies (Lepidoptera, Pieridae): a phylogeny using mitochondrial DNA. Heredity 80:611–616

Brunton CFA, Majerus MEN (1995) Ultraviolet colors in butterflies—intraspecific or inter-specific communication. Proc R Soc Lond B 260:199–204

Brunton CFA, Russel PJC, Majerus MEN (1996) Variation in ultraviolet wing patterns of brimstone butterflies (*Gonepteryx*: Pieridae) from Madeira and the Canary Islands. Entomol Gaz 115:30–39

Bybee SM, Yuan F, Ramstetter MD, Llorente-Bousquets J, Reed RD, Osorio D, Briscoe AD (2012) UV photoreceptors and UV-yellow wing pigments in *Heliconius* butterflies allow a color signal to serve both mimicry and intraspecific communication. Am Nat 179:38–51

Chadyšiene R, Girgždys A (2008) Ultraviolet radiation albedo of natural surfaces. J Environ Eng Landsc Manag 16:83–88

Chen P-J, Awata H, Matsushita A, Yang E-C, Arikawa K (2016) Extreme spectral richness in the eye of the common bluebottle butterfly, *Graphium sarpedon*. Front Ecol Evol 4:1–12

Chown SL, Gaston KJ (2010) Body size variation in insects: a macroecological perspective. Biol Rev Camb Philos Soc 85:139–169

Cotton S, Fowler K, Pomiankowski A (2004) Condition dependence of sexual ornament size and variation in the stalk-eyed fly Cyrtodiopsis dalmanni (Diptera: Diopsidae). Evolution 58:1038–1046

Dalrymple RL, Kemp DJ, Flores-Moreno H, Laffan SW, White TE, Hemmings FA, Tindall ML, Moles AT (2015) Birds, butterflies and flowers in the tropics are not more colourful than those at higher latitudes. Glob Ecol Biogeogr 24:1424–1432

Dixon AFG, Honěk A, Keil P, Kotela MAA, Šizling AL, Jarošík V (2009) Relationship between the minimum and maximum temperature thresholds for development in insects. Funct Ecol 23:257–264

Eguchi E, Meyer-Rochow VB (1983) Ultraviolet photography of forty-three species of lepidoptera representing ten families. Annot Zool Jpn 56:10–18

Endler JA (1981) An overview of the relationships between mimicry and crypsis. Biol J Linn Soc 16:25–31

Ferris CD (1972) Ultraviolet photography as an adjunct to taxonomy. J Lepid Soc 26:210–215

Fischer K, Karl I (2010) Exploring plastic and genetic responses to temperature variation using copper butterflies. Clim Res 43:17–30

Fleishman LJ, Loew ER, Whiting MJ (2011) High sensitivity to short wavelengths in a lizard and implications for understanding the evolution of visual systems in lizards. Proc R Soc Lond B 278:2891–2899

Fordyce JA, Nice CC, Forister ML, Shapiro AM (2002) The significance of wing pattern diversity in the Lycaenidae: mate discrimination by two recently diverged species. J Evol Biol 15:871–879

Friberg M, Vongvanich N, Borg-Karlson A-K, Kemp DJ, Merilaita S, Wiklund C (2008) Female mate choice determines reproductive isolation between sympatric butterflies. Behav Ecol Sociobiol 62:873–886

Fukano Y, Satoh T, Hirota T, Nishide Y, Obara Y (2012) Geographic expansion of the cabbage butterfly (*Pieris rapae*) and the evolution of highly UV-reflecting females. Insect Sci 19:239–246

Galván I (2011) Ultraviolet-blue plumage colouration can be perceived as an indicator of fluctuating asymmetry by Blue Tits (*Cyanistes caeruleus*). J Ornithol 152:223–230

Ghiradella H, Aneshansley D, Eisner T, Silberglied RE, Hinton HE (1972) Ultraviolet reflection of a male butterfly: interference color caused by thin-layer elaboration of wing scales. Science 178:1214–1217

Gibbs M, Breuker CJ (2006) Effect of larval-rearing density on adult life-history traits and developmental stability of the dorsal eyespot pattern in the speckled wood butterfly, *Pararge aegeria*. Entomol Exp Appl 118:41–47

