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Review article

# Evolutionary convergences and divergences in sympatric species: *Morpho* butterflies as a case study

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**Abstract.** How do closely related species interact in sympatry? And how do these interactions influence the evolution of their traits and the dynamics of species diversification? In this review, we show how recent research on the evolution of *Morpho* butterflies contributes to address these questions. We first show how sympatric species have colonized different vertical strata in the neotropical forest and how this divergence has produced cascading adaptive effects on behavioural (flight) but also morphological traits, including wing size, shape and coloration. We then focus on the evolution of peculiar dorsal blue coloration within the genus *Morpho*. During flight, the blue iridescence produces bright flashes that confuse predators and likely enhance the escape abilities of these butterflies. In turn, predators learn the association between such conspicuous coloration and escaping capacities. Such learning favours the locally abundant colour pattern and promotes the local convergence in sympatric species. However, this tight resemblance also induces sexual interference between mimetic species. Capture–Mark–Recapture data uncovered that mimetic species do not fly at the same hours: competition seems to have driven the divergence in the timing of flight activity between species. Overall, sympatry therefore promotes the intricate evolution of convergent and divergent traits among tightly related species, that jointly facilitate their coexistence. Whether ecological speciation was involved in this evolution is an intriguing open question. At the genomic level, analyses revealed a faster evolution of the sexual chromosome Z as compared to the autosomes, with extensive rearrangements and molecular signals of positive selection: these data thus suggest an important role for the Z chromosome in adaptive evolution in *Morpho* and possibly in speciation. Paving the way for future research, these various, multilevel studies show that *Morpho* are not just those showy butterflies in the box: they can also teach us much about evolutionary processes.

**Keywords.** Evolution, Adaptation, Speciation, Sympatry, Butterflies, Flight, Sex chromosomes.

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The growing availability of genomic resources and next-generation sequencing (NGS) technologies has provided insights into the genetic and developmental basis of phenotypic diversity. Yet the evolutionary processes ultimately responsible for such diversity are still largely unknown. A cross-talk between

different disciplines, including developmental genetics and genomics, together with evolutionary biology, is needed to understand how genetic and developmental variations are shaped by neutral and selective processes driving the course of evolution. Such cross-talk currently only occurs in a handful of model organisms, limiting the generality of the discovered processes. Here, by presenting recent research on

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*Morpho* butterfly evolution, we aim at highlighting how the study of non-model communities of species can uncover new evolutionary processes.

*Morpho* butterflies are fascinating insects encountered in the tropical areas of central and South America [1]. A *Morpho* flying in the Amazonian forest understory is a memorable sight, because of the peculiar iridescent blue displayed on the dorsal sides of their wings, generating shiny flashes during flapping flight. While these butterflies have been collected by naturalists for more than two centuries and many taxonomists have struggled to define the different *Morpho* species [2], there were surprisingly few scientific studies investigating their ecology and evolution until recently. Nevertheless, the large morphological and behavioural disparity of closely related *Morpho* species living within the same localities (thus defined as sympatric species) opens multiple questions on the evolutionary processes involved in the diversification of traits and on the effect of ecological specialization in such diversification. Here, we aim at summarizing current knowledge brought by research on *Morpho* butterflies, and at highlighting how these findings may open up new research avenues in evolutionary ecology. We specifically focus on (1) the diversification of flight behaviour throughout the vertical strata of the forest, (2) the selective processes shaping the evolution of their wing colour pattern and (3) the genomic and ecological factors involved in the divergence into multiple species co-existing in sympatry.

