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1 **Convergence in sympatry: evolution of blue-banded wing pattern in *Morpho* butterflies**

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15 **Keywords:** Evolutionary convergence, escape mimicry, wing colour pattern, phenotypic

16 distance, Lepidoptera

17

18 **Abstract:**

19 Species interactions such as mimicry can promote trait convergence but disentangling this
20 effect from those of shared ecology, evolutionary history and niche conservatism is often
21 challenging. Here by focusing on wing color pattern variation within and between three
22 butterfly species living in sympatry in a large proportion of their range, we tested the effect of
23 species interactions on trait diversification. These butterflies display a conspicuous iridescent
24 blue coloration on the dorsal side of their wings and a cryptic brownish colour on the ventral
25 side. Combined with an erratic and fast flight, these color patterns increase the difficulty of
26 capture by predators and contribute to the high escape abilities of these butterflies. We
27 hypothesize that, beyond their direct contribution to predator escape, these wing patterns can
28 be used as signals of escape abilities by predators, resulting in positive frequency-dependent
29 selection favouring convergence in wing pattern in sympatry. To test this hypothesis, we
30 quantified dorsal wing pattern variations of 723 butterflies from the three species sampled
31 throughout their distribution, including sympatric and allopatric situations and compared the
32 phenotypic distances between species, sex and localities. We detected a significant effect of
33 localities on colour pattern, and higher inter-specific resemblance in sympatry as compared to
34 allopatry, consistent with the hypothesis of local convergence of wing patterns. Our results
35 provide support to the existence of escape mimicry in the wild and stress the importance of
36 estimating trait variation within species to understand trait variation between species, and to a
37 larger extent, trait diversification at the macro-evolutionary scale.

38

39

40 **Introduction**

41 Understanding the evolutionary forces driving trait diversification between species is
42 challenging, as it results from a combination of contingent historical events, neutral
43 divergence and adaptive evolution. Disentangling the effect of neutral divergence from the
44 effect of selective forces that could be either shared or contrasted between species is a major
45 question for evolutionary biologists. Selective forces shared by closely-related species living
46 in similar ecological niches can limit divergence in adaptive traits, resulting in niche
47 conservatism along phylogenies (Wiens *et al.* 2010). On the contrary, when closely-related
48 species live in sympatry, trait divergence can be promoted by selection caused by species
49 interactions, including competition for resources (Schluter 2000) or reproductive interference
50 (Gröning & Hochkirch 2008). Documenting the relative importance of niche conservatism
51 among closely-related species *vs.* character displacement is essential to comprehend trait
52 diversification between species, and to estimate how much species interactions shape macro-
53 evolutionary patterns of trait variation.

54 Closely-related species partly living in sympatry offer a great opportunity to investigate the
55 effects of species interactions on the evolution of their traits: in geographic areas where
56 several closely-related species live in sympatry, the evolution of traits within a species can be
57 influenced by the evolution of traits in sympatric species, while the selective forces generated
58 by species interaction are no longer acting in allopatry. The role of species interactions in trait
59 divergence in sympatry has been well-documented for mating cues and preferences, as for
60 instance in the flycatcher bird *Fiducela hypoleuca* where plumage coloration is divergent
61 from the ancestral dark coloration in a population where the dark sister-species *F. albicollis*
62 lives in sympatry, as the result of selection against hybrids (Strae *et al.* 1997). Other
63 antagonistic interactions such as resources competition have also been reported to drive
64 character displacement in sympatry, as illustrated by the change in beak size in island
65 population of the Darwin finch *Geospiza fortis* following the arrival of the competitor species
66 *G. magnirostris* (Grant & Grant 2006). While the effects of antagonistic interactions on trait
67 divergence in sympatric species have been well-documented, those of mutualistic interactions
68 remain scarcely studied. Evidences from a few obligate mutualisms such as fig-waps
69 pollination (Jousselin *et al.* 2003) or acacia-ants protection (Ward & Branstetter 2017) have
70 nevertheless highlighted that positive interactions can drive repeated evolution in traits
71 involved in the interaction, such as ostiole shape in figs or aggressive behavior in ants.
72 Nevertheless, the obligate mutualism implies full sympatry between the two partners,
73 preventing within species comparisons of traits variations in absence of the mutualistic

74 partners. A striking example of non-obligate mutualism driving trait convergence between
75 sympatric species is Müllerian mimicry, whereby individuals from different chemically-
76 defended species display similar warning color patterns (Müller 1879). This evolutionary
77 convergence is driven by the predators learning the association between warning coloration
78 and distastefulness, resulting in mutualistic relationships between sympatric species (Sherratt
79 2008). Mimetic interactions strongly depend on the local communities of defended species,
80 resulting in spatial variations in mutualistic interactions and geographical variations in
81 warning coloration (Sherratt 2006). In closely-related mimetic species, sharing a common
82 coloration in sympatry may nevertheless lead to reproductive interference, because warning
83 coloration is often used as a mating cue (Jiggins *et al.* 2001), and mimicry between species
84 may thus enhance heterospecific sexual interactions. The costs generated by reproductive
85 interference may thus limit convergence in warning colorations among closely-related
86 species. The evolution of warning coloration could then be influenced by the relative
87 abundance of sympatric species, modulating the positive effect of mimicry and the negative
88 effect of reproductive interference on convergence in warning coloration.

89 Here we focus on three closely-related Neotropical butterfly species, namely *Morpho helenor*
90 (Cramer, 1776), *M. achilles* (Linnaeus, 1758), and *M. deidamia* (Hübner, 1819), that exhibit
91 substantial variation in dorsal wing colour patterns both within and among species, and whose
92 large distribution ranges comprise situations of allopatry and sympatry (Blandin 2007). These
93 three closely-related species are found both in sympatry and allopatry, and therefore offer a
94 relevant framework to investigate the consequences of sympatry on phenotypic evolution,
95 both within and among species, generated by ecological interactions (*i.e.* competition and/or
96 mimicry). Bright coloration of wings is often associated with protection against predators
97 either through chemical defenses or resemblance to chemically-defended species (Briolat *et*
98 *al.* 2018). Chemical defenses have not been reported in the three *Morpho* species studied and
99 their caterpillars all feed on non-toxic, Fabacea hostplants (Blandin *et al.* 2014). Nevertheless,
100 their iridescent blue band against a black background of the dorsal side of the wings is very
101 conspicuous and strikingly contrasts with the cryptic colour pattern displayed by the ventral
102 side (Debat *et al.* 2018; Debat *et al.* 2020). The flap-gliding flight behavior observed in these
103 species (Le Roy *et al.* 2019) generates alternative phases of (1) flashes of light when wings
104 are open and (2) vanishing when wings are closed. Associated with fast and erratic flight
105 trajectories, these contrasted dorsal and ventral wing colour patterns make these butterflies
106 difficult to locate and catch by humans and birds (Young 1971; Pinheiro 1996; Murali 2018).
107 Wing colour patterns may thus induce predator confusion, enhancing escape capacities

108 (Pinheiro *et al.* 2016). It was also suggested that such colour pattern might in turn act as a
109 signal of such high escape capacities, further limiting predation attempts: Pinheiro & Campos
110 (2019) observed that *M. achilles* and *M. helenor* butterflies were indeed frequently sight-
111 rejected by wild jacamars. Since wild jacamars are important butterfly predators occurring in
112 rainforests where these *Morpho* species are found, we hypothesize that these predators could
113 already have associated their wing patterns with high escape abilities. Behavioural
114 experiments in controlled conditions have also shown empirically that predators learn to
115 refrain their attacks towards preys displaying conspicuous coloration similar to that of
116 previously missed preys (Gibson 1980). As in chemical-defense, escape ability can thus be
117 associated with coloration by predators. Mimicking such a signal displayed by a prey with
118 high escape abilities could thus provide protection against predators. Individuals sharing a
119 locally abundant coloration associated with high escape capacities might thus benefit from
120 increased protection against predators in the wild, favouring the persistence of similar colour
121 pattern in sympatric species, as observed in chemically protected species (Müller 1879). Such
122 ‘escape mimicry’ has been hypothesized in *Morpho* but never formally tested (Pinheiro *et al.*
123 2016). The three *Morpho* species studied here display geographic variation in colour patterns
124 within species and their ranges largely overlap, *M. helenor* showing a more expanded range in
125 both central America and the Atlantic Forest region, as compared to the other two species
126 (Blandin & Purser 2013). *M. helenor* and *M. achilles* are sister species whereas *M. deidamia*
127 belongs to a more divergent clade (Chazot *et al.* 2016) but nevertheless displays similar
128 variation in dorsal color pattern. This situation allows comparing intra and inter-specific
129 variations and testing the effect of sympatry on the evolution of traits across those closely
130 related species. When these species occur in sympatry, they share the same micro-habitat
131 (DeVries *et al.* 2010) and thus probably face similar communities of predators, enabling the
132 evolution of mutualistic species interaction. In this study we tested whether colour pattern
133 variations across geographical areas observed in these three species are consistent with the
134 hypothesis that local selection exerted by these shared predators promotes the evolution of
135 convergent wing colour patterns, possibly *via* escape mimicry.

136 Based on the collection of *Morpho* held at the National Museum of Natural History in Paris
137 (France), we finely quantified dorsal wing colour pattern of 723 specimens sampled
138 throughout the whole distribution of the three species. We then tested the effect of sympatry
139 on colour pattern variation within and between species. In the absence of geographic variation
140 in natural selection acting on colour pattern, we do not expect any local increase in
141 resemblance among species within localities. Contrastingly, if escape mimicry promotes local

142 convergence in colour pattern, the geographic variation should be parallel in the three species.
143 First, we investigated colour pattern similarity between pairs of the three species in the
144 localities where the three species coexist (13 *sympatric* zones, see fig.1): we compared
145 phenotypic distances between species within and between these zones, expecting lower
146 phenotypic distances between pairs of species within locality as compared to among localities.
147 Under the hypothesis of convergent evolution of wing colour patterns in sympatric species,
148 the level of intra-specific phenotypic variation within sampling zones should be similar in the
149 three sympatric species. This is expected as a result of positive frequency-dependent selection
150 favouring similar phenotypes in the three sympatric species. Furthermore, for each species,
151 the protection gained by each phenotype depends on the range of phenotypes encountered by
152 predators, which directly depends on the variation within the other two mimetic species.
153 Significant correlations in the level of intra-specific variation in colour pattern within
154 localities would thus be consistent with the hypothesis of convergent evolution of wing colour
155 pattern in sympatry. Then, because in some geographic regions, *M. helenor* is not sympatric
156 with *M. achilles* and *M. deidamia* (9 *allopatric* zones, see fig.1), we specifically compared
157 colour pattern variation within *M. helenor* populations where the other two species co-occur
158 (*i.e.* in *sympatric* zones) to the variation observed in *M. helenor* populations where the other
159 two species are absent (*i.e.* in *allopatric* zones) (fig.1). Under the convergence hypothesis, we
160 predicted that the phenotypic variance within *M. helenor* sympatric populations should be
161 reduced, because of the constraining effects of the selection imposed by the other two species.

162

163 **Material and Methods**

164 *Sampling zones and specimens*

165 The genus *Morpho* is distributed through three biogeographical regions: the Atlantic Forest
166 region, the *cis*-Andean region (the Amazon and Orinoco basins, and the Guiana shield), and
167 the *trans*-Andean region (central and western Colombia, western Ecuador and north-western
168 Peru, Panama Isthmus and Central America) (Blandin 2007; Blandin & Purser 2013). *M.*
169 *helenor* is the only species covering the whole geographic range of the genus, from northern
170 Argentina to a large part of Mexico. It is also the most diverse species, with more than 40
171 described subspecies (Blandin 2007). In contrast, *M. achilles* and *M. deidamia* exist only in
172 the *cis*-Andean region. These two species are always sympatric to each other, and to *M.*
173 *helenor* at the understory level in rainforests, from sea level (in the Orinoco delta) to more
174 than 1000 m in Andean slopes. The three species are generally abundant and coexist in
175 virtually the same ecological conditions and at the same periods of the year. However, *M.*

176 *helenor* exists alone in dryer contexts (Blandin 2007; Neild 2008), notably in the middle
177 Marañon valley (Peru) and in eastern Venezuela (PM and EP sampling zones respectively, see
178 fig.1). The sister species *M. helenor* and *M. achilles* (Chazot et al. 2016) are morphologically
179 very similar, yet they can be readily distinguished by details of the ventral ornamentation (Le
180 Moulton & Réal, 1962 ; Blandin, 2007, and see supplementary material S1). *Morpho deidamia*
181 is more divergent, with a much more complex ornamentation of its ventral side
182 (supplementary material S1).

