Convergent morphology and divergent phenology promote the coexistence of *Morpho* butterfly species

Camille Le Roy, Camille Roux, Elisabeth Authier, Hugues Parrinello, Héloïse Bastide, Vincent Debat & Violaine Llaurens

The coexistence of closely-related species in sympatry is puzzling because ecological niche proximity imposes strong competition and reproductive interference. A striking example is the widespread wing pattern convergence of several blue-banded *Morpho* butterfly species with overlapping ranges of distribution. Here we perform a series of field experiments using flying *Morpho* dummies placed in a natural habitat. We show that similarity in wing colour pattern indeed leads to interspecific territoriality and courtship among sympatric species. In spite of such behavioural interference, demographic inference from genomic data shows that sympatric closely-related *Morpho* species are genetically isolated. Mark-recapture experiments in the two most closely-related *Morpho* species unravel a strong temporal segregation in patrolling activity of males. Such divergence in phenology reduces the costs of reproductive interference while simultaneously preserving the benefits of convergence in non-reproductive traits in response to common ecological pressures. Henceforth, the evolution of multiple traits may favour species diversification in sympatry by partitioning niche in different dimensions.
Natural communities are composed of multiple species involved in diverse ecological interactions either facilitating or impairing their coexistence. These interactions strongly depend on the level of phylogenetic divergence between the involved species\cite{1,2}. In particular, the coexistence of closely-related species is often impaired by their ecological and morphological similarities\cite{3,4,5}, generating either elevated interspecific competition and/or reproductive interference\cite{6,7,8}. Close relatedness and shared phenotypes are indeed identified as important factors enhancing behavioural interference in sympatric animal species\cite{9}. Nevertheless, inherited traits shared by closely-related species may also provide individual fitness benefits, for instance when facing common predators either by sharing a common warning signal\cite{10,11,12}, or by responding to alarm cues emitted by heterospecífic\cite{12,13}. Such interspecific positive density-dependence is predicted to favour species coexistence\cite{14}. Here, we investigate how shared and divergent traits can facilitate the coexistence of closely-related species.

Mimetic butterflies are a striking case of sympatric species with phenotypic convergences strongly promoted by natural selection. The convergence observed for warning wing patterns in defended species is frequently followed by convergence in other traits like flight height\cite{15,16,17} or host-plant\cite{17} since sharing a microhabitat increases the similarity of encountered predatory communities, thereby enhancing protection\cite{18}. Nevertheless, the benefits conferred by overlapping visual signals and ecological niches in mimetic species may, in turn, incur fitness costs through increased heterospecific rivalry, heterospecific female harassment and the expression of Dobzhansky-Müller incompatibilities in hybrids\cite{19,20,21}. These costs might ultimately either limit the coexistence of closely-related mimetic species\cite{22} or promote the evolution of alternative cues involved in species recognition\cite{23}. The conflicting ecological interactions between mimetic species, therefore, question their persistence in sympathy when they are closely related and point to the evolution of alternative divergent traits.

Here we investigate how trait evolution might favour the coexistence of closely-related species by focusing on three species of the butterfly genus *Morpho* that are sympatric over most of the Amazon\cite{24,25} and occupy the same understory niche in the tropical forest. Multiple local convergences in wing colour patterns are observed among these species, throughout their geographical range\cite{25}. Although not chemically defended, these butterflies are very difficult to capture because of their fast, erratic flight. The contrast between their dorsal bright iridescent blue and ventral cryptic brownish wing surfaces induces a flash pattern during flapping flight, that was suggested to confuse predators, further increasing their difficulty of capture\cite{26}. Predators may then learn to avoid such elusive prey harbouring the conspicuous blue patterns. The local convergence in the three closely-related *Morpho* species probably stems from frequency-dependent selection generated by predator behaviour, in a similar way as in Mullerian mimics\cite{25}. The iridescent blue colouration of *Morpho* butterflies shared by sympatric species may thus reduce individual predation by advertising escape ability (the ‘escape mimicry hypothesis’, see ref. 27). Males and females typically display the same colour pattern shared between sympatric species and may thus benefit from increased protection against the same predator community. While females are rarely observed in the field, males from these different closely-related species typically patrol within the same habitats. Male–male interactions are then frequently observed in these butterflies, one of the males eventually being chased away. Male territoriality may therefore occur both within and among species, potentially leading to important reproductive interferences\cite{28,29,30,31}. The local convergence in colour pattern might further enhance heterospecific rivalry and impair mate recognition in these sympatric species. The persistence of closely-related species sharing similar colouration would thus be enabled if intraspecific benefits of maintaining a convergent colour pattern outweigh the costs associated with reproductive interference.

