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Why are *Morpho* Blue?

9.1. Introduction

Their large size and the often brilliant blue of their wings put butterflies from the *Morpho* genus among some of the most spectacular insects in South America. Often mentioned in the reports of explorers in the 19th Century (see [FRU12]), they have been prominent in curiosity cabinets and natural history displays and are still today the subject of much commerce. We would logically expect the biology of such sought-after species to be well known and understood, but this is not the case [NEI 08]. Understanding of the origins and evolution of the iridescent blue color in particular is very limited. Why are *Morpho* (often) blue?

The problem of causality in biology can be tackled on three levels: structural, historical and functional [GOU 02]. (1) A *structural* explanation of the phenotype focuses on its physical or biochemical properties, thus answering the question of “how”. It also covers the genetic and developmental origin of the observed phenotype by describing in detail its development during the growth of the individual. This proximate explanation of the phenotype has a temporal dimension, the short time of development, and concerns not only the phenotype itself, but also the structures and processes that generate it. This approach can notably detect if

different structures are capable of producing the same phenotype. (2) The evolution of the phenotype (and of its genetic and developmental bases) can also be placed in its phylogenetic (*historical*) context. The study of phenotypic variation on a macro-evolutionary scale makes it possible to test the influence of evolutionary processes and of a wide array of factors on the evolution of phenotypes, while also taking into account the extent to which different species are related, i.e. the overlap of evolutionary history between species. (3) Finally, we can try to detect which evolutionary pressures influence the emergence, continuation or loss of the phenotype. Ecological or behavioral studies (and also biomechanical) allow us to determine in particular the role of selection, natural or sexual, in the evolution of the phenotype (*functional* explanation). In practice, these approaches are often somewhat combined: evo-devo is in most cases developmental genetics compared between related species, thus bringing together the structural and historical approaches; in much the same way, functional morphology combines the study of physical (structural) properties and ecology. Hence, the comparative method makes it possible to test adaptive hypotheses in a phylogenetic framework. However, it is still rare for these three approaches – structural, historical and functional – to be fully integrated [KLI 06]. In this review, we will give an overview of the nature and evolution of *Morpho* coloration across these three levels of analysis. We will attempt to identify the unanswered questions of most interest and propose possible avenues of research to answer them.

Are *Morpho* really blue? In the present case, we are interested in the color of the wings of adult butterflies (we will therefore put aside the egg, caterpillar and pupa stages, none of which – at least in those species of which these stages are known – shows any blue coloration [RAM 14, BEN 16]). Although iridescent blue is rightly associated with *Morpho*, it is not present throughout the genus. In fact, there are several dark species, predominantly brown, sometimes a greyish ocher, or even with a long wing proportion of orange ocher, as in the giant *M. hecuba* (Figure 9.1(b)). There are also three white-winged species, such as *M. polyphemus* (see Figure 9.1(c) and the phylogeny in Figure 9.2). Sometimes, for instance, in *M. telemachus* (Figure 9.1(a)), the blue is not widespread, only very weakly iridescent and much duller. In typically blue species, the coverage of this color varies from one species to another, always fringed by a black part around its edges and often at the base of the wings (Figure 9.1(d), (e), (f), (h), (i)). Even in those species most famous for their iridescent blue, females are often brown or orange (in *M. rhetenor*, for example, Figure 9.1(f) and (g)).

If the question “*why are Morpho blue?*” is undeniably interesting, the subsequent question “*Why are certain Morpho not blue?*” is just as interesting and makes it possible to investigate the factors involved in the acquisition and/or loss of this coloration. Finally, butterfly wings have two sides, dorsal and ventral, and the latter, excluding very rare cases of developmental aberration, is never blue: all species (see Figure 9.1(h), (i), (j), (k)) have a brownish ventral side (except the three white species), with eyespots varying in size, color and number – these famous marks that look like a vertebrate’s eye. The evolutionary origin of this more subtle coloration and of the presence of these eyespots will be briefly discussed.

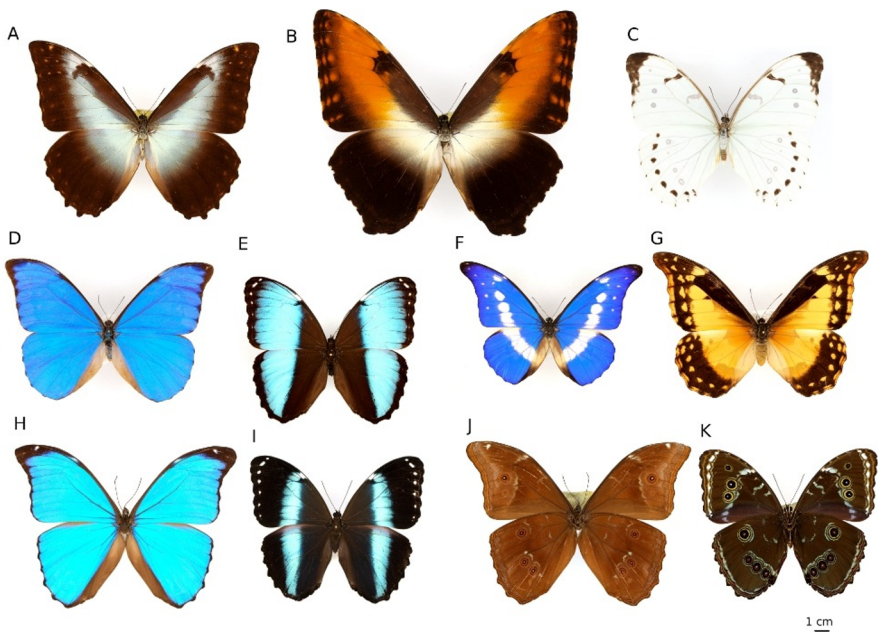


Figure 9.1. *Diversity of colors in Morphos. a) Male Morpho telemachus, b) Male Morpho hecuba, c) Male Morpho polyphemus, d) Male Morpho anaxibia, e) Male Morpho helenor papirius, f) Male Morpho rhetenor, g) Female Morpho rhetenor, h) Male Morpho menelaus, i) Male Morpho helenor, j) Male Morpho menelaus (ventral side), k) Male Morpho achilles (ventral side). For a color version of this figure, see www.iste.co.uk/grandcolas/biodiversity.zip*

