

# Refining mimicry: phenotypic variation tracking the local optimum

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1	Mimicry refinement: Phenotypic variations
2	tracking the local optimum
3	
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14	
15	Running title: Mimicry refinement in butterflies
16	
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19	
20	Summary
21	1. Müllerian mimicry between chemically defended preys is a textbook example of
22	natural selection favouring phenotypic convergence onto a shared warning signal.
23	Studies of mimicry have concentrated on deciphering the ecological and genetic
24	underpinnings of dramatic switches in mimicry association, producing a well-known
	1

mosaic distribution of mimicry patterns across geography. However, little is known
 about the accuracy of resemblance between natural co-mimics when the local
 phenotypic optimum varies.

- In this study, using analyses of wing shape, pattern and hue, we quantify multimodal
   phenotypic similarity between butterfly co-mimics sharing the so-called postman
   pattern in different localities with varying species composition.
- 31 3. We show that subtle but consistent variation between populations of the localised 32 species, *Heliconius timareta thelxinoe*, enhance resemblance to the abundant co-33 mimics which drive the mimicry in each locality.
- 4. Those results suggest that rarer co-mimics track the changes in the phenotypic
  optimum caused by gradual changes in the composition of the mimicry community,
  providing insights into the process by which intra-specific diversity of mimetic pattern
  may arise. Furthermore, our results suggest a multimodal evolution of similarity, with
  coordinated convergence in different features of the phenotype such as wing outline,
  pattern and hue.

5. Finally, multilocus genotyping allows estimating local hybridization rates between *H*. *timareta* and co-mimic *H. melpomene* in different populations, raising the hypothesis
that mimicry refinement between closely-related co-mimics may be enhanced by
adaptive introgression at loci modifying the accuracy of resemblance.

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- 45

#### 46 Introduction

Chemically-defended animal species often show striking convergence in their colour patterns 47 48 with other prey coexisting in the same habitat. This convergence may be explained, since the work of Müller (1879), by natural selection favouring superficial resemblance, and operated 49 by visual predators, a phenomenon called Müllerian mimicry. Theory proposes that predators 50 51 learn upon experience the association of prey distastefulness and prey visual appearance, 52 generally distinctive warning patterns. Mimicking a locally abundant warning signal, well known by local predators, constitutes a benefit to a defended prev species by decreasing 53 54 predation risk. Mimicry benefits associated with a given warning signal depend on the relative numbers of prey sampled vs. available to learning predators, and are usually driven by the 55 most toxic or the most abundant prev species (Mallet & Joron 1999). 56

57

Müller's general principle has been largely supported by theoretical models and empirical 58 experiments (Turner 1977; Sheppard et al. 1985; Turner 1987; Ruxton, Sherratt & Speed 59 2004). Field transplant experiments have confirmed selection favouring local patterns (Mallet 60 & Barton 1989; Kapan 2001; Chouteau & Angers 2011; Merrill et al. 2012) and the strong 61 frequency-dependent selection acting on warning signals in diverse communities (Chouteau, 62 Arias & Joron 2016). Natural selection for local mimicry explains local polymorphism of 63 distinct colour patterns (Kapan 2001; Joron & Iwasa 2005), as well as the maintenance of 64 geographical races with sharply distinct patterns, for instance the so-called postman vs. rayed 65 patterns of Heliconius erato and H. melpomene (Mallet & Barton 1989). Most of those studies 66 investigated the mimicry benefits associated with alternative warning strategies using 67 completely distinct patterns, corresponding to mimetic optima and describing an adaptive 68 landscape with adaptive peaks separated by valleys of low fitness (Leimar & Mallet 2012). 69 However, for a given morph, variations may be found between individuals, between sexes and 70

between localities. Fewer studies have addressed the significance of mimicry variation around a given adaptive peak, or the underpinnings of precise resemblance within a given mimicry ring. Mimetic communities often involve assemblages of species which differ between localities, and species indeed vary in the level of mimicry precision to others (Penney *et al.* 2012), but it is unclear what determines the level of mimicry accuracy in those communities or to which extent the mimicry optimum may vary through space or time.

77

The ability of predators to generalize the signals of defended prey is an important determinant 78 of selection on resemblance in a mimicry system (Rowe, Lindstrom & Lyytinen 2004; 79 Ihalainen et al. 2012). Sharing key components of a warning signal with co-mimics can 80 sometimes enhance protection and may allow crossing a valley of low fitness (Beatty, 81 Beirinckx & Sherratt 2004; Balogh & Leimar 2010). For instance, jacamars trained to avoid 82 83 butterflies with an orange patch were less likely to attack butterflies with a similar pattern painted red than those painted black (Langham 2004), suggesting that a red patch may be 84 85 sufficient to reduce predation regardless of the differences in hue. Nevertheless, in that example red butterflies still received higher predation than orange controls (Langham 2004), 86 suggesting that small deviations of colour hue are, to a certain extent, perceived by predators 87 and translate into fitness differences. Increased predation against imperfect mimics has also 88 been shown in lab experiments (Ihalainen et al. 2008), meaning that even if coarse 89 resemblance is attained, selection may still favour the improvement of mimicry. Those 90 findings support the classical scenario for the evolution of mimicry, first through a major 91 phenotypic change allowing coarse resemblance for certain key warning feature, followed by 92 the gradual improvement of mimicry under selection by narrowly generalizing predators 93 (Sheppard et al. 1985; Turner 1987; Franks & Sherratt 2007; Balogh & Leimar 2010; Leimar 94 & Mallet 2012). 95

97 The strength of selection for resemblance is affected by the complexity and diversity of the prey community. For instance, discrimination against imperfect mimics is less accurate when 98 the community of prey is complex, i.e. composed of several distinct warning signals 99 (Ihalainen et al. 2012). However, whether variations of pattern within a mimicry ring itself 100 affect the intensity of selection for resemblance has rarely been tested, since experimental 101 102 predators usually are only trained on a single prey type. Quantifying variations in resemblance of wild individuals within and between species of a given mimicry ring allows investigating if 103 and how the accuracy of resemblance evolves. 104

105

Phenotypic similarity is influenced not only by natural selection favouring accurate mimicry 106 but also by the genetic architecture underlying variation in phenotype. For instance, 107 108 phenotypic variation remaining within the generalization range of predators might be little influenced by mimicry selection and more by genetic correlations or environmental trade-offs. 109 110 Drift, mutations, environmental plasticity or hybridization are different sources of variation which can affect the accuracy of similarity and dissimilarity of phenotypes within and 111 between species of the mimicry ring. In addition, specific genetic architectures can favour 112 convergence and a good level of resemblance. In Heliconius butterflies, variations in mimetic 113 colour patterns are largely controlled by a few Mendelian loci of large effect, often coined the 114 "colour pattern toolbox", an architecture which may facilitate secondary convergence in wing 115 patterns (Reed et al. 2011). The most striking example of mimicry achieved through a shared 116 architecture is when alleles at colour-patterning loci are shared among co-mimics via adaptive 117 introgression. This was documented, for instance, in the pair of species H. timareta and H. 118 melpomene (Heliconius Genome Consortium 2012; Pardo-Diaz et al. 2012). In this case, 119 colour pattern resemblance reflects the shared origin of adaptive alleles in both species, but 120

selection for mimicry may also play a role, first by facilitating the invasion of introgressed alleles in the receiving population, and second by favouring further refinement of resemblance in the new genome. Describing variations in resemblance between species is therefore required to disentangle the relative importance of shared genetics and mimicry selection in the evolution of accurate resemblance.

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In this study, we investigate selection for mimicry perfection by quantifying phenotypic
similarity among multiple species forming the so-called "postman" mimicry ring in Northern
Peru.

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The "postman" wing pattern is a very strong warning signal working as a major (and possibly 131 ancient) mimicry attractor in this region (Hines et al. 2011). However, subtle geographic 132 variation in colour pattern was reported for one of the co-mimics (Heliconius timareta 133 thelxinoe), between localities separated by about 175 km (Mérot et al. 2013). The two 134 localities display some differences in the assemblage of species participating in the postman 135 mimicry. In the Alto Mayo, H. timareta thelxinoe co-occurs mostly with H. telesiphe while, in 136 the Cordillera Escalera, the 'postman' community is dominated by *H. erato favorinus* and *H.* 137 melpomene amaryllis (Fig.1). Theoretical simulations suggest that the most abundant species 138 of a mimicry ring generally drives the evolution of phenotypic resemblance in other species 139 (Turner 1977; Mallet 1999; Franks & Sherratt 2007; Ruxton et al. 2008). We therefore 140 hypothesise that the subtle variation in colour pattern found in certain species participating in 141 the mimicry ring might be the footprint of selection for different mimetic optima, reflecting 142 spatial changes in the phenotypic composition of the different communities. 143

To test whether the local mimetic community may influence the strength and the nature of 145 146 selection for mimicry, we investigate whether geographic variations for various modalities of the warning signals (colour pattern, reflectance of the colour patch, wing shape) within 147 participating species appear to track variations in composition of the mimetic community. We 148 use Colour Pattern Modelling (Le Poul et al. 2014), geometric morphometrics, and spectral 149 colour measurements to quantify phenotypic similarity between co-mimics, and analyse it in 150 151 the light of neutral molecular variation, hybridization rates, and species composition in the distinct communities. 152

#### 153 Methods

#### 154 Species studied, specimen collection and density.

The four species of the "postman" mimicry ring (Fig.1, *H. melpomene amaryllis* Feder & Feder, *H. timareta thelxinoe* Lamas & Mérot, *H. erato favorinus* Hopffer and *H. telesiphe* Doubleday, further abbreviated with their species name only) were sampled in two tropical montane areas separated by 175km, the Escalera and the Alto Mayo (San Martín, Peru). Sampling localities were chosen along an altitudinal continuum, which ranges from 400m to 1300m in the Escalera ("E", 06°27′28″S; 76°17′53″W) and from 1100m to 1800m in the Alto Mayo ("A", 05°39′58″S; 77°44′35″W).

162

To estimate the relative frequencies of each species, we used collection data corrected by the number of collecting days. On collecting days, all butterflies from the four species encountered were caught with entomological nets. A subset of this sample was used for genetic and phenotypic analysis (Table.S1&S2). We considered two populations of *H. melpomene* in the Escalera ("Low E", below 1000m; and "Escalera", above 1000m, sympatric with *H. timareta*). We also included an additional population of *H. melpomene* from Moyobamba (06°05′13°S; 76°59′36°W, Peru) to investigate geographic variation in *H. melpomene* between area of sympatry and allopatry with *H. timareta*.

#### 171 Phenotypic description and analyses

#### 172 Data acquisition

Images of ventral (v) and dorsal (d) forewings (FW) and hindwings (HW) were captured in
normalized light conditions (CIE Standard Illuminant D50) using a Nikon D90 digital camera
with a Nikon micro 105/2.8GEDVR lens.

176

Measurements of wing reflectance were done with a spectrometer (AvaSpec-3648, Avantes) 177 and a deuterium-halogen light source (DH-200, Avantes) connected to a 1.5mm diameter 178 sensor (FCR-7UV200-2-1.5x100, Avantes) inserted in a miniature black chamber (an opaque 179 black plastic tube surrounding the reflectance probe to exclude ambient light from the 180 measurement). Reflectance spectra were taken at 90° incidence relative to a 99% reflectance 181 182 standard (300-700nm spectralon) and to dark current. Spectra were recorded with the software Avasoft 7.0 using an average of 5 measures with an integration time of 23 ms. On all wing 183 surface (FWv, FWd, HWv, HWd), we recorded reflectance of colour patches and black area at 184 the same location for each specimen. 185

#### 186 Wing colour pattern

Colour pattern was analysed using Colour Pattern Modelling (Le Poul *et al.* 2014) which allows quantifying colour pattern variation and similarity across the entire wing. Detailed methods are given in supplementary material. Briefly, wing outline was extracted individually from the background. Within this area, the RGB colours are categorized into three colour classes (black, red or yellow). We called "yellow" the bar on all species hindwing so that pattern itself would be comparable, despite some hue variation between species and between individuals of different ages or wear. All individual wings were aligned by rotation,
translation, scaling and normalization based on an iterative process. Variations in patch
boundaries can then be fully described and compared between individuals.