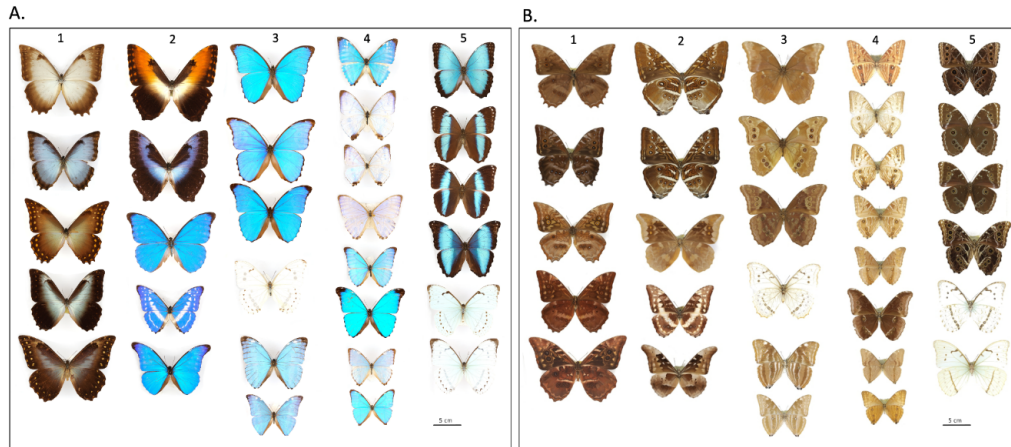
## 1. Adaptive evolution of flight in *Morpho* butterflies?

The genus *Morpho* has diverged from its sister genus *Caerois* circa 38 million years ago, and contains 30 currently-recognized species [3]. The phylogenetic relationships between species are well supported by morphological and molecular data [3,4]. Most of the species of the *Morpho* genus are distributed into two main clades, opposing species observed in two different microhabitats, i.e. understory and canopy. Two species (*Morpho eugenia* and *Morpho marcus*) belong to an outgroup that split from the rest of the genus earlier than the divergence between the two main clades (see phylogenetic relationships in Figure 2). Such a small phylogenetic scale is both a weakness and a strength for evolutionary inference:

on the one hand, it limits the power of phylogenetic comparative tests, impeding the identification of the factors shaping diversification and phenotypic evolution; on the other hand, the small size of the group allows to aim for an exhaustive sampling in phenotypic and genomic analyses. Most importantly, provided that enough field investigations are conducted, a good knowledge of the ecology of most species is in reach, allowing to identify the selective forces at play.

Striking morphological divergence is observed among these closely related species (see Figure 1). While all *Morpho* are large butterflies, their wingspan varies considerably among species, from 6–7 cm for the smallest to almost 20 cm for the largest. This morphological diversity also includes coloration: while the wings observed in most *Morpho* species are blue, some are orange, white, or brown. Even across species displaying blue wings, there is a marked diversity of blues, from bright to dark, shiny to matte, and the relative extent of blue and black on the wings also varies extensively. The disparity in wing shape is also striking in this small butterfly genus: while the wings are short and rounded in some species, in others they are elongated and sharp. What are the evolutionary drivers of such stunning morphological diversity across this small number of closely related species?

While the ecology of most *Morpho* species has been poorly investigated so far ([5] for a review), our limited knowledge nevertheless suggests some effects of ecological specialization on the morphological evolution of *Morpho*. For example, [3] have suggested that adaptation to different host plants may impact wing size evolution: the shift from dicotyledon to monocotyledon (*Chusquea* bamboo) of a small clade of species was associated with a sharp reduction in wing size (Figure 2; see also columns A4 and B4 in Figure 1). A smaller wing size could be favoured in these species, in particular in females that are often seen flying and ovipositing in very dense bamboo thickets [6–9]; alternatively, bamboos might constitute a poorly nutritive food, impacting development and body size. Many observations have also reported markedly different flight behaviours across species, opposing some species observed only high in the canopy, with individuals performing extensive gliding phases, to species observed mostly in the understory, with individuals relying on flapping flight [10]. Could such vertical ecological



**Figure 1.** Male specimens of the thirty *Morpho* species illustrating the disparity in wing sizes, colours and shapes. (A) dorsal view; (B) ventral view; columns 1–2: species observed in the canopy; columns 3–5: species observed in the understory. Note the striking contrasts between the dorsal and the ventral surfaces, especially in species where the dorsal side is blue.

stratification within the *Morpho* genus influence the diversification of traits, and in particular wing shape?