9.2. Structural explanation: the iridescent blue in *Morpho* is a physical color

Here we aim to identify the structures and physical properties of wings that are responsible for blue iridescence, and the genetic and cellular mechanisms involved in their formation. To approach this problem, we must first define precisely what we mean by “iridescent blue” and identify the visual properties we want to explain. We can thus define several parameters to describe visual appearance; these can be measured thanks to a spectrophotometer and compared between individuals or species (see Box 9.1). (1) *Hue*. Why do the wings of *Morpho* species reflect light at the wavelengths between 450 and 490 nm – corresponding to blue? In general, blue in animals is rarely caused by pigmentation – blue pigments are more complex, unstable and energetically costly than other pigments (e.g. [BUL 04]; see [BAG 07] and [UMB 13] for review). In fact, the origin of the blue in *Morpho* is not pigmentary but structural, i.e. linked to properties of the scales’ surface (we will later see how pigmentation still plays a role). (2) *Brightness*. Some *Morpho* are bright while others are darker. (3) *Glossiness*. While some *Morpho* are noticeably glossy (e.g. *M. cypris*), others are more matte (e.g. *M. anaxibia*). (4) *Saturation*. For a similar hue, certain *Morpho* show a more intense, saturated blue. (5) *Iridescence*. If a *Morpho* is manipulated, and the angle from which it is illuminated and/or observed varies, its color changes, ranging from blue to violet, or even to green and orange. Iridescence is defined as the change in color with the angle of illumination or observation (see Box 9.2 for a description of the physical origin of iridescence). The dominant wavelength being that of blue, *Morpho* wings are thus generally seen as iridescent blue.

The initial question of the origin of blue in *Morpho* therefore becomes more specific through the different parameters describing the visual impression generated, such as hue, brightness, glossiness, saturation and iridescence (see Boxes 9.1 and 9.2). We have to explain the physical origin of these different visual properties, and also what makes them vary between species: what structural differences explain these different properties?

Different parameters of color can be illustrated using a reflectance spectrum, which gives the amount of reflected light as a function of the wavelength (Figure 9.2).

Hue – color in the common meaning of the term (blue, green, yellow, red). It often corresponds to the maximum value in the reflectance spectrum of the object.

Brightness – average reflectance (average proportion of reflected light), corresponding to the level of grayness of a color (i.e. gray-spotted space under the curve or line in Figure 9.2).

Saturation (or Chroma) – characterizes the color's purity. It is linked to the spectral length of the object's peaks of reflection or transmission: the broader the peak, the less pure the color, and vice versa (see yellow arrow in Figure 9.2). It ranges from 1 for a monochromatic light (laser) to 0 for a white color.

Iridescence – change of hue as a result of change of angle of observation and/or illumination (i.e. here, shift in reflectance peak quantified by the purple arrow in Figure 9.2). The physical origin of iridescence is shown in Box 9.2.

Glossiness cannot be quantified with one reflectance spectrum alone and requires more complex methods of study. It corresponds to reflection in a particular direction (anisotropy). Matte surfaces, on the contrary, reflect light in all directions (isotropy).

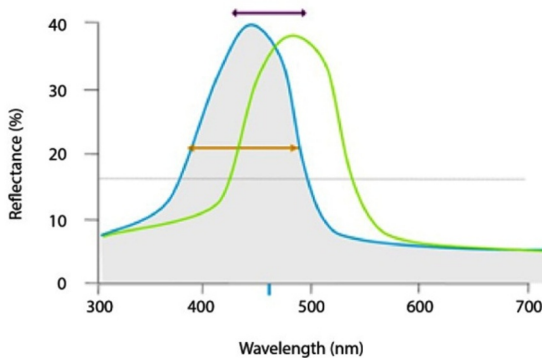


Figure 9.2. Reflectance spectra as a function of wavelength, illustrating how different parameters of color can be computed: the position in wavelength of the main peak defines hue (here, blue, between 450 and 490 nm); peak width at half height (yellow arrow) defines saturation; the surface under the curve, or the average reflectance (dotted) defines brightness; and a change in curve position (moving from the blue curve to the green curve: purple arrow) as a function of the angle of lighting or observation, defines iridescence. For a color version of this figure, see www.iste.co.uk/grandcolas/biodiversity.zip

Box 9.1. Different parameters of color

Iridescence (also called goniochromism) is a color change of an object as a result of the angle of observation and/or illumination (Figure 9.3).



Figure 9.3. Iridescence of *Morpho menelaus*. Here, the angle of observation is fixed (perpendicular to the wing) and only the angle of lighting is varied. The values in brackets indicate respectively height and azimuth. For a color version of this figure, see www.iste.co.uk/grandcolas/biodiversity.zip

Iridescence is caused by two distinct mechanisms that work in tandem in *Morpho*:

1) *Thin-film interference* – this is the most widespread phenomenon in nature. It results from the reflection of light on two sides of a thin transparent film. When these two waves are in phase, the amplitudes add up. They cancel each other out when they are out of phase. A simple trigonometric calculation tells us this happens when:

$$k\lambda = 2nec\cos\theta_r$$

where k is an integer, λ the wavelength, e the film thickness, n its refraction index and θ_r the angle of refraction (see Figure 9.4). As the cosine is a decreasing function between 0 and $\pi/2$, wavelength λ decreases as the angle of incidence increases and the color seen shifts toward blue (“blue shift”). In *Morpho*, this iridescence by interference takes place mostly on the basal scales, on the level of the corrugated lamellae (Figure 9.6 on right). It is important to note that thin film interferences are a phenomenon of reflection: the angles of illumination and observation must be varied simultaneously and by the same value.

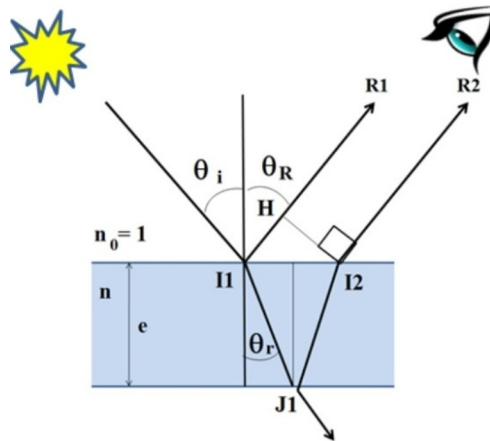


Figure 9.4. *Thin stratum interference.* According to Descartes, θ_i and θ_R are equal; to observe iridescence, the angle of observation must be changed as the angle of incidence of the light varies. For a color version of this figure, see www.iste.co.uk/grandcolas/biodiversity.zip

2) *Diffraction by grating* – a grating is a grouping of objects (lines, gaps, etc.) regularly arranged. Each of these objects refracts incident light and only certain wavelengths are in phase, and therefore visible, in certain directions. Calculations show us that this occurs when:

$$nk\lambda = \sin i + \sin i'$$

where k is an integer, n the number of objects per meter (the opposite of the step of grating, a), i the light's angle of incidence and i' that of observation (see Figure 9.5). We now have a sine law, a function that increases between 0 and $\pi/2$, that shows us red hues are observed at larger angles than blue ones (“red shift”), contrary to interference. This phenomenon occurs in *Morpho* on the level of the striations that run along the length of the scales (Figure 9.6) and act as a diffracting pattern. We should also note that, in this case, iridescence is only observed by changing the angle of observation.