196

Each wing surface (FWd, FWv, HWd, HWv), was characterized by a set of pixels with homologous position across specimens. Each pixel was associated with three presence/absence binary values for black, red and yellow, which allows colours to be treated separately. Variations in patch boundaries were then analysed for each surface separately with a principal component analysis applied to the set of pixels. The resulting components describe a morphological space used for subsequent analyses after the dimensionality reduction proposed by Baylac and Friess (2005).

204

Small colour elements located in the basal part of the wing were scored following Mérot *et al.* (2013) with an index describing variation at two qualitative characters: the red line on the ventral forewing (0=absent to 5=a well-marked line, Fig.S1a) and the extension and the number of basal red spots on the ventral hindwing (0=no dots to 5=large spots, Fig.S1b).

209

#### 210 Wing outline

Wing outline, extracted and aligned through the first step CPM procedure, was further analysed with elliptical Fourier analysis using custom scripts developed in Matlab (Jones *et al.* 2013), following the directions of (Neto & Samal 2006). A PCA was applied to the first twenty Fourier harmonics and allowed describing a morphological space for wing outline.

#### 216 Wing venation: analysis by geometric morphometrics

Wing venation was described using 15 (FW) and 14 (HW) landmarks, placed at vein 217 intersections and vein termini on the ventral side, as described in Mérot et al. (2013). 218 Standard tests of repeatability were done by taking the landmarks five times per wing on 219 subsamples of five butterflies from a single species, population and sex. Landmark 220 coordinates were digitalized using TpsDig2 (Rohlf 2010) and superimposed using a general 221 Procrustes analysis (Bookstein 1991; Zelditch et al. 2004). For each set of landmarks, 222 superposition includes all samples in a multidimensional Procrustes space whose tangent 223 space is a Euclidian morphological space for wing venation. A principal component analysis 224 225 was applied on this wing venation data followed by the dimensionality reduction proposed by 226 Baylac and Friess (2005). Wing size was measured using log-transformed centroid size (Bookstein 1991). 227

228

#### 229 Colour spectra and visual models

Colour spectra were analysed using Avicol V.6 (Gomez 2006). Colour spectra obtained
between 300 and 700 nm were smoothed using a local Fourier correction at 650 nm
(Fig.S5&S7). A mean colour spectrum was calculated for each colour patch of each individual
by averaging measurements on the left and the right wing. Each kind of colour patch was
further analysed separately.

235

Analyses of spectra were carried out using models of animal vision, which take into account the observer's vision and the illuminating light. Each photoreceptor is characterized by a sensitivity function which determines the wavelength of reflected light perceived by the eye. Analyses in the main text are with a tetrachromatic V-type bird visual system (Peafowl, *Pavo cristatus*, (Hart 2002)) and a light environment corresponding to large sunny gaps in a tropical

forest (Théry, Pincebourde & Feer 2008). Analysis with other visual system and other incident 241 lights gave consistent results and are presented in supplementary materials (Fig.S9). A 242 physiological model of Endler and Mielke (2005) was applied to visualize colour distribution 243 in an unconstrained space, a tetrahedron whose vertices corresponds to the four 244 photoreceptors (Fig.S6). Within this colour space, the relative location of each individual's 245 patch colour can be compared and the overlap between two clouds of points (Table.S6) can be 246 247 calculated. Relative excitation of each photoreceptor were treated as multivariate data and analysed with PCA analyses. 248

249

#### 250 Statistical analysis

Within each species, differences in colour pattern, colour spectra, wing outline and venation between geographic populations were tested by a one-way MANOVA on each subset of PCs with geographic origin as a factor. Then, within each species and for each trait, we tested discrimination between groups defined by geographical populations with a linear discriminant analysis. We compared the cross-validation values to discrimination between simulated populations of similar size following the methods described in Evin *et al.* (2013).

257

Phenotypic similarity between each species and each population of *H. timareta* was quantified 258 259 with several methods within each morphological space. First, to measure the magnitude of phenotypic similarity between individuals, Euclidian distances in the morphological space 260 were computed between all pairs of specimens. For colour spectra, we used perceptual 261 chromatic distances between all pairs of specimen, expressed in JNDs (just noticeable 262 differences) in a perception model of Vorobyev and Osorio (1998). This model takes into 263 account the noise due to errors in photoreceptor response and estimates more accurately 264 whether the discriminability between colours could really be perceived by predators. 265

Perceptual and Euclidian distances between a mimic and each population of *H. timareta* (Alto 266 267 Mayo vs. Escalera) were analysed with a mixed model with geographic population as factor and identity of the compared specimen as random factors. Second, to compute an indicator of 268 269 similarity between groups, Mahalanobis distances were calculated between the three species and the two populations of *H. timareta*. Contrary to Euclidian distances, this measure applies 270 in a transformed space and is scaled by intra-group variation. Third, to confirm our similarity 271 estimates, we calculated an index of cross-classification by performing a linear discriminant 272 analysis on a subset of two groups (for example, H. melpomene/H. timareta (A); H. 273 melpomene/H. timerata (E)). Since the latter two analyses are sensitive to unbalanced sample 274 275 sizes (Kovarovic 2011), we performed them on subsets of similar sizes, by randomly drawing 30 specimens from each group, repeating the procedure 1000 times and averaging over the 276 277 repetitions.

278

All analyses were performed in R 3.0.3. (R Core Team 2014) using the packages *ade4*, *nlme*, *Mass* and *Rmorph* (Baylac 2012).

281

#### 282 Multilocus microsatellite analysis

The multilocus microsatellite genotype, performed on *H. melpomene* and *H. timareta*, is based 283 284 on 11 loci, and is an extension of the genotyping performed in Mérot et al (2013) with the addition of 31 H. melpomene amaryllis and 69 H. timareta thelxinoe. Detailed methods can be 285 found in supplementary materials; genetic diversity and statistics in Table.S3. F-statistics were 286 calculated using GENETIX 4.05 (Belkhir et al. 1996-2004). We used STRUCTURE 287 (Pritchard, Stevens & Donnelly 2000), a multilocus Bayesian clustering method, to determine 288 population structure, to confirm the assignment of individuals to species, and to detect 289 290 admixed genotypes. STRUCTURE was run with 500,000 updates of the Markov chain after

an initial burn-in of 50,000 updates, to achieve chain convergence for a set of models with 291 292 different numbers of clusters (K=1-6). Since *H. melpomene* and *H. timareta* can hybridize and disperse, we used the 'admixture model' and 'correlated allele frequencies'. The most likely K 293 was chosen using the maximum likelihood value in STRUCTURE and the  $\Delta K$ -method 294 (Evanno, Regnaut & Goudet 2005) and then evaluated following recommendations from 295 STRUCTURE documentation. Over the entire set of specimens included, the likelihood 296 reaches a maximum plateau between K=2 and K=3. When K=2, the two clusters correspond 297 to the two species (identified phenotypically), so K=2 bears a clear biological meaning and 298 was retained. K=3 further splits H. timareta into two clusters with no obvious association with 299 300 biological variables (such as geographic populations), nor with any identifiable experimental bias. Further STRUCTURE analyses run on each species separately support the absence of 301 intraspecific clustering. Posterior probabilities of being member of a cluster were estimated 302 303 and allowed detecting potential hybrids. Hybrid detection was also run separately with NewHybrids 1.0 (Anderson & Thompson 2002). Relying on the results from simulated 304 hybrids (Mérot et al. 2013) and hybrids from controlled crosses (Mérot et al. 2015), 305 individuals were considered as "pure" if the posterior probability was above 0.9 in 306 STRUCTURE and above 0.7 in NewHybrids. 307

308

#### 309 **Results**

#### 310 *Geographical variation within species*

Populations of *H. timareta* from the Escalera and the Alto Mayo exhibit significant differences in colour spectra, pattern, wing outline and venation (Table.1). This result was consistent whether males and females were treated together or separately. A linear discriminant analysis on *H. timareta* allows discriminating the two populations, with a low level of misclassifications, better than the range of mis-classification obtained from randomly simulated population (Table.1). Centroid sizes did not exhibit significant differences between
the two populations of *H. timareta* (ANOVA FW p=0.12, HW p=0.77).

318

Within *H. melpomene* and within *H. erato*, neither colour spectra, wing outline nor size displayed any significant geographical phenotypic variations (Table.S4&S5). For pattern and venation, slightly significant differences were observed between populations of *H. melpomene* and *H. erato*. However, discrimination between geographic populations reaches 41-71% of misclassifications, which is within the misclassification range from randomly simulated populations of similar sample size.

325

#### 326 *Relative frequency of postman co-mimics*

The Escalera and the Alto Mayo areas display differences in their geography and topography, 327 their community composition and the altitudinal ranges of local species (Fig.1C). The 328 Escalera is a relatively thin, mid-elevation Eastern cordillera jutting from adjacent Amazonian 329 lowlands. On its slopes below 1000m, H. erato and H. melpomene are the only species with a 330 331 postman pattern and are generally very abundant, at our collection places and elsewhere in the foothills (>200 specimen of each species in 65 days, approximately 1:1 ratio, Table.S2). In the 332 highest areas, between 1000 and 1300m, H. erato becomes less abundant while H. melpomene 333 co-occurs with *H. timareta*. The Escalera is the southernmost tip of the known distribution of 334 *H. timareta* and the latter can be as abundant as *H. melpomene*, but only locally, as observed 335 at our collection places (1:1 ratio, Table.S2). At a finer scale, *H. timareta* gradually increases 336 in frequency relative to H. melpomene (Fig.1C). Despite intensive collection (83 days, 337 Table.S2), only two specimens of *H. telesiphe* were found in the Escalera, and only at the 338 highest collection station (1300m). 339

The Alto Mayo represents a wider, higher and more continuous area of montane forest surrounded by Andean areas. There, *H. melpomene* and *H. timareta* display less overlap since the transition is more abrupt and occurs at higher elevation (1300m, Fig.1C). *H. timareta* was never found below 1200m and was locally abundant at one collection station (1400-1600m). Over 1300m, *H. telesiphe* is an abundant species in the Alto Mayo and the commonest postman-patterned species. *H. erato* was not found in our standardized collection stations of the Alto Mayo although it is occasional up to 1200m, and thus parapatric with *H. timareta*.

348

#### 349 Variation in wing phenotype in the postman mimetic community

#### 350 Wing colour pattern

For forewing colour pattern, the first axis of the PCA (Fig.2a) displays variation from the 351 large rounded red patch of *H. erato* and *H. melpomene*, to the dislocated, slender patches *H*. 352 telesiphe. H. timareta is intermediate on this axis with the Escalera population closer to H. 353 melpomene and H. erato. H. telesiphe is sitting in a quite distant position overall, because it 354 355 exhibits a small additional subapical red patch on the forewing, missing in all other species. Nevertheless, the Alto Mayo population of *H. timareta* is the closest population to *H.* 356 357 *telesiphe* in the colour pattern space because of its somewhat dislocated, zigzagging red patch shape, reminiscent of the main red patch of *H. telesiphe*. 358

359

On the hindwing, all four species display a yellow/white pattern barring both sides of the hindwing longitudinally. The first PC shows variation in the width of the bar. *H. melpomene* and *H. erato* have a rather wide and long yellow bar while *H. telesiphe* has a thinner and shorter bar (Fig.2b). Again, *H. timareta* appears in intermediate position along this direction of variation, with the Escalera population having a wide yellow bar like *H. melpomene*, while Alto Mayo populations show a thinner bar like *H. telesiphe*. The second PC shows a continuous gradient from a long and pointed bar seen in *H. erato* to the thin bar characteristic
of *H. timareta* from the Alto Mayo, straight and wide in the proximal part and curved
posteriorly in the distal part. *H. melpomene* and *H. timareta* from the Escalera sit in an
intermediate position.