To test whether convergent wing patterns generate reproductive interference between species, we investigated the behaviour of *Morpho* towards flying dummy butterflies harbouring various wing patterns, in a series of experiments performed in the wild. To infer whether these heterospecific reproductive behaviours underpin genetic exchange, we then estimated the gene flow among them, their level of genomic divergence and investigated their demographic history. To explore whether interference could be mitigated by temporal partitioning, we finally monitored male flight activity using capture-mark-recapture experiments.

Our study reveals strong genetic isolation between species despite reproductive interference, probably facilitated by a marked difference in flying hours between *Morpho* species. This work highlights how joint convergent and divergent evolution of different traits may promote species diversification in sympathy.

**Results and discussion**

We focused on a single locality from Amazonian Peru where three *Morpho* species with strikingly similar colour patterns (*Morpho achilles*, *Morpho helenor* and *Morpho deidamia*), live in sympatry. In this field site, males from these three closely-related species display a typical patrolling behaviour along the river bed, allowing us to investigate species interactions in *natura*.

**Heterospecific interactions lead to reproductive interference among sympatric species.** To test whether the strong convergence in colour pattern among these sympatric *Morpho* species leads to heterospecific rivalry and courtship, we investigated the response of patrolling males to butterfly dummies placed in the field. We built realistic moving butterfly dummies using the actual wings of captured *Morpho*, set up on a solar-powered fluttering device (Fig. 1a and Supplementary Video 1; see also Supplementary Figs. 1, 2). To ensure that only visual cues were triggering the interactions, the wings were washed in hexane prior mounting, ruling out any effect of pheromones\cite{29}. We built ten different dummies with the wings of specimens from different species and sexes. Eight dummies were built from butterflies caught in the same Peruvian site, using both sexes of four sympatric species: the three mimetic species *M. helenor*, its sister species, *M. achilles*, and the more distantly-related *M. deidamia*, all exhibiting an iridescent blue band bordered by proximal and distal black areas, and the phenotypically distinct *Morpho menelaus*, exhibiting fully blue iridescent wings (referred to as local dummies: *n* = 8) (Fig. 1b, c and Supplementary Fig. 1). The last two dummies were built from *M. helenor* and *M. achilles* males captured in French Guiana (referred to as exotic dummies: *n* = 2), exhibiting a narrower blue band relative to local—Peruvian—individuals (Fig. 1b and Supplementary Fig. 1). We tested all dummies (*n* = 10) following a randomised design: a different dummy was placed each morning at the same site and left fluttering on the river bank for 5 h. This was replicated four times per dummy. The dummy was continuously filmed using a camera (Gopro Hero5 Black set at 120 images per second) and monitored by a human observer who recorded the timing of responses displayed by wild butterflies.