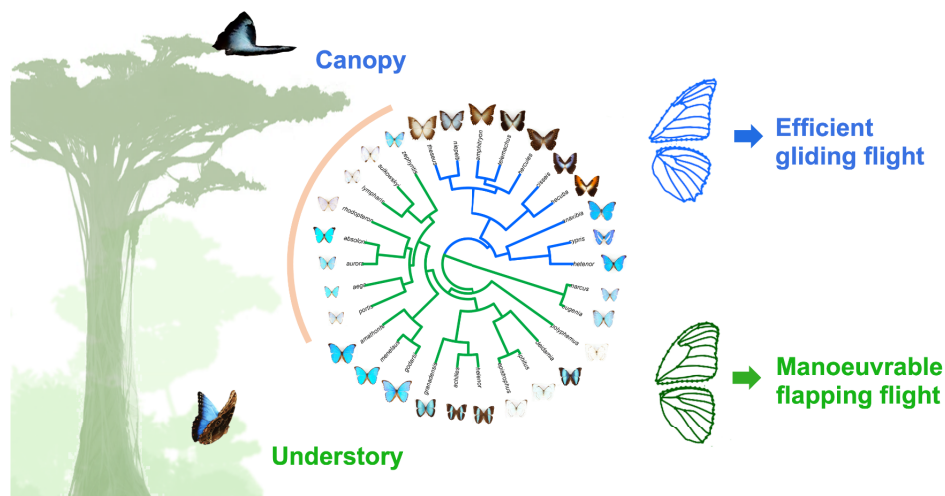
### 1.1. Evolution of wing shape across microhabitats

The rich collections of the Paris Museum of Natural History have enabled quantifying the wing shape of males and females from all 30 *Morpho* species, using a geometric morphometric approach, accounting for the phylogenetic relationships inferred from molecular data [3,4]. This approach relies on the position of homologous landmarks defined on wing veins and outlines, generating a precise quantification of shape variations and a visualization of shape differences (see Figure 2). The wing shape variation between species clearly matched the predicted opposition between microhabitats (canopy vs. understory) and types of flight (gliding vs. flapping): sharp triangular wing shapes are observed in canopy species, while shorter rounded wings are found in understory species. This morphological difference was thus stronger than expected under a Brownian evolution model accounting for the phylogenetic relatedness between species: this thus suggests that the divergence in wing shape is not explained by neutral evolution only but can also be significantly influenced by selection pressures that differ in the canopy vs. understory microhabitats, in line with

previous suggestions [10]. Elongated wings are typically associated with good gliding performances, while short rounded wings should favour manoeuvrable flapping flight, as reported in various flying organisms (bats (e.g. [11]), birds (e.g. [12]) and butterflies (e.g. [13])). This divergence of wing morphology might thus reflect a behavioural adaptation to the contrasted microhabitats: gliding might be advantageous in the open canopy, while manoeuvrability might be favoured in the cluttered Amazonian understory. Considering that species belonging to the outgroup clade (*M. eugenia* and *M. marcus*) are mainly observed in the understory (Figure 2), as do species from the sister-groups *Caerois* and *Antirhea*, the colonization of the canopy is likely a secondary event, possibly impacting the evolution of flight and related traits in these high-flying species.

### 1.2. Adaptive coevolution of wing shape and flight

Investigating the putative coevolution of flight behaviour and morphology then required to combine (1) the precise characterization of flight behaviour in both canopy and understory species to (2) the estimation of flight performance associated with different wing shapes using aerodynamic modelling. Field work in northern Peru allowed recording flight data for 12 *Morpho* species (eight from the understory



**Figure 2.** Divergence of wing shape among *Morpho* species flying in contrasted microhabitats (canopy vs. understory). The divergence is shown by the reconstructed extreme shapes along a canopy-understory gradient and suggests aerodynamic effects of wing shape variations on flight performances. The orange line indicates the shift from dicotyledon to monocotyledon host plants (bamboo), associated with a reduction in wing size.