183 We used the collections of the National Natural History Museum of Paris to study the
184 variation of colour pattern in these three species throughout their geographical range. These
185 collections cover the whole geographical range of the genus, and include large series of
186 specimens of *M. helenor*, *M. achilles*, and *M. deidamia*, from many different localities. In
187 order to study the variation of colour pattern in the three species throughout their geographical
188 ranges, we selected sampling zones based on the distribution of subspecies of *M. helenor*,
189 these subspecies being defined according to colour pattern differences (Le Moulton & Réal,
190 1962; Blandin, 2007; Neild, 2008). We chose 17 *M. helenor* subspecies, distributed from
191 northern Argentina to Central America. Some of these subspecies occupy vast regions, of
192 which limits are generally not precisely known. The collected specimens, from various
193 localities and different times by different collectors, nevertheless present constant diagnostic
194 characters allowing clear assignation. Other subspecies are much less widespread, sometimes
195 for ecological reasons, as for example *M. h. charapensis* Le Moulton & Réal, 1962, endemic of
196 the dry middle basin of the Marañon river in northern Peru (Blandin, 2007). In the *cis*-Andean
197 region, where the three species are sympatric, the subspecies *M. h. helenor*, *M. h. theodorus*,
198 and *M. h. coelestis* have very large geographical distributions (Blandin 2007). To capture the
199 putative geographic variation within these subspecies, we thus respectively sampled 2, 4, and
200 2 localities within their ranges. The total geographical sample was thus composed of 22 areas,
201 here designated as sampling zones: 13 *cis*-Andean where the three species are sympatric,
202 referred to as *sympatric zones*, and 9 areas where *M. helenor* occurs alone, referred to as
203 *allopatric zones* : 2 *cis*-Andean; 4 in the Atlantic Forest region, and 4 *trans*-Andean (fig. 1;
204 see S1 for comments on the sampling zones). We then selected specimens from the other two
205 species (*M. achilles* and *M. deidamia*) collected in these *sympatric* sampling zones. Assuming
206 that there is no local selection shared by the three species, the variations of colour patterns
207 within each of the three species should be independent. Significant resemblance between
208 species within locality would therefore point at an evolutionary convergence of colour pattern
209 driven by local selection or mimicry. We selected 723 specimens of *M. helenor* (n = 413), *M.*

210 *achilles* ($n = 156$) and *M. deidamia* ($n = 154$). We used both males ($n = 524$) and females
211 ($n = 199$; females are less numerous in collections because they are less frequently caught).
212 For each sampling zone, we selected specimens in the Museum collections, focusing on
213 intact, well-conserved individuals (see S1, table 1 for detailed numbers). By gathering a large
214 number of samples from different collectors and different time period, without the purpose of
215 testing geographical variations in colour pattern, Museum collections are likely to limit
216 potential effects of local or temporal fashion for a given species or collector bias. The three
217 species studied are very abundant and not particularly precious to collectors and were thus
218 kept in important numbers in the collections. Species from a given locality are often sampled
219 together in large numbers (most local collectors sample blue-banded *Morpho* without species
220 discrimination); we thus assume that it is unlikely to bias our convergence test.

221

222 *Quantifying colour pattern variation*

223 Pictures of collection specimens were taken in controlled standard white light conditions. The
224 four wings were first manually separated using Adobe Photoshop Element. Wing images were
225 then analysed following the Colour Pattern Modelling approach (Le Poul *et al.* 2014)
226 implemented in Matlab. This method allows precise comparison of colour pattern while
227 accounting for wing shape and venation that might differ between species. It has been shown
228 to be especially relevant to quantify similarity and differences in color pattern within and
229 across species (Le Poul *et al.* 2014; Huber *et al.* 2015; McClure *et al.* 2019). Briefly, the
230 algorithm detects the four wings on the white background and segments the colour pattern in
231 different categories based on pixel densities of the RGB values. The number of colours is then
232 set manually: here we chose to consider three colours, namely black, blue and white. Some
233 individuals (as for instance *M. deidamia* samples from French Guiana), display a gradient of
234 blue (see sup. Fig. 1) that is often detected as a different colour category by CPM. Dark blue
235 was nevertheless treated as blue in our analyses. This is probably a conservative assumption
236 regarding convergence in colour patterns, because the dark blue area of *M. deidamia* has a
237 similar location to the basal black area in *M. helenor* and *M. achilles*, and look very dark from
238 far-distance. After segmentation, wings were aligned by adjusting translation, rotation and
239 scale in order to maximize similarity, allowing the colour value for each pixel of the wings to
240 be compared.

241

242 *Testing phenotypic convergence between species when living in sympatry*

243 We first run a PCA based on colour values for each pixel on the four wings, creating a

244 morphospace where individuals located close to each other have a similar colour pattern. A
245 MANOVA on pixel values observed on the 723 individuals from the three species was
246 performed to test the effects of species, sex and sampling zone, as well as the interactions
247 between all these variables.

248 Under the hypothesis of convergent evolution of wing colour patterns in sympatric species,
249 the levels of intra-specific phenotypic variation within sampling zones should be similar in the
250 three sympatric species: this is expected as a result of positive frequency-dependent selection
251 favouring similar phenotypes in the three sympatric species, but also because for each species,
252 the protection gained by each phenotype depends on the range of phenotypes encountered by
253 predators, which directly depends on the variation within the other two mimetic species. We
254 used the trace of the within species covariance matrix based on the PCA axes as an estimate of
255 the level of phenotypic variation within species within each sampling zone. We then
256 computed the Pearson correlations between phenotypic variances observed in pairs of species
257 across the 13 sampling zones where the three species co-occur. Significant correlations would
258 be consistent with the hypothesis of convergent evolution of wing colour pattern in sympatry.

259
260 The phenotypic resemblance between species in sympatry was estimated by computing the
261 average Euclidian distance between species within and across sampling zones in the PCA
262 space (using the 15 first PCA axes, each explaining more than 0.5% of the total variance). To
263 test whether species were more similar within a sampling zone than expected by chance, we
264 generated a null distribution of distances between pairs of species by permuting the sampling
265 zones within each species independently, so that the sympatry/allopatry relationships between
266 inter-specific pairs of populations were randomized. We performed 10,000 simulations and
267 computed within each simulation the average phenotypic distance between the three species
268 pairs within sampling zones. This allowed generating for each pair of species, an estimated
269 distribution of interspecific phenotypic distances within sampling zone under the null model
270 assuming independent geographic variation in colour pattern within each species. We then
271 assessed significance by counting the proportion of inter-specific distances under this null
272 model that exhibited a lower value than the observed Euclidian distances between species
273 within sampling zone. The observed interspecific distance was considered significantly
274 smaller within sampling zone when the observed value was lower than 95% of the resampled
275 values.

276

277 *Testing the effect of sympatry on wing pattern in M. helenor*

278 Because *M. helenor* distribution range exceeds that of the two other species, it can be found in
279 isolation in a significant proportion of its distribution (notably in Central America and in the
280 East Coast of Brazil – see fig. 1). This situation allowed us to test the effect of sympatry on *M.*
281 *helenor* colour pattern. We thus contrasted sampling zones where it co-occurs with the other
282 two species (*i.e.* sympatric populations) with sampling zones where it occurs alone (*i.e.*
283 allopatric populations). This effect of sympatry *vs.* allopatry on colour pattern was first
284 explored in a PCA and then tested using a MANOVA, controlling for the effect of sex and
285 sampling zone.

286 Under the convergence hypothesis, we also predicted that the phenotypic variance within *M.*
287 *helenor* populations should be reduced when they are in sympatry with the two other species,
288 because of the constraining effects of the selection imposed by other two species. We thus
289 compared the level of phenotypic variation in *M. helenor* between sympatric and allopatric
290 sampling zones. We thus computed the phenotypic variance within each *M. helenor*
291 population using the trace of the covariance matrix of PCA coordinates and test whether the
292 variances observed in the 13 sympatric groups of populations had smaller values than those
293 observed in the 9 allopatric groups of populations using a simple *t*-test.

294 All analyses were performed using the software R (R Core Development Team 2005).

295

296 **Results**

297 *Geographic variation of wing colour pattern across the three species*

298 The PCA based on colour variation in wing pixels shows that individuals sampled within a
299 sampling zone tend to display a similar wing colour pattern (fig. 2). This effect was confirmed
300 by the MANOVA: We detected a strong and significant effect of sampling zone
301 (*Pillai* = 13.21, *F* = 2.87, *df* = 81, *P* < 0.001), species (*Pillai* = 1.27, *F* = 19.94, *df* = 2,
302 *P* < 0.001) and sex (*Pillai* = 0.80, *F* = 46.51, *df* = 1, *P* < 0.001), as well as significant
303 interactions between species and sex (*Pillai* = 0.67, *F* = 5.84, *df* = 2, *P* < 0.001), species and
304 sampling zone (*Pillai* = 4.74, *F* = 1.86, *df* = 36, *P* < 0.001), as well as sex and sampling zone
305 (*Pillai* = 5.72, *F* = 2.06, *df* = 40, *P* < 0.001). The observed geographic variation is thus
306 consistent with a greater resemblance between individuals within a sampling zone as
307 compared to individuals sampled in different sampling zones, modulated by some sexual
308 differences and variations between species.

309 In the 13 localities where the three species co-occur, the phenotypic variance within species
310 was highly correlated between *M. helenor* and *M. achilles* (Pearson correlation: *cor* = 0.83,
311 *P* = 0.0015) and *M. helenor* and *M. deidamia* (Pearson correlation: *cor* = 0.83, *P* = 0.0012),

312 and moderately correlated between *M. deidamia* and *M. achilles* (Pearson correlation:
313 $cor = 0.66$, $P = 0.028$). This suggests that within sampling zones, the level of within species
314 phenotypic variation is similar across species. This could stem from a sample bias among
315 sampling zones, but is also consistent with the convergence hypothesis, whereby phenotypic
316 variation within a species indirectly impacts the phenotypic variation in sympatric species by
317 modifying the selective pressure.

318

319 *Convergence of colour patterns between species within sampling zones*

320 To investigate the hypothesis of local convergence among the three species more directly, we
321 then specifically tested whether the mean Euclidian distance between species within sampling
322 zone was lower than expected when assuming an independent geographic differentiation in
323 colour pattern within each species. Considering the whole dataset (both sexes together and
324 including all sampling zones), the average phenotypic distances between each pair of species
325 within sampling zone were all significantly smaller than under the simulated null distribution
326 (fig. 4). These tests were also significant when excluding the sampling zones where *M.*
327 *helenor* occurs alone (see supplementary figure 1), and when carried out separately on males
328 and females (see supplementary figures 2 and 3 respectively), confirming the significant
329 convergence between species in sympatry. The observed inter-specific phenotypic distances in
330 sympatry were lower between *M. helenor* and *M. achilles* than between the other two pairs of
331 species, consistent with their closer phylogenetic distance (fig. 4). Despite this strong signal
332 of convergence of colour pattern in sympatry, some sampling zones departed from this general
333 trend, notably the zones situated in Venezuela (VT, VB and RA) and Bolivia (LP).