370

Results were consistent on the ventral colour pattern (Fig.S2) and whether males and femaleswere treated together or separately (Fig.S3).

373

374 Small elements of pattern (Fig.S1)

All specimens of *H. telesiphe* exhibit bright and large red spots on the ventral hindwing and a 375 well-marked red line on the ventral forewing (index=5). By contrast, H. erato nearly never 376 displays red dots or lines except in a few specimens (4-18%, depending on population) that 377 378 possess small faded spots or a slight line (index=0-1). Along this gradient between H. erato and *H. telesiphe*, most *H. melpomene* samples are closer to the *H. erato* phenotype, with no 379 red line (89-100% depending on the population) and small faded spots (89-96%). Then, 45 to 380 381 47% of *H. timareta* samples from the Escalera exhibit small red spots (index=2-3) and no or a tiny red line (index=0-3), making them closer to the *H. erato* and *H. melpomene* phenotypes. 382 On the contrary, a well-marked red line and large red dots (index=4-5) are observed in more 383 384 than 93% of *H. timareta* samples from the Alto Mayo (vs. only 53-55% from the Escalera population). 385

386

#### 387 Wing venation and outline

For forewing shape, results were congruent whether described by venation or by wing outline. For both venation and outline, the first PCs display continuous variations from the rather rounded forewings (Fig.3ac) of *H. melpomene*, shared by *H. timareta* from the Escalera, towards the thinner and more elongated wings of *H. telesiphe*. By contrast, *H. timareta* from
the Alto Mayo display resemblance to *H. telesiphe* through its more elongated wing shape. In
the venation analysis, which takes into account the discal cell and the distal veins, the second
PC recapitulates phylogenetic relationships, separating *H. erato* and *H. telesiphe* from *H. melpomene* and *H. timareta*.

396

For the hindwing shape, the first PCs of both the venation and outline analysis are mostly associated with sexual dimorphism (presence/absence of male androconia), but the second PCs display the same variation as the forewing, from the rounded wings of *H. melpomene* to the thin, elongated wings of *H. telesiphe* (Fig.3bd). Both *H. timareta* populations are in intermediate position.

402

#### 403 Colour spectra

For both forewing and hindwing, the reflectance of colour patches also follows a continuum 404 between the four species from H. erato and H. melpomene to H. telesiphe (Fig.4). The two 405 populations of *H. timareta* occupy different positions along this continuum in the colour 406 407 space. On the one hand, the Escalera population displays similar colours to H. melpomene and *H. erato* (red-orange and bright yellow), with which it overlaps in the three-dimensional 408 409 colour space (Fig.S6, Table.S6). On the other hand, the position of *H. timareta* from the Alto Mayo comes closer to *H. telesiphe* for the red forewing patches (with a deeper red) and, to a 410 lesser extent, for the whitish hindwing patch. 411

412

It is notable that colours are generally lighter on the ventral side than on the dorsal side and
exhibit more intra- and inter-specific variability. On the ventral side, both red and yellow
patches of *H. timareta* and *H. telesiphe* reflect more short-wavelengths and UV than *H. erato*

and *H. melpomene*. The higher variability in colour on the ventral side is also shown by the
analyses of perceptual distances, since discrimination was higher for the ventral side than for
the dorsal side. On the ventral side, visual contrast within species was smaller than between
species, for both red and yellow and reaches 2 to 6 JND (Fig.5ij). On the dorsal side, within
species and between species visual distances spanned a similar range (2 JND, Fig.5gh), except
between *H. telesiphe* and all others for the white dorsal hindwing patch.

422

#### 423 Similarities in wing phenotype in the postman mimetic community

For forewing and hindwing colour patterns, forewing shape and hue of all patches, 424 425 resemblance indicators suggest that the Escalera population of *H. timareta* is more similar to 426 the most abundant local co-mimics of the Escalera (H. erato, H. melpomene) than the Alto Mayo population is. Euclidian and perceptual distances as well as Mahalanobis distances 427 between H. timareta and H. erato or H. melpomene are smaller for the Escalera population 428 than for the Alto Mayo population (Table.S7, Fig.5). The reverse was found for distances with 429 *H. telesiphe*, suggesting that the Alto Mayo population of *H. timareta* is more similar to *H.* 430 telesiphe than the Escalera population is. Those results are supported by the differences in 431 cross-classification rates from the discriminant analysis (Table.S8). Results were consistent 432 433 for males and females taken independently and together.

434

For hindwing shape, similarity estimates do not show any consistent trend differentiating thetwo geographic populations of *H. timareta*.

437

#### 438 Genetic divergence and population structure

439 No genetic differentiation was found between the two geographic populations of *H. timareta*.
440 The F<sub>ST</sub> value over all loci was estimated at 0.025 and the two populations do not split into

different genetic clusters. For *H. melpomene*, no genetic differentiation was found either,  $F_{ST}$ reaches only 0.009 between the four populations and they do not display any genetic clustering.

444

Genetic differentiation between *H. melpomene* and *H. timareta* was significant in the two geographic areas and of similar magnitude (A:  $F_{ST}$ =0.148; E:  $F_{ST}$ =0.157).

447

#### 448 Hybrid detection

The level of admixture follows the expected trend, with slightly higher rates in the Escalera, where *H. timareta* and *H. melpomene* populations show more altitudinal overlap and are the most phenotypically similar. However, this difference was not significant, possibly due to our limited samples sizes (test of equal proportions: p=0.67).

453

Bayesian clustering analyses with STRUCTURE detected 4.2% of admixed individuals in the 454 Escalera (Fig.S11, 2 back-crosses to melpomene, 2 back-crosses to timareta and 3 F1 hybrids 455 456 out of 167 specimens), and 3.2% in the Alto Mayo (1 back-cross to melpomene, 1 F1 or back-457 cross to timareta out of 63 specimens). Surprisingly, given that H. timareta does not occur below 1000m, one admixed specimen was detected in the low Escalera population at about 458 700m of elevation. Assignment tests with New Hybrids1.0 found the same admixed 459 individuals except in one case, but also pointed out six more putative back-crosses, raising the 460 proportion of admixed individuals to 8.7% in the Escalera and 6.3% in the Alto Mayo. 461

#### 463 **Discussion**

#### 464 Tracking variations in the local prey environment

465 Our results show that despite an absence of genetic differentiation at neutral markers, 466 geographic populations in one of the species, *H. timareta*, display subtle but consistent 467 variations in wing phenotype, associated with enhanced similarity to locally abundant co-468 mimics. Our results therefore denote a geographic shift in mimicry association consistent with 469 quantitative changes in the composition of the mimicry community.

470

471 Geographic mosaics of co-varying warning pattern are commonly observed in animal clades involved in mimicry associations, from butterflies to frogs (Thompson 2005; Twomey et al. 472 2013). An iconic case is the faithful co-variation of Heliconius melpomene and H. erato 473 throughout their shared range, the two butterflies switching in concert from red postman 474 pattern (in certain Andean valleys, south-eastern Brazil, and Carribean area) to orange-rayed 475 476 Amazonian patterns (Sheppard et al. 1985; Reed et al. 2011). Our results suggest that comimetic species not only exhibit adaptive shifts of colour pattern but can also track one 477 another geographically for more subtle variations within a type of pattern. Quantitative 478 479 variations in pattern and hue appear adaptive and match local specificities of the surrounding communities of co-mimetic prey. Such partial phenotypic divergence represents an 480 intermediate situation between undifferentiated populations and colour pattern races 481 participating in disjoint mimicry assemblages, opening a window on the process by which 482 diversity within species may evolve. This also brings support to the hypothesis that the 483 484 variation in abundance of certain key species can influence the local mimicry optimum in a way that translates into changes in the selected phenotypes of other species, a process which 485 was proposed to contribute to mimicry diversification in the face of selection for resemblance 486 487 (Turner & Mallet 1996).

489 Geographic variations were not found, at our scale, within *H. melpomene amaryllis* nor *H.* erato favorinus, two species which are generally abundant and widespread compared to H. 490 491 timareta. This observation fits the prediction that the commoner species should be less influenced by selection for perfect resemblance in a Müllerian association (Ruxton, Sherratt & 492 Speed 2004; Ruxton et al. 2008). However, in other cases where H. melpomene is not the 493 494 dominant species, such as in the Amazon lowlands, subtle geographic variations are reported where they match the local butterfly communities. For instance, among the orange-rayed 495 Amazonian forms, local populations from the Marañon valley exhibit a partial lack of orange 496 497 rays, which presumably enhance their resemblance to the commonest local species, H. himera (J. Mallet, pers. com.). Similarly, the two parapatric races named H. melpomene aglaope and 498 H. m. malleti represent a geographic continuum of populations whose wing pattern varies 499 500 quantitatively in the width and shape of the yellow forewing patch, matching similar variations in the patterns of local *H. erato* populations and likely other co-mimetic species. 501 502 Together with those examples, our results suggest that coordinated quantitative co-variations of the elements of the warning signal are adaptive and, despite receiving little quantitative 503 attention, reflect the tracking of a geographically changing optimum defined by a community

of multiple mimetic species. 505

506

504

#### The multimodality of traits involved in mimicry 507

Resemblance between populations of *H. timareta* and the local co-mimetic species appear 508 congruent over the different traits measured in our study: colour pattern, hue and wing shape, 509 suggesting a multimodal and coordinated evolution of resemblance. This is consistent with 510 recent studies showing that, although colour might have a greater influence on predator 511

decision, pattern features also play a significant role in signalling (Finkbeiner, Briscoe &Reed 2014).

514

Whether wing shape is also under selection for mimicry is unclear. Wing venation itself 515 cannot presumably be seen by predators but it may be indirectly under selection since wing 516 motion and wing outline are associated with mimicry (Srygley 1994; Jones et al. 2013). Long 517 and narrow forewings (high aspect ratio) are generally associated with fast and extended flight 518 while broader wings may be better suited for slow and more fluttery flights (Betts & Wootton 519 1988), and *H. telesiphe* displays a notably faster and more elusive flight than *H. erato* and *H.* 520 521 *melpomene* (pers. obs.). Flight behaviours may be perceived by bird predators as informative characters for prey identification and may participate in the definition of the local mimicry 522 optimum (Srygley & Ellington 1999), so the similarity in shape of H. timareta to H. telesiphe 523 524 in the Alto Mayo and to *H. melpomene* in the Escalera may represent an adaptive response to selection on flight patterns. 525

526

A striking result from the spectral analysis is that, although both wing sides show a signal of 527 local mimicry adaptation, hue on the dorsal side is less variable (within and between species) 528 than on the ventral side. In butterflies, ventral and dorsal patterns can be genetically correlated 529 as shown by experimental evolution lines of Bicyclus anynana (Beldade, Koops & Brakefield. 530 2002) but also strikingly different, as in Morpho for instance, which exhibit a bright blue 531 dorsal side and a camouflaged ventral side. Pattern differences between the ventral and dorsal 532 sides then generally result from different selective pressures. Here, the lower variance 533 measured on dorsal hue presumably reflects the fact that dorsal patterns in Heliconius are 534 more frequently visible to predators in full light during sunny hours, when the butterflies are 535 flying or basking with wings spread out, translating into stronger natural selection on hue 536

resemblance. By contrast, ventral patterns are exhibited when resting in the shadow or
roosting at night or at dusk or dawn (Mallet 1986; Finkbeiner, Briscoe & Reed 2012), in a
light environment where colour differences are less noticeable than in sunlight (Théry,
Pincebourde & Feer 2008). Inter-specific differences in ventral hue, especially in UV
reflectance, might also be associated with intra-specific social and sexual communication
(Silberglied & Taylor 1978).