and four from the canopy): *Morpho* were first filmed while free flying in their natural habitat to characterize natural flight behaviour. Using a large insectary equipped with a multicamera videographic system then permitted to finely characterize *Morpho* flight in standardized conditions. The reconstruction of 3D trajectories then provided estimations of a series of general flight parameters, including speed, acceleration, sinuosity, and more specific parameters characterizing gliding behaviour. For instance, the position of the wings during gliding phases was measured for the different species studied, a critical piece of information for aerodynamic modelling. Observations in both natural and captivity environments showed the same trends: gliding behaviour was much more prevalent in canopy than in understory species. In turn, significantly more powerful flapping flight was detected in understory species as compared to understory ones. These behavioural results were used to parametrize computational fluid dynamics (CFD) simulations. The aerodynamic modelling showed that the wing shapes of canopy species are associated with a higher lift-to-drag ratio, i.e. higher gliding performances, as compared to the aerodynamic properties emerging from the wing shapes of the understory species. This original combination of observations of natural behaviours, quantification of

flight behaviour in captivity, and aerodynamic modelling suggests an adaptive co-divergence of flight and wing shape across the two microhabitats in *Morpho* butterflies [14]. But did the microhabitat shift promote the adaptive evolution of new flight behaviour and morphology, or was it rather facilitated by a pre-existing variation in wing shape within the understory species? Supporting the first hypothesis, a classic view posits that adaptive syndromes are often initiated by behavioural shifts followed by morphological fine-tuning [15,16]. However, the reverse might also be true (see [17], and [18] for general discussions): precisely quantifying variation in flight height and flight behaviour across species might help solve this question.

The drivers of this vertical shift towards the canopy are also unknown. Sexual interference, i.e. the occurrence of costly interspecific fights or courtships, seems to be strong in current *Morpho* species, as suggested by experimental data ([19], see below). Colonizing the canopy might have been advantageous, by alleviating this competition in the understory, but the low number of species at that time (only two; see [3]) might rather point at an alternative, neutral scenario. Whether this niche partitioning was involved in sympatric speciation, or rather separated already formed species is thus

currently unknown and is an exciting open question. Further diversification then took place within the canopy, filling the new niche, and possibly prevented further secondary colonization.

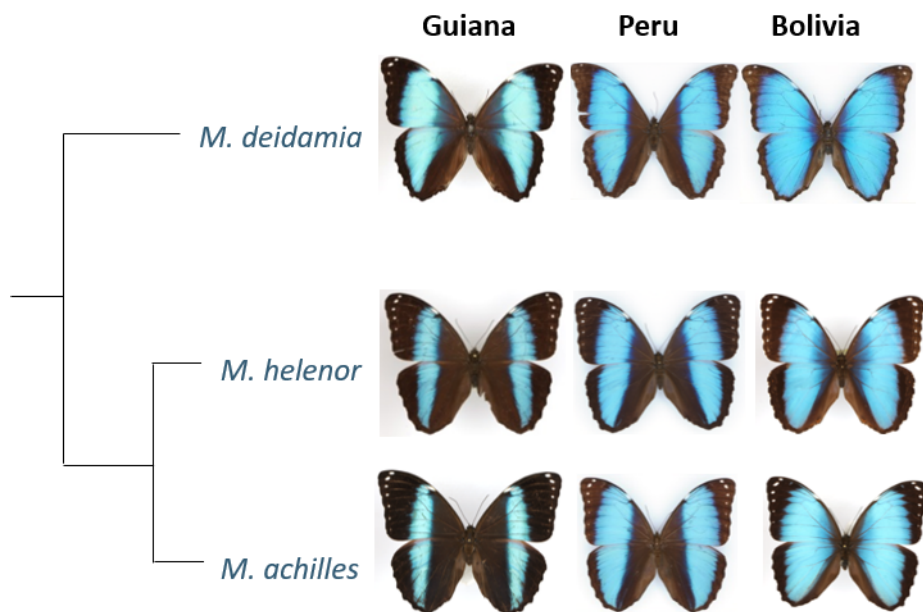
The divergence of flight behaviour between canopy and understory species might also have consequences on the evolution of coloration. Out of the ten canopy species, seven have indeed lost the blue iridescence and present duller colorations (Figure 1), suggesting that gliding flight might promote less conspicuous appearance. Interestingly, flight data on *Morpho rhetenor*, a canopy species where males exhibit striking shiny iridescent-blue coloration, revealed a powerful flapping flight, in sharp contrast with the gliding behaviour observed in other canopy species displaying dull colorations. These observations support an association between fast flapping flight and blue iridescence, and suggest a complex history of coevolution of morphological traits during the diversification of *Morpho* butterflies.