334

335 *Effect of sympatry on colour pattern variation in M. helenor*

336 We then compared the colour pattern of *M. helenor* from sampling zones where it co-occurs
337 with *M. deidamia* and *M. achilles* to that of sampling zones where it occurs alone, using a
338 PCA on sympatric and allopatric individuals of the three species ($n = 723$). Interestingly, some
339 allopatric populations of *M. helenor* (in particular populations located in the Atlantic coast of
340 Brazil, *i.e.* BJ and BRJ) show wing patterns that differ from individuals living in sympatry in
341 other geographical areas, as highlighted by their distributions in the wing colour pattern
342 morphospace (fig.3). Using a MANOVA on colour pattern variations in *M. helenor* only
343 ($n = 413$), a significant effect of sympatry was detected ($Pillai = 0.95$, $F = 155.77$, $df = 1$,
344 $P < 0.001$), controlling for the effect of sex ($Pillai = 0.71$, $F = 19.41$, $df = 1$, $P < 0.001$) and
345 sampling zone ($Pillai = 7.81$, $F = 5.66$, $df = 19$, $P < 0.001$).

346 The levels of phenotypic variation in *M. helenor* were also slightly higher in allopatric
347 populations (*mean variance* = 4766) as compared to sympatric populations (*mean*
348 *variance* = 2571) ($t = 1,997$, $df = 18.96$, $P = 0.06$). Although neutral divergence among these
349 geographically distant localities might contribute to the observed effects, these comparisons
350 between sympatry and allopatric populations of *M. helenor* are consistent with a substantial
351 effect of species interactions on the evolution of wing colour pattern in *M. helenor*.

352

353 **Discussion**

354 Ecological interactions between closely related species living in sympatry are often negative,
355 dominated by competition, usually leading either to divergent character displacement (Brown
356 & Wilson 1956; Grant 1972), or at the extreme, to competitive exclusion. In the best studied
357 cases of positive interspecific interactions, like butterfly Müllerian mimicry, sympatric
358 speciation is frequently associated with a shift in mimetic colour pattern (Mallet 2009) so that
359 sympatric species with convergent colour patterns are in most cases more distantly related
360 (Kozak *et al.* 2015). Here, we show convergence in wing colour pattern between the sister-
361 species *M. helenor* and *M. achilles*, despite their close relatedness. This suggests a strong
362 selective pressure driving this convergence, consistent with an effect of escape mimicry. This
363 striking phenotypic similarity in wing pattern between sympatric species likely induces some
364 degree of reproductive interference that might be balanced by a divergence in other traits like
365 pheromones or temporal niches that remains to be investigated. Our results therefore settle
366 these *Morpho* butterflies as new and insightful model allowing investigating the ecological
367 conditions promoting convergence in sympatry.

368 We also argue that escape mimicry would deserve more attention, as it constitutes a
369 potentially important driving force of the evolution of intra and interspecific variation under
370 predation pressure, with possibly large relevance to insect evolution (e.g. Guerra 2019) and
371 beyond (Baker & Parker 1979)(Götmark & Unger 1994).

372

373 *Estimating phenotypic variation based on Museum collections*

374 Our study of phenotypic variation within and among species was enabled by the rich *Morpho*
375 collection held in the Museum of Natural History in Paris, containing large number of
376 specimens collected throughout the whole geographic distribution of the three species studied
377 here (see S1 for more details). Nevertheless, estimations of phenotypic variation based on
378 museum collections can be biased: (1) females are generally under-sampled, because they are
379 less frequently encountered in the wild; (2) phenotypic variation can be overestimated,

380 because collectors tend to prefer specimens with unusual colour patterns. Concerning the first
381 bias, the sexual dimorphism in colour pattern is very limited in the three studied species (fig.
382 2B) and the signal of convergence observed was similar when considering males and females
383 separately, suggesting that the convergence observed is likely to occur similarly in both sexes.
384 Concerning the second bias, an overestimation of phenotypic variation is likely to decrease
385 the signal of convergence; therefore our approach based on collection specimens is probably
386 conservative relatively to phenotypic convergence.

387

388 *Local similarity of colour pattern between species*

389 By precisely quantifying colour pattern variation across a large sample of *Morpho* butterflies,
390 we detected a significantly increased resemblance between individuals from different species
391 living in sympatry as compared to allopatry. This convergence is stronger between the two
392 sister species *M. helenor* and *M. achilles* than for the more distantly related species *M.*
393 *deidamia*. The larger phylogenetic distance might involve stronger developmental constraints
394 limiting the convergent evolution in *M. deidamia*. This convergence trend is confirmed for a
395 majority of sampling zones located in the Amazonian rainforest. Despite the similar
396 ecological conditions encountered throughout the Amazonian basin, wing colour patterns
397 displayed by butterflies from different species are more similar within localities as compared
398 to across localities, suggesting that convergent evolution of colour patterns might be promoted
399 by local interactions among species.

400 In Bolivian and two Venezuelan sampling zones (LP and BC; RA and VT, see fig. 1), a large
401 diversity of colour pattern was observed within species. Although such a high level of
402 intraspecific variation is found in the three species (fig. 2C), it is likely responsible for the
403 non-significance of our test comparing inter-specific phenotypic distance within and among
404 sampling zones (fig. 4). However, the colour patterns displayed by all three species are rather
405 similar, and quite different from those observed in other geographic regions (fig. 2C). The
406 important variation within each species in these populations might thus still be consistent with
407 the convergence hypothesis.

408 Overall, (1) the greater resemblance between the three species within localities in the largest
409 part of their common range, together with (2) the divergence in colour pattern displayed by *M.*
410 *helenor* butterflies in localities where the other two species do not occur, point at a role of
411 species interactions in the evolution of dorsal wing pattern in the three species.

412

413 *Convergences shaped by escape mimicry*

414 How can we account for the similar variation among populations of the three *Morpho*
415 species? One hypothesis would be that similar environments result in shared selective
416 pressures acting locally on the three species, leading to the observed similarity of colour
417 patterns, independently from interactions occurring in sympatry. What local selective
418 pressures might be involved is however unclear. It has been shown that the evolution of
419 warning coloration can be influenced by selective forces independent from mimetic
420 interactions. For instance, the light environment may modify the conspicuousness of colour
421 patterns (Rojas *et al.* 2014) so that variation in light environment in different localities may
422 select for different wing colour patterns. However, the three *Morpho* species studied here
423 mostly fly in the understory, regardless of the geographic regions, suggesting that the different
424 populations may be evolving in similar light environment. Another hypothesis could stem
425 from the role of melanin in thermoregulation (e.g. in *Colias* butterflies, Ellers & Boggs 2004)
426 that may result in contrasted selective pressures in different geographic regions. The variation
427 in dorsal pattern observed among *Morpho* populations indeed mostly affects the proportion of
428 melanic patches on the wing, the populations of Surinam and French Guiana being the darkest
429 (SFG locality on fig. 1). Variation in melanic surface on butterfly wing has been related to
430 adaptation to cold environments in butterflies (e.g. in *Parnassius phoebus*, Guppy 1986).
431 However, populations of *M. helenor*, *M. achilles*, and *M. deidamia* with very reduced black
432 areas occur at sea level in the Orinoco delta, as well as around 700-800 m.a.s.l. in Bolivian
433 valleys. Moreover, the darkest specimens occur at low altitudes in French Guiana, while
434 populations with wider blue bands occur in some Peruvian valleys at more than 1000 m.a.s.l.
435 The extension of black areas in these *Morpho* species therefore does not seem to occur in
436 colder environments, making inconsistent the hypothesis of an effect of adaptation to
437 temperature on black colour pattern evolution.

438 Although we cannot rule out that an unidentified local selection might promote the evolution
439 of a similar colour pattern in the three species, the observed repeated local convergence is also
440 consistent with the escape mimicry hypothesis. In Müllerian mimetic species such as the
441 butterfly species *Heliconius melpomene* and *H. erato*, multiple geographic races with striking
442 colour pattern variations are maintained within species, with strong resemblance to races from
443 the other species (Jiggins 2017). These multiple locally convergent colour patterns are
444 maintained by positive frequency dependent selection due to increased protection of mimetic
445 colour patterns, reinforced by sexual preferences toward locally mimetic mates (Merrill *et al.*
446 2012). The geographic variations of these mimetic coloration then mainly stem from
447 stochastic processes, because the colour pattern may not necessarily provide a selective

448 advantage *per se*, but can be favored once it becomes frequent within a given locality where
449 predators learn to avoid it (Mallet 2010). These evolutionary forces documented to drive
450 variations in mimetic coloration in defended species might explain the multiple convergences
451 observed in the three *Morpho* species throughout their geographical range. While warning
452 colour can be physiologically linked with the presence of toxins (Blount *et al.* 2012), making
453 them an honest signal of defenses (Blount *et al.* 2009), specific colour pattern in species
454 involved in escape mimicry can also contribute to escaping capacities. By generating sporadic
455 flashes during flap-gliding flight, iridescent coloration confuses predators, therefore
456 increasing survival chance in case of attack (Murali 2018). *Morpho* iridescent patterns would
457 thus not only act as a signal that can be memorized by predators, but might also directly
458 increase their escaping capacities. The selective advantage related to the relative extent of the
459 iridescent blue and black bands on the wings is currently unknown. The local convergence
460 might be only driven by local positive frequency-dependent selection favouring the most
461 common phenotype – the most avoided by predators. By providing strong evidence for
462 multiple convergences in colour pattern between palatable *Morpho* species, our results are
463 thus shedding light on the poorly documented phenomenon of escape mimicry, whereby,
464 similarly to chemical defenses, the high escape capacities promote local convergence of
465 colour patterns. Escape mimicry, also referred to as evasive mimicry, has been described in
466 the XXth century as an evolutionary outcome of predation pressure in some butterflies (Van
467 Someren & Jackson 1959), as well as in other insects (Thompson 1973). Its contribution to
468 the evolution of colour pattern in different prey species is however largely unknown. The
469 quantitative demonstration presented here might stimulate new research on escape mimicry
470 and shed light on the joint evolution of colour pattern and behaviour shaped by predation.

471

472 *Mechanism of convergent evolution in closely-related species*

473 Because the three *Morpho* species studied here are closely related, their local resemblance is
474 probably facilitated by some common developmental bases of colour pattern variation,
475 explaining why convergence is weaker in the more distantly related species *M. deidamia*. The
476 convergence between *M. achilles* and *M. helenor* could be facilitated by ancestral standing
477 genetic variation affecting color pattern jointly inherited by the two species or by
478 introgression. Introgression is indeed recognized as an important mechanism favouring
479 convergence in sympatric species of *Heliconius* butterflies involved in Müllerian mimicry
480 (Dasmahapatra *et al.* 2012; Pardo-Diaz *et al.* 2012; Wallbank *et al.* 2016). The relative
481 contribution of ancestral variation *vs.* introgression in convergence between related species is

482 still debated but both mechanisms require strong selection for mimetic alleles to be retained in
483 genomes despite genetic drift. Interestingly, in the butterfly genus *Heliconius*, sister-species
484 living in sympatry usually display divergent colour patterns, and therefore belong to different
485 mimicry rings. Shift in mimicry is indeed invoked as an ecological factor promoting
486 speciation through both pre-zygotic isolation due to colour-pattern based mate choice, but also
487 post-zygotic isolation due to counter-selection exerted on non-mimetic hybrids by predators
488 (Merrill *et al.* 2012). The striking parallel evolution of colour pattern in the sister species of
489 *M. achilles* and *M. helenor* contrasts with this general trend, and questions the ecological
490 mechanisms involved in speciation and co-existence in sympatry of mimetic species.
491 Escape mimicry might explain the observed similarity between species within some localities.
492 The evolution of colour pattern in these *Morpho* species would then strongly depend on the
493 history of their sympatry in different geographic areas. When the different species are
494 ancestrally allopatric, the evolution of colour pattern would follow an advergence scenario:
495 the evolution of recently settled species wing patterns would be strongly constrained by that
496 of the locally already abundant species. Alternatively, parallel geographic diversification in
497 colour patterns might have occurred if these three species have been sympatric throughout
498 their evolution and expanded their geographical range simultaneously. The history of wing
499 pattern diversification in the three species thus strongly depends on their biogeographic
500 history and in particular, on the history of speciation in this clade. Because wing colour
501 patterns are frequently used in butterflies as a mating cue, the high resemblance between
502 closely-related species might lead to costly reproductive interference (i.e. interspecific
503 courtship or even mating). Such costs of reproductive interference are thus predicted to limit
504 the convergence triggered by mimicry: divergence in colour pattern would thus be favoured
505 during speciation or in case of secondary contacts (Lukhtanov *et al.* 2005). Because both
506 mimicry and reproductive interference depend on the relative species and phenotype
507 abundances, this study illustrates the importance of assessing phenotypic variation both within
508 and among species to understand phenotype and species diversification in sympatry at larger
509 evolutionary scale.

