

Cryptic differences in colour among Müllerian mimics: how can the visual capacities of predators and prey shape the evolution of wing colours?

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Abstract

Antagonistic interactions between predators and prey often lead to co-evolution. In the case of toxic prey, aposematic colours act as warning signals for predators and play a protective role. Evolutionary convergence in colour patterns among toxic prey evolves due to positive density-dependent selection and the benefits of mutual resemblance in spreading the mortality cost of educating predators over a larger prey assemblage. Comimetic species evolve highly similar colour patterns, but such convergence may interfere with intra-specific signalling and recognition in the prey community, especially for species involved in polymorphic mimicry. Using spectrophotometry measures, we investigated the variation in wing coloration among comimetic butterflies from distantly related lineages. We focused on seven morphs of the polymorphic species *Heliconius numata* and the seven corresponding comimetic species from the genus *Melinaea*. Significant differences in the yellow, orange and black patches of the wing were detected between genera. Perceptions of these cryptic differences by bird and butterfly observers were then estimated using models of animal vision based on physiological data. Our results showed that the most strikingly perceived differences were obtained for the contrast of yellow against a black background. The capacity to discriminate between comimetic genera based on this colour contrast was also evaluated to be higher for butterflies than for birds, suggesting that this variation in colour, likely undetectable to birds, might be used by butterflies for distinguishing mating partners without losing the benefits of mimicry. The evolution of wing colour in mimetic butterflies might thus be shaped by the opposite selective pressures exerted by predation and species recognition.

Introduction

The evolution of traits is often influenced by interactions between species, including mutualistic interactions such as the reproductive mutualism between *Ficus* species and their pollinating wasps (Jousselin *et al.*, 2003)

or antagonistic interactions such as the host–parasite evolutionary arms race between *Daphnia magna* and the bacterium *Pasteuria ramosa* (Decaestecker *et al.*, 2007). The evolution of prey colour in relation to the visual capacities of predators is a fascinating example of an antagonistic relationship shaping the evolution of traits involved in predation–prey interactions (e.g. evolution of cryptic patterns shaped by birds' detection behaviour; Bond & Kamil, 2002). Many prey also exhibit odours or toxins which are repulsive to predators, and tend to evolve conspicuous colorations as warning signals for predators (Ruxton *et al.*, 2004). Similar warning signals may be used by multiple toxic species, an adaptive

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resemblance known as Müllerian mimicry which enhances protection against educated predators (Müller, 1879). Comimetic prey share the mortality cost due to attacks by uneducated predators over a larger community, leading to positive density-dependent selection on similar colour patterns (Turner, 1984). Müllerian mimicry involves warning patterns often composed of distinctive shapes and colours, as is seen for instance in poisonous frogs (Chouteau *et al.*, 2011) or in venomous snakes (Sanders *et al.*, 2006). Mimicry works if prey can be perceived as similar by predators. Therefore, the level of colour or pattern similarity reached by mimetic species should depend tightly on the visual discrimination capacities of predators (Ruxton *et al.*, 2008).

Colour patterns can also play a key role in mate recognition (e.g. in frogs Summers *et al.*, 1999; in fish, Couldridge & Alexander, 2002; in butterflies, Costanzo & Monteiro, 2007) and are subject to sexual selection (e.g. in butterflies, Kemp, 2008; in lizards, Chen *et al.*, 2012; or in birds, Dakin & Montgomerie, 2013). More specifically, colour patterns influence mate recognition in several mimetic species from the butterfly genus *Heliconius* (Nymphalidae) (Jiggins *et al.*, 2001; Mavarez *et al.*, 2006), making *Heliconius* a model to jointly study the influence of mimicry and mate recognition on wing colour patterns. Mimetic convergence in colour patterns between distantly related species might indeed interfere with mate recognition cues based on wing colours, as was shown in *Heliconius* (Estrada & Jiggins, 2008). Briscoe *et al.* (2010) argued that the visual system of *Heliconius erato* has probably co-evolved with wing pigmentation, suggesting that certain small colour differences between comimics could be detected by the butterflies and aid in reducing interferences with mate recognition. Bybee *et al.* (2012) demonstrated that colour differences in yellow scales in *Heliconius* and the closely related genus *Eueides* were more likely to be detected by butterfly observers than by birds. Colour evolution in mimetic butterflies should therefore be influenced by selection in opposing directions, favouring sufficient resemblance on the one hand to allow generalization by birds and sufficient distinctiveness on the other hand to be distinguished by the butterflies themselves. Colour differences between comimics could then be cryptic to predators' vision but still serve as mating cues for butterflies.