Over a 2-months period, we recorded the patrolling behaviours of all males passing through the river on sunny mornings, resulting in 2700 responses to dummies. We specifically focused on the behaviour of butterflies from the two mimetic sister species *M. helenor* and *M. achilles*, which represented the large majority of the passing males (35% and 47% respectively, Supplementary...
Fig. 1 Behaviour of *Morpho* towards flying dummy butterflies of conspecifics and congeners. a Experimental set up used to study flight interaction. The dummy butterfly was placed at the centre of a cubic area materialised by four 1 m$^3$-sticks, and fixed to a solar-powered fluttering device reproducing butterfly flying behaviour. Interaction between the visitor and dummy butterfly (defined as a visitor entering in the cubic area) were recorded using a stereoscopic high-speed videography system, allowing to quantify flight trajectory during the interaction (Fig. 2). b Frequency of interaction with conspecific and congener male dummies (blue colour) in the two mimetic sister species *Morpho helenor* (left column) and *M. achilles* (right column). c Frequency of interaction with the female dummies (yellow colour). Raw data << nb of interactions/nb of approaches >> are indicated on each bar. Proportions were compared using Fisher Exact probability tests. Only significant differences are shown (P < 0.05). For proportions differing from all the others, the highest P-value is reported. Source data are provided as a Source Data file.

Fig. 3). Visiting species were identified by sight, except for *M. helenor* and *M. achilles* that cannot be reliably discriminated in flight. We used an indirect method to distinguish these two strikingly similar species (see ‘Methods’ section and Supplementary Fig. 13). During these sessions, we defined two behaviours: (1) approaches, when a marked change in the trajectory of the passing butterfly towards the dummy was observed and (2) interactions, when the butterfly entered a 1 m$^3$ zone around the dummy (Fig. 1a). We controlled for the effect of cloud cover on these behaviours (see ‘Methods’ section and Supplementary Fig. 4). This procedure allowed us to test whether patrolling *Morpho* males (1) are more strongly attracted by the colour pattern of their conspecifics as compared to that of other species, (2) discriminate between sexes and (3) are more attracted by local colour patterns than by exotic ones, thereby assessing the strength of behavioural interference between sympatric species.

About half of the patrolling individuals deviated from their flight path to approach the setup (see Supplementary Figs. 5, 6 and Supplementary Tables 2–4 for more details). Wing area and proportion of iridescent blue on the dummy wings had limited effect on the percentage of approach (Supplementary Fig. 7 and Supplementary Table 7): the long-range blue signal emitted by the different wing patterns displayed in the different species thus appeared similarly attractive to patrolling *M. helenor* and *M. achilles* males, suggesting they may approach anything roughly recognised as a potential mate or rival.

In both *M. helenor* and *M. achilles*, about 40% of approaches resulted in interactions, the visitor typically flying in circles around the dummy (Supplementary Video S1). Surprisingly, interactions with conspecific dummies were not significantly higher than with sympatric heterospecifics. Overall, no strong discrimination between sympatric conspecifics and sympatric heterospecifics, nor between female and male dummies was found, suggesting that interspecific interactions are frequent among wild *Morphos* in sympathy (Fig. 1b, c). Some differences were nevertheless detected. Dummies with a larger wing area and a greater proportion of iridescent blue colouration were more likely to trigger interactions (Supplementary Fig. 7), suggesting that these characteristics are used by patrolling males to discriminate encountered individuals. *M. helenor* males were found to approach dummies (all sexes and species pooled) significantly more than *M. achilles* males (mean % of approach in *M. helenor* = 48.3 ± 15.7; *M. achilles* = 39.7 ± 14.8; P = 0.01). They interacted as frequently with *M. achilles* females as with their conspecific females (Fig. 1b). In contrast, interactions occurred in markedly lower proportions with the exotic dummies, that were largely ignored (Fig. 1b). *Morpho* males, therefore, do distinguish different colour patterns (i.e. large blue band in local individuals, vs. narrower blue band in exotic ones), but yet they largely engage in interactions with congeners bearing locally known signals, including signals sharply dissimilar from their own displayed by more distantly-related species (e.g. fully blue wings in *M. menelaus*). For males, interspecific interactions with both males and females could be promoted because mating opportunities are limited by strong male–male competition. Defending territory against any males harassing a potential mate, or courting any females would then be a favoured behaviour. The costs of missed mating opportunities are probably elevated for males when species recognition is impaired by the phenotypic resemblance between species.