510

511 **Conclusions**

512 By extensively studying wing colour patterns in three closely-related *Morpho* butterflies
513 species throughout their geographic range, we detected significant resemblance among
514 species within some localities and parallel variations across localities. Those results are in line
515 with the escape mimicry hypothesis, which assumes that the similar high escape capacities of

516 these three species may promote mimicry in their dorsal colour pattern. Besides providing
517 evidence pointing at a major role of escape mimicry in the convergence in colour patterns in
518 *Morpho* our study more generally highlights the effect of sympatry on phenotypic evolution
519 across species, stressing the need to jointly consider intra and interspecific variations to
520 understand phenotypic and species diversification in sympatry.

521

522 **Acknowledgments**

523 All authors declare having no conflict of interest.

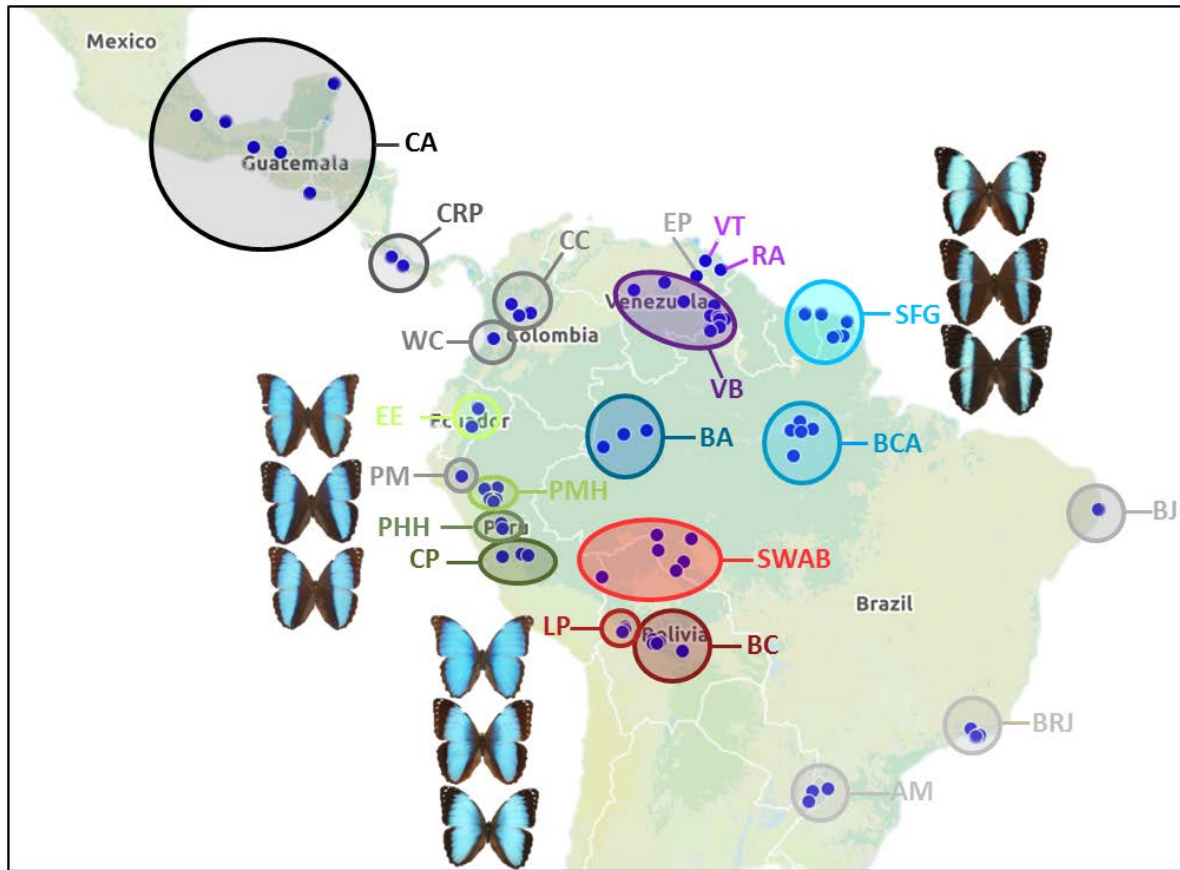
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525 **Data Availability Statement**

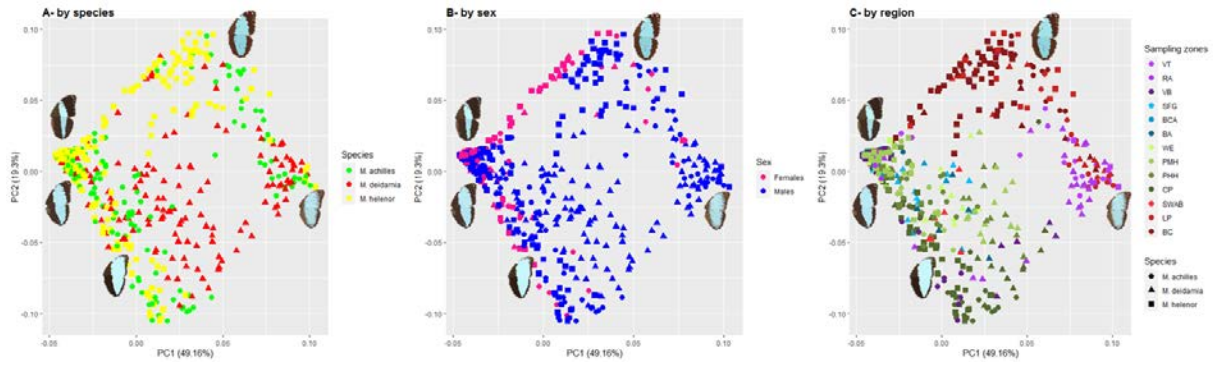
526 The PCA coordinates describing the dorsal wing colour pattern variations of each of the 723
527 *Morpho* butterflies used in this study, with information on their species, subspecies, sex and
528 sampling locality are provided on the dryad repository [doi:10.5061/dryad.6q573n5xb](https://doi.org/10.5061/dryad.6q573n5xb).

529

530 **Figure list:**



531 **Figure 1: Geographic location of specimens, showing the 22 defined sampling zones** (see
532 supplementary table 1 for detailed numbers of specimens per sampling zone). The 13
533 sympatric sampling zones where the three species (*M. achilles*, *M. deidamia* and *M. helenor*)
534 co-occur, and share the same micro-habitat, are shown with different colours (EE: Eastern
535 Ecuador, PMH: Peru Middle Huallaga, PHH: Peru High Huallaga, CP: Central Peru, SWAB:
536 South-Western Amazonian basin, LP: Bolivia La Paz, BC: Bolivia Cochabamba, BA: Brazil
537 low Amazon, BCA: Brazil Central Amazon, SFG: Surinam and French Guiana, VB:
538 Venezuela Bolivar, RA: Venezuela Rio Aguirre, VT: Venezuela Tucupita. The 9 allopatric
539 sampling zones where only *M. helenor* occurs are shown with grey levels (CA: Central
540 America, CRP: Costa Rica and Panama, WC: Western Colombia, CC: Central Colombia, EP:
541 Venezuela El Pao, PM: Peru Marañon, BJ: Brazil Joao Pessoa, BRJ: Brazil Rio de Janeiro,
542 AM: Argentina Misiones). Examples of butterflies from the three species - *M. deidamia* (top)
543 *M. helenor* (middle) and *M. achilles* (bottom) - sampled in the middle Huallaga in Peru (top
544 left triplet), Bolivia (bottom left triplet) and French Guiana (top right triplet) are shown.
545
546



547

548

Figure 2: Colour pattern variations among individuals living in sympatry, from different

549

species (A), sexes (B) and sampling zones (C), captured by the PCA based on pixels colour

550

variations analysed by CPM. Only sampling zones where the three species live in sympatry

551

are represented here (n = 557 individuals). To explicit phenotypic variations, male specimens

552

of *M. achilles* sampled in different sampling zones are shown closed to their location on the

553

morphospace. Symbols differ among species, with dots for *M. achilles* individuals, triangles

554

for *M. deidamia* and squares for *M. helenor*. The colours of symbols differ among species

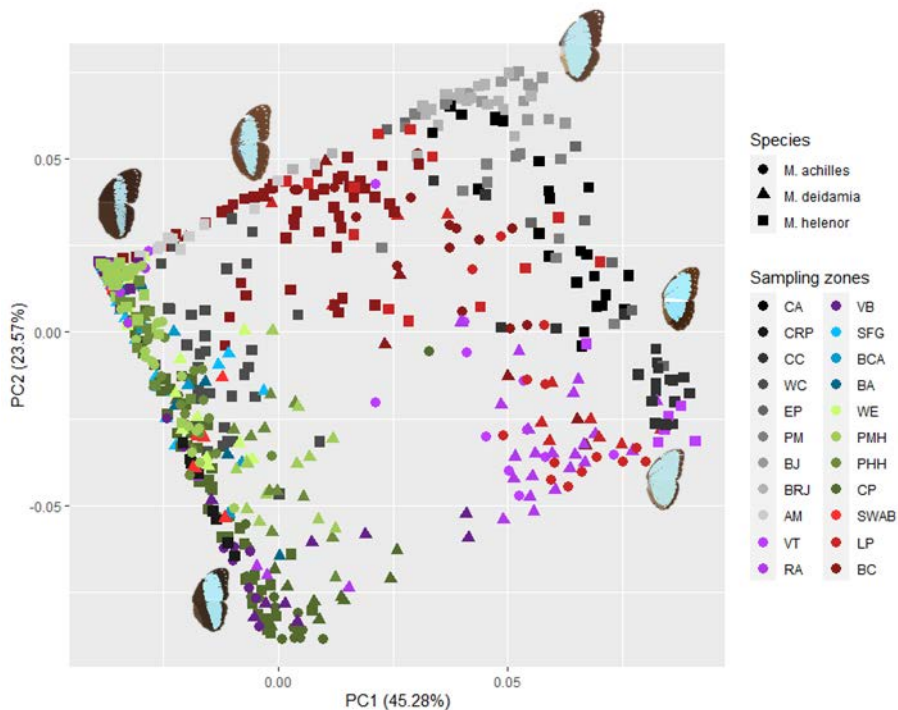
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(left plot A), sex (central plot B) and sampling areas (right plot, C). Note the colours of