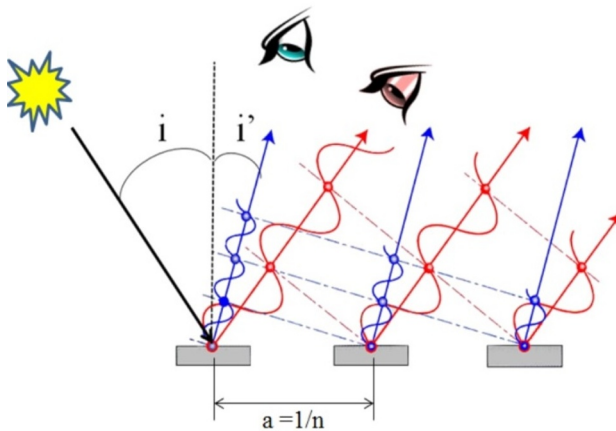


Figure 9.5. Diffraction by a grating of spacing a . Here we have only shown an order of 1 ($k = 1$); the same phenomenon occurs at larger angles ($k = 2, 3\dots$). For a color version of this figure, see www.iste.co.uk/grandcolas/biodiversity.zip

The iridescence observed in *Morpho* is therefore complex, since it combines these two antagonistic effects. Each scale acts as a striation pattern whose spacing, in the range of $1 \mu\text{m}$, depends on the species and causes a color shift towards red (Figure 9.6, left). But each striation is made of corrugated lamellae roughly 100nm thick that produce the color blue, and also a shift towards blue when the angle of incidence and observation increases.

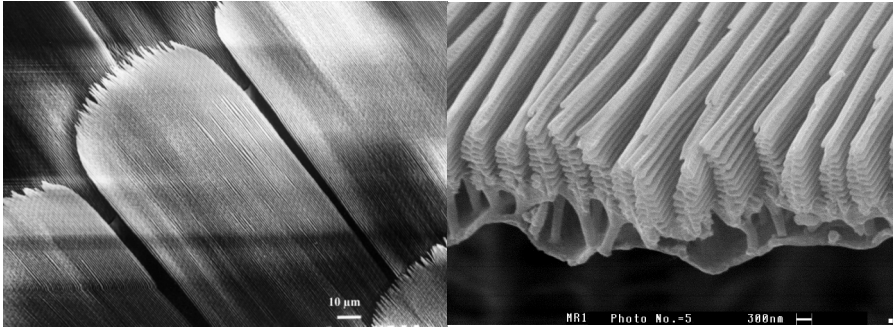


Figure 9.6. On the left, basal scale of *Morpho menelaus* showing the striation pattern, with a step of approximately $1\mu\text{m}$. On the right, corrugated blades (known as “Christmas trees”, see also Figure 9.7) producing blue interferences

Box 9.2. Iridescence

9.2.1. Scale structure

Butterfly wings, like that of other insects, are made of two cellular layers on top of one another: the dorsal layer and the ventral layer. What is particular to butterflies (and by definition to all Lepidoptera) is that the ventral and dorsal sides present two layers of scales that more or less overlap, called cover scales and basal scales (e.g. [NIJ 91]). The relative size of these scales and the degree of overlap of these two layers are variable between species of *Morpho* and can influence the visual properties of their wings [BER 10]. Unlike other iridescent butterflies, the structure of basal scales, and not that of cover scales, produces most of the photonic phenomena in *Morpho* species (e.g. [ING 08]): thus the light passes through a layer of transparent scales before reaching the iridescent scales (and passes through it again after reflection). The understanding of the optic properties of *Morpho*'s wings has been made possible by the technical progress of microscopy, which has allowed the identification of the micro- and nano-structures responsible for them (e.g. [GHI 72]; see [YOS 04, ING 08, BER 10] for historical descriptions).

Scales are marked longitudinally, from the root to the apex, by microscopic parallel striations (at least on the main part of the scale; Figures 9.6 and 9.7(a–b)). These striations are made of corrugated lamellae of chitin, of varying quantity depending on species (but very constant within each species), slightly tilted from scale surface. Striation combined with

lamellae three-dimensional organization (Figure 9.7) is responsible for scale photonic properties, notably iridescence [ING 08, BER 10] (see Box 9.2). In particular, lamellae corrugation amplifies the amount of light reflected, which becomes stronger (the color becomes more saturated) as lamellae number increases [BER 10, GIR 16]. Thus, intensely colored species such as *M. rhetenor* and *M. cypris* show striations made of ten to twelve corrugated lamellae, while striations in *M. helenor*, which displays a less striking blue, only contain three or four corrugated lamellae [GIR 16]. This corrugation is also the cause of the blue coloration, through an effect of interference (see Box 9.2 and Figure 9.7(d)). In fact, each lamella partly reflects the light that reaches its surface: each successive ray of reflected light interferes, sometimes positively (which increases the amplitude of certain wavelengths, here those in the blue range) and sometimes negatively (which reduces the amplitude of other wavelengths). Distance between lamellae (in “Christmas tree” organization, see Figures 9.6 and 9.7(c)) plays a large part in determining the wavelength reflected (and therefore the hue).

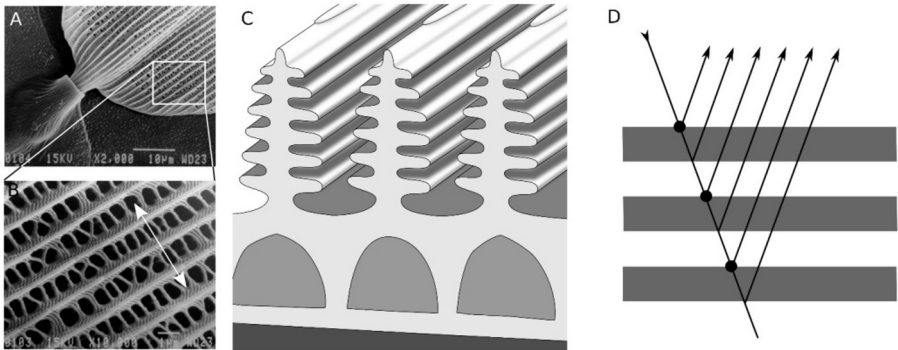


Figure 9.7. Micro- and nano-structure of scales responsible for blue iridescence. a) View in electronic microscopy of scale base showing the pedicel. The longitudinal striations covering the scale are clearly visible. b) Detailed view of striations under strongest magnification (electronic microscope). The double-ended arrow indicates the plane of the cross-section corresponding to (c). c) Schematic representation of perpendicular cross-section of a Morpho scale. The overlap of chitin lamellae results in this “Christmas tree” organization (see also Figure 9.6). d) Illustration of the phenomenon of reflection/positive interference, linked to the superposition of lamellae separated by air. (a) and (b) from [BER 10]

As well as their peculiar physical structure, the basal scales contain pigments. Their dark pigmentation (melanin) reduces unwanted light reflection and thus increases color saturation [ING 08; BER 10, GIR 16].