Overall, the strong visual attraction and the low species discrimination suggest that both heterospecific interactions—contests among males and mating or mating attempts with females—can frequently happen. This indicates that reproductive interferences occur among these sympatric *Morpho* species.

Patrolling *M. achilles* males had limited interactions with the co-mimetic *M. helenor* female, but interacted indiscriminately with the dummies of the other sympatric species (i.e. *M. deidamia*
and *M. menelaus* (Fig. 1c). Such a specific, acute visual discrimination towards its — albeit phenotypically closest — sister species possibly evolved in *M. achilles* as a result of reinforcement selection against genetic incompatibilities expressed in hybrids. Whether reinforcement selection does occur in those closely-related species would however require further study. Reinforcement process may also occur through divergence in olfactory cues enabling discrimination among species, although this remains to be investigated in *Morpho*.

This specific effect detected in female interactions suggests that patrolling males can discriminate sexes based on short-distance visual cues. To test whether the behaviour of *M. achilles* males indeed differs when interacting with conspecific male and female dummies, we equipped our set up with a stereoscopic high-speed videography system, enabling us to quantify the three-dimensional flight kinematics of visiting butterflies in natural conditions on a sub-set of sessions (Figs. 1a and 2a). Behavioural differences were detected between the interactions toward males and females (*n* = 14 flights analysed for each sex): wild males circled closer to the female compared to the male dummy (significant interaction between distance from dummy and the sex of the dummy on the proportion of time spent within distance intervals: *F*₁₁,₃₁₂ = 1.9; *P* = 0.03, Fig. 2b, see also Supplementary Fig. 8), ensuring a steady speed when close to the female, whereas they displayed more erratic accelerations around the male dummy (significant interaction between the distance from dummy and the sex of the dummy on acceleration within distance intervals: *F*₁₁,₁₇₇ = 1.9; *P* < 0.001, Fig. 2c). These aerial interactions suggest that *M. achilles* males adjust their flight behaviour according to the sex of their conspecific, showing that, at least in *M. achilles*, *Morpho* males can identify females using visual cues alone.

The limited attraction of *M. achilles* towards the females of its sister species *M. helenor* and the contrasted attraction to local and exotic colour patterns suggest that sympatric interactions might have promoted discrimination capacities in the closest related species, thereby reinforcing pre-zygotic barriers. Overall, the behavioural interferences among these sympatric *Morpho* species as well as the putative reinforcement effect detected in *M. achilles* question their stable coexistence and reproductive isolation.

**Limited genetic exchanges between species despite close relatedness and reproductive interference.** To test the level of reproductive isolation despite such a strong behavioural interference, we then used genomic data obtained for the three mimetic species *M. achilles*, *M. helenor* and *M. deidamia*. Patterns of genetic polymorphism (Supplementary Figs. 9 and 10) and divergence (Supplementary Fig. 11) throughout the genome were investigated using RAD-sequencing. Eight categories of contrasted scenarios of speciation and models of linked selection (Supplementary Fig. 12) were then proposed to be statistically evaluated using a modified version of DILS 1.0.0 adapted to three gene pools. Each category was simulated using four sub-models depending on whether the effective population size is assumed to be homogeneous or heterogeneous along the genome, as well as for introgression rates. Consistent with the phylogeny, demographic inferences performed on our Peruvian populations revealed a more recent time of the split between the sister species *M. achilles* and *M. helenor* than between these two species and *M. deidamia*. Our hierarchical approach of eight categories of models according to temporal patterns of migration between all three species provided strong statistical support for current genetic isolation between *M. deidamia* and both *M. helenor/M. achilles* (*F*₄₉ ± std *M. helenor − M. achilles* = 0.30 ± 0.226; *M. helenor − M. deidamia* = 0.82 ± 0.11; *M. achilles − M. deidamia* = 0.85 ± 0.11) (Fig. 3; posterior probability = 0.94). Current isolation was also strongly supported between *M. helenor* and *M. achilles*, with gene flow restricted to the early times of speciation (posterior probability = 0.80). Genomic or behavioural barriers are thus likely to strongly limit gene flow between these closely-related sympatric species (Fig. 3 and Supplementary Tables 8–10). Note that if our demographic inferences show that models with ancestral migration reproduce the molecular data better than models with ongoing introgression, we cannot rule out the possibility that
more complex, untested models might provide a better fit to the data (see ‘Methods’ section). This inferred strict genetic isolation despite close relatedness and reproductive interference between *M. achilles* and *M. helenor* nevertheless suggests that ecological factors might prevent gene flow in sympatry.