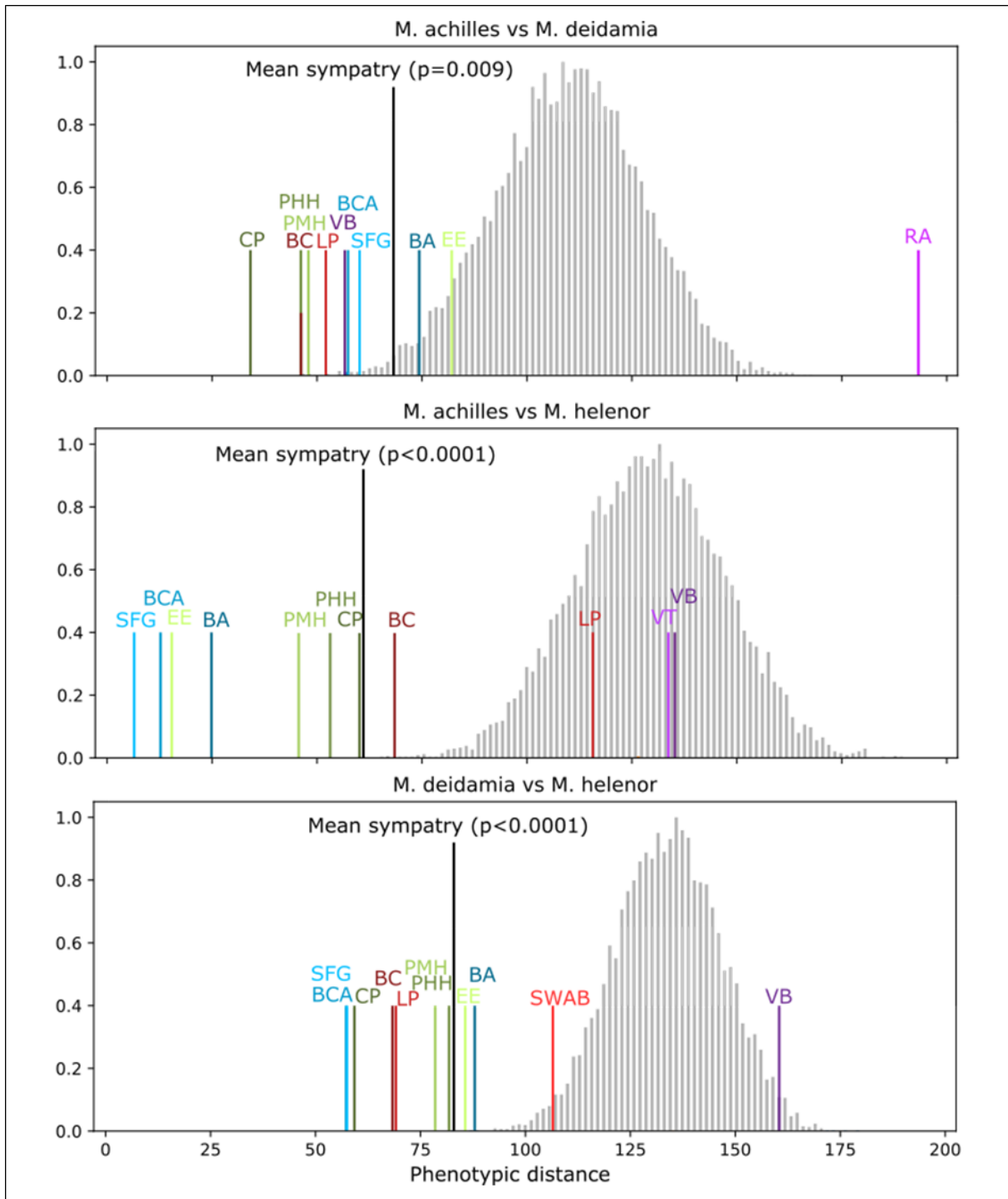
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sampling areas on plot C match the colour code used on the geographic map (fig.1).

557



558
 559 **Figure 3: Colour pattern variations among individuals in sampling zones where *M.***
 560 ***helenor* is in sympatry with the other two *Morpho* species (colored symbols) and in**
 561 **sampling zones where *M. helenor* does not co-occur with the other two species (grey-**
 562 **scale symbols), from the three different species represented by the first two axes of the PCA**
 563 **based on pixels colour variations analysed by CPM ($n = 723$). To explicit phenotypic**
 564 **variations, male specimens of *M. helenor* sampled in different sampling zones are shown**
 565 **closed to their location on the morphospace. Symbols differ among species, with dots for *M.***
 566 ***achilles* individuals, triangles for *M. deidamia* and squares for *M. helenor*. Note the colours of**
 567 **sampling zones match the colour code used on the geographic map (fig.1).**
 568



569

570 **Figure 4: Phenotypic distances between species in sampling zones where they are found**
 571 **in sympatry (colored bars) and predicted distribution obtained using 10,000 bootstraps,**
 572 **randomly reallocating the different sampling zones within each species (grey bars).** The
 573 black bar shows the mean phenotypic distance observed between pairs of species in the
 574 sympatric sampling zones; top plot: distances between *M. achilles* and *M. deidamia*, middle
 575 plot: distances between *M. helenor* and *M. achilles*, and bottom plot: distances between *M.*
 576 *deidamia* and *M. helenor*. The *p*-value is based on the number of simulations where the

577 phenotypic distance between species is higher than the mean value of inter-specific distances
578 observed in sympatry. Note the colours and codes of sampling zones match the colour code
579 used on the geographic map (fig.1).

580

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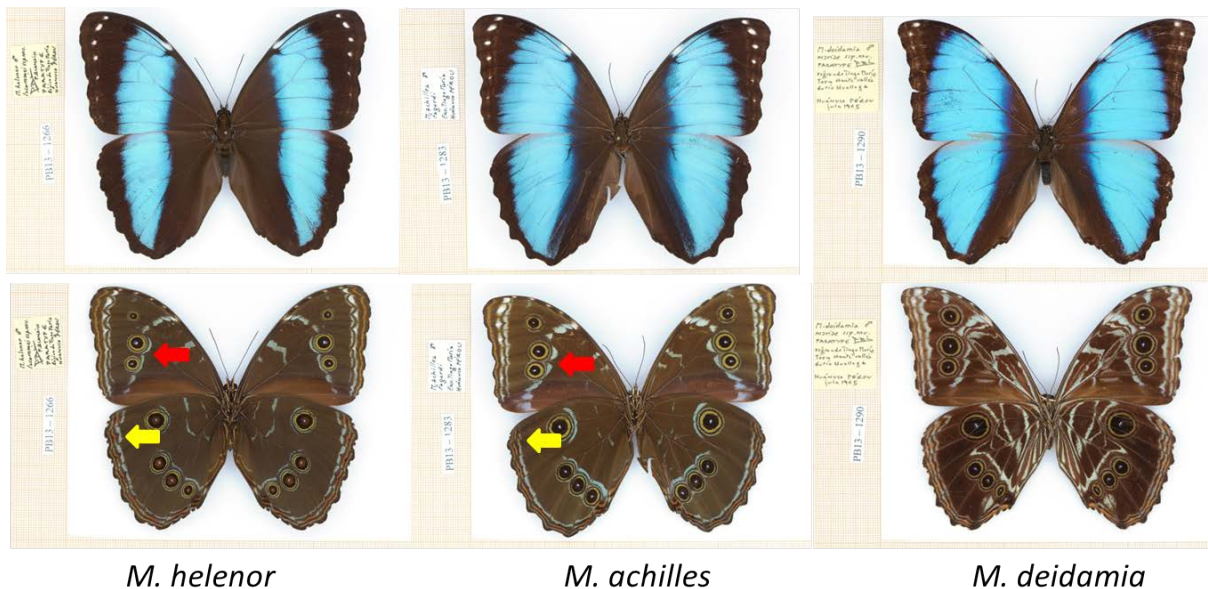
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Supplementary material

S1 Identification and selection of the specimens in the collection of the Muséum national d'Histoire naturelle (Paris)

Identification of *Morpho helenor*, *M. achilles*, and *M. deidamia*

Morpho helenor and *M. achilles* are very similar sister species. From their initial description, in the 18th Century, to the mid-20th Century, much confusion occurred, amplified by nomenclatural problems. Diagnostic characters were definitely identified by Le Moult & Réal (1962), and confirmed by Blandin (2007), in details of the ventral ornamentation (fig. 1). These characters allow unambiguous identification of specimens throughout the cis-Andean geographical range of the two species. *Morpho deidamia* belongs to the sister clade of the *M. helenor* clade (Chazot et al. 2016) ; it presents a very different ventral surface (fig. 1).



Supplementary fig. 1. Comparison of the dorsal (top row) and ventral (bottom row) ornamentalations of *Morpho helenor* (left-column), *M. achilles* (central column), and *M. deidamia* (right column). Arrows indicate the characters allowing to distinguish sympatric *M. helenor* and *M. achilles* specimens: the shape of the whitish mark behind the medium ocellus in forewings (more triangular in *M. achilles*) (red arrows), and the width of the anterior end of the submarginal whitish band (strongly reduced in *M. achilles*) (yellow arrows).

The *Morpho* collection of the Muséum national d'Histoire naturelle

Collections of major museums were built along decades – since the 19th or even the 18th Century, thanks to many collectors, donations or bequests from donors, and various research

programs. They provide the best geographical coverage for many taxa, even if regions of difficult access remained poorly or not explored by lepidopterists, even during the 20th Century. With around 9000 specimens, the *Morpho* collection of the Muséum of Paris is one of the world largest public collections. Notably for common species, series from different localities have been gathered without any selection, provided by different collectors and donors at different times along the 20th Century. Therefore, the impact of possible collector's biases is probably negligible. However, rare individual varieties of high commercial value were often looked for by *Morpho* collectors. This may result in an over-estimation of intra-populational variability. *Morpho helenor*, *M. achilles* and *M. deidamia* being widely distributed and abundant, they are more often collected without species discrimination (for instance, many local collectors are unaware of the subtle differences between *M. helenor* and *M. achilles*.) Besides, the availability, at least for some areas, of old and recent specimens allows to verify the stability of characters.

Definition of sampling zones and selection of specimens in the collection

We selected 723 specimens from sampling zones were mostly based on the geographical ranges of 17 subspecies of *M. helenor* which represent the range of variation of its dorsal colour pattern (Supplementary table 1). In the *cis*-Andean region, where the three species are sympatric, the subspecies *M. h. helenor*, *M. h. theodorus*, and *M. h. coelestis* have very large geographical distributions (Blandin 2007), we thus respectively sampled 2, 4, and 2 localities within their ranges to capture the putative geographic variation within these subspecies.

The distribution of *M. helenor* subspecies generally matches up to the endemic centres that were identified and named by Brown (1987) after a detailed study of subspecies distribution of Heliconiinae and Ithomiinae mimetic butterflies. However, depending on the distribution of *M. helenor* subspecies, we subdivided, or on the contrary we grouped, samples corresponding to some endemic centres, and a few sampling zones do not correspond to any of Brown's endemic centres.

The majority of selected specimens were collected during the 1960-1990 decades by local collectors. More recently, along the 1990-2000 decades, specimens were collected in the middle Huallaga valley (Peru), and some ones in Bolivar State (Venezuela). In the Orinoco delta and along the río Aguirre (eastern Venezuela), specimens were collected between 1920 and 1933. At that time, intense collecting was performed for the French insect trader Le Moul. Later, this area of difficult access remained almost unexplored.