The recognition of colour patterns as a signal of unpalatability for birds or as a signal of species identity for butterflies might depend on variations in both pattern and colour. Both pattern and colour have been shown to be used in mate recognitions (e.g. in fish, Houde, 1997) although colour appears to be subject to stronger selection in some species (see for instance in darters, Williams & Mendelson, 2013). Similarly, experiments performed with birds have shown that colours rather than pattern can play a preponderant role in the recognition of unpalatable prey (Aronsson & Gamberale-

Stille, 2008) stressing the importance of studying colour variation among comimetic species.

Here, we study the evolutionary convergence in wing colours among distantly related, defended comimics in pairs of butterflies in the Nymphalidae, a family where Müllerian mimicry have been extensively documented (Sherratt, 2008). We focused on seven pairs of Müllerian mimics involving morphs of the polymorphic species *Heliconius numata* and their respective comimics in the genus *Melinaea*. In *Heliconius numata*, several distinct morphs coexist, each of them characterized by different wing colour patterns, and potentially complicating species recognition based on wing colour pattern. *Heliconius* and *Melinaea* lineages diverged over 90 million years ago (Wahlberg *et al.*, 2009), but the wing colour patterns of *Melinaea* species are highly similar to the forms of the polymorphic *Heliconius numata* (see Fig. 1). The densities of the different morphs in *Heliconius numata* and their comimetic *Melinaea* species have been shown to be correlated (Joron *et al.*, 1999), confirming the positive density-dependent selection acting on mimicry. These two genera thus provide an excellent framework to jointly investigate the influence of intra- and inter-specific signalling on the variations in wing colours. Here, we thus evaluated the variations in black, yellow and orange colours on the wings of both *Heliconius* and *Melinaea*, in order to (i) characterize the variation in colour spectra within and between mimetic species and (ii) estimate the capacity of birds and butterflies to distinguish the colour differences between species.

Materials and methods

Specimens

Wild butterflies were collected between 1998 and 2012 in the Amazonian basin, mainly in Peru and French Guiana. The wings were detached from the body and preserved in paper envelopes stored in boxes containing silica gel. We focused on seven mimetic morphs from the species *Heliconius numata* and on corresponding mimetic species from the genus *Melinaea* (Fig. 1), with the aim of measuring 20 individuals per morph in *H. numata* and per species in *Melinaea* with an equilibrated sex ratio (actual sample sizes were as follows: *H. n. arcuella*, $n = 23$; *H. n. aurora*, $n = 21$; *H. n. bicoloratus*, $n = 21$; *H. n. silvana*, $n = 19$; *H. n. tarapotensis*, $n = 20$; *H. n. timaeus*, $n = 7$; *H. n. elegans*, $n = 6$; *M. marsaeus phasiana*, $n = 20$; *M. marsaeus rileyi*, $n = 20$; *M. marsaeus mothone*, $n = 23$; *M. ludovica ludovica*, $n = 24$; *M. menophilus ssp. Nv*, $n = 34$; *M. menophilus hicetas*, $n = 16$; and *M. satevis cydon*, $n = 20$).

Reflectance spectrophotometry

Measurements on butterfly wings were collected using a spectrophotometer (AvaSpec-3648, Avantes, Apeldoorn,

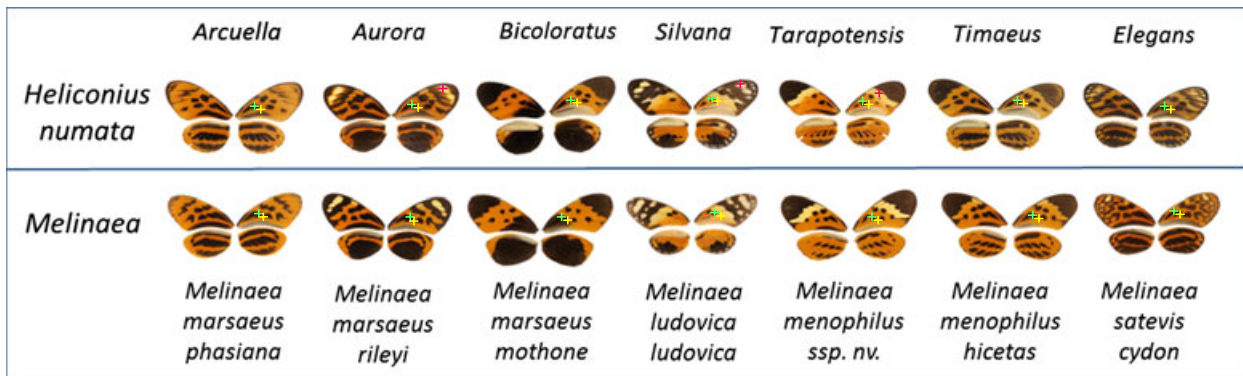


Fig. 1 Examples of the wings used for the spectrophotometry measurements. Wings of the seven comimic species from the genera *Heliconius* (top row) and *Melinaea* (bottom row) are presented for each butterfly lineage with their dorsal side on the left and their ventral side on the right. The names of the *H. numata* morphs are displayed on the top, whereas names of the different *Melinaea* species are displayed on the bottom of the figure. Crosses indicate the location of the measurements on the wings: green and yellow crosses indicate the locations of the measurement taken for black and orange, respectively; pink crosses indicate the location of the measurement taken for yellow, which differed for the *H. numata* f. *tarapotensis* and its corresponding comimic because of the location of the yellow band. Note that the locations are shown on the ventral sides but measurements were performed at symmetrical locations on the dorsal sides.