**Temporal segregation between sympatric sister species.** The analysis of the temporal variations of flight activity in a mark-recapture experiment revealed a striking difference in patrolling time among species (Kruskal–Wallis test: Chi-square = 179.7, *P* < 0.001, df = 3), with little overlap between the sister species *M. achilles* and *M. helenor* (Fig. 4). Males *M. helenor* patrolled earlier than *M. achilles* (mean patrolling time ± s.d. = 11:14 ± 00:45 vs. 12:35 ± 00:40, respectively). Patrolling time in *M. deidamia* (12:40 ± 00:46) however overlapped with *M. achilles*. Time of (re) capture of the same individual in *M. achilles* was notably stable (correlation between time of first vs. second capture: *r* = 0.40; *P* = 0.05), suggesting a regularity in patrolling time at the individual level in this species (Fig. 4). Whether such individual temporal regularity is genetically determined or reflects a plastic behaviour remains to be investigated.

Reproductive and/or aggressive interference are generally expected to promote spatial or temporal habitat segregation between species because it reduces the cost of negative interspecific interactions. The observed shift in flight activity peaks suggests that reproductive interferences among the morphologically convergent *Morpho* species have favoured the evolution of divergent temporal niches in *M. achilles* and *M. helenor*. Remarkably, this temporal segregation extended to the more distantly-related *Morpho menelaus*, that flies earlier in the morning (Fig. 4). In particular, no overlap between its activity range and that of *M. achilles* was detected, mitigating the potentially strong interference between these species suggested by the attraction of patrolling *M. achilles* towards *M. menelaus* dummy (Fig. 1b). Whether the temporal segregation observed between these sympatric *Morpho* species was involved in a sympatric speciation process or stem from reinforcement after secondary contact between these closely-related species remains to be elucidated, as well as the genetic vs. plastic origin of this behavioural trait.

Temporal segregation in response to resource competition or reproductive interference has been reported in other taxa, but mostly at a larger temporal scale (e.g. seasonal segregation). Peaks of patrolling activity between sister *Morpho* species are shifted by less than 2 h, representing a remarkably fine temporal scale for a shift in reproductive behaviour. Nonetheless, comparable fine-scale temporal partitioning of sexual activity has been observed in other moth and butterfly species, suggesting that it may be relatively common in Lepidoptera.

Altogether, our results suggest that there is a potential for strong interspecific competition among males from *Morpho* species in sympatry, but that this competition is mitigated at least in part by their temporal segregation. Heterospecific courtships may also be limited, if the temporal segregation observed in *Morpho* males is mirrored by a similar temporal partitioning of...
females’ activities. This could not be tested here as females were almost never seen flying along the riverbank: future studies specifically exploring females behaviour should thus be performed. Synchronisation of mating activity between sexes is however likely\textsuperscript{41,42}, because any deviation of males relative to females activity would reduce the probability of intraspecific mating\textsuperscript{3,43,5}. In contrast, a shift in mating time among species would act as a powerful isolation mechanism\textsuperscript{39}, explaining how co-mimetic \textit{Morpho} species can maintain a similar colour pattern in the same habitat while remaining sexually isolated.