Biogeographic region	Species co-occurrence	Sampling zone	Code	<i>M. helenor</i>			<i>M. achilles</i>		<i>M. deidamia</i>		Total ♂	Total ♀	Total
				Subspecies	♂	♀	♂	♀	♂	♀			
<i>trans-Andean</i>	Allopatry	Central America	CA	<i>montezuma</i>	17	7	-	-	-	-	17	7	24
	Allopatry	Costa Rica/Panama	CRP	<i>limpida</i>	9	1	-	-	-	-	9	1	10
	Allopatry	Western Colombia	WP	<i>macrophthalmus</i>	30	8	-	-	-	-	30	8	38
	Allopatry	Central Colombia	CC	<i>peleides</i>	15	5	-	-	-	-	15	5	20
<i>cis-Andean</i>	Sympatry	Eastern Ecuador	EE	<i>theodorus</i>	5	2	8	0	10	1	23	3	26
	Allopatry	Peru Middle Marañon	PM	<i>charapensis</i>	10	4	-	-	-	-	10	4	14
	Sympatry	Peru Middle Huallaga	PMH	<i>theodorus</i>	17	4	18	2	14	6	49	12	61
	Sympatry	Peru High Huallaga	PHH	<i>lacommei</i>	11	10	16	9	11	5	38	24	62
	Sympatry	Central Peru	CP	<i>papirius</i>	30	16	10	9	10	3	50	28	78
	Sympatry	Bolivia La Paz	LP	<i>coelestis</i>	10	4	10	4	7	5	27	13	40
	Sympatry	Bolivia Cochabamba	BC	<i>coelestis</i>	35	15	10	5	5	3	50	23	73
	Sympatry	South-Western Amazon Basin	SWAB	<i>helenor</i>	10	1	0	0	6	0	16	1	17
	Sympatry	Brazil Central Amazon	BCA	<i>theodorus</i>	6	3	3	0	5	1	14	4	18
	Sympatry	Brazil Lower Amazon	BA	<i>helenor</i>	1	1	4	4	6	3	11	8	19
	Sympatry	Venezuela Bolívar	VB	<i>tepuina</i>	24	14	7	2	8	2	39	18	57
	Sympatry	Venezuela Tucupita	VT	<i>tucupita</i>	5	1	11	1	0	0	16	2	18
	Sympatry	Venezuela Rio Aguirre	RA	<i>extremus</i>	0	0	7	2	26	1	33	3	36
	Allopatry	Venezuela El Pao	EP	<i>ululina</i>	6	2	-	-	-	-	6	2	8
Sympatry	Surinam/French Guiana/Northern Pará	SFR	<i>helenor</i>	16	11	9	5	11	5	36	21	57	
Atlantic	Allopatry	Argentina Misiones	AM	<i>achillides</i>	9	2	-	-	-	-	9	2	11
	Allopatry	Brazil Rio de Janeiro	BR	<i>achillaena</i>	16	7	-	-	-	-	16	7	23
	Allopatry	Brazil João Pessoa	BJ	<i>anakreon</i>	10	3	-	-	-	-	10	3	13
Total					292	121	113	43	119	35	524	199	723

Supplementary table 1: Numbers of specimens from the Natural History Museum of Paris (France) photographed in this study, with their geographic areas and their sampling zones, and the occurrence of *M. helenor* in allopatry (in blue) or in sympatry (in black) with *M. achilles* and *M. deidamia*. The subspecies names for *M. helenor* in each sampling zone follow Blandin (2007). The numbers of male (M) and female (F) specimens are given (note that females are harder to catch and are therefore less numerous in Museum collections).

In Mexico, Honduras, El Salvador, Guatemala, and Nicaragua *M. helenor* is represented by the common and weakly variable subspecies *montezuma* Guenée, 1859, characterized by the blue colour covering most of the dorsal surface. The selected specimens were collected in Mexico and El Salvador (**Central America: CA**), within the Guatemala endemic centre. Two other subspecies with limited ranges along the Pacific coasts were not selected.

Morpho helenor is highly diversified in Costa Rica and Panama, from where 8 subspecies have been described (Blandin, 2007; Blandin, 2017). We only selected the subspecies *limpida* Butler, 1872, as it presents a pattern different from *montezuma*, having reduced blue bands. This subspecies exist on the Pacific side of south-eastern Costa Rica, with limited extension in Panama. The sampling zone, on both sides of the border (**Costa Rica-Panama: CRP**), corresponds to the Chiriqui endemic centre.

Along the Pacific coast and the Andean foothills, from Colombia to north-western Peru, *M. helenor* diversified in 5 subspecies (Blandin, 2007). We selected the subspecies *macrophthalmus* Fruhstorfer, 1907, from one locality in north-western Colombia (**Western Colombia: WC**), thus corresponding to the Chocó endemic centre. It differs from the others subspecies as the blue colour does not spread on the wing bases, which are black with more or less visible deep violet-blue sheens.

In central Colombia (**Central Colombia: CC**), we selected the common subspecies *peleides* Kollar, 1850, associated to the Magdalena endemic centre, in which the blue colour covers most of the dorsal surface. The specimens were collected in a limited area, in the valley of the Magdalena river, notably in the classical localities Muzo and Otanche.

Along the Ecuadorian eastern Andes, *M. helenor* is represented by the subspecies *theodorus* Fruhstorfer, 1907, which occurs from eastern Colombia to Bolivia in the south-western Amazon basin (Blandin, 2007). Everywhere in this vast area, it is sympatric with *M. achilles* and *M. deidamia*. We selected specimens from Napo province (**Eastern Ecuador: EE**), corresponding to the Napo endemic center.

The middle Marañón valley, between the Peruvian eastern and western cordilleras, is very dry. Only *M. helenor charapensis* Le Moul & Réal, 1962 occurs in this area, in forest galleries. This subspecies differs from *theodorus* by having the blue colour covering the wing bases. All specimens were collected in the surroundings of the town of Jaén (**Peru, Middle Marañón: PM**), within the Marañón endemic centre.

In northern Peru, Brown (1987) identified a single endemic center in the Río Huallaga valley. However, data on various *Morpho* species suggest distinguishing the middle and the high valley (Blandin, 2007). Therefore, we defined two sampling zones: the **Middle Huallaga (PMH)**, from Tarapoto to Juanjui surroundings, where *M. helenor* is represented by the subspecies *theodorus*, and the **High Huallaga (PHH)** (area of the town Tingo María), where *M. helenor* is represented by the subspecies *lacommei* Blandin, 2007, characterized by a blue band statistically wider than in *theodorus*.

In central Peru, we chose a sampling zone (**Central Peru: CP**), that covers a part of the basin of the río Perene, from the Chanchamayo area to the surroundings of Satipo. This zone corresponds to the Chanchamayo endemic center, where *Morpho helenor* is represented by the subspecies *papirius* Hoppfer, 1874, characterized by wide blue bands and narrow, black margins. In the surroundings of the town Satipo, the specimens are more variable than in the upper valley of the río Perené. A similar situation occurs in *M. achilles* and *M. deidamia* sympatric populations.

Brown (1987) identified a single, wide endemic center along the northern slopes of Bolivian Andes, the Yungas endemic centre, but he recognized two sub-centres, in La Paz and Cochabamba departments. *Morpho helenor*, *M. achilles* and *M. deidamia* are sympatric everywhere. The former species is represented in both sub-centres by a single subspecies, *coelestis* Butler, 1866, in which the blue colour spreads towards the body, becoming more or less darker. On the contrary, *M. achilles* and *M. deidamia* are represented by different subspecies in La Paz sub-centre (*songo* Weber, 1944 and *electra* Röber, 1903 respectively), and Cochabamba sub-centre (*vitrea* Butler, 1866 and *steinbachi* Le Moulton & Réal, 1962 respectively). Consequently, we chose a sampling zone in each of the sub-centres. In La Paz (**LP**), most of the specimens were collected in the surroundings of Caranavi (upper basin of the río Beni). In Cochabamba (**BC**), most of the specimens were collected near Villa Tunari, but we added a few ones from other localities, including from Buena Vista, in the neighboring Santa Cruz department.

Brown (1987) identified several endemic centres through the Brazilian Amazon basin: the Tapajós, Tefé, Madeira, and Rondônia centres. The three species co-occur throughout the Amazon basin, without obvious geographical variations of their blue bands. Possibly, eastern and western subspecies should be differentiated (*M. h. helenor* – *M. h. theodorus*; *M. a. achilles* – *M. a. phokylides* Fruhstorfer, 1912; *M. d. neoptolemus* Wood, 1863 – *M. d.*

grambergi Weber, 1944), but their supposed geographical limits are arbitrary (see Blandin, 2007). We tentatively considered three distant sampling zones. In the sampling zone **Brazil Lower Amazon (BA)**, corresponding to the Tapajós endemic center, specimens were collected in localities south of the Amazon river (Santarem, Itaituba, Juruti, rio Arapiuns). In the sampling zone **Brazil Central Amazon (BCA)**, specimens were collected in localities located between the Brazilian Tefé and the Peruvian Loreto endemic centres (Fonte Boa, São Paulo de Olivença, Tonantins). In the sampling zone **South-Western Amazon Basin (SWAB)**, specimens were collected in localities from Rondônia State (thus corresponding to the Rondônia endemic centre), but we added specimens from the surroundings of Puerto Maldonado (Peru, Madre de Dios department), hypothesizing that there are no significant geographical variations of the three species in the south-western part of the Amazon basin.

In eastern Venezuela, two endemic centres have been identified: Imataca in the north, Pantepui in the south (Brown, 1987; Neild, 2008). However, the distribution of *M. helenor* subspecies implied a somewhat different definition of sampling zones. Many specimens of *M. h. tucupita* Le Moulton, 1925 were collected within the Orinoco delta, in the surroundings of the town Tucupita. This subspecies is characterized by the blue colour covering most of the wings except the narrow black distal margins. On the contrary, in the mainland south of the delta, notably along the río Aguirre, specimens belong to *M. h. extremus* Le Moulton, 1933, in which the blue colour is restricted to a transversal band. However, on mainland, there are many intermediate specimens, which suggest the existence of gene flows between the two subspecies. A similar situation is observed in *M. achilles* and *M. deidamia* (Blandin, 2007). Therefore, we considered two sampling zones: **Venezuela Tucupita (VT)**, and **Venezuela río Aguirre (RA)**.

Between the río Caura to the west and the eastern border of Venezuela, the three species co-occur. *Morpho helenor* is represented by a remarkably variable subspecies, *tepuina* Forbes, 1942, in which the blue band can be strongly reduced, or even missing on the hindwings. As available data suggest that its distribution overlaps both Imataca and Pantepui endemic centres, we considered a single sampling zone, **Venezuela Bolívar (VB)**.

South of the lower Orinoco river, around the small town El Pao, there is a rather dry area with semi-deciduous forests where only *M. helenor* occurs, represented by the subspecies *ululina* Le Moulton & Réal, 1962; it is characterized by the blue colour widely spreading on the wing

bases. This limited ecological area was not identified as an endemic centre, but we considered it as a distinct sampling zone, **Venezuela El Pao (EP)**.

The nominotypical subspecies of *M. helenor*, *M. achilles*, and *M. deidamia* co-occur in Surinam, French Guiana, and also in the northern part of the Brazilian Pará state, on the left side of the Amazon river. Therefore, we considered this vast area as a unique sampling zone (**Surinam/French Guiana/Northern Pará: SFG**) that corresponds approximately to the Oyapok endemic centre.

