From optical properties to ecology: what are the evolutionary forces shaping the diversity of iridescence in *Morpho* butterflies?

State of the art – problematics

Iridescence is the variation in the reflectance spectrum of an object, depending on the illumination and/or observation angles. Iridescent colorations have evolved multiple times in animals, and in particular in butterflies (Lloyd & Nadeau 2021). The visual appearance of iridescent animals is very labile, as their color change, in particular when they are moving. Iridescent patterns are often involved in intraspecific (e.g. sexual selection) and interspecific (predator avoidance, species recognition, mimicry) interactions. The perception of iridescence by predators and sexual partners is thus a key factor in the evolution of such coloration in animals.

Morphos are large neotropical butterflies famous for their blue iridescence, which is emblematic of bio-photonics (Vukusik & Sambles 2003; Berthier 2010). The nanostructures on the scales covering their wings produce this iridescence (Figure 1). While the optical properties of wings have been studied in a few Morpho species (e.g. Giraldo et al 2016), their diversity has not been assessed throughout the whole genus, nor at the intraspecific level. The visual effects on predators or on Morphos themselves of such diversity of iridescence have never been investigated. The genus *Morpho* comprises 30 species, with a large diversity in color patterns (Figure 1). Some species are not blue and there can be strong differences in coloration among sexes (Chazot et al 2016). In blue species, there is still a large diversity of hue, brightness and iridescence (Debat et al 2018). What are the physical bases of iridescence variation in the genus? What are the main ecological factors shaping the evolution of iridescence? Do iridescence and its perception co-evolve in the genus Morpho ?

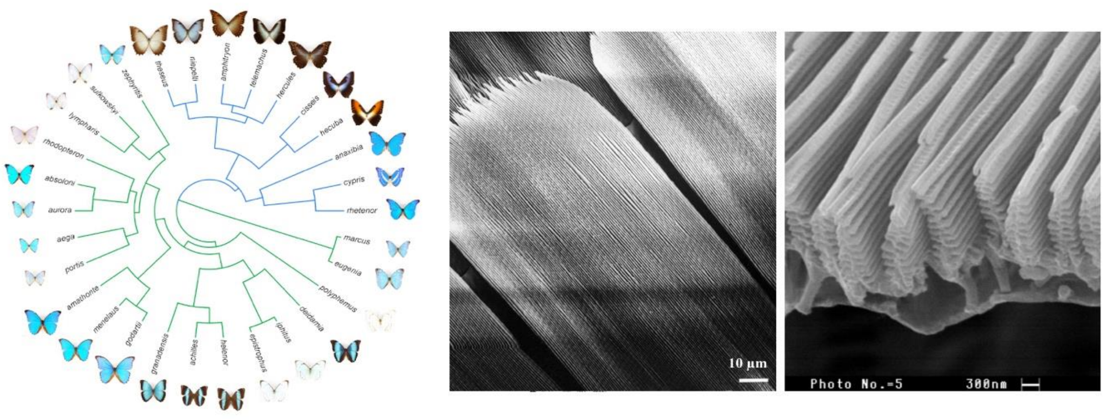

Figure 1: Phylogeny of Morpho. Blue : canopy species, green understory species. Microscopy and SEM showing the scales and the nanostructures producing iridescence in *M. rhetenor*

Several selective forces, all related to the visual perception of coloration, can influence the evolution of iridescence in Morphos. (1) **Predation**: iridescent blue patterns, combined with fast erratic flight, can have an antipredator effect. In many Morpho species, wings are indeed blue on the dorsal side, but their ventral side is brown. Flapping flight alternatively exposes those two sides, producing blue flashes that make the butterfly trajectory difficult to predict, enhancing the difficulty of capture (Murali 2018). Insectivorous bird attacks are indeed often unsuccessful (Pinheiro et al. 2016). Birds also seem to associate Morpho blue iridescence with this difficulty of capture, as they often renounce attacking them (Pinheiro & Campos 2019). The convergence of color patterns among some sympatric Morpho species could thus be triggered by the behavior of predators. The degree of convergence in iridescence would then be conditioned by the color discrimination capacities of predators. (2) **Sexual selection**: in most blue species, sexual color dimorphism is observed, females being less colorful than males (Chazot 2016). This suggests that blue iridescence might be involved in mate choice and could be under sexual

selection. (3) **Species recognition**: the high resemblance in wing color patterns among species (Llaurens et al 2021), can induce competitive and sexual interference among sympatric species (Le Roy et al. 2021a; Boussens-Dumon & Llaurens 2021). Such a negative effect could promote the evolution of cryptic signals allowing an efficient visual recognition by butterflies while retaining protection from predators. The ability to discriminate subtle variations in iridescent blue colors might thus evolve in Morphos as a result of negative interspecific interactions. If iridescence indeed plays a role in intra-specific communication (sexual selection and/or species recognition), we predict a co-evolution between wing colors and their visual discrimination in Morphos.
This PhD project aims at identifying the morphological bases and selective factors involved in the evolution of iridescence and its visual perception, by combining the physical characterization of iridescence and the molecular analysis of visual pigments. Analyzing the covariation between iridescence and vision will be performed at two evolutionary levels: (1) at the whole genus level, to characterize the diversity of iridescence and its perception and (2) in a triplet of species (*M. helenor*, *M. achilles* and *M. deidamia*) presenting strongly convergent color patterns in sympatry (Llaurens et al. 2021). Analyzing variation in iridescence at the micro-evolutionary scale will allow identifying the effect of interspecific interactions on this evolution: we predict the iridescence of closely related species to diverge in localities where they co-occur, allowing species recognition by sexual partners.