These recent findings thus point at the cascading evolutionary effects of interspecific interactions in sympatry: the putative competition among *Morpho* species might have favoured the colonization of the canopy level, and the very different ecological conditions encountered there might have released selection on flapping flight speed and manoeuvrability and promoted the evolution of gliding flight, imposing new selective pressures on wing size and shape, thereby also modifying selection on wing coloration. Such coevolution of multiple phenotypic traits, behavioural (flight) and morphological (wing shape and colour), is a promising direction for future research. A precise quantification of flight behaviour and flight height beyond the binary opposition of canopy and understory, as well as a more detailed analysis of the ecological interactions among species within microhabitats will be necessary to identify the temporal sequence of these phenotypic shifts and assess their causal relationships.

## 2. Mimicry among closely related species living in sympatry

Several species of *Morpho* display an iridescent blue colour on the dorsal side of their wings, contrasting with a dull brownish colour on the ventral side (Figure 1). This dorso-ventral contrast results in a series of flashes during flapping flight. Capture-mark

experiments carried out in the Atlantic forest of Brazil with *Morpho helenor* manipulated to display blue colours on both wing sides show lower levels of recapture of individuals with blue colours on both wing sides, as compared to wild-type ones displaying iridescent blue only on the dorsal side [21]. This suggests a positive influence of the dorsal-ventral contrast producing flash effects during flight on the survival of iridescent blue *Morphos*. The visual effect of the contrasted dorso-ventral contrast associated with an erratic flight is indeed likely to enhance the difficulty of predators in capturing them. [22] then tested the ability of humans to catch a moving stimulus, comparing a situation where the stimulus was visually constant to a situation where it varied in colour while moving. This experiment showed that the colour change during the movement was associated with decreased attack success as compared to white or background matching stimuli. This experiment is thus consistent with the hypothesis of an effect of the peculiar dorso-ventral pattern of *Morpho* butterflies in limiting predation. Furthermore, this specific colour pattern may also act as a signal of escape ability and hence deter predators from chasing butterflies displaying such coloration. This hypothesis was suggested by [23], and experiments carried out with wild birds were generally consistent with his hypothesis [24]: *Morpho* butterflies were sight-rejected 11 out of 13 times by wild jacamar birds while flying. Although these experiments were based on a limited sample size, they suggest that predators may (1) recognize and avoid butterflies displaying the contrasted blue signal and (2) consume these butterflies, therefore confirming their palatability. These observations suggest that the evolution of blue colour patterns in *Morpho* butterflies could be influenced by bird cognitive capacities: in a given locality, colour patterns commonly displayed by evasive prey would be recognized and avoided by local birds. Such predator behaviour likely favours positive selection of colour patterns commonly displayed by evasive prey, therefore promoting evolutionary convergence in colour patterns in sympatric prey species. Such evolutionary convergence in colour patterns has been confirmed multiple times in toxic species, including butterflies (i.e. Müllerian mimicry, [25]). Convergence in colour patterns could then be promoted by selection from the local communities of birds [23]. In an experiment in controlled



**Figure 3.** Representative examples of parallel geographic variations of dorsal wing colour patterns observed in *Morpho helenor*, *Morpho achilles* and *Morpho deidamia*, documented in [20], with a schematic view of their phylogenetic relationships. Pictures were taken in the collections of the Natural History Museum of Paris.

conditions, Paez *et al.* [26] indeed showed that blue tits can associate a colour pattern with evasive capacities. Comparing the learning curve of birds when associating the same colour pattern with either a distasteful experience or an evasive experience showed that birds tended to learn the colour pattern faster when associated with evasive prey. The lack of success of the birds in catching the prey is thus likely to trigger a faster learning response as compared to unpalatability. The speed of colour-pattern learning by birds likely contributes to the strength of selection: the faster each bird does learn in the wild, the lower the risk there is for a given butterfly to encounter a bird that would be unaware of the difficulty of capture associated with the visual signal. The fast learning speed of birds facing evasive prey may therefore provide a very strong advantage to mimetic coloration in prey with high escaping skills.