543

#### 544 The evolution of accurate mimicry

Most variations in resemblance quantified between co-mimics here are quite subtle. All 545 populations belong to the general "postman" wing pattern but the precision of mimicry is 546 enhanced between taxa from the same locality, as expected if predators select for mimicry 547 refinement. A long-standing debate is whether evolution takes place by small gradual or large 548 549 changes (Punnett 1915; Fisher 1927; Nicholson 1927; Fisher 1930). Mimicry evolution is classically suggested to occur first via a large phenotypic change which allows coarse 550 resemblance, then by a phase of more subtle refinement (Sheppard et al. 1985; Turner 1987; 551 552 Franks & Sherratt 2007; Balogh & Leimar 2010; Leimar & Mallet 2012). Introgression of 553 alleles of the optix locus from H. melpomene, controlling the presence of the red forewing patch, is thought to have been a major step allowing *H. timareta* to join the postman mimicry 554 555 ring (Heliconius Genome Consortium 2012; Pardo-Diaz et al. 2012). Our results describe the second phase of mimicry evolution in which small variations in shape, colour and pattern 556 557 improve the match to the local optimum. Those mimicry improvements may have evolved through the selection of allelic variants at optix and other unlinked loci modifying wing 558 morphology and pattern. In H. melpomene, the dislocated vs. rounded shape variation of the 559 red forewing patch is associated with a quantitative trait locus (QTL) unlinked to optix 560 (Baxter, Johnston & Jiggins 2008). Differential selection towards different mimetic optima 561

determined by changes in the local species communities would then be predicted to affect
such "secondary" mimicry-refining loci which act epistatically with the major mimicry
switches already identified.

565

#### 566 *A role for gene flow?*

The process of gradual evolution is particularly efficient in populations with a rich supply of 567 genetic variation for traits contributing to the warning signal (Ruxton et al. 2008). Apart from 568 mutation and intra-specific migration, gene flow from closely-related species through 569 hybridization may be an important source of variation (Grant & Grant 1994). Hybridization 570 with local species may bring new, locally adapted alleles and our data show that a certain 571 percentage of F1 hybrids and admixed individuals between H. melpomene and H. timareta are 572 found in natural populations. Genome-wide signatures of past and ongoing gene flow are 573 documented between H. melpomene and H. timareta, and introgressed alleles of the red-574 patterning gene optix from H. melpomene determine the general postman wing pattern in H. 575 timareta of Northern Peru (Heliconius Genome Consortium 2012; Pardo-Diaz et al. 2012; 576 Martin et al. 2013). The occurrence and timing of the introgression need to be clarified more 577 578 finely to assess its role in the differentiation we report between the two locations. For instance, one hypothesis is that resemblance may have had more time to evolve in one 579 population if the postman pattern is older there. Similarly, recurrent interspecific gene flow 580 for adaptive alleles at *optix* and other loci readily favoured by selection may participate in the 581 refinement of mimicry in places where *H. melpomene* determines the position of the optimum 582 and overlaps with H. timareta. 583

585 One intriguing result here is that traits which might not be readily perceived by predators 586 (small red spots, costal line, forewing venation) also display a higher similarity between *H*.

*timareta* and *H. melpomene* in the Escalera than in the Alto Mayo. If those traits are indeed subject to weaker selection by predators (cf. the stronger resemblance on dorsal phenotypic traits), their similarity might reflect local gene flow between hybridizing *H. melpomene* and *H. timareta*. Our genetic analysis does not support higher gene flow in the Escalera than in the Alto Mayo, but the resolution is limited by the small number of markers. We suggest it would be worth exploring the contribution to resemblance of inter-specific gene flow in this and similar systems.

594

#### 595 **Conclusion**

Here we showed that subtle variations in wing pattern across short geographic distances allow 596 populations of co-mimics to track gradual changes in the mimicry optimum, which suggest 597 that predators do not generalise widely and that selection is operating to maximise mimicry 598 efficiency even between relatively similar warning signals. This demonstrates that fitness 599 peaks in the morphological (signalling) space are sharp and that the quantitative movements 600 601 of the position of the optimum are of strong adaptive significance for certain species of the community. Effectively, this amounts to H. timareta switching mimetic association and to our 602 knowledge, this is the first quantification of such a mimicry switch in a community context. 603 All traits quantified here display coordinated variations, suggesting multimodal and gradual 604 evolution of multiple traits improving the level of resemblance and describing the second 605 606 phase of colonisation of a new fitness peak. Our study also raises the possibility that the observed geographic pattern of adaptation and the accuracy of mimicry might partly be 607 enhanced by interspecific gene flow providing adaptive alleles readily favoured by selection. 608

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- 619

#### 620 Data accessibility

- 621 Data available from the Dryad Digital Repository: <u>http://dx.doi.org/10.5061/dryad.h4j6c</u>
- 622
- 623 **References**
- Anderson, E.C. & Thompson, A. (2002) A model-based method for identifying species
  hybrids using multilocus genetic data. *Genetics*, 160, 1217-1229.
- Balogh, A.C.V., Gamberale-Stille, G., Tullberg, B. S. & Leimar, O. (2010) Feature Theory
  and the Two-step Hypothesis of Müllerian Mimicry Evolution. *Evolution*, 64, 810-822.
- Baxter, S.W., Johnston, S.E. & Jiggins, C.D. (2008) Butterfly speciation and the distribution
   of gene effect sizes fixed during adaptation. *Heredity*, 1-9.
- Baylac, M. (2012) Rmorph: a R geometric and multivariate morphometrics library. *Available from the author: baylac@mnhn.fr.*
- Baylac, M. & Friess, M. (2005) Fourier descriptors, procrustes superimposition, and data
  dimensionality: an example of cranial shape analysis in modern human populations.
  In: Slice DE, ed. Modern morphometrics in physical anthropology,part 1 theory and
  methods. New York, NY: Kluwer Academic/Plenum Publishers, 142-165.
- Beatty, C.D., Beirinckx, K. & Sherratt, T.N. (2004) The evolution of mullerian mimicry in
   multispecies communities. *Nature*, 431, 63-67.
- Beldade, P., Koops, K. & Brakefield., P. (2002) Developmental constraints versus flexibility
   in morphological evolution. *Nature*, 416, 844-847.
- Belkhir, K., Borsa, P., Chikhi, L., Raufatse, N. & Bonhomme, F. (1996-2004) GENETIX 4.05,
  logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome,
  Populations, Interactions, CNRS UMR 5171, Université de Montpellier II,
  Montpellier (France).

- Betts, C.R. & Wootton, R.J. (1988) Wing shape and flight behaviour in butterflies
  (Lepidoptera: Papilionoidea and Hesperioidea): a preliminary analysis. *Journal of Experimental Biology*, 138(1), 271-288.
- Bookstein, F. (1991) Morphometrics tools for landmark data: geometry and biology. New
  York, NY: Cambridge University Press.
- Chouteau, M. & Angers, B. (2011) The Role of Predators in Maintaining the Geographic
   Organization of Aposematic Signals. *American Naturalist*, **178**, 810-817.
- Chouteau, M., Arias, M. & Joron, M. (2016) Warning signals are under positive frequency dependent selection in nature. *Proceedings of the national Academy of Sciences*, 113, 2164-2169.
- Endler, J.A. & Mielke, P.W. (2005) Comparing entire colour patterns as birds see them.
   *Biological Journal of the Linnean Society*, 86, 405-431.
- Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals
  using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14, 26112620.
- Evin, A., Cucchi, T., Cardini, A., Vidarsdottir, U.S., Larson, G. & Dobney, K. (2013) The long
  and winding road: identifying pig domestication through molar size and shape. *Journal of Archaeological Science*, 40, 735-743.
- Finkbeiner, S.D., Briscoe, A.D. & Reed, R.D. (2012) The benefit of being a social butterfly:
   communal roosting deters predation. *Proceedings of the Royal Society B-Biological Sciences*, 279, 2769-2776.
- Finkbeiner, S.D., Briscoe, A.D. & Reed, R.D. (2014) Warning signals are seductive: Relative
  contributions of color and pattern to predator avoidance and mate attraction in
  Heliconius butterflies. *Evolution*, 68, 3410-3420.
- Fisher, R. (1930) The genetical theory of natural selection. A complete variorum edition.
   Oxford Univ. Press, Oxford, UK.
- Fisher, R.A. (1927) On some objections to mimicry theory; statistical and genetic. *Trans. R. Entomol. Soc.*, **75**, 269-278.
- Franks, D.W. & Sherratt, T.N. (2007) The evolution of multicomponent mimicry. *Journal of Theoretical Biology*, 244, 631-639.
- 674 Gomez, D. (2006) AVICOL, a program to analyse spectrometric data. Last update october
  675 2011. Free executable available at <u>http://sites.google.com/site/avicolprogram/</u> or from
  676 the author at dodogomez@yahoo.fr.
- Grant, P.R. & Grant, B.R. (1994) Phenotypic and genetic effects of hybridization in Darwin's
   finches. *Evolution*, 48, 297-316.
- Hart, N.S. (2002) Vision in the peafowl (Aves : *Pavo cristatus*). *Journal of Experimental Biology*, 205, 3925-3935.
- Heliconius Genome Consortium (2012) Butterfly genome reveals promiscuous exchange of
   mimicry adaptations among species. *Nature*, **487**, 94-98.
- Hines, H.M., Counterman, B.A., Papa, R., de Moura, P.A., Cardoso, M.Z., Linares, M.,
  Mallet, J., Reed, R.D., Jiggins, C.D., Kronforst, M.R. & McMillan, W.O. (2011) Wing
  patterning gene redefines the mimetic history of Heliconius butterflies. *Proceedings of the National Academy of Sciences, USA*, 108, 19666-19671.
- Ihalainen, E., Lindstrom, L., Mappes, J. & Puolakkainen, S. (2008) Can experienced birds
   select for Mullerian mimicry? *Behavioral Ecology*, **19**, 362-368.
- Ihalainen, E., Rowland, H.M., Speed, M.P., Ruxton, G.D. & Mappes, J. (2012) Prey
   community structure affects how predators select for Mullerian mimicry. *Proceedings of the Royal Society B-Biological Sciences*, 279, 2099-2105.
- Jones, R., Poul, Y.L., Whibley, A., Mérot, C., FFrench-Constant, R. & Joron, M. (2013) Wing
   shape variation associated with mimicry in butterflies. *Evolution*, 67(8), 2323-2334.