Methodology and aims

1) *Physical characterization of iridescence, its morphological bases and its evolution*

The first aim of the project is the comprehensive quantification of iridescence and of its physical bases, throughout the genus. A standardized method for quantifying iridescence was recently set-up in collaboration with the CRC team (Gruson et al 2019), and validated on a few *Morpho* species. Iridescence will be measured throughout the whole wing using a hyperspectral camera, varying the illumination and the observation angles. The protocol will be applied to specimens of both sexes throughout the 30 species of the genus, and to a large number of individuals from different populations within each of the three convergent species. Animal vision models will then be used to test whether the measured differences are likely to be perceived by birds and butterflies (e.g. Llaurens et al 2014).
In Morphos, iridescence is produced by the joint effects of (1) a diffraction grating composed of the juxtaposition of longitudinal ridges along the scales and (2) a multilayer interference produced by the stacking of chitinous lamellae (Figure 1). In some species lacking lamellae, the interference is produced by the stacking of scales rather than lamellae, suggesting that iridescence evolved at least twice in the genus. The morphological bases of iridescence will be systematically examined using SEM to assess the physical origin of the differences in measured optical properties.
Phylogenetic comparative analyses will be used to estimate the phylogenetic signal of iridescence and to test the divergence between canopy and understory species. Predator communities, as well as light conditions, are indeed very different between canopy and understory, so that the selective pressures acting on coloration might differ among different micro-habitats. The gliding flight typically performed by canopy species (Le Roy et al 2021b) likely undermines the antipredator effect of the dorso-ventral contrast. A marked divergence in iridescence is thus predicted between canopy and understory species. The link between coloration and flight behavior will be quantified, the flight data being already available in the ISYEB team (Le Roy et al 2021b). The detailed geographic variation in the three convergent species will be assessed, testing if iridescence plays a role in species recognition or rather evolves under the primary effect of predation.

2) *Molecular analysis of opsins – covariation with wing colour*

In many animals, the ability to discriminate colors strongly depends on opsins – eye proteins that interact with the chromophore and thus react to different wavelengths. In most butterfly species, there are three main types of opsins sensitive to UV (UV-opsin), short (S-opsin) and long (L-opsin) wavelengths respectively. A duplication of the L-opsin was detected in some blue butterfly species, possibly increasing the discrimination of blue wavelengths (Frentiu et al 2007). Our recent assembly of *M. helenor* genome has revealed the existence of three L-opsin copies, displaying non-synonymous variations as compared to the other copies, potentially associated with shifts in wavelength sensitivity. We will test the variations of the full sequence of the three opsins (UV, S, L) and their potential copies among the 30 *Morpho* species as well as among populations of the three sympatric species. This will allow to assess the differences in color sensitivity across species and to test whether visual abilities covary with wing color and depend on species interactions in sympatry. We predict (1) at the genus level, a difference in blue sensitivity between blue and non-blue species, as well as a divergence of visual performance between canopy and understory species linked to the difference in light environments; (2) within blue species, a higher capacity blue sensitivity in localities where these species are observed in sympatry.

Thesis supervision and feasibility

Sampling iridescence diversity within and among species is secured by the access to the MNHN collection (the world largest public collection of Morpho butterflies). Non-destructive quantification of iridescence will be performed at the CRC, using a hyperspectral camera, with the support of the engineer Aurélie Tournié who has a large experience in the acquisition and analyses of these data (Tournié et al. 2019). The protocol for opsin sequencing has been established in 2021 and the availability of a reference genome recently assembled in the ISYEB team will greatly facilitate the molecular work. The PhD will be directed at ISYEB by Vincent Debat, a biologist specialized in morphological evolution of insects wings, and Violaine Llaurens, a biologist specialized in the evolution of butterfly colour patterns; and at CRC by Christine Andraud, a physicist specialized in materials optical properties, in particular transparency (McClure et al. 2019, Pinna et al 2021) and iridescence (Gruson et al. 2018) of butterfly wings. Research expertise of the two teams is particularly complementary: the ISYEB team provides a strong expertise in evolutionary biology, from genetics to ecology, in particular on the evolution of butterfly color patterns (e.g. Nadeau et al 2016) and of Morphos specifically (e.g. Debat et al. 2018 ; 2020 ; Le Roy et al 2021a ; Llaurens et al. 2020). At CRC, the recruited student will benefit from the strong expertise in optics and from the experimental infrastructures. Combining these two disciplines, evolutionary biology and photonics, will shed a new light on the morphological bases of *Morpho* iridescence and on the neutral and selective processes shaping the evolution of color in these emblematic neotropical butterflies.

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