Divergence in daily phenology in butterfly mating activities may be a widespread process enabling the persistence of diversity-rich assemblages, as suggested by several reports of temporally structured sexual activities in other butterflies\textsuperscript{33–35}, including closely-related species\textsuperscript{46}. Our study shows that the coexistence of closely-related species can generate complex ecological interactions, both mutualistic (mimicry) and antagonistic (reproductive interference), that could be mitigated by shifts in temporal niches. The evolution of multiple traits, morphological and behavioural, may thus favour species diversification in sympathy by partitioning niche along different ecological dimensions.

\textbf{Methods}

\textbf{Study site and population.} The study was conducted between July and October 2019 in the North of Peru. We focused on populations of coexisting \textit{Morpho} species present in the regional park of the Cordillera Escalera (San Martin Department) near the city of Tarapoto. Both the capture-recapture and the dummy experiment were performed at the exact same location, on the bank of the Shilcayo river (60°27′14.366″S, 76°20′45.852″W).

\textbf{DNA extraction and RAD-sequencing.} Thirty-one wild males caught on the study site were sequenced to perform population genomic analyses (\textit{M. achilles} – \(n = 13\), \textit{M. helenor} – \(n = 10\) and \textit{M. deidamia} – \(n = 8\)). DNA was extracted from each sample from a slice of the thorax, using Qiagen kit DNeasy Blood & Tissue. DNA quantification (using the microfluorimetric method) and quality controls (using electrophoresis and spectrophotometric method) were performed prior to sequencing. RAD-library preparation and sequencing were performed at the MGX-Montpellier GenomiX platform (Montpellier, France). DNA was digested with the Pst1 enzyme and the library was prepared according to Baird and Etter\textsuperscript{\textregistered} ow cell of an Illumina HiSeq2500 in a rapid mode so that reads (125 bp) tend to the data, but it would be surprising to detect a strong support for the model assuming a lack of recent gene flow, if the most recent secondary contact of such cyclicity induced elevated gene flow.

On each sampled, 50,000 simulations using random permutations of parameters were performed. Parameters were drawn from uniform prior distributions. Population sizes were sampled from the uniform prior \([0–1,000,000]\) (in diploid individuals); the older time of split was sampled from the uniform prior \([0–8,000,000]\) (generations); ages of the subsequent demographic events were sampled in a uniform prior between 0 and the sampled time of split. Migration rates were sampled from the uniform prior \([0–50]\). Both migration rates and effective population sizes are allowed to vary throughout the genomes as a result of linked selection, following refs.\textsuperscript{53}.

On each simulated dataset, we calculated a vector of means and standard deviations for different summary statistics: intraspecific statistics (\(\pi\) for \textit{M. helenor}, \(\pi\) for \textit{M. achilles}, \(\pi\) for \textit{M. deidamia}, \(\theta_y\) for \textit{M. helenor}, \(\theta_y\) for \textit{M. deidamia}, Tajima’s \(D\) for \textit{M. helenor}, \(D\) for \textit{M. deidamiapillars} and interspecific statistics (gross divergence, net divergence and \(F_{ST}\) for all three possible pairs; ABBA-BABA D). Our version of DILS includes part of the DaDi\textsuperscript{19} and Moments\textsuperscript{27} strategy involving the identification of the best model proposed by demographic model from the molecular patterns of polymorphism and divergence (proportion of shared polymorphisms, fixed differences between species, exclusive polymorphisms, etc.), excluding monomorphic loci. Thus, only loci containing at least one SNP in an alignment of the three species studied are considered, including singletons. Importantly, each locus carrying at least one SNP in a triotypic alignment is simulated with a mutation rate of 1.0 × 10\textsuperscript{−9} mutations per generation and per base pair to convert demographic parameters into demographic units from coalescent lineages.