the Netherlands) and a deuterium–halogen light source (DH-2000, Avantes) connected to a 1.5-mm-diameter sensor (FCR-7UV200-2-1.5 × 100, Avantes) inserted in a miniature black chamber. Reflectance spectra were taken at 90° incidence relative to a 99% reflectance standard (300–700 nm Spectralon) and to dark current. The small size of the sensor facilitated the measurement of small patches and the avoidance of wing veins. Spectra were recorded with the software Avasoftware 7.0 using an average of 5 measures with an integration time of 23 ms. Patches of black, orange and yellow were measured at the same location of the forewing of each specimen (Fig. 1). For yellow, the same patch was used for the mimetic pairs but was different among morphs due to the variation in the location of the yellow patches in the different morphs (see Fig. 1 for more details). In some *H. numata* morphs (*elegans*, *arcuella*, *timaetus* and *bicoloratus*) and corresponding *Melinaea* species (*M. satevis cydon*, *M. marsaeus phasiana*, *M. menophilus hicetas* and *M. marsaeus mothone*), wings do not exhibit any yellow patches and only orange and black were measured. Measures of the 2 or 3 distinct colours were performed on the forewing on both dorsal and ventral faces (i.e. 4–6 measures per butterfly). In total, 1454 measurements were taken.

Analyses of colour spectra

Colour spectra were analysed using Avicol v.6 (Gomez, 2006). Colour spectra obtained for the 300–700 nm wavelength range were compared using classical parameters computed on the reflectance curves, namely brightness (*B*), hue (*H*) and chroma (*C*). To estimate these parameters, we used the segmentation model of Endler (1990), with 100 nm range segments throughout the

300- to 700-nm interval. Variations in the three colour parameters (*B*, *H* and *C*) were analysed using a general linear model (GLM) computed by the software R 2.13.0. (R Core Development Team, 2013) assuming a Gaussian distribution, with wing side, colour patch, genus, sex and the corresponding interactions as factors.

Models of animal vision

Models of animal vision allow inferences about the colour variations that can be perceived by different animals, taking into account the sensitivities of photoreceptors present in their eyes. Photoreceptors are characterized by sensitivity functions which determine the wavelengths of reflected light perceived by the eye. Photoreceptor types are generally defined by the wavelength of their maximal sensitivity. Depending on the species considered, the number of photoreceptor types can vary: for instance, most birds have four receptor types (tetrachromat), whereas humans have only three (trichromat). The relative densities of these photoreceptors can also vary and modify the ability of animals to discriminate colours. Finally, the presence of oil droplets in birds' photoreceptors and the transmittance of the ocular media also modify the perception of light by the eye and were therefore taken into account in the models.

As the photoreceptor sensitivities of the birds composing the predator community in this study system are not known, the calculations were based on the two main vision systems found in birds. We used the data obtained for the peafowl (*Pavo cristatus*) to model the violet-sensitive (VS) bird visual system (Hart, 2002) and the blue tit (*Parus caeruleus*) to model the UV-sensitive (UVS) bird visual system (Hart *et al.*, 2000). In both

cases, the sensitivity of photoreceptors includes visual pigments absorbances, relative cone densities, and oil droplets and ocular media transmittances.

To model butterfly vision, the photoreceptors sensitivity was computed using the visual sensitivity peaks reported for *Heliconius erato* (see Briscoe *et al.*, 2010 for a summary). Following the approach of Stavenga (Stavenga, 2010; Stavenga & Arikawa, 2011), the template reported by Stavenga *et al.* (1993) was used for those photoreceptors that present a peak at wavelengths lower than 400 nm. For photoreceptors which presented sensitivity at higher wavelengths, the template calculated by Govardovskii *et al.* (2000) was applied.

Detailed studies regarding the relative density of photoreceptors in *Heliconius* eyes have not been reported. However, studies in butterflies have shown that densities of the different photoreceptors in the eye were broadly similar (Qiu & Arikawa, 2003). For this reason, we assume the same proportional density for the four different kinds of photoreceptors in *H. erato*.

Finally, photoreceptor activity is characterized by the signal-to-noise ratio, which is generally computed from its inverse, the Weber fraction (Vorobyev *et al.*, 1998), and which was set to 0.05 for all models in this study.