- Joron, M. & Iwasa, Y. (2005) The evolution of a Mullerian mimic in a spatially distributed
   community. *Journal of Theoretical Biology*, 237, 87-103.
- Kapan, D.D. (2001) Three-butterfly system provides a field test of müllerian mimicry. *Nature*,
   409, 338-340.
- Kovarovic, K., Aiello, L.C., Cardini, A., Lockwood, C.A., (2011) Discriminant function
   analyses in archaeology: Are classification rates too good to be true? . *Journal of Archaeological Science*, 38.
- Langham, G.M. (2004) Specialized avian predators repeatedly attack novel color morphs of
   Heliconius butterflies. *Evolution*, 58, 2783-2787.
- Le Poul, Y., Whibley, A., Chouteau, M., Prunier, F., Llaurens, V. & Joron, M. (2014)
   Evolution of dominance mechanisms at a butterfly mimicry supergene. . *Nature Communications*, 5.
- Leimar, O., Tullberg, B. S. & Mallet, J. (2012) The Adaptive Landscape in Evolutionary
   Biology
- Mallet, J. (1986) Gregarious roosting and home range in *Heliconius* butterflies. *National Geographic Research*, 2, 198-215.
- Mallet, J. (1999) Causes and consequences of a lack of coevolution in mullerian mimicry.
   *Evolutionary Ecology*, 13, 777-806.
- Mallet, J. & Barton, N.H. (1989) Strong Natural-Selection in a Warning-Color Hybrid Zone.
   *Evolution*, 43, 421-431.
- Mallet, J. & Joron, M. (1999) Evolution of diversity in warning color and mimicry:
   Polymorphisms, shifting balance, and speciation. *Annual Review of Ecology and Systematics*, **30**, 201-233.
- Martin, S.H., Dasmahapatra, K.K., Nadeau, N.J., Salazar, C., Walters, J.R., Simpson, F.,
  Blaxter, M., Manica, A., Mallet, J. & Jiggins, C.D. (2013) Genome-wide evidence for
  speciation with gene flow in *Heliconius* butterflies. *Genome Research*, 23, 1817-1828.
- Mérot, C., LePoul, Y., Théry, M., Joron, M. 2016. Data from: Mimicry refinement:
   Phenotypic variations tracking the local optimum. Dryad Digital Repository.
   doi:10.5061/dryad.h4j6c
- Mérot, C., Frérot, B., Leppik, E. & Joron, M. (2015) Beyond magic traits: Multimodal mating
   cues in *Heliconius* butterflies. *Evolution*, 69, 2891-2904.
- Mérot, C., Mavarez, J., Evin, A., Dasmahapatra, K.K., Mallet, J., Lamas, G. & Joron, M.
  (2013) Genetic differentiation without mimicry shift in a pair of hybridizing *Heliconius* species (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society*, 109, 830-847.
- Merrill, R.M., Wallbank, R.W.R., Bull, V., Salazar, P.C.A., Mallet, J., Stevens, M. & Jiggins,
   C.D. (2012) Disruptive ecological selection on a mating cue. *Proceedings of the Royal Society B-Biological Sciences*, 279, 4907-4913.
- Müller, F. (1879) *Ituna* and *Thyridia*: A remarkable case of mimicry in butterflies.
   *Transactions of the entomological society. London.*
- Neto, J.C., Meyer, G. E., Jones, D. D. & Samal, A.K. (2006) Plant species identification
  using Elliptic Fourier leaf shape analysis.*Computers and Electronics in Agriculture*,
  50, 121-134.
- Nicholson, A.J. (1927) A new theory of mimicry in insects. *Australian zoologist*, 5, 10-104.
- Pardo-Diaz, C., Salazar, C., Baxter, S.W., Merot, C., Figueiredo-Ready, W., Joron, M.,
  McMillan, W.O. & Jiggins, C.D. (2012) Adaptive Introgression across Species
  Boundaries in *Heliconius* Butterflies. *PLOS Genetics*, 8, 13.
- Penney, H.D., Hassall, C., Skevington, J.H., Abbott, K.R. & Sherratt, T.N. (2012) A
  comparative analysis of the evolution of imperfect mimicry. *Nature*, 483, 461-U110.

- Pritchard, J.K., Stevens, M. & Donnelly, P. (2000) Inference of population structure using
   multilocus genotype data. *Genetics*, 155, 945-959.
- 745 Punnett, R.C. (1915) *Mimicry in butterflies*. Cambridge Univ. Press, Cambridge.
- R Core Team (2014) R: A language and environment for statistical computing. R Foundation
   for Statistical Computing, Vienna, Austria. URL <u>http://www.R-project.org/</u>.
- Reed, R.D., Papa, R., Martin, A., Hines, H.M., Counterman, B.A., Pardo-Diaz, C., Jiggins,
  C.D., Chamberlain, N.L., Kronforst, M.R., Chen, R., Halder, G., Nijhout, H.F. &
  McMillan, W.O. (2011) *Optix* drives the repeated convergent evolution of butterfly
  wing pattern mimicry. *Science 333: 1137-1141*.
- Rohlf, F. (2010) *TPSDig 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY.*
- Rowe, C., Lindstrom, L. & Lyytinen, A. (2004) The importance of pattern similarity between
   Mullerian mimics in predator avoidance learning. *Proceedings of the Royal Society B- Biological Sciences*, 271, 407-413.
- Ruxton, G.D., Franks, D.W., Balogh, A.C.V. & Leimar, O. (2008) Evolutionary Implications
   of the Form of Predator Generalization for Aposematic Signals and Mimicry in Prey.
   *Evolution*, 62, 2913-2921.
- Ruxton, G.D., Sherratt, T.N. & Speed, M.P. (2004) Avoiding attack: the evolutionary ecology
   of crypsis, warning signals and mimicry. *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry.*, pp. i-xii, 1-249.
- Sheppard, P.M., Turner, J.R.G., Brown, K.S., Benson, W.W. & Singer, M.C. (1985) Genetics
   and the evolution of muellerian mimicry in *Heliconius* butterflies. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **308**, 433-613.
- Silberglied, R.E. & Taylor, O.R. (1978) Ultraviolet reflection and its behavioral role in
   courtship of sulfur butterflies *Colias eurytheme* and *C. philodice* (Lepidoptera,
   Pieridae). *Behavioral Ecology and Sociobiology*, 3, 203-243.
- Srygley, R.B. (1994) Locomotor mimicry in butterflies? The associations of positions of
   centers of mass among groups of mimetic, unprofitable prey. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 343, 145-155.
- Srygley, R.B. & Ellington, C.P. (1999) Discrimination of flying mimetic, passion-vine
   butterflies Heliconius. *Proceedings of the Royal Society B-Biological Sciences*, 266,
   2137-2140.
- Théry, M., Pincebourde, S. & Feer, F. (2008) Dusk light environment optimizes visual
   perception of conspecifics in a crepuscular horned beetle. *Behavioral Ecology*, 19, 627-634.
- Thompson, J.N. (2005) *The geographic mosaic of coevolution*. University of Chicago Press.

Turner, J.R. (1987) The evolutionary dynamics of batesian and muellerian mimicry:
 similarities and differences. pp. 81-95. Wiley Online Library.

- Turner, J.R. & Mallet, J.L. (1996) Did forest islands drive the diversity of warningly coloured
   butterflies? Biotic drift and the shifting balance. . *Philosophical Transactions of the Royal Society B-Biological Sciences*, **351**, 835-845.
- Turner, J.R.G. (1977) Butterfly mimicry the genetical evolution of an adaptation. .
   *Evolutionary Biology*, 10, 163-206.
- Twomey, E., Yeager, J., Brown, J.L., Morales, V., Cummings, M. & Summers, K. (2013)
   Phenotypic and Genetic Divergence among Poison Frog Populations in a Mimetic
   Radiation. *Plos One*, 8.
- Vorobyev, M. & Osorio, D. (1998) Receptor noise as a determinant of colour thresholds.
   *Proceedings of the Royal Society B-Biological Sciences*, 265, 351-358.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. (2004) Geometric Morphometrics
   for Biologists. A Primer. Elsevier Academic Press, San Diego.

#### 795 **Figure legends**

# Figure.1 Distribution and phenotype of the four species belonging to the postman mimicry ringin Northern Peru.

(a) Overview of the phylogenetic relationship between the four co-mimics (left: dorsal side, right:
ventral side). Note that this phylogeny is simplified to present the four taxa of interest and the postman
pattern is not shared through common ancestry. (b) Map of the study area with an example of phenotypic variation observed in *H. timareta* (T) between the two regions (Escalera and Alto Mayo). (c)
Abundance of each species at different localities (AV=Aguas Verdes; Se=Serranoyacu; Af=Afluente;
V=Venceremos; Sh=Shilcayo; RV=Rancho Vista; U=Urahuasha; T=Túnel; An=Antena, Table.S1)
sorted by elevation, corrected by sampling effort.

805

#### 806 Figure.2 Variation in colour pattern between and within species of the postman mimicry ring.

807 PCA based on colour pattern of the dorsal side and visualisation of the variations in the morphospace.

808 (a-b:FW, c-d:HW). Open dots are females and solid dots are males.

809

#### Figure.3 Variation in wing shape between and within species of the postman mimicry ring.

PCA based on wing venation (**a**:FW, **b**:HW) and wing outline (**c**:FW, **d**:HW). Variation in shape along the axis is represented with the red solid lines at the positive part of the axis and blue dotted lines at the negative value of the axis. Open dots are females and solid dots are males.

814

#### Figure.4 Colour variation between and within species of the postman mimicry ring.

PCA based on the excitation of the four photoreceptors in the physiological model of Endler & Mielke. The spot on each wing corresponds to the area where the reflectance measurement was taken
(a:FWd, b:HWd, c:FWv, d:HWv).

819

# Figure.5 Pairwise phenotypic distances between groups (co-mimetic species and geographic populations of *H. timareta*)

- 822 Euclidian distances in each PCA morphospace (a-f). Perceptual distances in the physiological model
- 823 of Vorobyev & Osario (g-j). Coloured boxes describe phenotypic distances between a given co-mimic
- 824 (E= *H. erato*, M= *H. melpomene*, Te= *H. telesiphe*) and each population of *H. timareta* (dark blue:
- Alto Mayo T(A) light blue: Escalera T(E)). Statistical differences between pairwise distances to a
- given mimic were tested using a mixed model with the geographic population as factor (A vs. E) and
- identity of the compared specimen as random factors (\*\*\*: $P \le 0.001$ , \*\*: $P \le 0.01$ , \*: $P \le 0.05$ , ns: $P \ge 0.05$ ).
- 828 White boxes provide distances between the remaining groups.

Table.1 MANOVA on phenotypic variation and mis-classification rate from the linear discriminant analysis between geographical populations of *H. timareta* (E vs. A)

	001 11				· /	
						mis-classification
		dF	F	Pillai	Р	(LDA)
	dorsal red FW	1,132	13.5	0.24	< 0.001	28%
Colore en ester	ventral red FW	1,133	8.9	0.17	< 0.001	39%
Colour spectra	dorsal yellow HW	1,134	8.7	0.17	< 0.001	29%
	ventral yellow HW	1,135	7.9	0.15	< 0.001	34%
	dorsal red patch FW	1,136	16.5	0.66	< 0.001	13%
Colour pattern	dorsal yellow bar HW	1,137	18.9	0.70	< 0.001	11%
	FW	1,138	7.1	0.68	< 0.001	30%
Wing venation	HW	1,139	7.0	0.69	< 0.001	27%
	FW	1,133	5.7	0.29	< 0.001	23%
Wing outline	HW	1,120	5.3	0.30	< 0.001	21%











# Mimicry refinement: Phenotypic variation tracking the local optimum

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# **Supplementary materials**

### Multilocus microsatellite analysis (Mérot et al, 2013)

Multilocus genotypes were derived by examining variation at eleven microsatellite loci developed for *Heliconius*, using primers and PCR conditions adapted from (Flanagan *et al.*, 2002) and (Mavarez & Gonzalez, 2006). A preliminary set of specimens of 59 *H. m. amaryllis* and 28 *H. t. thelxinoe* was analysed using GeneMapper with the Genescan Rox-500 size standard for allele size determination (Applied Biosystems). A secondary set of specimens of 68 *H. m. amaryllis* and 128 *H. t. thelxinoe*, including one putative hybrid and four reference individuals per species from the first set, was analysed using GeneMarker 2.2.0 with the Genescan-500Liz size standard. Linkage disequilibrium and departure from Hardy-Weinberg within each population within each species were tested using exact tests implemented in GENEPOP 4.1.4 (Rousset, 2008). We used FSTAT 2.9.3 (Goudet, 2001) to survey within-species genetic diversity in terms of expected heterozygosity ( $H_E$ ), observed heterezygosity ( $H_O$ ) and allelic richness (A), estimated on the smallest sample size per locus per population (N=22). Allelic frequency and *F*-statistics (Weir & Cockerham, 1984) were calculated using GENETIX 4.05 (Belkhir *et al.*, 1996-2004).

### Colour pattern modelling (Le Poul et al, 2014)

*Normalized photographs.* First, for each specimen, both sides of each forewing and hindwing were photographed in normalized light conditions (CIE Standard Illuminant D50), with a high colour rendering light source (Philips Master TL-D 90 Graphica pro). A scale indicator was included in each picture, and the white balance was normalized. A Nikon D90 digital camera with a Nikon micro 105/2.8G ED VR lens was used to capture high resolution images with accurate colour rendering.