Glaeser G, Paulus HF, Nachtigall W (2017) Criteria of evolution. In: Glaeser G, Paulus HF, Nachtigall W (eds) The evolution of flight. Springer, Cham, pp 87–122

Graham J, Özener B (2016) Fluctuating asymmetry of human populations: a review. Symmetry 8:154

Graham JH, Raz S, Hel-Or H, Nevo E (2010) Fluctuating asymmetry: methods, theory, and applications. Symmetry 2:466–540

Hart NS, Vorobyev M (2005) Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191:381–392

Herman JR, Krotkov N, Celarier E, Larko D, Labow G (1999) Distribution of UV radiation at the Earth's surface from TOMS-measured UV-backscattered radiances. J Geophys Res Atmos 104:12059–12076

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978

Hiyama A, Taira W, Otaki JM (2012) Color-pattern evolution in response to environmental stress in butterflies. Front Genet 3:15

Hunt S, Cuthill IC, Bennett AT, Church SC, Partridge JC (2001) Is the ultraviolet waveband a special communication channel in avian mate choice? J Exp Biol 204:2499–2507

Imafuku M (2008) Variation in UV light reflected from the wings of *Favonius* and *Quercusia* butterflies. Entomol Sci 11:75–80

Iwasa Y, Pomiankowski A (1995) Continual change in mate preferences. Nature 377:420–422

Kemp DJ (2006a) Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. Evol Ecol Res 8:515–527

Kemp DJ (2006b) Ultraviolet ornamentation and male mating success in a high-density assemblage of the butterfly *Colias eurytheme*. J Insect Behav 19:669–684

Kemp DJ (2007) Female butterflies prefer males bearing bright iridescent ornamentation. Proc R Soc Lond B 274:1043–1047

Kemp DJ (2008a) Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). Behav Ecol 19:1–8

Kemp DJ (2008b) Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. Evolution 62:2346–2358

Kemp DJ, Macedonia JM (2006) Structural ultraviolet ornamentation in the butterfly *Hypolimnas bolina* L. (Nymphalidae): visual, morphological and ecological properties. Aust J Zool 54:235–244

Kemp DJ, Rutowski RL (2007) Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. Evolution 61:168–183

Kemp DJ, Rutowski RL (2011) The Role of coloration in mate choice and sexual interactions in butterflies. Adv Study Behav 43:55–92

Kemp DJ, Rutowski RL, Mendoza M (2005) Colour pattern evolution in butterflies: a phylogenetic analysis of structural ultraviolet and melanic markings in North American sulphurs. Evol Ecol Res 7:133–141

Kemp DJ, Vukusic P, Rutowski RL (2006) Stress-mediated covariance between nano-structural architecture and ultraviolet butterfly coloration. Funct Ecol 20:282–289

Kemp DJ, Herberstein ME, Grether GF (2012) Unraveling the true complexity of costly color signaling. Behav Ecol 23:233–236

Kleisner K, Kočnar T, Tureček P, Stella D, Akoko RM, Třebický V, Havlíček J (2017) African and European perception of African female attractiveness. Evol Hum Behav 38:744–755

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Resour 11:353–357

Klingenberg CP (2015) Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. Symmetry 7:843–934

Klingenberg CP (2016) Size, shape, and form: concepts of allometry in geometric morphometrics. Dev Genes Evol 226:113–137

Klingenberg CP, McIntyre GS (1998) Geometric morphometrics of developmental instability: analyzing patterns of fluctuatingasymmetry with Procrustes methods. Evolution 52:1363–1375

Klingenberg CP, Barluenga M, Meyer A (2002) Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56:1909–1920

Knuttell H, Fiedler K (2000) On the use of ultraviolet photography and ultraviolet wing patterns in butterfly morphology and taxonomy. J Lepid Soc 54:137–144

Komárek S (2003) Mimicry, aposematism and related phenomena: mimetism in nature and the history of its study. Lincom Europa, München

Kudrna O (1975) A revision of the genus Gonepteryx Leach (Lep., Pieridae). Entomol Gaz 26:3–37