The perception of differences in colour was estimated using the physiological model of Vorobyev and Osorio for tetrachromatic vision (Vorobyev & Osorio, 1998; Vorobyev *et al.*, 1998). A light environment corresponding to small gaps in a forest canopy (referred as SG light) was assumed to model a realistic situation with respect to the natural populations of *H. numata* and *Melinaea* which live in the Amazonian forest and are often caught in small light gaps. This light spectrum was obtained by computing the average of 100 small gap irradiance spectra measured in primary forest in French Guiana (Thery *et al.*, 2008). Models based on a spectrum of large gap light and forest shade light obtained in the same conditions were also used to confirm the generality of our results.

We computed the chromatic (δS) and achromatic (δQ) contrasts between pairs of spectra within each individual, that is, yellow on black background and orange on black background using Avicol v.6 (Gomez, 2006). Variations in contrasts are usually measured in just noticeable difference (JND) units, assuming that 1 JND is the minimum detection threshold. Both chromatic and achromatic contrasts were compared using a GLM assuming a Gaussian distribution, controlling for sex, using the software R 2.13.0. (R Core Development Team, 2013).

Discrimination capacities analysis

We studied further the abilities of the three types of observers (butterfly, VS bird and UVS bird) to discriminate colours displayed by comimics. For each observer,

we estimated the perception of wing colour using the ratio between colour (yellow or orange patches) and background (black patch) quantum catches for each of the four photoreceptors, assuming a SG light (see above). The coordinates of each spectrum in a three-dimensional tetrahedron of colour space were then computed as described by Kelber *et al.* (2003). The coordinates of the different individuals were separated using a linear discriminant analysis (LDA) with genus as separating class, following the method proposed by Bybee *et al.* (2012). The percentage of individuals correctly assigned to the actual genus was then computed for each observer, to estimate their discrimination capacities using the library MASS in the software R 2.13.0. (R Core Development Team, 2013).

Results

Analyses of raw colour spectra

Differences between dorsal and ventral wing surface

The wings of the butterflies from all species studied here exhibit similar patterns on both dorsal and ventral sides, but the pattern usually appears less vivid on the ventral surface (see Fig. 1). We thus tested whether colour spectra differ significantly between sides. As expected, brightness was significantly higher on the ventral than on the dorsal side ($F_{1, 1422} = 190.07$, $P < 0.001$), controlling for colour patch, sex and genus. No interaction with colour patch or genus was detected, confirming that brightness is generally higher on the ventral side regardless of the colour considered. No difference in hue was detected between sides ($F_{1, 1422} = 1.32$, $P = 0.25$). Chroma was significantly higher on the dorsal side controlling for sex, genus and colour patch ($F_{1, 1422} = 153.56$, $P < 0.001$). More specifically, this difference was significant in yellow ($F_{1, 328} = 27.17$, $P < 0.001$) and orange patches ($F_{1, 544} = 316.27$, $P < 0.001$) but not in black patches ($F_{1, 544} = 2.07$, $P = 0.15$).

Sex differences

Subsequent analyses were carried out on the dorsal surface only, as we assumed that this surface is the most exposed to birds during flight, basking and feeding. Differences in the shape of spectra were investigated by comparing brightness, hue and chroma within colour patches, testing for the effect of genus, sex and the interaction between sex and genus. A significant effect was found for sex on chroma in yellow, black and orange patches (Table 1). For yellow patches, chroma was significantly lower for *Heliconius* females as compared to males ($t = -9.70$, $df = 46.94$, $P < 0.001$), whereas the effect was significant in the opposite direction in *Melinaea* ($t = 4.07$, $df = 86.29$, $P < 0.001$). For orange patches, sex had a just significant effect in *Heliconius* only ($t = -2.41$, $df = 63.50$, $P = 0.02$). On the contrary,

Table 1 Effect of genus, sex and the interaction between sex and genus on brightness, hue and chroma estimated from colour spectra obtained for yellow, orange and black patches on dorsal side of 278 individuals from the genera *Heliconius* and *Melinaea*. Effects were tested with a general linear model, assuming a Gaussian distribution.

Effect	Yellow		Black		Orange	
	$F_{1, 163}$	P	$F_{1, 271}$	P	$F_{1, 270}$	P
Brightness						
Genus	14.667	<0.001	0.0018	0.966	0.8952	0.344928
Sex	1.1653	0.282	0.0005	0.983	0.025	0.874426
Genus/Sex	1.4108	0.237	0.8997	0.344	9.8205	0.001918
Hue						
Genus	19.1641	<0.001	0.1891	0.664	60.923	<0.001
Sex	1.5067	0.221	0.3981	0.529	3.3568	0.06804
Genus/Sex	1.7896	0.183	1.8268	0.178	0.401	0.5271
Chroma						
Genus	19.63	<0.001	41.1266	<0.001	33.7737	<0.001
Sex	19.544	<0.001	9.5371	0.002	1.9288	0.16605
Genus/Sex	135.991	<0.001	18.2664	<0.001	6.8955	0.00914