This pigmentation is absent in the two most basal species of the *Morpho* phylogeny, *M. marcus* and *M. eugenia*, in the three white species (*M. polyphemus*, *M. epistrophus* and *M. iphitus*) and in species from the *sulkowskyi* clade, apart from *M. absoloni* (i.e. *M. aega*, *M. portis*, *M. aurora*, *M. rhodopteron*, *M. zephyritis*, *M. lympharis* and *M. sulkowskyi*) (see Figure 9.7; [BER 10]). This absence of melanin contributes to the generally less intensely blue appearance of these species.

Glossiness diversity of *Morpho* species (comparing, for example, the very glossy *M. rhetenor* or *M. sulkowskyi* to the more matte *M. helenor* or *M. menelaus*) is influenced by the cover scales [VUK 99, YOS 04]. In the glossiest species, cover scales are either reduced or provide little coverage, while they are more developed in other species, where they may act as an “optic filter”, reducing wing glossiness while producing more diffuse reflected light [YOS 04] (see Box 9.1). Scale shape can also play a role: in *M. anaxibia*, a species of a deep but quite matte blue, scales are convex, which reduces glossiness [BER 10].

Two species, *M. eugenia* and *M. marcus* – the most basal species of the group – show optical properties that stem from fundamentally different causes than other species of *Morpho*. In these two species, scale striations are made of only one chitin lamella: iridescence is therefore not produced by the corrugation of lamellae but by that of the scales themselves [BER 10]. These two different organisations have very little influence on hue but directly affect the spatial distribution of reflected waves. Distance between scales is far greater than the length of the wave packet, or coherence length, which is of circa 1 μ m for solar light. This prevents any coherent effect, in particular diffraction by the striation pattern. We can therefore suppose that iridescence appeared in *Morpho* twice independently early during their diversification.

There is another way of producing blue colors in nature: fluorescence (see [LAG 15] for review). By a nonlinear process, high-energy radiations (generally UV) are absorbed by fluorescent molecules, and re-emitted at a lower energy, usually as blue or green. *M. sulkowskyi* deserves particular attention in this regard (e.g. [KUM 94]). It is the only species of the *Morpho* genus to show noticeable fluorescence. In this species, the basal scales lack the melanins that absorb ultraviolet rays and limit fluorescence. Instead, they contain purines that fluoresce. The emission spectrum shows a blue peak at 480 nm. The output, while remaining relatively weak, adds a noticeable base of blue to this otherwise very pale species [VAN 11a, VAN 11b].

A final optic property of *Morpho* wings is polarization. In a wave model, light is an electromagnetic wave where electric and magnetic fields are crosswise, i.e. perpendicular to the direction of propagation. Natural light is unpolarized. Fields oscillate in all directions perpendicular to the light ray. Various devices called polarizers select one direction of oscillation. Any wave that has undergone an oblique reflection, for example on foliage or on water surface, is partially polarized. This is also the case for light reflected by the scales of certain *Morpho*, as a result of raised striations of lamellae [VUK 99, BER 10]. Imperceptible to the human eye, this effect is interestingly visible to various insects (e.g. [KEL 99]), such as butterflies. Kelber [KEL 01] showed that butterflies use polarization, combined with color information, to choose their site of oviposition (see also [DOU 07] for a discussion on the biological role of polarization). The ecological importance of polarization for *Morpho* is unknown.

9.2.2. Scale development

If the structural origin of *Morpho* coloration has been relatively well identified, the genetic and development origin of the structures involved are less well known. Colors of butterflies are, however, the subject of numerous research programs, notably in evo-devo (e.g. [ALL 08, BEL 02, HEL 12, JOR 11, LEP 14, NAD 16]). However, most genetic and developmental studies are interested in pigment synthesis as well as the positioning of color patterns and contain little or no information on scale structure and development (e.g. [BEL 02, NAD 16]). Yet, it has been suggested that these two aspects, pigmentation and structure, are linked (e.g. [GIL 88]). There is therefore little work available on the development of butterfly scales [OVE 66, GHI 02, GHI 76, GAL 98, CHO 12, DIN 14].

It has been shown that scales are homologous with the sensory bristles of other insects (notably of *Drosophila*; [GAL 98]), the development of which is well studied (e.g. [SIM 90, SKE 91]). Galant *et al.* [GAL 98] showed that, in the *Precis coenia* (Nymphalidae) butterfly, the scales are formed at the start of pupation by two waves of cellular division: the first, followed by massive apoptosis, organizes the cells in successive rows along the proximo-distal axis of the wing; the surviving cells undergo a second wave of division which in the case where each dividing cell gives rise to one cell that produces the scale and one cell producing the pedicel of the scale (“socket cell”, Figure 9.7(a)). The similarity with sensorial bristle

development can be seen at a morphological level but is even more striking at the genetic level (notably by the expression pattern of the *achaete scute* homologous gene, a gene playing a central role in the differentiation of bristles in *Drosophila* (see [GAR 09] for review). These similarities support the hypothesis that scales are modified sensorial bristles, co-opted by Lepidoptera in the development of colored scales [GAL 98]. The scale itself is formed from skeletal cellular material; microtubules undergo special growth at the end of wing development and form fiber bundles that are particularly important to the formation of the striations on scale surface [OVE 66]. In a recent study, Dinwiddie *et al.* [DIN 14] showed that actin filaments play an important role in scales morphogenesis, and in particular in the development of dorsal striations that cause most of the optic phenomena discussed. Finally, ploidy might also play a role in scales development: cells that produce scales are polyploid, and the ploidy level is, at least in *Manduca sexta* (Sphingidae), correlated with scale size [CHO 12].

Certain aspects of the three-dimensional structure of *Morpho* scales described here, in particular the corrugated lamellae in “Christmas tree” organization, are also present in other butterfly species, but on the cover scales and not the basal scales (see [VUK 00]). This is the case for numerous Pieridae species (e.g. the genera *Eurema*, *Colias* or *Gonepteryx*; [GHI 76, WIL 11]). These are not blue, but they nonetheless show a significant component of physical color in the ultraviolet range (e.g. [GHI 76, WIL 11]). Similarly, the three-dimensional structure of scale striations in *Trogonoptera brookiana* (Papilionidae) is very similar to that of *Morpho* [WIL 16].