We first conditioned the mutations occurring during coalescent simulations by using Ne \(= 1,000\) (i.e. \(\lambda\) is the effective size, \(N_e\) is the population size, \(r\) is the mutation rate per nucleotide and per generation; \(\lambda\), the length of locus \(I\) ). The number of simulated segregating sites for a given locus strongly depends on the coalescent history (i.e the total length of the simulated coalescent tree), occasionally generating monomorphic loci. To confirm that the inferences are not impacted by differences in the number of monomorphic loci in the simulated datasets, we then used an alternative simulation approach, by randomly placing in simulated coalescent trees a fixed number of mutations corresponding to the observed number of SNPs for each locus. Thus, a randomly simulated dataset consists of 2740 loci whose lengths (ranging from 339 to 894 nucleotides) and number of
SNPs (ranging from 1 to 91) individually match the properties of the observed loci in the actual dataset. Since the results drawn from both approaches were similar, we report only the results provided by the simulations based on the actual number of SNPs. Comparisons between the two approaches can be found in Supplementary Tables 8, 9.

Statistical comparisons between simulated and observed statistics were performed using the R package abfcr version 1.8.1.98

Mark-recapture experiment. To estimate the timing of patrolling activity among *Morpho* species, we performed capture-mark-recapture between 9 a.m. and 2 p.m. (flight period) on a daily basis in order to avoid overheating at high ambient temperature (above 30°C) during the middle of the day. Although on a few days, capture was cancelled because of bad weather annihilating butterfly activity, the 17 capture sessions were mostly consecutive, as they were performed in a 22 days period (Supplementary Table 1 and Supplementary Fig. 15). All butterflies were captured with hand nets, identified at the species level, and numbered on their dorsal wing using a black marker. The exact time of each capture was annotated. Butterflies captured while inactive, such as those laying on a branch or on the ground were excluded from the analysis to focus exclusively on actively patrolling individuals. We measured patrolling time for a total of 295 occasions, including 78 recaptures (i.e. 217 individuals were processed using a linear Kalman filter65, providing smoothed temporal dynamics of spatial position, velocity and acceleration of the body centroid. Based on these data, we investigated how spatial position, speed and acceleration of the visitor butterfly varied over the course of the interaction. We proceeded by dividing space into 10 cm spherical intervals around the dummy position ranging from 0 to 1.2 m powering the dummy. Differences of distance between the butterfly and the dummy were assessed by testing the effect of species on time of capture using a mixed-effects model, potentially affecting the butter results (Supplementary Fig. 13). After the 17 nearly-consecutive days of capture, one day of capture was repeated every 2 weeks during 2 months in parallel to the dummy experiment (described below), to verify that temporal activity was stable over time (Supplementary Fig. 13).

Estimating population size from mark-recapture data. Based on capture-recapture histories, we estimated individual abundance for each species using a loglinear model implemented in the R package Rcapture version 1.4.3.60 (Supplementary Fig. 15). Given the short duration the sampling period (22 days) relative to the longevity of adult *Morpho* butterflies (several months61), we used a closed-population model with no effect of births, deaths, immigration or emigration. Abundance was estimated in *Morpho helenor* and *M. achilles* only, as capture and recapture events were too few in the other species (*M. didamia* and *M. menelaus*) to allow estimating population size (Supplementary Table 1).