Significant P-values appeared in bold.

for black patches, sex had a significant effect in *Melinaea* only ($t = 5.14$, $df = 159.75$, $P < 0.001$). Finally, the interaction between sex and genus was also significant on the brightness of orange patches (Table 1). In *Melinaea*, brightness was significantly higher in females ($t = 1.99$, $df = 134.05$, $P = 0.049$), whereas in *Heliconius*, brightness was lower in females ($t = -2.38$, $df = 70.27$, $P = 0.02$). For all other parameters, significant effects were neither observed on sex nor on the interaction between sex and genus. To sum up, sex differences can thus be detected on the different patches of these butterflies' wings, mainly on chroma, and these appear to differ between the two mimetic genera.

Differences between genera

Mean colour spectra differed in shape for the dorsal side of *Heliconius* and *Melinaea* specimens (Fig. 2). The most striking differences were found for yellow and

orange patches, where brightness, hue and chroma all exhibited significant differences (see Table 1). In particular, brightness was higher in *Heliconius* than *Melinaea* for these two colour patches.

In contrast, spectra obtained for black patches were very similar for *Heliconius* and *Melinaea*, only chroma was found to be significantly different between the genera (Table 1): *Heliconius* black patches tended to be slightly redder than those in *Melinaea* (see Fig. 2).

Colour contrast perception

In most cases, both chromatic and achromatic contrasts between colour pairs perceived by the different observers (birds or butterflies) appear to be significantly higher on the dorsal side than on the ventral side (see Table S1). However, these differences were all smaller than one unit of just noticeable difference (JND), suggesting

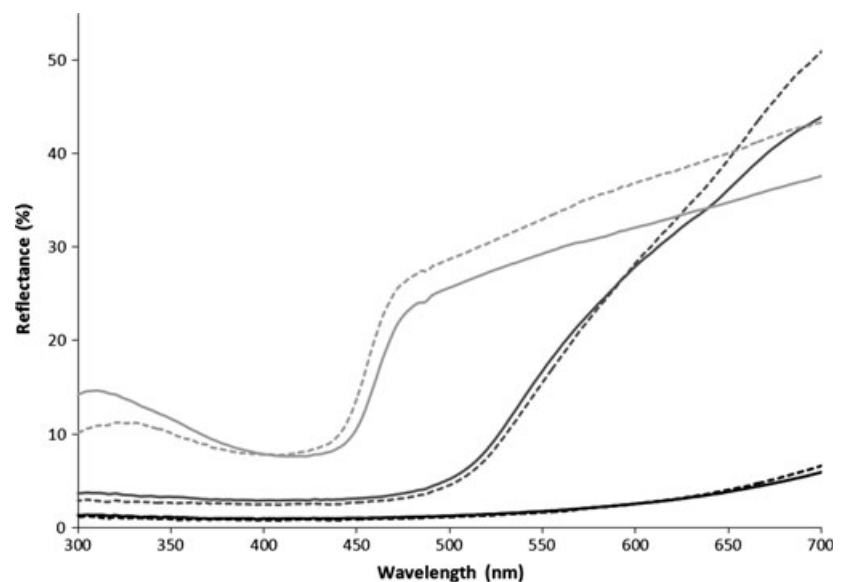


Fig. 2 Average reflectance spectra measured in the 300–700 nm wavelength range obtained on the dorsal side of 278 wings. Light grey: yellow patches; dark grey: orange patches; black: black patches. Solid lines: *Melinaea* specimens; dotted lines: *Heliconius* specimens

that they might not be easily detected by both birds and butterflies observers in SG light conditions.

We then focused on the difference in colour contrast between *Heliconius* and *Melinaea*, restricting the analysis to the dorsal side, and controlling for sex (Fig. 3). Consistent with the difference in colour spectra, contrasts of yellow on a black background showed significant differences, whereas contrasts between orange and black did not (see Table S1 for detailed statistics). The yellow–black contrast was more striking in *Heliconius* than in *Melinaea* regardless of the observer type. However, the average contrast differences perceived between genera were generally below one JND and thus not easily detectable by the three observers in small gap light (Fig. 3). However, the difference in achromatic yellow–black contrast (δQ) between *Heliconius* than in *Melinaea* as detected by butterflies was above 1 JND (Fig. 3) and thus more likely to be considered different by a butterfly observer. Estimations of colour contrasts using a large gap light (data not shown) showed that achromatic contrasts perceived by the different observers were largely above 1 JND, the contrast perceived by butterflies still being significantly higher than in the other observers, confirming the higher sensibility of the butterfly visual system to wing colour contrasts. Results on the yellow–orange contrast were similar to the yellow–black contrast due to the variation in yellow between comimics (data not shown).