*Wing extraction.* The first step of CPM relies in identifying and extracting outline of wings on pictures. Wings were automatically detected in the images using their colour difference with the homogenous white background, and were then precisely extracted using the marker-based watershed transformation (Meyer & Beucher, 1990) along the image colour gradient (Meyer, 1992). This segmentation method finds the maximum intensity of the colour transition between the marked wings and the marked background, which was then considered to be the wing outline.

*Colour number reduction and colour attribution.* Then, the pattern was modelled by considering explicitly the mosaic distribution of colour across the wings, which allows describing efficiently the variation in patches boundaries. A set of discrete colours characteristic to each wing was first identified using an algorithm based on colour histograms (Cheng & Sun, 2000; Kurugollu *et al,* 2001). After simplifying the spatial structures of wing images (Nikolaev & Nicolayev, 2004), pixels were then attributed to each colour (black, red and yellow) using a simple threshold.

Colours classes were extracted from the histogram by the following procedure. First, to smooth the colour distribution on the histogram and simplify colour histogram processing, the image was projected from the 3-dimensional RGB colour space to a 2-dimensional (2D) colour space, where

dimension 1 corresponds to luminance (the Y component in the Ycrbr colour space), and dimension 2 to the major colour variation axis using all wing images. This projection preserved about 97% of colour variance in the images. A 2D-histogram, representing the distribution of pixel colour, was computed in the same 2D-projected colour space for all wings. Each separate colour on the wing was defined as a local maximum on the 2D-histogram. These local maxima are always numerous because of the complexity of the natural image. To prevent over-segmentation, minor peaks were automatically removed by consideration of their proximity to and separation from neighbouring peaks. We performed a watershed transformation on the additive inverse of the 2D-histogram to partition the colour space among the major peaks (Shafarenko *et al*, 1998). At the end of this process, each wing could be associated with a set of characteristic colour partitioning the colour space and accounting for the colour variation actually present on the wing RGB image.

In order to preserve the patch structure of colour patterns, we also performed a routine to merge neighbouring pixels of homogenously-coloured regions in the images (Nikolaev & Nicolayev, 2004). The scale indicator within each image was first used to rescale images to an output length of around 512 pixels, leading to a mean spatial resolution of 10.9px/mm. Each reduced image was then transformed into a mosaic of homogeneously-coloured spatial zones. A watershed transformation of the image colour gradient was used to carry out the mosaicking (Meyer, 1992). Each homogenously-coloured region was then attributed a colour according to the classification given by the segmented histogram.

Finally, the attribution to the different colours (black, red, and yellow) was done automatically using a threshold on RGB values, followed by manual checking to correct errors, which were mostly due to minor damage to parts of the wings, resulting in the final segmented image.

*Alignment*. For the wing images to be efficiently comparable pixel by pixel, a proper homology of pixel positions was needed. This match was obtain by transforming each set of processed images into a common coordinate system which maximize similarity between each wing pattern and wing outline to a wing model (*i.e.* the 'mean' of all individuals), treating each wing surface separately. Similarity was measured by the Mattes implementation of mutual information metric (Thévenaz & Unser, 2000; Mattes *et al*, 2001; Mattes *et al*, 2003), which is minimal when colour patches and outlines are aligned in an optimal compromise. The one+one evolutionary optimizer (Styner & Gerig, 1997; Styner *et al*, 2000), implemented within the ITK free image proceeding library in C++ (Yoo *et al*, 2002; Ibañez, 2003; Martin & Hoffman, 2003); , was used to find the scale, rotation and translation parameter set that minimized this mutual information value. This procedure created an initial registration set based on wing shape, which allowed generation of the wing pattern model. Each wing was then recursively aligned to the model, until the variance of the metric stabilized (variance varying less 1% (Rohlf & Slice, 1990). At the completion of this process, all wings could be considered to be positioned in the same physical space, with pixel locations and colour values among wings being comparable among all individuals.

## Table S2. Number of specimen used in each analysis

	Density estimates	Density	Scored for genotype				Score	d for phe	notype			
		(butterflies/day of collection)	(microsatellites)	Band/ spots	Venation	Outline (FW)	Outline (HW)	Pattern (FW v)	Pattern (FW d)	Pattern (HW v)	Pattern (HW d)	Reflectance
Alto Mayo	180 (25 days)		59	200	151	118	105	121	124	109	112	118
H. timareta	83	3.3	38	71	54	42	31	42	44	34	36	43
H. melpomene	54	2.2	20	60	39	27	25	28	28	25	25	31
H. erato	0	0.0		5	5	5	5	5	5	5	5	5
H. telesiphe H. melpomene	43	1.7		63	53	44	44	46	47	45	46	39
x H. timareta ?			1									
Escalera	473 (83 days)		172	324	148	187	172	195	195	190	191	98
H. timareta	203	2.4	115	167	70	91	89	97	97	98	97	73
H. melpomene	175	2.1	54	118	67	61	55	64	63	62	63	16
H. erato	93	1.1		34	10	33	26	32	33	28	29	8
H. telesiphe H. melpomene	2	0.0		2	1	2	2	2	2	2	2	1
x H. timareta ?			3									
Escalera_low	517 (65 days)		32	163	78	128	119	132	132	121	124	44
H. melpomene	293	4.5	32	103	55	77	72	81	80	73	73	22
H. erato	224	3.4		60	23	51	47	51	52	48	51	22
Moyobamba			11	54	37	47	44	50	48	45	45	22
H. melpomene			11	35	25	30	29	32	30	30	30	16
H. erato				19	12	17	15	18	18	15	15	6
Raised												
H. melpomene x H. timareta			11	11								9
Total			285	752	414	480	440	498	499	465	472	291

#### Table S3: Genetic polymorphism of the studied sample.

Allelic richness (A) is estimated for the smallest population (N=22).  $H_0$  represents the observed heterozygosity and  $H_E$ , the expected heterozygosity. Significant deviations from Hardy-Weinberg expectations are indicated by asterisks (P < 0.05 \*; P < 0.01 \*\*). Loci come from Flanagan et al (2002)[1], and Mavarez et al (2006)[2].

					H. timareta	a thelxinoe (Alto M	ayo)					H. tir	nareta thelxii	noe (Esca	alera)		
		GenBank	No. of	No. of	Allelic		•	HW		No. of	No. of	Allelic	Size range		HW		
Locus	Primer sequence (5'-3')	accession no.	sample	alleles	Richness	Size range (bp)	Ho	DEFICIT	He	sample	alleles	Richness	(bp)	Ho	DEFICIT	He	
Hel2 (1)	TCAAAATGTTGCAGACCGAG	AF481467	40	4	3.6	186-202	0.20	**	0.52	113	5	4.3	196-202	0.50	**	0.58	
	TGCACTTCATTGTAAGGCGT																
Hel4 (1)	CGTTGCCGCTTATACTTTCC	AF481469	40	7	6	268-282	0.34	**	0.62	109	10	6.8	262-302	0.37	**	0.77	
	GGAACGGAGTGCCCTAAAAC																
Hel5 (1)	TGCTGTCCATACCCAACTCA	AF81470	40	3	2.6	314-330	0.24		0.32	115	5	2.9	314-330	0.21	**	0.22	
	CGAACTCACAACCATCAGTCA																
Hm02 (2)	TATTTGCACGATGGAAACCC	DQ020073	40	6	5.5	180-200	0.54	**	0.75	112	11	7.2	180-200	0.71	**	0.72	
	GCGAGGTGGAGACAAAAGAC																
Hm03 (2)	GACGTCACAGCGGGGAAC	DQ020074	40	9	7.5	292-352	0.49	*	0.65	110	10	6.8	312-348	0.59	*	0.72	
	AGAGGGGAACGGAGTGTCAT																
Hm04 (2)	CCTGGCTTATCTACGACGACA	DQ020075	40	10	8	410-458	0.66	**	0.79	111	7	5	406-452	0.49		0.60	
	ATGCAGCTTACTCGCTGGTT																
Hm05 (2)	GCGGTAAGGTAAAACCGTGA	DQ020076	40	8	7.2	284-302	0.76		0.71	112	12	8.3	274-300	0.71	**	0.82	
	CAGAAGAAAATGGTTGGATGG																
Hm06 (2)	AAATAGTGTGCGGCGGAATA	DQ020077	40	4	4	240-246	0.49	**	0.73	111	5	4.9	238-246	0.59	**	0.74	
	TGGAGTAGAAATGCGGGTTTA	-															
Hm13 (2)	TCACTAGTTTTCGGCTTATCG	DQ020083	40	6	5.9	168-188	0.78		0.82	112	9	6.5	168-190	0.65	**	0.77	
	AAGGCTAAATGATGCCTAAAG	-															
Hm19 (2)	CGCTAATTCAAAGGAAAGAGGA	DO020088	40	5	4	182-216	0.22		0.25	110	9	5	182-216	0.14	**	0.32	
- ~-/	AGTGCTGTCATGGCTAACGA	<b>C</b>	-														
Hm22 (2)	CCTCGTCCAAACTCCAAAAC	D0020092	40	3	2.8	252-256	0.51		0.53	113	6	3.5	252-264	0.54		0.53	
	AACAATGTCACAACCATCGC	- 20-0072		U		202 200	0.01		0.00		0	0.0	202 201	0.0		0.00	

					H. melpome	<i>ne amaryllis</i> (Alto I	Mayo)					H. melj	pomene amar	yllis (Esc	calera)	
		GenBank	No. of	No. of	Allelic			HW		No. of	No. of	Allelic	Size range		HW	
Locus	Primer sequence (5'-3')	accession no.	sample	alleles	Richness	Size range (bp)	Ho	DEFICIT	He	sample	alleles	Richness	(bp)	Но	DEFICIT	He
Hel2 (1)	TCAAAATGTTGCAGACCGAG	AF481467	22	5	5	184-192	0.55	**	0.65	53	6	5	184-192	0.54	*	0.63
	TGCACTTCATTGTAAGGCGT															
Hel4 (1)	CGTTGCCGCTTATACTTTCC	AF481469	22	12	12	236-312	0.46	**	0.88	50	22	14.7	236-322	0.29	**	0.90
	GGAACGGAGTGCCCTAAAAC															
Hel5 (1)	TGCTGTCCATACCCAACTCA	AF81470	22	11	11	310-358	0.82		0.85	53	21	14.9	296-358	0.77	*	0.90
	CGAACTCACAACCATCAGTCA															
Hm02 (2)	TATTTGCACGATGGAAACCC	DQ020073	22	7	7	184-200	0.46	**	0.64	53	10	7.3	170-198	0.64	**	0.69
	GCGAGGTGGAGACAAAAGAC															
Hm03 (2)	GACGTCACAGCGGGGAAC	DQ020074	22	7	7	314-352	0.46		0.55	53	111	8.2	302-348	0.56		0.67
	AGAGGGGAACGGAGTGTCAT															
Hm04 (2)	CCTGGCTTATCTACGACGACA	DQ020075	22	11	11	402-444	0.64	*	0.78	53	14	10.9	396-428	0.71	**	0.80
	ATGCAGCTTACTCGCTGGTT															
Hm05 (2)	GCGGTAAGGTAAAACCGTGA	DQ020076	22	13	13	274-320	0.77		0.85	52	14	11.5	274-306	0.75	*	0.87
	CAGAAGAAAATGGTTGGATGG															
Hm06 (2)	AAATAGTGTGCGGCGGAATA	DQ020077	22	4	4	240-246	0.77		0.76	53	6	5.5	238-248	0.64		0.73
	TGGAGTAGAAATGCGGGTTTA															
Hm13 (2)	TCACTAGTTTTCGGCTTATCG	DQ020083	22	12	12	144-186	0.73	*	0.88	53	13	10.2	170-198	0.56	**	0.76
	AAGGCTAAATGATGCCTAAAG															
Hm19 (2)	CGCTAATTCAAAGGAAAGAGGA	DQ020088	22	13	13	154-208	0.64	**	0.85	53	20	14.8	150-210	0.52	**	0.88
	AGTGCTGTCATGGCTAACGA															
Hm22 (2)	CCTCGTCCAAACTCCAAAAC	DQ020092	22	13	13	246-279	0.68	**	0.91	52	15	11.8	246-280	0.67	**	0.85
	AACAATGTCACAACCATCGC															