Iridescence may be adaptive in different contexts, but can also evolve in a neutral way from non-iridescent scales of simple structure. In an artificial selection experiment on *Bicyclus anynana* (Nymphalidae), Wasik *et al.* [WAS 14] obtained individuals with partially iridescent wings in the violet range after only six generations of artificial selection from brown-winged ancestors. This study highlights the presence of genetic variation for this trait in this species. Several species in the genus *Bicyclus* show violet iridescent scales, the evolution of which seems to have occurred independently and by different means (iridescence of basal scales or cover scales according to the species). Wasik *et al.* [WAS 14] therefore suggested that the adaptive evolution of structural colors may be easier than that of pigmentary colors: unlike the latter, which often requires diet changes to obtain new pigment(s), changes involved in the evolution of structural colors are of a quantitative nature, occurring by modulation of the quantity of chitin secreted by wing

cells. Finally, Ghiradella and Radigan [GIR 76] suggested that self-organizing processes in intra-cellular structures could play an important role in the development of butterfly scales, which could explain why few genetic changes are necessary to modify their structure, and therefore their color.

9.3. Historical explanation: evolutionary origin of blue color in *Morpho*

Morpho are part of the Satyrinae sub-family, where they form, along with *Antirrhoea* and *Caerois*, the *Morphini* tribe [DEV 85, PEN 06], the sister tribe of the *Brassolini* (including the renowned owl-butterflies from the *Caligo* genus) [FRE 04, WAH 09]. The *Antirrhoea* and *Caerois* do not have the spectacular iridescence of some *Morpho*, but they do quite often show zones of violet–blue iridescence (personal observation; see [DAB 84]). These butterflies fly in the understory, mostly in shadow (never in environments fully exposed to sunlight), and most often at the ground level [DEV 85]. Their ecology seems quite different from that of *Morpho*, which comprise high-flying species, flying in or above the canopy, and species flying mainly in the understory [MIC 11, DEV 10, CHA 16]). The *Morpho* genus comprises 30 species, for which numerous sub-species have been described (e.g. [LAM 04, BLA 07, BLA 12]). Their phylogeny has been the subject of much work in recent years [PEN 02, CAS 10, CAS 12, PEN 12, BLA 13, CHA 16] and is now well established (Figure 9.8 modified according to [CHA 16]).

9.3.1. Color variation in the genus *Morpho*

The most basal lineage of the genus comprises two species, *Morpho marcus* and *M. eugenia*, which both show an iridescent blue color in males, while the females are brown-black with a yellow band across the two wings (Figure 9.7). These two species are described as flying mainly in the understory [PEN 02, CHA 16]. The rest of the genus is then divided into two clades: a clade with canopy-flying large species with lengthened triangular forewings, a trait suspected to be an adaptation to gliding in open spaces [DEV 10, CHA 16], and the second clade being generally associated with flight in the understory. The “canopy clade” is split in two: first, a group of large to very large butterflies whose flight pattern is typically gliding, and in which the iridescence characteristic of the genus is either reduced or absent

(the “*telemachus*” group; see Figure 9.1, *M. telemachus* and *M. hecuba*; see also Figure 9.7); second, a group of three species, *M. anaxibia*, *M. cypris* and *M. rhetenor*, all of which are blue, and the latter two highly iridescent. These three species are sexually dimorphic in color: in *M. anaxibia* females, the blue covers less surface than in males; in *M. rhetenor* females, the dorsal side is ocher-orange with brown-black patterning (Figure 9.1(f) and (g)); the same is true of *M. cypris*, a species in which there are, however, females with the brilliant blue characteristic of males on part of their wings.

In the “understory clade”, color diversity is also large. A white species, *M. polyphemus*, forms the base of the clade, which is divided into two sub-clades. The first sub-clade itself contains two groups, one made of three relatively large blue species, *M. amathonte*, *M. menelaus* and *M. godartii*, all three of which are fairly dimorphic, the females showing brown-black edges with clear marks (and therefore a smaller blue area than in males). The second group contains eight small species, often very glossy, but whose hue is a pale blue, and even slips into white in certain species, such as *M. sulkowskyi*. Two species in this group show particularly remarkable sexual dimorphism: *M. aega*, the females of which are polymorphic, one forming an ocher-orange color with brown-black patterning, another with a large covering of iridescent blue and a third intermediary form; and *M. zephyritis*, the females of which are ivory with very weak iridescence, while the males are particularly shiny blue. *Morpho absoloni*, the males of which are also a particularly glossy blue, are unique, differing from other species of the clade as the females have large brown-black margins, while the proximal half of the wings are blue.

The second understory sub-clade contains the type species of the genus *Morpho achilles*, and is divided into two groups, one comprising *M. achilles*, *M. helenor* and *M. granadensis*, three species characterized by two black parts, proximal and distal, whose width varies geographically. Hence, the blue patch between the black parts can be reduced to a very narrow band (see Figure 9.1(i)), or it can almost completely cover the wings. The second group contains three species, of which two are white (*M. iphitus* and *M. epistrophus*) and resemble the basal species *M. polyphemus* very closely. The third species in this group is *M. deidamia*, whose dorsal side looks quite similar to that of *M. helenor*, *M. achilles* and *M. granadensis*, with geographical variations, while the ventral side shows a more complex color pattern. This second understory sub-clade is characterized by a very limited color dimorphism, and a fairly consistent yet

definitively less glossy and less iridescent blue than in more striking species such as *M. rhetenor* [BER 10].

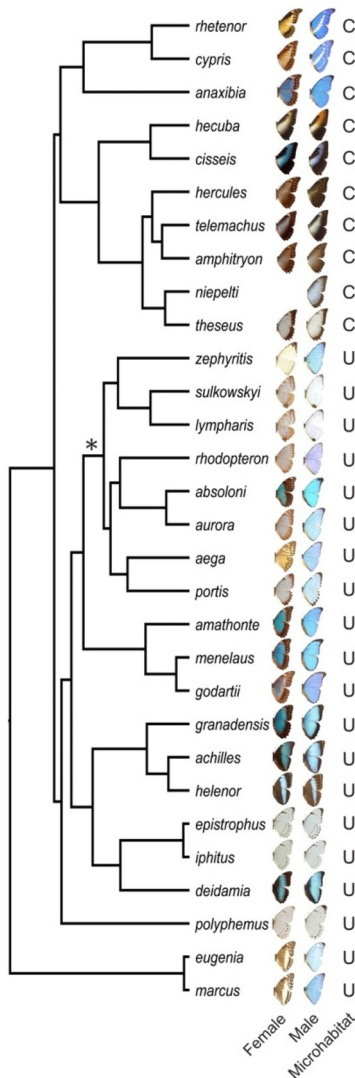


Figure 9.8. Phylogeny of the genus *Morpho*, showing the right wings of males and females. U = Understory and C = Canopy. Figure modified according to [CHA 16]. For a color version of this figure, see www.iste.co.uk/grandcolas/biodiversity.zip

This description of color variation on the scale of the genus is inevitably schematic and does not take into account the numerous variations, notably on an intra-specific scale (i.e. the existence of various sub-species and geographical variants). It underlines the inter- and intra-specific diversity (sexual dimorphism) of coloration in *Morpho*, in the clades that live in the understory as well as in the canopy, suggesting a complex evolutionary history of coloration in this genus.