Observers' discrimination capacities

To compare the capacities of the different observers (butterflies and birds) to discriminate between genera, we performed a linear discriminant analysis based on the simulations of the four visual cone types for the two colour contrasts (yellow on black background and orange on black background) measured on the dorsal side under small gap light (Table 2). The ability of the three observers to discriminate between genera was higher for the yellow–black contrast (above 70% of correct assignment) than for the orange–black contrast (below 70% of correct assignment). For the yellow–black contrast, butterfly eyes (*Heliconius erato*) showed the highest discrimination rate (83% correct assignment), whereas VS birds had the lowest discrimination capacity (73% correct assignment). On the contrary, for the orange–black contrast, VS birds exhibited the highest visual ability to discriminate among mimetic genera (66% correct assignment), and UVS birds the lowest (59% of correct assignment), although the overall low values of assignment success suggested that black–orange contrast was unlikely to provide a relevant cue to infer genus. These results did not change qualitatively depending on the light considered, with large gap light and forest shade showing the same discrimination order among observers.

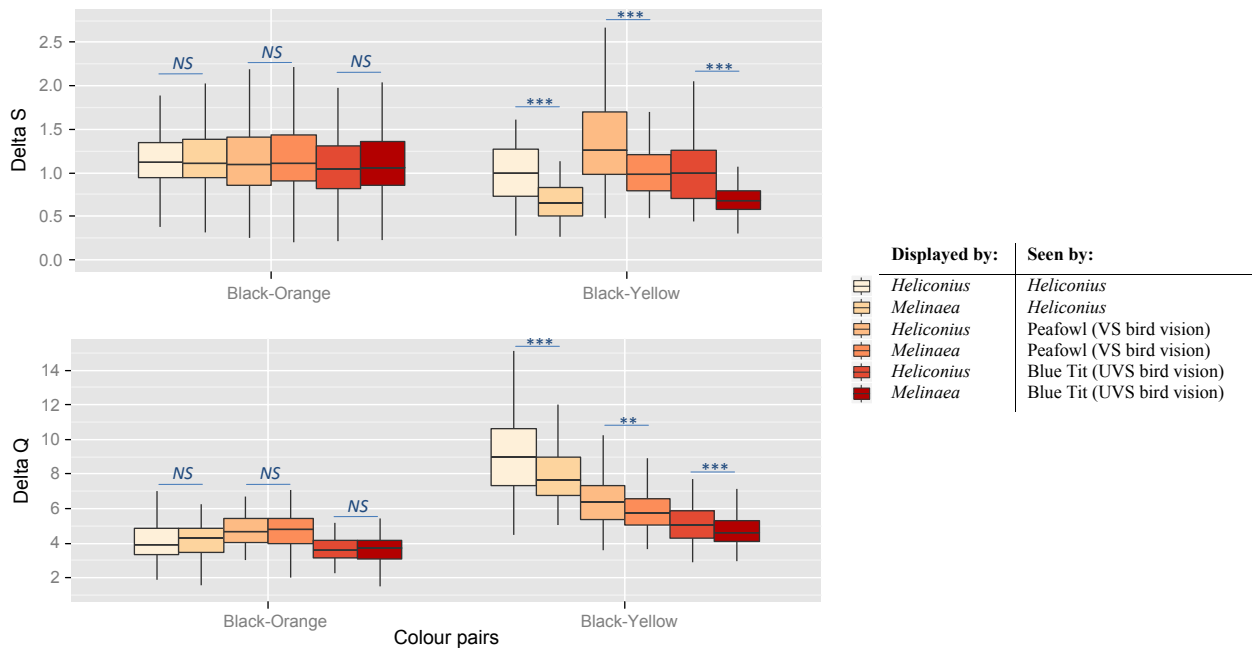


Fig. 3 Comparison of dorsal colour contrasts between *Heliconius* and *Melinaea* specimens. Chromatic (Delta S) and achromatic (Delta Q) contrasts were computed assuming small gap light for three visual systems (butterfly vision modelled from data obtained on *Heliconius erato*, VS bird vision modelled from data obtained on peafowl and UVS bird vision modelled from data obtained on blue tit). Statistical differences between genera were tested using a GLM controlling for sex (***: $P \leq 0.001$, **: $P \leq 0.01$, NS: $P \geq 0.05$).

Table 2 Percentage of correct classification with respect to genus, estimated by a linear discriminant analysis based on the coordinates of the colour contrasts (yellow–black and orange–black) measured on the dorsal side of the wing in the tetrachromatic colour space for the three modelled visual systems (butterfly (*Heliconius erato*), bird UV-type (blue tit) and bird V-type (peafowl)), assuming a small gap light.