Consistent with this hypothesis, we recently demonstrated a striking parallelism in the phenotypic diversification of three blue *Morpho* species throughout their sympatric distribution (Figure 3). Using samples of the closely related species *Morpho achilles*, *M. helenor* and *Morpho deidamia* from the

collections of the Natural History Museum in Paris (France), we quantified the variations in black and blue dorsal wing patterns displayed in individuals collected throughout the geographic range of the three species. Using permutation tests, we showed that pairwise phenotypic distances between individuals from different species were significantly more similar within geographic localities, as compared to between localities. The high resemblance between species within localities is likely resulting from multiple local convergence in the width of their dorsal blue band [20]. The effect of predation on the evolution of resemblance is currently the most likely hypothesis, but it has not been directly tested yet, so that we cannot rule out the effect of contrasted selective regimes occurring in different geographic localities. Interestingly, evidence for evasive mimicry is currently still scarce, but this phenomenon is probably more important for the evolution of coloration than previously acknowledged. On top of butterflies [27], evasive mimicry has indeed been hypothesized in various prey taxa, including beetles [28], grasshoppers [29] and flies, and may involve various predators from birds to mantids [30]. The colours

displayed by the evasive prey are likely conspicuous, allowing recognition by potential predators in a similar manner as in Müllerian mimicry. However, the iridescent blue of the wings in *Morpho* butterflies likely directly contributes to the difficulty of capture, not only because of the dorso-ventral contrast, but also because of the unstable visual appearance of the iridescent surface, possibly enhancing predator confusion. The evolution of the colour pattern might thus be constrained by the direct survival advantage provided by the visual effects generated during escape flight. Differences in colour pattern among species might be blurred by motion, as the blue dorsal side is only visible during flight, possibly reducing the strength of selection on minute details of the colour pattern. In sharp discrepancy with this hypothesis, the very close similarity between *M. achilles* and *M. helenor* suggests that this potential blurring does not limit the precision of the convergence. Alternatively, their close phylogenetic proximity might impose a tight parallelism due to genetic and developmental constraints. Interestingly, the more distant but also convergent species *M. deidamia* displays a slightly different colour pattern suggesting an effect of phylogenetic distances constraining the level of resemblance, and consistent with the motion blurring effect allowing imperfect mimicry.

Recent findings on the evolution of colour pattern in *Morpho* butterfly communities shed light on the potential significance of evasive mimicry in shaping the joint evolution of dorso-ventral coloration and flight behaviour: how did these evasive syndromes evolve? Does divergence in flight morphology and behaviour evolve before changes in coloration, or the other way around? It also raises new questions on the parallel evolution of colour patterns in closely related species: are similar molecular pathways involved in these multiple convergence events, or alternatively does introgression between closely related species facilitate such parallelism?

### 3. Reproductive interferences and evolution of circadian activities

Mimicry among butterflies frequently occurs among distantly related species [31,32]. Precise quantification of phenotypic distances in Papilionidae butterflies recently suggested that mimicry might be more prevalent in distantly related species rather

than closely related ones, especially in males [33]. This pattern may be due to the opposite effect of natural and sexual selection, since the wing colour pattern is also involved in mate recognition [34]: the evolution of colour patterns in sympatric species could indeed be influenced by predation favouring convergence in toxic or evasive prey, but also by sexual selection favouring divergence in closely related species, because of the costs associated with heterospecific interactions, such as heterospecific mating or rivalry for instance (see [35] for a review on heterospecific behavioural interactions generating reproductive interferences across animal species). Convergence in wing colour patterns could thus be more likely to happen across distantly-related species, where multiple alternative pre-mating barriers have already accumulated. In *Morpho* butterflies, however, we documented striking resemblance in patterns [20], as well as iridescent blue [36] within locality, among the closely-related species *M. achilles* and *M. helenor*. Because iridescent coloration has been shown to be involved in mate choice in other animals [37], the dorsal colour pattern displayed in *Morpho* could have an effect on sexual attraction and may result in heterospecific interactions. Using solar-powered mobile dummy butterflies placed in a field site in Amazonian Peru, we found that wild males of all these three species were attracted to the blue colour of dummies outfitted with wings from all these three different species [19]. The interactions between wild males patrolling in the field and the dummies provide evidence of an enhanced attraction toward the locally displayed colour pattern as compared to patterns displayed in other localities. Wild males were thus attracted by the local colour patterns of males and females from the three species, suggesting intense heterospecific male–male competition and courtship. Behavioural experiments in cages also confirmed the lack of visual discrimination of conspecific vs. heterospecific females by wild males from two sister species, *M. achilles* and *M. helenor* [36].