Leamy LJ, Klingenberg CP (2005) The genetics and evolution of fluctuating asymmetry. Annu Rev Ecol Evol Syst 36:1–21

Lens LUC, Van Dongen S, Kark S, Matthysen E (2002) Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? Biol Rev 77:27–38

Lim MLM, Land MF, Li D (2007) Sex-specific UV and fluorescence signals in jumping spiders. Science 315:481

Losey GS, Cronin TW, Goldsmith TH, Hyde D, Marshall NJ, McFarland WN (1999) The UV visual world of fishes: a review. J Fish Biol 54:921–943

Lukhtanov VA, Kandul NP, Plotkin JB, Dantchenko AV, Haig D, Pierce NE (2005) Reinforcement of prezygotic isolation and karyotype evolution in Agrodiaetus butterflies. Nature 436:385–389

Mardia KV (2005) Statistical assessment of bilateral symmetry of shapes. Biometrika 92:249–250

Matsuno H (1988) The ultraviolet reflectance pattern of the genus *Gonepteryx* (Lepidoptera, Pieridae) and ist adaptive significance. Trans Lepid Soc Jpn 39:149–165

Mazokhin-Porshnyakov GA (1957) Reflecting properties of butterfly wings and the role of ultra-violet rays in the vision of insects. Biophysics 2:285–296

Meslin C, Cherwin TS, Plakke MS, Small BS, Goetz BJ, Morehouse NI, Clark NL (2017) Structural complexity and molecular heterogeneity of a butterfly ejaculate reflect a complex history of selection. Proc Natl Acad Sci USA. https://doi.org/10.1111/geb.12368

Meyer-Rochow VB (1991) Differences in ultraviolet wing patterns in the New Zealand lycaenid butterflies *Lycaena salustius*, *L. rauparaha*, and *L. feredayi* as a likely isolating mechanism. J R Soc NZ 21:169–177

Meyer-Rochow VB, Järvilehto M (1997) Ultraviolet colours in *Pieris napi* from Northern and Southern Finland: arctic females are the brightest! Naturwissenschaften 84:165–168

Møller AP, Pomiankowski A (1993) Fluctuating asymmetry and sexual selection. Genetica 89:267–279

Morehouse NI, Rutowski RL (2010) Developmental responses to variable diet composition in a butterfly: the role of nitrogen, carbohydrates and genotype. Oikos 119:636–645

Morehouse NI, Vukusic P, Rutowski R (2007) Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. Proc R Soc Lond B 274:359–366

Mouchet SR, Vukusic P (2018) Structural colours in lepidopteran scales. In: Ffrench-Constant RH (ed) Butterfly wing patterns and mimicry. Elsevier, Amsterdam, pp 1–53

Nekrutenko YP (1964) The hidden wing-pattern of some Palearctic species of *Gonepteryx* and its taxonomic value. J Res Lepid 3:65–68

Nekrutenko YP (1968) Phylogeny and geographical distribution of the genus *Gonepteryx* (Lepidoptera, Pieridae): an attempt of study in historical zoogeography. Naukova Dumka, Kiev

Nekrutenko YP (1972) A new subspecies of *Gonepteryx amintha* (Pieridae) from Yunnan, mainland China, with comparative notes. J Res Lepid 11:235–240

Obara Y (1970) Studies on the mating behavior of the White Cabbage Butterfly *Pieris rapae crucivora* Boisduval III. Near-ultra-violet reflection as the signal of intraspecific communication. Z Vgl Physiol 69:99–116

Obara Y, Majerus MEN (2000) Initial mate recognition in the British cabbage butterfly, *Pieris rapae rapae*. Zool Sci 17:725–730

Obara Y, Ozawa G, Fukano Y (2008) Geographic variation in ultraviolet reflectance of the wings of the female cabbage butterfly, *Pieris rapae*. Zool Sci 25:1106–1110

Obara Y, Watanabe K, Satoh T (2010) UV reflectance of inter-subspecific hybrid females obtained by crossing cabbage butterflies from Japan (*Pieris rapae crucivora*) with those from New Zealand (*P. rapae rapae*). Entomol Sci 13:156–158

Ödeen A, Håstad O (2013) The phylogenetic distribution of ultraviolet sensitivity in birds. BMC Evol Biol 13:36