Colour contrast	Morph	Visual system		
		Butterfly	Bird UV-type	Bird V-type
Yellow/black	<i>Silvana</i>	0.81	<u>0.83</u>	<u>0.85</u>
	<i>Aurora</i>	0.83	<u>0.76</u>	0.66
	<i>Tarapotensis</i>	<u>0.90</u>	<u>0.83</u>	<u>0.82</u>
	ALL	0.84	0.79	0.73
Orange/black	<i>Silvana</i>	<u>0.81</u>	<u>0.69</u>	<u>0.67</u>
	<i>Aurora</i>	0.58	0.49	0.61
	<i>Tarapotensis</i>	<u>0.78</u>	<u>0.69</u>	<u>0.63</u>
	<i>Bicoloratus</i>	<u>0.74</u>	<u>0.74</u>	<u>0.70</u>
	<i>Timaeus</i>	<u>0.74</u>	<u>0.83</u>	<u>0.74</u>
	<i>Arcuella</i>	<u>0.76</u>	<u>0.86</u>	<u>0.81</u>
	<i>Elegans</i>	<u>0.91</u>	<u>0.91</u>	<u>0.95</u>
	ALL	0.61	0.59	0.66

Underlined results indicate that the discrimination capacities computed within morphs were higher than the discrimination capacities computed in the pool of all morphs.

Interestingly, the discrimination capacities computed within morphs were generally higher than in analyses considering all morphs together (see underlined results in Table 2), suggesting greater divergence in colour within comimics than between genera.

To compare the strength of mimicry between sexes, we computed a linear discriminant analysis within each sex. We found that assignment to the correct genus for the yellow–black contrast was higher within male wings (89.01% and 81% when perceived by UVS and VS birds, respectively) than within female wings (75.6% and 73%). This suggested that birds would be more prone to mix up females comimics than males and thus that colour mimicry might be more accurate in females. In contrast, the discrimination of colour between comimics when observed by *Heliconius* did not differ greatly with respect to sex (86% for male wings and 84% for female wings), suggesting that the assignment to the correct genus was similarly performed for both sexes.

Discussion

Aposematism

Although the ventral and dorsal sides of the wings showed similar colour patterns in both *Heliconius* and *Melinaea*, our results confirm that the wing colour pattern is paler on the ventral than the dorsal side and shows a lower achromatic contrast. The aposematic value

of those patterns is thus presumably higher on the dorsal sides, with more conspicuous and contrasted patterns. During daylight, the butterflies are seen flying or feeding on flowers, and the dorsal side is more exposed to the sight of predators. These butterflies often rest with wings open during the day, exposing the dorsal side of their wings. Selection for the warning function of those patterns is thus probably higher on dorsal side, which might explain the higher conspicuousness of dorsal patterns. However, wings are closed during night-time and roosting, leading to a higher exposure of the ventral side when roosting (Mallet & Gilbert, 1995).

The difference in wing pattern conspicuousness between the sides of wings can also be linked to difference in selective regimes. For instance, in the genus *Morpho* (Lamas, 2004), the dorsal side often exhibits intense blue coloration in flight and is thought to be involved in sexual selection, whereas the cryptic colour patterns on the ventral side are exposed in the resting position and may be selected for camouflage against visual predators. The ventral sides of many *Heliconius* species are enriched in white scales (e.g. in *H. melpomene* (Baxter *et al.*, 2009); *H. timareta* (Mérot *et al.*, 2013); in *H. numata silvana*, *H. erato*, pers. obs.), which reflect UV and might have a role in courtship (Kemp, 2008). Both sides of the wings are potentially important cues to predators, but the trade-off between courtship and predation risk is probably resolved differently in flight vs. resting modes.

Colour mimicry

Our results show that differences in coloration can be observed in the main three colours composing the wing patterns of *Heliconius numata* and its *Melinaea* comimics. However, the most important colour difference was observed for yellow patches, more specifically in the UV wavelength range. As suggested by the discrimination analysis, this difference appeared more striking when seen by butterflies rather than by birds with a VS visual system. The best-documented predators of *Heliconius* butterflies are jacamars (e.g. the rufous-tailed jacamar *Galbula ruficauda*) (Chai & Srygley, 1990; Langham, 2004), birds belonging to the Galbuliformes order. The visual system of Galbuliformes is only known from the species *Nystalus maculatus*, which is VS according to opsin sequence similarity (Odeen & Hastad, 2003). Other Galbuliformes such as jacamars may be expected to have a VS visual system too and therefore to be unable to perceive spectral differences in the UV wavelengths and discriminate *H. numata* vs. *Melinaea* colours.

In contrast, birds with UVS visual system are probably better at distinguishing the slight colour difference in yellow from the two genera. However, even then this difference is on average lower than the just noticeable difference threshold, presumably making the two butterflies hardly distinguishable based on colour. Colour

mimicry among species belonging to very distantly related lineages (with an estimated divergence time of 90 million years Wahlberg *et al.*, 2009) therefore appears to be very good and might prevent birds from discriminating between them. Behavioural experiments with live birds are now needed to test the accuracy of their vision with respect to these cryptic differences in colour.