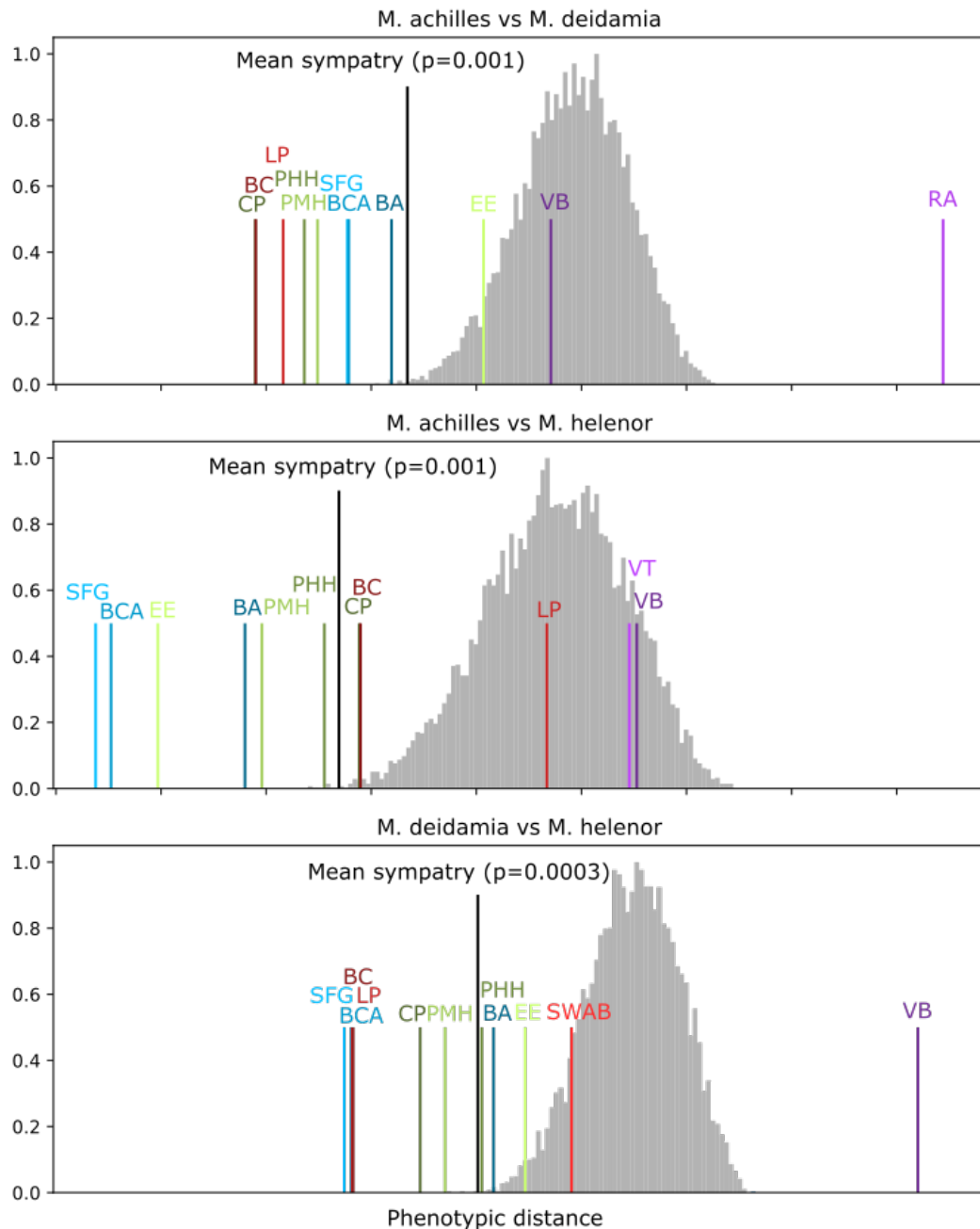
In the Atlantic Forest region, only *M. helenor* occurs. Ten subspecies have been described (Blandin, 2007). We selected three sampling zones corresponding to subspecies representative of the two main morphological types: i- *M. h. achillaena* (Hübner, [1823]) (**Brazil Rio de Janeiro (BR)**), and *M. h. anakreon* Fruhstorfer, 1910 (**Brazil João Pessoa (BJ)**), which both have the blue colour covering the wing bases; ii- *M. h. achillides* C. Felder & R. Felder, 1867, which has narrow blue-violet bands and black wing bases with more or less pronounced violet sheens (**Argentina Misiones (AM)**). The two first sampling zones correspond to the Rio de Janeiro endemic centre and Pernambuco endemic centre respectively. Brown (1987) did not identify an endemic centre in Misiones, neither in Paraguay, where *M. h. achillides* also exists.

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S2 - Euclidian distances in subsets

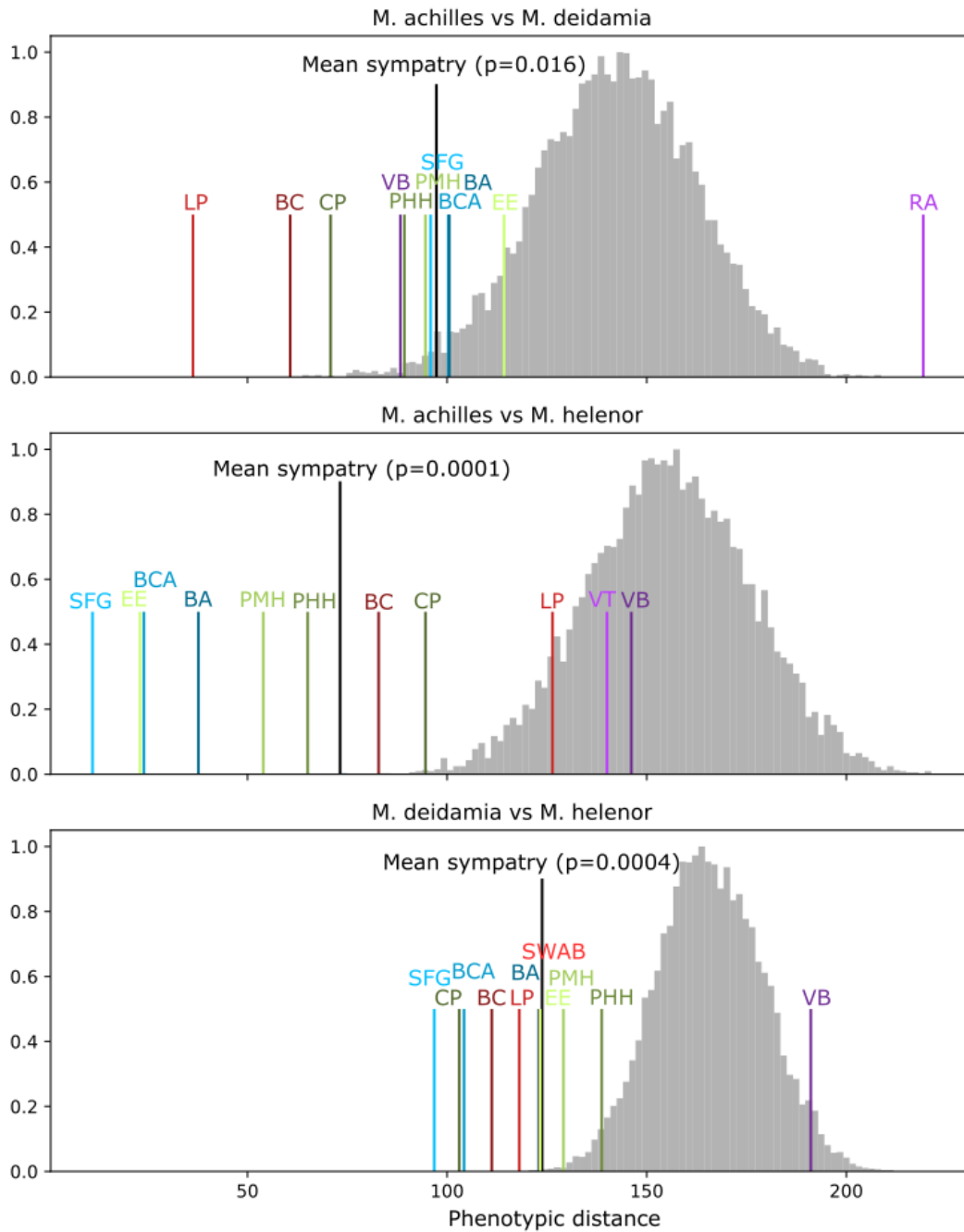
To test whether the phenotypically highly divergent allopatric populations of *M. helenor* increased the signal of convergence described in the main text, we repeated the simulations of pairwise Euclidian distances between species without those *M. helenor* allopatric populations. The convergence in sympatry was also found significant in this subset (see supplementary figure 1 below).



Supplementary figure 1: Phenotypic distances between species in sampling zones where they are found in sympatry (colored bars) and predicted distribution obtained using 10,000 bootstraps, randomly reallocating the different sampling zones within each

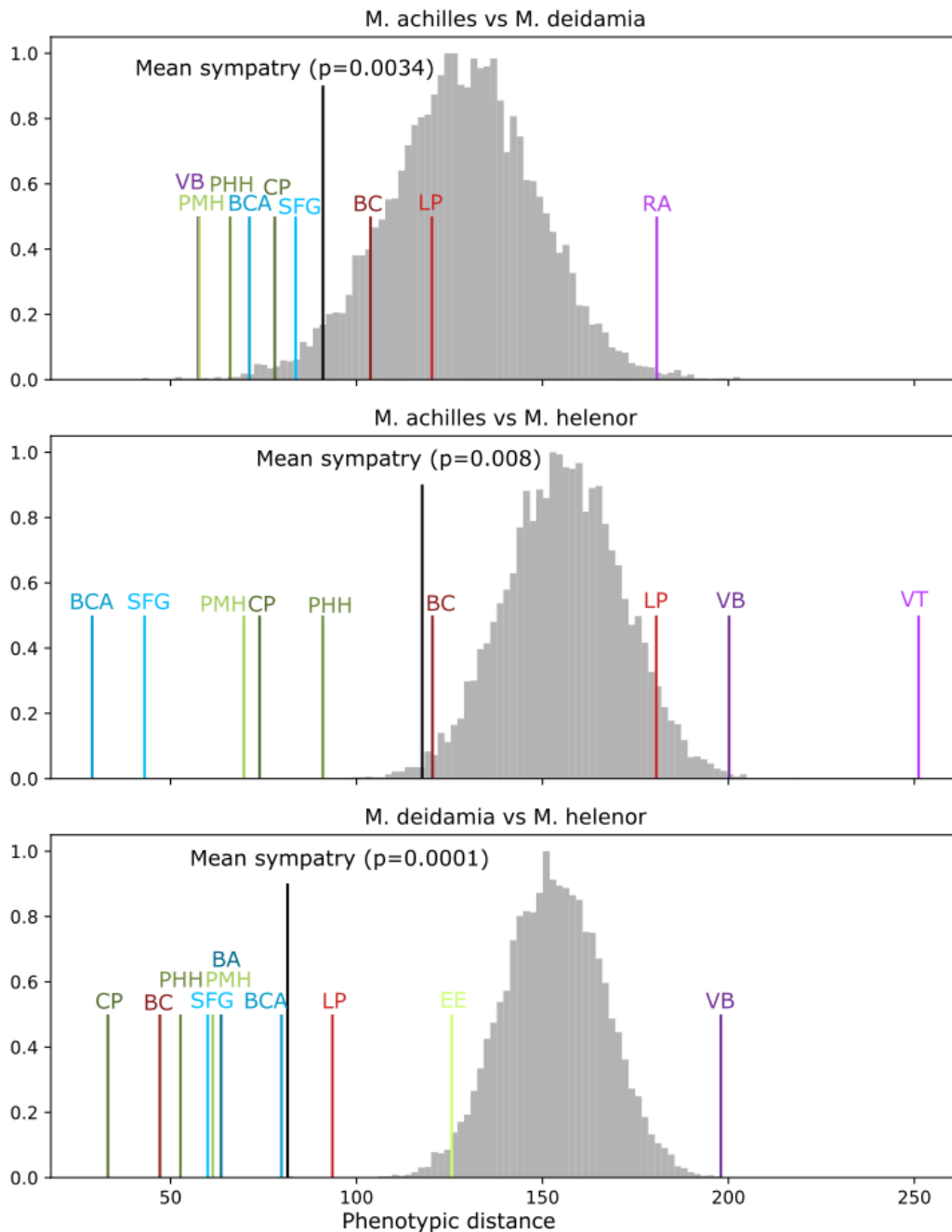
species (grey bars), excluding the sampling zones where *M. helenor* does not co-occur with *M. achilles* and *M. deidamia*. The black bar shows the mean phenotypic distance observed between pairs of species in the sympatric sampling zones; top plot: distances between *M. achilles* and *M. deidamia*, middle plot: distances between *M. helenor* and *M. achilles*, and bottom plot: distances between *M. deidamia* and *M. helenor*. The *p*-value is based on the number of simulations where the phenotypic distance between species is higher than the mean value of inter-specific distances observed in sympatry. Note the colours and codes of sampling zones match the colour code used on the geographic map (fig.1).

To test whether the slight phenotypic differences detected between males and females could interfere in the signal of convergence described in the main text, we performed the simulations of pairwise Euclidian distances between species in two separated datasets including males only and females only respectively. The convergence in sympatry was also found significant in the subset containing males only (see supplementary figure 2 below), as well as females only (see supplementary figure 3 below).



Supplementary figure 2: Phenotypic distances between species in sampling zones where they are found in sympatry (colored bars) and predicted distribution obtained using 10,000 bootstraps, randomly reallocating the different sampling zones within each species (grey bars), including males only from all sampling zones. The black bar shows the mean phenotypic distance observed between pairs of species in the sympatric sampling zones; top plot: distances between *M. achilles* and *M. deidamia*, middle plot: distances between *M. helenor* and *M. achilles*, and bottom plot: distances between *M. deidamia* and *M. helenor*. The

p -value is based on the number of simulations where the phenotypic distance between species is higher than the mean value of inter-specific distances observed in sympatry. Note the colours and codes of sampling zones match the colour code used on the geographic map (fig.1).