Nevertheless, sequencing individuals of the two sister-species *M. achilles* and *M. helenor* sampled in the same locality in Peru allowed to reconstruct the history of gene flow between these sympatric species. Demographic inference models based on these genomic variations within and between species are consistent with current genetic isolation [19].

This suggests that pre- or post-zygotic mechanisms might prevent gene flow between these two species, despite limitations in species recognition based on coloration. Interestingly, capture/mark/recapture experiments carried out in the same Peruvian locality revealed that males from these two mimetic species patrol at different times of the day [19]. This divergent temporal window may favour the evolution of divergent temporal niches, if male behaviour is consistent with variations in female mating availability during the day. In those species, female behaviour makes them less likely to be observed because they are typically found deeper in the understory, close to their host plants, while males patrol along river beds. Males transfer spermatophores to females during mating, and these spermatophores can provide sperm to fertilize multiple eggs and usually prevent successive matings with other males. Females are thus probably only mating rarely throughout their life, while males are probably able to mate multiple times. The availability of virgin females is thus probably the limiting factor shaping the reproductive success of males, so that variation in the timing of adult emergence could shape the temporal niche of reproductive activities in these species. Using a theoretical model, [38] indeed showed that male–male competition to access virgin females can promote the divergent evolution of temporal niches in sympatry. A similar effect has also been hypothesized to contribute to allochronic speciation in *Spodoptera* moths [39]. Such a process could be further promoted by genetic incompatibilities evolving within populations.

Allochrony (differences in breeding time) is generally suggested to be an important pre-zygotic mechanism promoting speciation, or evolving as a trait reinforcing barrier to gene flow between recently diverged species secondary getting in contact [40]. Seasonal variations in phenology are documented as a mechanism involved in sympatric speciation, especially in plants [41,42]. However, daily variation in reproductive timing is much less documented except for some insects, such as *Drosophila* [43] or some Lepidoptera [44]. In neotropical skipper butterflies, for instance, observations of more than 400 species from different genera show significant differences in flight time between males of closely related species [45]. In butterflies and other insects, daily activity is controlled by a series of genes and transcription factors that regulate the circadian clock

[46]. Circadian genes, as *vrille* or *period*, have been suggested to control breeding time in moth species as *Spodoptera frugiperda* or *Plutella xylostella*. In the case of *S. frugiperda*, the gene *vrille* could be responsible for the pre-zygotic isolation of the two strains of the species, as the differential transcription of the gene is associated to the allochronic differentiation of the two strains [44,47]. The sequencing and annotation of reference genomes for eleven *Morpho* species recently allowed to study the evolution of eight circadian genes throughout the *Morpho* genus [48]. Signal of positive selection was detected in the circadian gene *period*, suggesting that this gene could be acting in the diversification of flight time in this genus.

By highlighting the divergence in temporal niches and putative genetic bases for the evolution of daily allochrony in *Morpho* butterflies, recent studies show the significance of the diel niche in the diversification of ecological niches, specifically in communities where multiple closely related species co-occur in sympatry, as in tropical areas. It also calls for further research on the biotic and abiotic factors shaping these daily temporal niches, as well as on the respective contributions of genetic and developmental effects to such divergence in circadian activities.

#### 4. Genomic barriers to gene flow and Z-chromosome evolution

##### 4.1. Reduced gene flow in sympatry despite mimicry

Other mechanisms acting at the post-zygotic stage can also prevent gene flow and promote speciation between closely related species in sympatry. These mechanisms occur post-fertilization, resulting in the production of hybrid zygotes with lower viability or fitness compared to non-hybrid zygotes. Chromosomal rearrangements, such as inversions, may serve as post-zygotic barriers by reducing recombination in the inverted regions, thereby fuelling divergence between haplotypes and contributing to reproductive isolation [49]. Inversions are common across various biological groups, including insects, teleosts, birds, and mammals [50].