What we can take, however, from this brief description, as well as from looking at the phylogeny (Figure 9.8), is an idea of the importance of phylogeny in determining coloration. In other words, phylogenetically close species seem, in general, to be more similar in terms of coloration (this is called phylogenetic signal). This amounts to the null hypothesis of neutral morphological divergence along the branches of the phylogeny (the Brownian model; e.g. [GAR 94]). In answer to the initial question, “*why are Morpho blue?*”, this model would answer “*because they inherit their blue color, or the absence of it, from their ancestors*”, without suggesting any particular adaptive mechanism. But does phylogenetic history suffice to explain the precise distribution of color in this genus? In particular, the evolution toward a very clear, even white, color is intriguing. This has occurred several times, likely independently: other than the white species, species belonging to different clades (*M. theseus*, *M. godartii*, *M. aurora*, *M. helenor*) contain very pale or white sub-species. These colorations may have been favored by ecological factors. Similarly, some species show a marked blue coloration, in contrast to their closest relatives (e.g. *M. absoloni* vs. *M. aurora* or *M. zephyritis* vs. *M. sulkowskyi*), which suggests natural selection may play a role in the evolution of this color. These hypotheses must be explicitly tested, either experimentally or in a comparative framework. Finally, the evolutionary origin of iridescence itself is yet to be explored. The study of wing structures responsible for iridescence and the study of their diversity among *Morpho* species would allow us to identify phenomena of convergence. This could also shed light on the ecological implications of this trait and on selection pressures affecting its evolution (see [BER 10, GIR 16] for the comparative analysis of some of the species of the genus).

9.4. Functional explanation: the role of selection in the evolution of *Morpho* color

The above discussion demonstrates that answering the question “*why are Morpho blue?*” also implicates the identification of the selective processes involved in the evolution of this color and its diversification.

Color diversity in animals is often discussed in terms of signals received by predators and/or sexual partners or competitors (e.g. [GOM 07]). Butterfly coloration is therefore generally considered first and foremost as a visual signal, both on an inter- and intra-specific level (see, for example, [END 78, SIL 84, NIJ 91] for general references). Other selective pressures act on coloration: butterfly wing scales are also involved in thermoregulation and hydrophobia.

9.4.1. Thermoregulation

In butterflies, the muscles involved in flight must reach a fairly high temperature to work properly, making flight possible (e.g. [KIN 85]). Thermoregulation is therefore particularly important for these insects. Coloration, because of its light-absorbing qualities, plays an important role in thermoregulation (e.g. [WAS 75, KIN 85]). However, this role is generally limited to the base of the wings, suggesting that apical patterns have little to do with thermoregulation ([WAS 75]; see [KIN 85, KIN 87, KIN 95] for a discussion on Pieridae). All variations in color patterning can therefore probably not be explained by their effects on body temperature. The possibility that iridescence may have a particular effect on thermoregulation is quite controversial [DOU 09], certain authors suggesting that high reflectivity tends to reduce the capacity for thermic absorption (e.g. [KOO 00]), others claiming on the other hand that scale structure and organization allow heat to be more effectively directed toward the veins and the hemolymph (e.g. [TAD 98]; see [WAS 75] for further discussion on the role of the hemolymph in thermoregulation). Data gathered about *Morpho* wings suggest that their high reflectivity has little effect on their absorption capacity [BER 10]. If it seems unlikely that blue iridescence in *Morpho* has evolved primarily in response to selective pressures linked to thermoregulation, it is nonetheless within the realms of possibility that the thermal effects of iridescence have played a role in its evolution. This hypothesis should be tested with greater accuracy by comparing the distribution of thermal absorption throughout the phylogeny in tandem with

coloration distribution (see [BER 10]). Moreover, literature on thermoregulation is for the most part focused on species in temperate climates [KIN 85], and tropical butterflies are therefore not at all understood in the context of thermoregulation.

9.4.2. *Hydrophobia*

When it rains, butterflies hide under cover, generally on a tree-trunk or under a leaf. In humid tropical climates, it is definitively more difficult to avoid getting wet than in temperate climates. The scales covering butterfly wings in much the same way as roof tiles generally create a very hydrophobic surface (e.g. [WAG 96]). Zheng *et al.* showed that in *Morpho* the existence of raised, overlapping chitin lamellae (Figure 9.3) increases hydrophobia to an extreme degree (see also [BER 10]). In addition, this super-hydrophobia is combined with directional adhesion, where water droplets are prevented from rolling toward the body and instead roll off wing outer edges [ZHE 07]. It is thus conceivable that the evolution of *Morpho* scales nanostructure has been influenced not only by their photonic properties, but also by natural selection favoring more hydrophobic morphologies. Nonetheless, *Morpho* do not usually fly in wet weather. They rather stay under vegetation with their wings closed, where the non-iridescent ventral side of their wings is exposed to the rain.

9.4.3. *Signaling to predators: a confusing effect?*

When it comes to coloration as a visual signal, it is difficult to identify the relative importance of the various visual parameters such as hue, iridescence, brightness, glossiness and saturation, all of which are in reality combined. Analysis of their covariation – or, on the other hand, their potential independence – would be very informative. We will consider these parameters in combination in the following discussion.

Morpho are relatively fast-flying butterflies often considered difficult to capture – at least by human predators ([YOU 71, PIN 96, PIN 16]; personal observation). Their coloration may play a role in making their capture even more difficult, as a result of the marked but intermittent signaling emitted by their wings, irregular and ever-changing in nature as they beat rapidly in flight, hiding and exposing successively the glossy dorsal side and the darker ventral side. The irregular flashes of changing colors (i.e. a dark–light

contrast, as well as variation in glossiness and iridescence), combined with a complex flight pattern and escape manoeuvres, make it difficult to locate the butterfly and to predict its flight trajectory. The intense, iridescent and glossy blue may therefore produce sensorial confusion in the predator (see [STE 07] for a general discussion on the sensorial effects of coloration on predators). The hypothesis that iridescence may have an anti-predator role ([ROB 96, HIN 73]; see for review [MEA 09]; see [CLE 66] for particular mention of butterflies) has received little to no experimental testing (see, however, [HIK 15]), and remains completely unexplored in *Morpho* (see [NEI 08] for discussion). Color evolution in *Morpho* would therefore seem to hinge both on light environment and on predator cognitive capacities. In addition, gliding, which seems favored in the canopy [DEV 10, CHA 16], would therefore limit the frequency of “anti-predator flashes”, which could explain why iridescent blue is less important in certain canopy species. In any case, these considerations highlight the strong link between the flight evolution and evolution of color patterning.