Experiment with dummy butterflies. We investigated the response of patrolling males to sympatric conspecifics, congeners and of exotic conspecifics, using butterflies placed on their flight path. Butterflies were built with real wings dissected and were processed with hexane to remove volatile compounds and cuticular hydrocarbons, ensuring to test only the visual aspect of the butterflies. We mounted the wings on a solar-powered flying device (Butterfly Solar Héliobil R029br) that mimics a flying butterfly, thereby increasing the attractiveness of the dummy. The dummy butterfly was positioned on the riverbank, and placed at the centre of a 1 m² space delimited with four vertical sticks (Fig. 1a). The dummy was continuously monitored by a human observer and filmed using a camera (Gopro Hero5 Black set at 120 images per second) mounted on a tripod. Patrolling *Morpho* butterflies that deviated from their flight path to approach the dummy but did not enter the cubic space were categorised as approaching. Any *Morpho* butterfly entering the cubic space was considered as interacting with the dummy. Those butterflies visiting (real) butterflies and cuticular hydrocarbons, ensuring to test only the visual aspect of the butterflies. We mounted the wings on a solar-powered flying device (Butterfly Solar Héliobil R029br) that mimics a flying butterfly, thereby increasing the attractiveness of the dummy. The dummy butterfly was positioned on the riverbank, and placed at the centre of a 1 m² space delimited with four vertical sticks (Fig. 1a). The dummy was continuously monitored by a human observer and filmed using a camera (Gopro Hero5 Black set at 120 images per second) mounted on a tripod. Patrolling *Morpho* butterflies that deviated from their flight path to approach the dummy but did not enter the cubic space were categorised as approaching. Any *Morpho* butterfly entering the cubic space was considered as interacting with the dummy. Those butterflies visiting (real) butterflies and cuticular hydrocarbons, ensuring to test only the visual aspect of the butterflies. We mounted the wings on a solar-powered flying device (Butterfly Solar Héliobil R029br) that mimics a flying butterfly, thereby increasing the attractiveness of the dummy. The dummy butterfly was positioned on the riverbank, and placed at the centre of a 1 m² space delimited with four vertical sticks (Fig. 1a). The dummy was continuously monitored by a human observer and filmed using a camera (Gopro Hero5 Black set at 120 images per second) mounted on a tripod. Patrolling *Morpho* butterflies that deviated from their flight path to approach the dummy but did not enter the cubic space were categorised as approaching. Any *Morpho* butterfly entering the cubic space was considered as interacting with the dummy. Those butterflies visiting (real) butterflies and cuticular hydrocarbons, ensuring to test only the visual aspect of the butterflies. We mounted the wings on a solar-powered flying device (Butterfly Solar Héliobil R029br) that mimics a flying butterfly, thereby increasing the attractiveness of the dummy. The dummy butterfly was positioned on the riverbank, and placed at the centre of a 1 m² space delimited with four vertical sticks (Fig. 1a). The dummy was continuously monitored by a human observer and filmed using a camera (Gopro Hero5 Black set at 120 images per second) mounted on a tripod. Patrolling *Morpho* butterflies that deviated from their flight path to approach the dummy but did not enter the cubic space were categorised as approaching. Any *Morpho* butterfly entering the cubic space was considered as interacting with the dummy. Those butterflies visiting (real) butterflies and cuticular hydrocarbons, ensuring to test only the visual aspect of the butterflies. We mounted the wings on a solar-powered flying device (Butterfly Solar Héliobil R029br) that mimics a flying butterfly, thereby increasing the attractiveness of the dummy. The dummy butterfly was positioned on the riverbank, and placed at the centre of a 1 m² space delimited with four vertical sticks (Fig. 1a). The dummy was continuously monitored by a human observer and filmed using a camera (Gopro Hero5 Black set at 120 images per second) mounted on a tripod. Patrolling *Morpho* butterflies that deviated from their flight path to approach the dummy but did not enter the cubic space were categorised as approaching. Any *Morpho* butterfly entering the cubic space was considered as interacting with the dummy. Those
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Author contributions

C.L.R., V.L. and V.D. designed the research plan and performed the field experiments. H.P. performed the RAD-library preparation and sequencing. E.A. and H.B. aligned the RAD-sequencing data. C.R. and E.A. performed the demographic inferences. C.L.R., V.L. and V.D. wrote the paper. All authors edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Camille Le Roy.

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