In some groups, inversions tend to accumulate more on sex chromosomes compared to autosomes. For instance, a study of 411 passerine bird species

found a higher probability of fixation for inversions on the Z chromosome than on the autosomes [51]. Sister species with overlapping ranges were also more likely to exhibit inversions in their genome than those in allopatry, suggesting an effect of the reinforcement process in favouring inversions between sympatric species [51]. Similarly, in the genus *Morpho*, comparisons between species pairs revealed extensive chromosomal rearrangements in the Z contig compared to the autosomes, especially between closely related species coexisting in sympatry and with overlapping temporal niches [48].

#### 4.2. Effect of the inversions and gene evolution in the Z chromosome

In species with chromosomal sex determination, it has usually been observed that loci harboured on sex chromosomes may evolve faster than similar loci on the autosomes [52], a phenomenon commonly known as faster-X or faster-Z evolution [52,53]. Traditionally, the faster Z or X effect has been attributed to the faster fixation of beneficial alleles by positive selection, because of immediate expression at the hemizygous stage [52]. In autosomes, loci with recessive effects are not exposed to selection because they are masked by the dominant ancestral allele. However, if these loci arise on the X or Z chromosome, their effects on fitness are fully expressed in the hemizygous sex (the sex with only one copy of the sex chromosome, such as males in species with XY sex determination or females in ZW systems) [53]. Then, recessive beneficial alleles will be more rapidly fixed by positive selection when they are located on the X or Z chromosome. However, the faster evolution of X/Z chromosomes can also result from the effects of genetic drift, which limit the purging of deleterious mutations. In each mating pair, there are four copies of autosomal genes but only three copies of X or Z-linked genes. Consequently, the effective population size ( $N_e$ ) for X or Z sex chromosomes is reduced compared to autosomes ( $3/4 N_e$ ). This reduced  $N_e$  may limit the elimination of slightly deleterious mutations within each species.

Empirically, a faster evolution of the X/Z chromosome has been observed in many groups with XY and ZW determination such as mammals, birds, butterflies, snakes, spiders, or the salmon louse *Lepeophtheirus salmonis* [54–62]. The frequent occurrence of

faster evolution of X/Z chromosomes suggests that they substantially contribute to key evolutionary processes such as speciation and adaptation.

In the case of *Morpho*, we analyzed the selective pressures acting on the genes located on the Z chromosomes and in the autosomes among 12 assembled genomes belonging to 11 species. We specifically compared the ratio of non-synonymous vs. synonymous mutations ( $dN/dS$ ) on the genes located in the autosomes and the genes located on the Z chromosome. We detected faster evolution for genes in the Z contig compared to the autosomes, suggesting a potential role of the Z chromosome in driving adaptive evolution in this group. The faster evolution of genes located on the Z chromosome was also confirmed when comparing closely related species pairs. We found significant differences in the  $dN/dS$  ratio between autosomal genes and Z-linked genes, with Z-linked genes showing higher average values than autosomal genes. This pattern was especially pronounced in species pairs that share the same habitat and have overlapping flight periods [48]. This suggests that divergent selection among sympatric species and/or reinforcement processes could contribute to the faster evolution of the Z chromosome among sympatric species.

Although our genomic analyses revealed a highly dynamic Z chromosome, characterized by the presence of inversions and an elevated  $dN/dS$  ratio, it is crucial to corroborate these findings at the population level. Population genomic analyses can determine whether these inversions are fixed in different populations of the species where they were initially identified. Additionally, such analyses can shed light on the roles of Z-linked genes in speciation and adaptation. Investigating the selective pressures on these genes in closely related species, particularly in contexts of sympatry and allopatry, would provide valuable insights into their evolutionary significance.

## 5. Conclusions

*Morpho* butterflies have long been considered for their beauty rather than their scientific interest, resulting in a paradoxical situation: they are heavily collected and sold, but their biology and ecology are poorly known. The recent works reviewed here suggest that *Morphos* are ideally suited to allow investigating fundamental questions of evolutionary

biology. Focusing on wild communities of closely related species is indeed a relevant way to identify key mechanisms involved in speciation, ecological specialization, as well as diversification of adaptive traits. The results obtained on *Morpho* butterflies highlight the relevance of combining investigations on the phenotypic, genomic and ecological variations in non-model organisms to shed light on the evolutionary mechanisms generating and maintaining natural communities in the wild.

### Declaration of interests

Views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them.

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