Supplementary figure 3: Phenotypic distances between species in sampling zones where they are found in sympatry (colored bars) and predicted distribution obtained using 10,000 bootstraps, randomly reallocating the different sampling zones within each species (grey bars), including females only from all sampling zones. The black bar shows

the mean phenotypic distance observed between pairs of species in the sympatric sampling zones; top plot: distances between *M. achilles* and *M. deidamia*, middle plot: distances between *M. helenor* and *M. achilles*, and bottom plot: distances between *M. deidamia* and *M. helenor*. The p -value is based on the number of simulations where the phenotypic distance between species is higher than the mean value of inter-specific distances observed in sympatry. Note the colours and codes of sampling zones match the colour code used on the geographic map (fig.1).

S3- Phenotypic distances estimated with Malahanobis distance

To compare the phenotypic resemblance between species within and across sampling zones, we also computed Malahanobis distance that takes into account the distribution of individuals within each compared groups. To test whether inter-specific similarity was higher within a sampling zone than across sampling zones, we then performed a custom 1,000 bootstrap of inter-specific phenotypic distances across localities. For each of the 13 sampling zones where the three *Morpho* species co-occur, for each species, we re-sampled individuals in a randomly-chosen different sampling zone, and computed mean Malahanobis distance among species on each re-sampled dataset. We thus obtained a null distribution of interspecific Malahanobis distance on re-sampled individuals matching the actual number of individuals from each species within sampling zone. We then compared the observed mean Malahanobis distances between species within sampling zone to this null distribution to assess statistical significance: the among species distance was considered significantly smaller within sampling zone than among sampling zones when the observed value was lower than 95% of the resampled values.

Supplementary Table 2: Observed and predicted phenotypic distances among individuals from different species within sampling zones. Mean predicted distances and p -values were obtained from a bootstrap analysis based on re-sampling of individuals from the different species in different sampling zones, matching the number of individuals from each species actually sampled in each sampling zone. This bootstrap analysis includes *M. helenor* populations where *M. achilles* and *M. deidamia* did not co-occur. Significant convergence was inferred when the observed mean phenotypic Mahalanobis distances between species within sampling zone was smaller than 950 predicted distances out of 1000 re-sampling across sampling zones. The sampling zone, and corresponding code and number of individuals sampled are reported. Note the colours of sampling zone codes match the colour code used on the geographic map (fig.1) and on the morphospace (fig.2).

Sampling zone	Code	#ind	Obs. mean	Pred. mean	P -value	Direction
Surinam / French Guiana/ Northern Pará	SFG	57	66.89	109.27	<0.001	Convergence
Eastern Ecuador	EE	26	64.53	117.32	<0.001	Convergence
Peru Middle Huallaga	PMH	61	76.51	110.16	<0.001	Convergence
Brazil Central Amazon	BCA	19	64.36	98.24	0.006	Convergence
Brazil Lower Amazon	BA	18	84.00	121.01	0.037	Convergence
Central Peru	CP	78	59.58	75.44	0.050	Convergence
South-Western Amazon Basin	SWAB	17	108.66	117.10	0.336	NS
Peru Upper Huallaga	PHH	62	75.59	82.43	0.428	NS
Bolivia Cochabamba	BC	73	99.53	103.18	0.442	NS
Bolivia La Paz	LP	40	113.08	109.58	0.583	NS
Venezuela Bolívar	VB	57	140.77	125.59	0.889	NS
Venezuela Tucupita	VT	18	136.28	65.68	0.997	Divergence
Venezuela Río Aguirre	RA	36	193.85	52.23	1	Divergence

Supplementary table 3: Observed and predicted phenotypic distances among individuals from different species within sampling zones, excluding populations of *M. helenor* populations where *M. achilles* and *M. deidamia* did not co-occur. Mean predicted distances and *p*-values were obtained from a bootstrap analysis based on re-sampling of individuals from the different species in different sampling zones, matching the number of individuals from each species actually sampled in each sampling zone. Significant convergence was inferred when the observed mean phenotypic Mahalanobis distances between species within a sampling zone was smaller than 950 predicted distances out of 1000 re-sampling across sampling zones. The sampling zones, with the corresponding codes and numbers of individuals sampled are reported. Note the colours of locality codes match the colour code used on the geographic map (fig.1) and on the morphospace (fig.2).

Locality	Code	#ind	Obs. mean	Pred. mean	<i>P</i> -value	Direction
Surinam / French Guiana	SFG	57	80.74	117.92	0.001	Convergence
Peru Middle Huallaga	PMH	61	91.65	119.58	0.001	Convergence
Brazil Central Amazon	BCA	19	77.57	108.84	0.009	Convergence
Eastern Ecuador	EE	26	87.21	127.61	0.019	Convergence
Brazil Lower Amazon	BA	18	97.45	134.95	0.093	Convergence
Central Peru	CP	78	75.02	85.58	0.213	NS
Bolivia La Paz	LP	40	116.98	121.55	0.408	NS
Bolivia Cochabamba	BC	73	108.16	112.20	0.479	NS
South-West Amazon Basin	SWAB		124.32	123.70	0.55	NS
Peru High Huallaga	PHH	62	95.50	93.64	0.608	NS
Venezuela Tucupita	VT	18	140.11	65.99	1	Divergence
Venezuela Rio Aguirre	RA	36	211.60	60.05	1	Divergence
Venezuela Bolivar	VB	57	180.58	129.68	1	Divergence

Supplementary table 4: Observed and predicted phenotypic distances among females from different species within sampling zones. Mean predicted distances and p -values were obtained from a bootstrap analysis based on re-sampling of females from the different species in different sampling zones, matching the number of females from each species actually sampled in each sampling zone. This bootstrap analysis includes *M. helenor* populations where *M. achilles* and *M. deidamia* did not co-occur. Note that the SWAB sampling zone is missing because no females from these geographical areas were held in the Museum collections. Significant convergence within females was inferred when the observed mean phenotypic Mahalanobis distances between species within a sample zone was smaller than 950 predicted distances out of 1000 re-sampling across sample zones. The sampling zones, with the corresponding codes and numbers of females sampled are reported. Note the colours of locality codes match the colour code used on the geographic map (fig.1) and on the morphospace (fig.2).

Locality	Code	#females	Obs. mean	Pred. mean	P -value	Direction
Brazil Central Amazon	BCA	8	80.98	120.89	0.008	Convergence
Brazil Lower Amazon	BA	4	74.03	134.45	0.024	Convergence
Peru Middle Huallaga	PMH	12	88.93	126.36	0.026	Convergence
Surinam / French Guiana/ Northern Pará	SFG	21	102.73	135.02	0.032	Convergence
Central Peru	CP	28	85.23	108.73	0.046	Convergence
Peru High Huallaga	PHH	24	71.22	75.40	0.395	NS
Eastern Ecuador	EE	3	129.04	143.76	0.402	NS
Bolivia Cochabamba	BC	23	119.21	121.42	0.482	NS
Bolivia La Paz	LP	13	146.52	120.94	0.900	NS
Venezuela Bolívar	VB	18	167.90	137.91	0.924	NS
Venezuela Rio Aguirre	RA	3	196.18	132.29	0.931	NS
Venezuela Tucupita	VT	2	272.58	177.55	0.949	NS

Supplementary table 4: Observed and predicted phenotypic distances among males from different species within sampling zones. Mean predicted distances and *p*-values were obtained from a bootstrap analysis based on re-sampling of males from the different species in different sampling zones, matching the number of males from each species actually sampled in each sampling zone. This bootstrap analysis includes *M. helenor* populations where *M. achilles* and *M. deidamia* did not co-occur. Significant convergence within males was inferred when the observed mean phenotypic Mahalanobis distances between species within locality was smaller than 950 predicted distances out of 1000 re-sampling across localities. The sampling zones, with the corresponding codes and numbers of males sampled are reported. Note the colours of locality codes match the colour code used on the geographic map (fig.1) and on the morphospace (fig.2).

Locality	Code	#males	Obs. mean	Pred. mean	<i>P</i> -value	Direction
Brazil Central Amazon	BCA	11	101.54	144.03	0.007	Convergence
Eastern Ecuador	EE	23	112.34	150.76	0.008	Convergence
Surinam / French Guiana/ Northern Pará	SFG	36	107.22	142.60	0.011	Convergence
Brazil Lower Amazon	BA	14	106.30	146.32	0.017	Convergence
Central Peru	CP	50	86.38	105.16	0.066	Convergence
Peru Middle Huallaga	PMH	49	117.31	132.66	0.074	NS
South-Western Amazon Basin	SWAB	16	123.66	135.33	0.255	NS
Bolivia Cochabamba	BC	50	87.17	95.92	0.343	NS
Peru High Huallaga	PHH	38	128.91	131.30	0.432	NS
Bolivia La Paz	LP	27	109.52	107.97	0.591	NS
Venezuela Bolívar	VB	39	164.83	149.96	0.926	NS
Venezuela Tucupita	VT	16	143.95	78.96	0.978	Divergence
Venezuela Rio Aguirre	RA	33	219.87	76.19	1	Divergence

Convergences in sympatry confirmed by tests based on Mahalanobis distances

To test the hypothesis of local convergence among the three species more directly, we specifically compared the mean Mahalanobis distances between species within and among sampling zones. Supplementary Table 1 shows the results for males and females together to increase our sample size, but the analyses performed on each sex independently show similar trends (see supplementary table 3 and 4 for results obtained within females and within males respectively).

Considering the whole dataset (both sexes together and including sampling zones where *M. helenor* does not co-occur with the other two species), smaller interspecific phenotypic distances within sampling zone was found for 9 out of 13 localities. However, this reduced divergence was statistically significant for only 6 of these localities, mostly situated in Ecuador, Peru, Brazil, French Guiana and Surinam (supplementary table 2). In these 6 localities, the reduced phenotypic distance between species is consistent with convergence in wing colour pattern among the three sympatric species. In 4 sampling zones, the divergence was higher within sampling zone than among sampling zones, but only significantly so for two of them. It is worth noting that these two sampling zones situated in Venezuela (they include the Orinoco delta and mainland south of the delta) show a particularly high level of variation within-species (fig.2C). No significant difference was thus detected in 5 sampling zones, mostly covering Bolivia and South-Western Brazil. A bootstrap analysis performed excluding the sampling zones where *M. helenor* occurs alone showed a similar trend (see supplementary table 3), although the marginally significant signal of convergence observed in central Peru (CP) was no longer significant, while the divergence in Bolivar (VB) this once is significant.

Effect of phylogenetic distance on convergence

To test whether convergence was stronger between the two sister species *M. helenor* and *M. achilles* than with the more distantly related species *M. deidamia*, we then computed the Mahalanobis distances between pairs of species within sampling zones, and tested whether the distances within sampling zones were smaller in *M. helenor*/*M. achilles* pairs as compared to the other two pairs (*M. helenor*/*M. deidamia* and *M. achilles*/*M. deidamia*), using an ANOVA, controlling for the effect of the sampling zone.

The comparison of Mahalanobis distances between species within sampling zones reveals that the phenotypic distances among *M. helenor* and *M. achilles* in sympatry were generally smaller than between the other two species pairs involving the more distantly related species *M. deidamia* ($F = 5.978$, $df = 1$, $P = 0.024$), controlling for the effect of sampling zones ($F = 3.585$, $df = 10$, $P = 0.008$). Despite substantial phenotypic variation among sampling zones, this suggests that phenotypic resemblance between species is stronger between the sister species *M. helenor* and *M. achilles*, as compared to the more distantly-related species *M. deidamia*.

Blandin P (2007) *The Systematics of the Genus Morpho Fabricius, 1807* Hillside Books, Canterbury.