## Geographic variation in H. melpomene and H. erato

Table	<b>S4.</b>	MANOVA	on	phenotypic	variation	and	mis-classification	rate	from	the	linear
discrir	nina	nt analysis b	oetw	een four geo	graphical ]	popul	ations of <i>H. melpo</i>	mene	("Low	Esca	alera",
"High	Esca	alera", "Alto	Ma	yo", "Moyok	oamba")						

						mis-classification
		dF	F	Pillai	Р	(CVA)
	dorsal red FW	3,95	2.2	0.20	0.02	65%
Calarra area ataa	ventral red FW	3,95	1.9	0.17	0.055	64%
Colour spectra	dorsal yellow HW	3,95	0.6	0.06	0.77	70%
	ventral yellow HW	3,95	1.4	0.13	0.19	71%
	dorsal red patch FW	3,194	1.7	0.36	0.004	58%
Colour pattern	dorsal yellow bar HW	3,198	2.6	0.52	< 0.001	52%
	FW	3,178	1.8	0.43	0.001	60%
Wing venation	HW	3,178	2.7	0.59	< 0.001	53%
	FW	3,193	1.2	0.16	0.27	59%
Wing outline	HW	3,177	1.8	0.26	0.009	59%

Table S5. MANOVA on phenotypic variation and mis-classification rate from the linear discriminant analysis between three geographical populations of *H. erato* ("Low Escalera", "High Escalera", "Moyobamba")

						mis-classification
		dF	F	Pillai	Р	(CVA)
	dorsal red FW	2,34	1.2	0.20	0.33	43%
Calary an astro	ventral red FW	2,34	1.2	0.20	0.32	43%
Colour spectra	dorsal yellow HW	2,34	0.5	0.09	0.80	41%
	ventral yellow HW	2,34	0.3	0.06	0.91	41%
	dorsal red patch FW	2,98	1.3	0.38	0.14	52%
Colour pattern	dorsal yellow bar HW	2,96	1.1	0.33	0.36	54%
	FW	2 34	1 /	0.08	0.18	57%
Wing venation		2,34	1.7	0.70	0.10	5170
ving venution	HW	2,34	1.7	1.09	0.06	60%
Wing outling	FW	2,99	1.0	0.17	0.52	48%
wing outline	HW	2,87	1.1	0.22	0.36	46%

#### Colour pattern analyses



**Figure S1: Distribution of the indices scoring the small ventral elements of pattern between and within species of the postman mimicry ring.** (a) Presence and size of a red line at the base of the costal vein of the ventral forewing. The index ranges from 0 (absence of red line) to 5 (large and well-marked red line). (b) Presence and size of red dots at the base of the ventral hindwing. The index ranges from 0 (no dot) to 5 (presence of four large smudgy spots). 1 to 4 correspond to a gradation between one or two minute dots and more numerous or larger spots.



**Figure S2: Colour pattern variation between and within species of the postman mimicry ring.** Principal component analysis based on colour pattern and visualisation of variation in the morphospace (ventral FW (a-b), ventral HW (c-d))



Figure S3: Wing colour pattern PCA within each sex.

Wing sketches show the pixels involved in the variation of colour pattern associated with each axis. A positive contribution (red) for one colour indicates that an increase in the score is associated with the appearance (presence) of this colour on the phenotype. On the contrary, a negative contribution (blue) is associated with a disappearance of this colour. (a) Red colour on male FW, (b) yellow colour on male HW, (c) red colour on female FW, (d) yellow colour on female HW.

Wing venation



**Figure S4: PCA on wing venation for each sex.** (a) FW for males, (b) HW for males, (c) FW for females, (d) HW for females.

#### **Colour analyses**

The best-documented predators of *Heliconius* include jacamars (Chai & Srygley 1990; Langham 2006), which belong to the order Galbuliformes. Within this order, only *Nystalus maculates* visual system has been studied and would have a tetrachromatic violet-type visual system according to opsin sequence similarity (Odeen & Hastad 2003). However, the guild of bird predators on those butterflies may include other birds, which possibly differ in the spectral sensitivities of their short-wavelength-sensitive cone visual pigments. Therefore, we included two tetrachromatic visual systems in our analysis: a bird with a V-type visual system (Peafowl, *Pavo cristatus*,(Hart 2002)), a bird with a UV-type visual system (Blue tit, *Parus caeruleus*,(Hart *et al.* 2000)). Because the results were not qualitatively different when analysed with different visual systems, we chose to present the results in the V-type vision model. Results in the UV-type visual system displayed consistent results with the V-type

system (Fig. S9). All models were run considering two kinds of incident light: a light environment corresponding to large sunny gaps in a tropical forest, and a light environment corresponding to small light gaps. Those light spectra were measured by M. Théry in primary forest in French Guiana (Théry, Pincebourde & Feer 2008). Results were consistent with both types of light so we only present models with large gap incident light here, which corresponds better to the natural habitat of the species considered here.

Surrounding colours can affect perception and it is usually important to take background colour into account. Here, all patches are large making the colour well-visible. The patches are surrounded by the black part of the wing, for which we analyzed reflectance. Colour contrasts calculated with the model of Vorobyev and Osorio (1998) indicate that differences between and within species were not perceptible for observers (below the usual value of 1 JND), with mean values around 0.5 JND in the large gap conditions and 0.2 JND in the small gap conditions (Fig. S8) Therefore, we focused on the comparison of actual patches' colour between species and populations.



**Figure S5: Mean raw spectra of colour patches for each taxon.** The x-axis represents the wavelength from 300 to 700 nm and the y-axis the reflectance in percentage of the white control. The shadows represent standard deviation.



**Figure S6: Location of wing colours in the tetrahedral chromatic visual space.** Based on a model Endler and Mielke (2005) with a bird V-type visual system (Peafowl) and a large gap light.

# Table S6. Percentage of overlap in the tetrahedral colour space between spectral variation of each species and each population of *H. timareta*.

The last column gives the overlap between the two populations of H. *timareta*. This is based on a physiological model Endler and Mielke (2005) with a bird V-type system (Peafowl) and a large gap light.

	Н. е	erato	H. mel	pomene	H. tel	esiphe	H. timareta
	H. timareta	A vs. E					
	A	E	Α	E	Α	E	
Red FW d	0.22	0.36	0.43	0.58	0.09	0.05	0.58
Red FW v	0.00	0.08	0.08	0.22	0.20	0.11	0.39
Yellow HW d	0.31	0.32	0.25	0.29	0.00	0.00	0.42
Yellow HW v	0.09	0.12	0.26	0.43	0.00	0.00	0.34



**Figure S7: Mean raw spectra of the black area for each taxon.** The x-axis represents the wavelength from 300 to 700 nm and the y-axis the reflectance in percentage of the white control. The shadows represent standard deviation.



Figure S8: Controlling perceptual distances in the black.

Colour perceptual distances for the black part of each wing in the model of Vorobyev & Osorio (1998) expressed in unit of just noticeable differences (JND). Bird V-type system (Peafowl) and a large gap light. Contrast between species blackness is below 1 JND.



Figure S9: Investigating other vision system (UV-type).

Colour perceptual distances in the model of Vorobyev & Osorio (1998) with a bird UV-type visual system (Blue tit) and a large gap light for the red patches (R) and the yellow patches (Y)



Figure S10: Principal component analyses based on the excitation of the four photoreceptors in the physiological model of Endler & Mielke for each colour patch

Bird V-type visual system and large gap light. (a) red patch on male dorsal FW, (b) yellow patch on male dorsal HW, (c) red patch on male ventral FW, (d) yellow patch on male ventral HW, (e) red patch on female dorsal FW, (f) yellow patch on female dorsal HW, (g) red patch on female ventral FW, (h) yellow patch on female ventral HW.

## Distances and cross-validation

		H. erato f	avorinus	H. melpome	ne amaryllis	H. te	lesiphe
		H. timareta	H. timareta	H. timareta	H. timareta	H. timareta	H. timareta
		A	E	A	E	A	E
	Red FW d	$4.0 \pm 1.0$	$1.2\pm0.5$	$2.3{\pm}~0.9$	$0.4 \pm 0.3$	$4.3 \pm 1.1$	$9.1 \pm 1.9$
	Red FW v	$12.4 \pm 2.1$	$7.5 \pm 1.8$	$6.7 \pm 1.7$	$3.1 \pm 1.2$	$2.9{\pm}~0.9$	$5.9 \pm 1.6$
our tra	Yellow HW d	$1.4 {\pm} 0.6$	$0.3 \pm 0.2$	$1.1\!\pm0.6$	$0.3 \pm 0.3$	$53.3 {\pm}~8.3$	$66.5 \pm 10.1$
Colc	Yellow HW v	$4.5 \pm 1.2$	$2.0{\pm}~0.8$	$2.0{\pm}~0.8$	$0.4 \pm 0.3$	$7.2\pm1.2$	$12.0{\pm}~2.2$
In Li	Red patch FW d	$82.6 \pm 11.8$	$64.4 \pm 11.1$	$34.3 \pm 4.0$	$17.4 \pm 2.9$	$561.7 \pm 62.4$	$579.4 \pm 62.4$
Colc	Yellow bar HW d	$25.2 \pm 4.1$	$18.7{\pm}\ 3.7$	$17.5 \pm 3.2$	$11.7 {\pm}~2.8$	$59.4{\pm}~7.6$	$78.7{\pm}~9.2$
g tion	FW venation	$22.6 \pm 2.7$	$21.4\pm2.5$	$15.1 \pm 2.3$	$10.3 \pm 1.9$	$37.2 \pm 4.4$	$42.0\pm4.8$
Win	HW venation	$25.9 \pm 2.9$	$24.6{\pm}~2.8$	$12.5{\pm}~2.0$	$12.1 \pm 1.9$	$34.4{\pm}~3.7$	$35.8 \pm 4.1$
g ine	FW outline	$18.9 {\pm} 4.6$	$16.5\pm3.9$	$13.4\pm3.9$	$12.1\pm3.5$	$32.9\pm7.4$	$36.8\pm8.2$
Win Outl	HW outline	$17.3 \pm 4.6$	$12.2\pm3.5$	$6.9 \pm 2.2$	$6.8 \pm 2.4$	$42.8{\pm}~8.0$	$38.5{\pm}7.5$

Table S7. Mahalanobis distances for each component of the phenotype between	populations	of
<i>H. timareta</i> and their co-mimics (mean of the bootstrap $\pm$ sd)		

		H. timareta A	H. erato	H. telesiphe	H. telesiphe
		H. timareta E	H. melpomene	H. melpomene	H. erato
_	Red FW d	$1.2 \pm 0.5$	$0.7 {\pm} 0.4$	$11.9 \pm 2.8$	15.9± 2.9
ectra	Red FW v	$0.9 {\pm} 0.5$	3.3±1.3	$15.5 \pm 3.2$	$26.1 \pm \ 4.0$
our sp	Yellow HW d	$1.3 {\pm} 0.7$	$0.5 \pm 0.4$	$64.1\!\pm\!10.1$	69.9± 10.3
Colc	Yellow HW v	$1.3 \pm 0.7$	$1.3 \pm 0.7$	$14.1 \pm 2.7$	$22.0\pm~3.2$
ur su	Red patch FW d	9.6±1.7	43.3±9.9	$597.4 \pm 61.4$	$617.3 \pm \ 62.6$
Colc	Yellow bar HW d	$5.0 \pm 1.1$	8.7±2.1	$84.7\!\pm\!10.6$	$88.1 \pm \ 10.9$
g ation	FW shape	$3.1 {\pm} 0.7$	16.6±2.3	$52.9\!\pm\!6.5$	33.2± 4.4
Win venâ	HW shape	$2.8 \pm 0.7$	$17.2 \pm 2.5$	$50.5\pm5.8$	$39.5\pm~3.9$
Je	FW outline	$4.7 \pm 1.7$	$10.3 \pm 3.2$	$48.4 \pm 10.6$	34.1± 7.2
Wing outlir	HW outline	$3.7 \pm 1.2$	$14.3 \pm 3.7$	$64.1 \pm 11.6$	$44.7 \pm 9.2$