Oliver JC, Monteiro A (2011) On the origins of sexual dimorphism in butterflies. Proc R Soc Lond B 278:1981–1988

Olofsson M, Vallin A, Jakobsson S, Wiklund C (2010) Marginal eyespots on butterfly wings deflect bird attacks under low light intensities with UV wavelengths. PLoS ONE 5:e10798

Osorio D, Vorobyev M (2008) A review of the evolution of animal colour vision and visual communication signals. Vis Res 48:2042–2051

Oudemans JT (1903) Etudes sur la position de repos chez les Lepidopteres. Verhandelingen der Koninklijke Akademie van Wetenschappen, Amsterdam

Painting CJ, Rajamohan G, Chen Z, Zeng H, Li D (2016) It takes two peaks to tango: the importance of UVB and UVA in sexual signalling in jumping spiders. Anim Behav 113:137–146

Palmer RA, Strobeck C (2003) Fluctuating asymmetry analyses revisited. In: Polak M (ed) Developmental Instability (DI): causes and Consequences. Oxford University Press, Oxford, pp 279–319

Papke R, Kemp D, Rutowski R (2007) Multimodal signalling: structural ultraviolet reflectance predicts male mating success better than pheromones in the butterfly *Colias eurytheme* L. (Pieridae). Anim Behav 73:47–54

Pecháček P, Stella D, Keil P, Kleisner K (2014) Environmental effects on the shape variation of male ultraviolet patterns in the Brimstone butterfly (*Gonepteryx rhamni*, Pieridae, Lepidoptera). Naturwissenschaften 101:1055–1063

Pirih P, Wilts BD, Stavenga DG (2011) Spatial reflection patterns of iridescent wings of male pierid butterflies: curved scales reflect at a wider angle than flat scales. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197:987–997

Polak M, Starmer WT (2005) Environmental origins of sexually selected variation and a critique of the fluctuating asymmetry-sexual selection hypothesis. Evolution 59:577–585

Poulton EB (1890) Colours of animals: their meaning and use, especially considered in the case of insects. D. Appleton & Company, New York

R Development Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Remington CL (1973) Ultraviolet reflectance in mimicry and sexual signals in the Lepidoptera. J N Y Entomol Soc 81:124

Rohlf FJ (2013a) TpsDig (version 2.17). Department of Ecology and Evolution, State University of New York at Stony Brook

Rohlf FJ (2013b) TpsRelw (version 1.53). Department of Ecology and Evolution, State University of New York at Stony Brook

Rothschild M (1981) The mimicrats must move with the times. Biol J Linn Soc 16:21–23

Rutowski RL (1977) The use of visual cues in sexual and species discrimination by males of the small sulphur butterfly *Eurema lisa* (lepidoptera, pieridae). J Comp Physiol 115:61–74

Rutowski RL, Kemp DJ (2017) Female iridescent colour ornamentation in a butterfly that displays mutual ornamentation: is it a sexual signal? Anim Behav 126:301–307

Rutowski RL, Gilchrist GW, Terkanian B (1987) Female butterflies mated with recently mated males show reduced reproductive output. Behav Ecol Sociobiol 20:319–322

Rutowski RL, Macedonia JM, Morehouse N, Taylor-Taft L (2005) Pterin pigments amplify iridescent ultraviolet signal in males of the orange sulphur butterfly, *Colias eurytheme*. Proc R Soc Lond B 272:2329–2335

Rutowski RL, Macedonia JM, Merry JW, Morehouse NI, Yturralde K, Taylor-Taft L, Gaalema D, Kemp DJ, Papke RS (2007) Iridescent ultraviolet signal in the orange sulphur butterfly (*Colias eurytheme*): spatial, temporal and spectral properties. Biol J Linn Soc 90:349–364

Schoch RR (2010) Riedl's burden and the body plan: selection, constraint, and deep time. J Exp Zool B Mol Dev Evol 314B:1–10

Scott TA (1972) Mating of butterflies. J Res Lepidoptera 11:99–127

Shawkey MD, Morehouse NI, Vukusic P (2009) A protean palette: colour materials and mixing in birds and butterflies. J R Soc Interface 6:221–231