Besides camouflage, which is a form of signaling limiting detection by predators, the often striking colors of butterflies may indicate to potential predators that an attack would be costly, for example, in the case of toxic butterflies (e.g. [END 88, SHE 08]). This is called an aposematic signal, which confers to those individuals carrying it protection against predators who learn, at their own expense, to avoid them. Avoidance by predators favors aposematism and can lead to convergence on the same aposematic signaling in different toxic species that are exposed to the same predators (Müllerian mimicry). Indeed, toxic species mimicking a signal already known by predators benefit from the protection associated with this signal and reduce even further the individual risk of predation which is spread over a larger number of individuals. Furthermore, non-toxic species can also evolve such aposematic signals, thus benefiting from the aposematic protection associated without entailing the metabolic costs associated with the production of chemical defenses (Batesian mimicry: see, for example, [MAL 99] for review).

The toxicity of butterflies is not the only factor incurring a potential cost for predators. The attack in itself is obviously costly in terms of energy, especially when the butterfly is difficult to capture – for instance if it flies fast or erratically. The energetic cost will be relatively higher for prey with lower calorie content. From the butterfly’s point of view, toxicity and difficulty of capture are two traits that limit the risk of being caught by predators.

Box 9.3. Aposematism

9.4.4. Signaling to predators: an aposematic blue?

Beyond its direct contribution to making them difficult to locate, the blue seen in *Morpho* could equally act as a signal of fast and/or erratic flight to predators, in particular birds. In much the same way as the bright colors of toxic butterflies (see Box 9.3), this color may inform predators about the cost of an attack, but this time in relation to the difficulty of capture rather than toxicity [YOU 71, PIN 96, SRY 99, PIN 16]. This hypothesis has been supported by experimental data: Pinheiro [PIN 96] exposed butterflies to a predator – a Jacamar, an insectivorous bird specialized in catching butterflies – and showed that *Morpho* are very rarely attacked, and in the event of an attack, they are very rarely caught. This conclusion is nonetheless contradicted by field observations concerning *M. rhetenor* ([NEI 08], p. 218) and *M. menelaus* ([GAY 16], pp. 16–17). Data in this area are generally quite rare and the correlation between the blue signal intensity and protection from attack has never been formally tested.

It has also been suggested that this escape aposematism could, like toxicity aposematism, trigger mimetic evolution (referred to as escape or evasive mimicry; [VAN 59]). In other words, if the visual signal associated with the difficulty of capture provides a selective advantage by discouraging attack, convergence toward the same signal could occur among fast flying species (Müllerian escape mimicry). On the contrary, species less difficult to capture could benefit from being similar in appearance to faster species (Batesian escape mimicry). Mallet and Singer [MAL 87] discussed this hypothesis and suggested it could be valid, particularly when the energy benefits of capture are low, such as with big butterflies with a “*thick, indigestible cuticle and solid wings*”, as well as with very small butterflies (low in nutrition), in particular Lycaenidae. This hypothesis has been criticized, mainly because of the lack of robust empirical data, and also on theoretical grounds: Brower [BRO 95] suggested that, unlike toxicity (unpleasant signal), the stimulus that would associate color with failure to catch the prey would not be strong enough to warrant long-term memorizing by the predator, making the evolution of escape mimicry, Batesian or Müllerian, unlikely. Ruxton *et al.* [RUX 05] modeled the evolution of Batesian and Müllerian escape mimicry. Their findings suggest such an evolution is possible in both cases, as long as an attack is costly for the predator, there is abundant alternative prey, and escape is costly for the butterfly.

These hypotheses of *Morpho* color as signaling difficulty of capture, and the subsequent hypotheses of the possible evolution of escape mimicry, have however never been empirically tested. It is nonetheless interesting to note that in *Morpho*, even of the largest species, the body is relatively small and likely poorly profitable as prey.

9.4.5. Sexual selection

The coloration of *Morpho* is widely considered to be a form of signaling in intra-specific communication (e.g. [VUK 99]), either toward individuals of the opposite sex in the context of mate choice or toward individuals of the same sex in the context of intra-sexual competition (e.g. [SIL 84]). This hypothesis is not specific to *Morpho*: the coloration of butterflies has generally been interpreted in this context (e.g. [END 78, KEM 11]), and notably by Darwin and Wallace ([KOT 80]; see [SIL 84] for further discussion). This is the case above all in species where there is a strong sexual dimorphism in color, the males being generally more colored, which suggests either sexual selection or ecological differences between sexes associated with different natural selection pressures [ALL 11]. Iridescence in particular has been the subject of much attention, because it allows for directional signaling. The polarization of reflected light has also been suggested as a way of producing easily detectable signals in situations where there is little light, for example, in the understory (e.g. [DOU 07]). It has been shown, in the neo-tropical butterfly *Heliconius cydno* (Nymphalidae), that the polarized iridescent blue of the wings is involved in signaling in sexual encounters [SWE 03]. What about *Morpho*?

Gomez and Théry [GOM 07] showed that light, yellow, white or blue signals create a strong visual contrast (for birds but also more generally for any tetrachromatic system), making it easier to communicate in the understory. They also suggest that a dark saturated blue is more visible in the canopy. These results are in accordance with the distribution of brightness of blue between canopy and understory species: indeed, the three blue canopy species (*M. anaxibia*, *M. rhetenor* and *M. cypris*) show an intense blue that is generally darker than that of understory species (see Figure 9.7), reinforcing the idea of coloration as a visual signal. In blue species, there is also a more or less marked sexual dimorphism in color, where the males always have a more intense blue that covers more of the wing relative to females ([CHA 16]; see Figure 9.7; note that these differences are not quantified, and

there is no quantitative data allowing us to compare iridescence and glossiness between sexes). This dimorphism is the strongest in *M. rhetenor*, *M. cypris* and *M. aega*. In the latter two, the females are polymorphic, the predominant morph being an ocher-orange color. It is of interest that color dimorphism is particularly low in non-blue species, which suggests that the blue color is either targeted by sexual selection or by a particularly contrasted natural selection between sexes. Certain species of *Morpho* are often said to be territorial, patrolling in regular fashion a specific area from which they “chase” other males fairly aggressively (e.g. *M. amathonte* [YOU 73]). This territoriality should, however, be considered with caution, as it is generally impossible to follow butterflies in the forest, which limits our understanding of their actual movements and forays into open spaces (rivers, paths and forest borders). In some species (e.g. *M. helenor*), on the contrary, it is not uncommon to observe several males feeding on the same fruit. Nonetheless, in all blue species, the males are attracted by the lure of metallic blue, which suggests either a territorial behavior, or more simply, an inability to discriminate from a distance the lure from a female (in species showing little dimorphism) or from a potential rival. Sex ratio is unknown for most species, but females are much more rarely observed than males, which may indicate a sex ratio that is biased in favor of males (difficult to explain from an evolutionary standpoint) or simply a behavioral difference, males being more visible due to their patrols in open parts of the forest, while females might remain in the close vicinity of host plants to lay eggs (but again, our understanding of behavior is very limited).