# Table S8. Percentage of cross-validation in the linear discriminant analysis for each component of the phenotype between each population of *H. timareta* and their co-mimics

	H. erato		H. melpomene		H. telesiphe	
	H. timareta					
	A	E	Α	E	Α	E
Red FW d	$0.82 ~\pm~ 0.04$	$0.69 ~\pm~ 0.05$	$0.73~\pm~0.05$	$0.55 ~\pm~ 0.08$	$0.83 ~\pm~ 0.03$	$0.92 \ \pm \ 0.03$
Red FW v	$0.99 ~\pm~ 0.01$	$0.92 ~\pm~ 0.03$	$0.90~\pm~0.03$	$0.74 ~\pm~ 0.05$	$0.78 ~\pm~ 0.04$	$0.87 ~\pm~ 0.03$
Yellow HW d	$0.69 ~\pm~ 0.04$	$0.54 ~\pm~ 0.07$	$0.66 ~\pm~ 0.05$	$0.50~\pm~0.08$	$1.00~\pm~0.00$	$0.99 ~\pm~ 0.01$
Yellow HW v	$0.85 ~\pm~ 0.03$	$0.69 ~\pm~ 0.05$	$0.75 ~\pm~ 0.05$	$0.52 ~\pm~ 0.09$	$0.98~\pm~0.01$	$0.98~\pm~0.01$
Red patch FW d	$1.00~\pm~0.00$	$0.99 ~\pm~ 0.01$	$0.97 ~\pm~ 0.02$	$0.92 ~\pm~ 0.04$	$1.00~\pm~0.00$	$1.00~\pm~0.00$
Red patch FW v	$1.00~\pm~0.01$	$0.99 ~\pm~ 0.01$	$0.97 ~\pm~ 0.02$	$0.90~\pm~0.04$	$1.00~\pm~0.00$	$1.00~\pm~0.00$
Yellow bar HW d	$0.94 ~\pm~ 0.03$	$0.94 ~\pm~ 0.03$	$0.89 ~\pm~ 0.04$	$0.85 ~\pm~ 0.05$	$0.98 ~\pm~ 0.01$	$1.00~\pm~0.01$
Yellow bar HW v	$0.99 ~\pm~ 0.01$	$0.98~\pm~0.02$	$0.96~\pm~0.02$	$0.95 ~\pm~ 0.03$	$0.99 ~\pm~ 0.01$	$1.00~\pm~0.01$
FW shape	$0.99 ~\pm~ 0.01$	$0.69~\pm~0.06$	$0.91 ~\pm~ 0.04$	$0.85 ~\pm~ 0.05$	$0.93 ~\pm~ 0.03$	$0.99 ~\pm~ 0.01$
HW shape	$1.00~\pm~0.01$	$0.67 ~\pm~ 0.06$	$0.89 ~\pm~ 0.04$	$0.90~\pm~0.04$	$0.90~\pm~0.04$	$1.00~\pm~0.01$
FW outline	$0.93 ~\pm~ 0.03$	$0.92 ~\pm~ 0.04$	$0.84 ~\pm~ 0.05$	$0.85 ~\pm~ 0.05$	$0.99 ~\pm~ 0.01$	$0.99 ~\pm~ 0.01$
HW outline	$0.89 ~\pm~ 0.04$	$0.86~\pm~0.05$	$0.74 ~\pm~ 0.05$	$0.76~\pm~0.06$	$0.98 ~\pm~ 0.01$	$0.98~\pm~0.02$

 $(1 = \text{complete discrimination between the two groups compared, 100% of the individuals can be re$ identified); mean of the bootstrap + sd

		H. timareta A	H. erato	H. telesiphe	H. telesiphe
		H. timareta E	H. melpomene	H. melpomene	H. erato
Colour spectra	Red FW d	$0.70 ~\pm~ 0.05$	$0.61 ~\pm~ 0.06$	$0.92 ~\pm~ 0.03$	$0.98 ~\pm~ 0.01$
	Red FW v	$0.64 ~\pm~ 0.05$	$0.80~\pm~0.05$	$0.97 ~\pm~ 0.02$	$1.00~\pm~0.00$
	Yellow HW d	$0.66 ~\pm~ 0.05$	$0.58 ~\pm~ 0.06$	$1.00~\pm~0.00$	$1.00~\pm~0.00$
	Yellow HW v	$0.65 ~\pm~ 0.05$	$0.63 ~\pm~ 0.06$	$0.98~\pm~0.01$	$1.00 ~\pm~ 0.00$
Colour pattern	Red patch FW d	$0.84 ~\pm~ 0.05$	$0.98~\pm~0.02$	$1.00 ~\pm~ 0.00$	$1.00~\pm~0.00$
	Red patch FW v	$0.85 ~\pm~ 0.05$	$0.97 ~\pm~ 0.02$	$1.00 ~\pm~ 0.00$	$1.00~\pm~0.00$
	Yellow bar HW d	$0.75 ~\pm~ 0.05$	$0.81 ~\pm~ 0.05$	$1.00 ~\pm~ 0.00$	$0.99 ~\pm~ 0.01$
	Yellow bar HW v	$0.79 ~\pm~ 0.05$	$0.91 ~\pm~ 0.04$	$1.00 ~\pm~ 0.00$	$1.00~\pm~0.00$
Wing venation	FW shape	$0.69 ~\pm~ 0.06$	$0.85 ~\pm~ 0.05$	$1.00 ~\pm~ 0.01$	$0.93 ~\pm~ 0.03$
	HW shape	$0.67 ~\pm~ 0.06$	$0.90~\pm~0.04$	$1.00 ~\pm~ 0.01$	$0.90~\pm~0.04$
Wing outline	FW outline	$0.68 ~\pm~ 0.06$	$0.84 ~\pm~ 0.05$	$0.99 ~\pm~ 0.01$	$0.98~\pm~0.02$
	HW outline	$0.66 ~\pm~ 0.06$	$0.89 ~\pm~ 0.04$	$1.00 \pm 0.01$	$0.98 ~\pm~ 0.02$

#### Hybrid detection and population structure



Figure S11. Multilocus Bayesian clustering and assignment analysis with STRUCTURE 2.3.1.

Each individual is represented by a column and the colour represents the relative genome contribution of each cluster. The blue arrows point to the specimens identified as F1 hybrids with other assignment analyses (NewHybrids).

#### References

- Belkhir, K., Borsa, P., Chikhi, L., Raufatse, N. & Bonhomme, F. (1996-2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier (France).
- Chai, P. & Srygley, R.B. (1990) Predation and the Flight, Morphology, and Temperature of Neotropical Rain-Forest Butterflies. *American Naturalist*, **135**, 748-765.
- Cheng H-D, Sun Y (2000) A hierarchical approach to color image segmentation using homogeneity. *Image Process IEEE Trans On* 9: 2071–2082.
- Endler, J.A. & Mielke, P.W. (2005) Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society*, **86**, 405-431.
- Flanagan, N.S., Blum, M.J., Davidson, A., Alamo, M., Albarran, R., Faulhaber, K., Peterson, E. & McMillan, W.O. (2002) Characterization of microsatellite loci in neotropical *Heliconius* butterflies. *Molecular Ecology Notes*, 2, 398-401.
- Goudet, J. (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from <u>http://www2.unil.ch/popgen/softwares/fstat.htm</u>. Updated from Goudet (1995)
- Hart, N.S. (2002) Vision in the peafowl (Aves : *Pavo cristatus*). *Journal of Experimental Biology*, **205**, 3925-3935.
- Hart, N.S., Partridge, J.C., Cuthill, I.C. & Bennett, A.T.D. (2000) Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *Journal of Comparative Physiology, A*, 186, 375-387.

Ibáñez L, Insight Software Consortium (2003) The ITK software guide. [Clifton Park, N.Y.]: Kitware.

- Kurugollu F, Sankur B, Harmanci AE (2001) Color image segmentation using histogram multithresholding and fusion. *Image Vis Comput* 19: 915–928.
- Langham, G.M. (2006) Rufous-tailed jacamars and aposematic butterflies: do older birds attack novel prey? *Behavioral Ecology*, **17**, 285-290.
- Le Poul, Y., Whibley, A., Chouteau, M., Prunier, F., Llaurens, V. & Joron, M. (2014) Evolution of dominance mechanisms at a butterfly mimicry supergene. . *Nature Communications*, **5**.
- Martin K, Hoffman B (2003) Mastering CMake: a cross-platform build system. [Clifton Park, New York]: Kitware Inc.
- Mattes D, Haynor DR, Vesselle H, Lewellen TK, Eubank W (2001) Nonrigid multimodality image registration. *Med Imaging* 4322: 1609–1620.
- Mattes D, Haynor DR, Vesselle H, Lewellen TK, Eubank W (2003) PET-CT image registration in the chest using free-form deformations. *Med Imaging IEEE Trans On* 22: 120–128.
- Mavarez, J. & Gonzalez, J. (2006) A set of microsatellites markers for *Heliconius melpomene* and closely related species. *Molecular Ecology Notes*, **6**, 20-23.
- Mérot, C., Mavarez, J., Evin, A., Dasmahapatra, K.K., Mallet, J., Lamas, G. & Joron, M. (2013) Genetic differentiation without mimicry shift in a pair of hybridizing *Heliconius* species (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society*, **109**, 830-847.
- Meyer F, Beucher S (1990) Morphological segmentation. J Vis Commun Image Represent 1: 21-46.
- Meyer F (1992) Color image segmentation. Image Processing and its Applications, 1992., International Conference on. pp. 303–306.
- Nikolaev DP, Nikolayev PP (2004) Linear color segmentation and its implementation. *Comput Vis Image Underst* 94: 115–139. doi:10.1016/j.cviu.2003.10.012.
- Odeen, A. & Hastad, O. (2003) Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. *Molecular Biology and Evolution*, **20**, 855-861.
- Rohlf FJ, Slice D (1990) Extensions of the Procrustes Method for the Optimal Superimposition of Landmarks. *Syst Zool* 39: 40–59. doi:10.2307/2992207.
- Rousset, F. (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103-106.
- Shafarenko L, Petrou H, Kittler J (1998) Histogram-based segmentation in a perceptually uniform color space. *Image Process IEEE Trans On* 7: 1354–1358.
- Styner M, Gerig G (1997) Evaluation of 2D/3D bias correction with 1+ 1ES-optimization. *Rapp Rech* 179.
- Styner M, Brechbuhler C, Szckely G, Gerig G (2000) Parametric estimate of intensity inhomogeneities applied to MRI. *Med Imaging IEEE Trans On* 19: 153–165.
- Théry, M., Pincebourde, S. & Feer, F. (2008) Dusk light environment optimizes visual perception of conspecifics in a crepuscular horned beetle. *Behavioral Ecology*, **19**, 627-634.
- Thévenaz P, Unser M (2000) Optimization of mutual information for multiresolution image registration. *Image Process IEEE Trans On 9*: 2083–2099.
- Yoo TS, Ackerman MJ, Lorensen WE, Schroeder W, Chalana V, et al. (2002) Engineering and algorithm design for an image processing api: a technical report on itk-the insight toolkit. *Stud Health Technol Inform*: 586–592.
- Vorobyev, M. & Osorio, D. (1998) Receptor noise as a determinant of colour thresholds. *Proceedings* of the Royal Society B-Biological Sciences, **265**, 351-358.
- Weir, B.S. & Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.