Sheehan MJ, Tibbetts EA (2011) Condition dependence and the origins of elevated fluctuating asymmetry in quality signals. Behav Ecol 22:1166–1172

Shimmi O, Matsuda S, Hatakeyama M (2014) Insights into the molecular mechanisms underlying diversified wing venation among insects. Proc R Soc Lond B 281:20140264

Silberglied RE (1979) Communication in the ultraviolet. Annu Rev Ecol Syst 10:373–398

Silberglied RE (1984) Visual communication and sexual selection among butterflies. In: Vane-Wright RI, Ackery PR (eds) The biology of butterflies. Academic Press, London, pp 207–223

Silberglied RE, Taylor OR (1973) Ultraviolet differences between the sulphur butterflies, *Colias eurytheme* and *C. philodice*, and a possible isolating mechanism. Nature 241:406–408

Silberglied RE, Taylor OR (1978) Ultraviolet reflection and its behavioral role in courtship of sulfur butterflies *Colias Eurytheme* and *C. Philodice* (Lepidoptera, Pieridae). Behav Ecol Sociobiol 3:203–243

Stavenga DG (2014) Thin film and multilayer optics cause structural colors of many insects and birds. Mater Today Proc 1:109–121

Stavenga D, Arikawa K (2006) Evolution of color and vision of butterflies. Arthropod Struct Dev 35:307–318

Stavenga DG, Giraldo MA, Hoenders BJ (2006) Reflectance and transmittance of light scattering scales stacked on the wings of pierid butterflies. Opt Express 14:4880–4890

Stella D, Faltýnek Fric Z, Rindos M, Kleisner K, Pecháček P (2018a) Distribution of ultraviolet ornaments in *Colias* butterflies (Lepidoptera: Pieridae). Environ Entomol 47:1344–1354

Stella D, Pecháček P, Meyer-Rochow VB, Kleisner K (2018b) UV reflectance is associated with environmental conditions in Palaearctic *Pieris napi* (Lepidoptera: Pieridae). Insect Sci 25:508–518

Stevens M (2005) The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. Biol Rev 80:573–588

Talloen W, Van Dyck H, Lens L, Mallet J (2004) The cost of melanization: butterfly wing coloration under environmental stress. Evolution 58:360–366

Tovée MJ (1995) Ultra-violet photoreceptors in the animal kingdom: their distribution and function. Trends Ecol Evol 10:455–460

Tsubaki Y, Matsumoto K (1998) Fluctuating asymmetry and male mating success in a sphragis-bearing butterfly *Luehdorfia japonica* (Lepidoptera: Papilionidae). J Insect Behav 11:571–582

Tuomaala M, Kaitala A, Rutowski RL (2012) Females show greater changes in wing colour with latitude than males in the green-veined white butterfly, *Pieris napi* (Lepidoptera: Pieridae). Biol J Linn Soc 107:899–909

Vane-Wright RI, Boppré M (1993) Visual and chemical signalling in butterflies: functional and phylogenetic perspectives. Philos Trans R Soc Lond B Biol Sci 340:197–205

Wahlberg N, Rota J, Braby MF, Pierce NE, Wheat CW (2014) Revised systematics and higher classification of pierid butterflies (Lepidoptera: Pieridae) based on molecular data. Zool Scr 43:641–650

Wickman PO (1992) Sexual selection and butterfly design—a comparative study. Evolution 46:1525–1536

Wijnen B, Leertouwer HL, Stavenga DG (2007) Colors and pterin pigmentation of pierid butterfly wings. J Insect Physiol 53:1206–1217

Wiklund C, Lindfors V, Forsberg J (1996) Early male emergence and reproductive phenology of the adult overwintering butterfly *Gonepteryx rhamni* in Sweden. Oikos 75:227–240

Windig JJ, Nylin S (1999) Adaptive wing asymmetry in males of the speckled wood butterfly (*Pararge aegeria*)? Proc R Soc Lond B 266:1413–1418

Zapletalová L, Zapletal M, Konvicka M (2016) Habitat impact on ultraviolet reflectance in moths. Environ Entomol 45:1300–1305