The hypothesis of sexual selection by females to explain the evolution of iridescence and sexual dimorphism of coloration has been put forward for some species with iridescent males (e.g. [CON 07]), and validated in experiments (notably for *Hypolimnas bolina* (Nymphalidae), *Eurema hecabe* (Pieridae) [KEM 07a, KEM 07b, KEM14] and *Bicyclus anynana* (Nymphalidae) [ROB 05]). To be demonstrated in *Morpho*, this hypothesis must establish that females choose males differently according to their color, which must therefore be more than a mere signal of species or sex recognition [KEM 11]. Sexually selected traits are generally costly and condition dependent (i.e. dependent on individual health condition, which is in turn linked with genetic quality (see [HIL 11] for discussion); e.g. [ALL 11]). We currently have no data on the condition dependence of coloration in *Morpho*. Kinoshita *et al.* [KIN 02] showed that the irregularity of lamella corrugation formations comprising the striations limits iridescence. High regularity in this area could therefore be important for

generating highly iridescent coloration patterns. Indeed, it is imaginable that maintaining such a high regularity is energetically costly during development (see [DOU 09] for review). Variations in the quality of iridescence between males of the same species could thus be linked to variations in their general health (their condition). In turn, iridescence could then be used by females as a mate choice criterion, and their preference could hence evolve through the selective advantage associated with paternal genetic quality transmitted to their offspring.

9.4.6. Different natural selection between sexes?

It is also possible that color dimorphism between sexes is linked to different natural selective pressures. This hypothesis is fairly consistent with Wallace's views on sexual dimorphism [KOT 80], which he thought resulted from the appearance of a trait in both sexes followed by its elimination by selection in the most cryptic sex (here, females). We have recently shown [CHA 16] that sexual dimorphism of wing shape was associated with color dimorphism. This association could come from different selective pressures between sexes, females, due to being more cryptic and generally heavier, adopting a different flight pattern from males. In particular, they might fly less than males, leading to reduced predation pressure from birds. This hypothesis goes against Darwin's, focused on sexual selection [KOT 80], here blue being sexually selected by females as an honest signal (i.e. a feature costly to maintain and signaling the good genetic quality of its carrier). However, these two points of view are not exclusive (see [ALL 11] for detailed discussion). In *Morpho*, coloration, if indicative of flight performance and capacity to escape predators, could be directly used as a mate choice criterion by females. On the other hand, coloration could offer no advantage against predators, but on the contrary a handicap, providing females with an indirect criterion of quality.

9.4.7. And the lack of blue?

Understanding the evolution of glossy iridescent blue can also be improved by identifying selective pressures that allow for the maintenance of different colorations. First, the ventral face of *Morpho* is not blue but brown-beige with eyespots, the number and size of which vary between species (Figure 9.1). This dorso-ventral contrast strongly suggests that the two faces are subject to different selective pressures – indeed, opposite pressures. If the evolution of high conspicuousness may be favored on the

dorsal face by predator behavior, selective pressures on the ventral face seem to promote camouflage. When butterflies are at rest, shaded by foliage with folded wings, the coloration of their ventral side makes them very difficult to spot. Eyespots, more or less visible and marked depending on the species, could divert predator attacks away from the most vital parts (see [STE 05] and [MON 15] on the role of eyespots in other species). It has also been suggested that eyespots may play a role in mate choice (e.g. [STE 05]). These hypotheses have never been tested in *Morpho*.

So what about non-blue species? In the case of the “*telemachus*” group, we should note that males of this species fly at great heights, at the very top of the trees, and descend very rarely to the ground [MIC 11, DEV 10, PEN 12, CHA 16]. It is therefore tempting to suppose that selective pressures that would favor iridescent blue individuals would no longer be relevant above the canopy or would be counterbalanced by deleterious effects (see section 9.4.3 for discussion on luminous flashes emitted by escape flight, probably less effective in gliding scenarios). Moreover, a number of ecological parameters change with height – such as habitat openness, increased sunlight or the nature and number of predators – which can influence the selective value of a phenotype. Three species said to be “canopy butterflies” have, however, intense and iridescent blue color (*M. cypris*, *M. rhetenor* and *M. anaxibia*), which shows that the canopy/understory dichotomy cannot exclusively explain the presence or absence of blue.

In the case of the three white *Morpho* species, who do not belong to one sole clade, their close resemblance suggests some sort of convergence, but the ecological factors involved are unknown; they are unlikely to be linked to flight height since *M. polyphemus* flies high, even at the canopy level [YOU 72]. As regards the two palest species of the group, “*sulkowskyi*” (*M. sulkowskyi* and *M. lympharis*), males have wings with virtually no melanin. They fly in fairly open environments and often above the vegetation, displaying a pale yellow color which, at least theoretically, should make them rather inconspicuous [GOM 07]. These two species are found at high altitudes, most often approximately 2000–2500 m. This association between loss of melanin and high altitude is quite surprising, given the importance of melanin in thermoregulation, at least in temperate climates, where species generally have more melanin when they live at higher altitudes (e.g. [KIN 85]).

Finally, in some species, females show no blue coloration: as previously discussed, this situation could result from a difference of ecological context between sexes, females being selected for their cryptic colors (Wallace's hypothesis). It has been suggested that the ocher-orange colors associated with the brown-black patterning in the females of certain species (*M. rhetenor*, *M. cypris* and *M. aega*) could indicate cases of Batesian mimicry, where the models to be imitated may be toxic species from the *Danainae* family [GAY 16]. However, the mimicry is not very accurate, and *M. rhetenor* and *M. cypris* females are much larger than their supposed models, which would contradict this hypothesis.

9.5. Conclusions and open questions

The resounding message of this review is that despite their iconic status, *Morpho* remain relatively unknown especially for the evolution of their coloration. While their phylogeny is well established and the nano-structural basis of their color fully identified, the ecological and genetic factors of these traits are almost entirely unexplored. The hypotheses commonly called upon to explain the evolution of the iridescent blue color, whether in terms of sexual selection or escape from predators, have never been explicitly tested. These gaps leave us with a number of open questions: are non-blue species subject to different selective pressures (thermoregulation, hydrophobia, predation, communication)? Do they fly more slowly or simply in a different way than their blue relatives? Are there, within each species, differences in flight behavior between sexes? In the hypothesis regarding the importance of blue as a sexual signal, do *Morpho* show evidence of particular visual receptors? Are they sensitive to polarized light? All of these questions require experiments to be carried out that would allow us to study flight modalities and numerous behavioral traits. But they point above all, despite more than a century of collecting, toward the lack of understanding of the ecology of *Morpho*.

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