Colour and mate recognition

Sexual differences in colour were detected in our study, suggesting cryptic sexual dimorphism in colour. Furthermore, females appeared to be better mimics than males, based on the discrimination capacities of yellow–black contrasts in birds. Selection exerted on mimicry might differ between sexes in *H. numata*, as suggested earlier based on sexual differences in flight behaviour (Joron, 2005); for instance, males tend to fly higher while searching for mates, whereas females stay mostly in the understory or in small gaps seeking *Passiflora* host plants, showing habitat preferences closer to their *Melinaea* comimics during much of the day (Joron, 2005). Sexual dimorphism in colour patterns can happen in mimetic butterflies with male and female morphs belonging to different mimicry rings (e.g. *Godyris zavaleta caesiopicta*; Young, 1974) perhaps due to ecological or behavioural differences. In several mimetic species, mimicry is also restricted to females only (e.g. in *Papilio*, see Kunte, 2009). Based on predation marks on wings, Ohsaki (2005) showed that the predation risk was indeed higher for females, and proposed that positive selection for mimicry could be higher for females. Mimicry in males may also be limited by intrasexual competition among males in *Papilio polyxenes* (Lederhouse & Scriber, 1996). Behavioural differences between sexes therefore seem to interact with mimicry in many butterfly species, so we could hypothesize that the subtle variations in mimicry between sexes in *H. numata* may be influenced by differences in male and female behaviours.

However, cryptic sexual dimorphism in colour in *H. numata* might also result from sexual selection. In Lepidoptera, intense colorations are associated with mating success (see Allen *et al.*, 2011 for a review), which often leads to males being more colourful than females. Females are generally observed to be darker than males in *Heliconius numata* (Jones *et al.*, 2012), whereas this relationship seems to be reversed between the *Melinaea* sexes. The significant difference in sexual dimorphism found here between the two lineages may suggest a potential role of colour in mate recognition in those butterflies. The use of colour as a signal for species recognition is also suggested by our results showing that differences between *Heliconius* and *Melinaea* in yellow–black achromatic contrasts are more easily detected by butterflies than by birds. Furthermore, the ability to discriminate comimics seems to be higher for

the butterfly visual system than for bird visual systems, suggesting that lineage identity would be more easily determined based on wing colour for the butterflies themselves. This is in accordance with previous results obtained within the *Heliconiini* tribe, where differences in yellow were shown to be more visible for butterflies than for avian predators (Bybee *et al.*, 2012).

Mimicry involving colours which are distinguishable by the butterflies but not by bird predators could be the result of dual selective pressures exerted on wing colours by different selective agents. Species discrimination among comimetic taxa is important for butterflies to avoid the costs of inappropriate courting. In contrast, avian predators have no interest in distinguishing toxic comimics. In the polymorphic species *H. numata* in particular, the diversity of wing colour patterns might enhance the error rate in partner recognition based on visual cues. However, our results on discrimination capacity within morph tend to show that differences in colours are pronounced within a given pattern and could be detected by butterflies, suggesting that colour variation could help butterflies to identify species among comimics. Co-evolution between the UVS visual system and coloration in *Heliconius* has been documented by Briscoe *et al.* (2010) who suggest that selection could act to improve colour-based discrimination in those butterflies. The evolution of slight differences in mimetic wing colours which are hard to distinguish by predators but easily distinguishable by the butterflies themselves might therefore be the outcome of a selective process which balances the opposing evolutionary pressures exerted by mimicry and mate recognition.

Conclusions

Our study reveals that comimetic species with highly similar wing colour patterns can show cryptic differences, in this case in the black, orange and yellow colours shared between the *Heliconius* and *Melinaea* genera. The perception of subtle colour differences was shown to depend strongly on the visual system of the observers and implied that butterflies should have higher visual abilities than birds to detect differences between comimetic species. Predatory selection leading to mimicry on one hand and selection for reliable species recognition on the other hand might have shaped both the colour patterns and the visual abilities of mimetic butterflies. Of course, colour differences are only one modality used by mimetic butterflies during mate choice, which is also influenced by other mating cues like pheromones and behaviour. In the butterfly *Bicyclus anynana*, visual and chemical cues appear to have a similar importance in female choice (Costanzo & Monteiro, 2007). Variations in both colour and odour in mimetic butterflies remain to be investigated to clarify the relative strength of opposing selective pressures acting on their wing colour patterns.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Analysis of chromatic and achromatic contrasts (*DeltaS* and *DeltaQ* respectively) for yellow–black and orange–black, using a GLM using wing side, genus and sex as factors (no significant interactions were detected neither between side and genus nor between side and sex; those interactions have thus been removed from the models for simplicity).

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