Journal of Zoology. Print ISSN 0952-8369

A bird's eye view of two mimetic tropical butterflies: coloration matches predator's sensitivity

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Keywords
avian visual models; Batesian mimicry; coloration; *Mimoides*; *Heliconius*; JNDs; aposematism; UV vision.

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Editor: Robert Knell

Received 22 June 2015; accepted 30 September 2015

doi:10.1111/jzo.12305

Abstract

Unprofitable prey with conspicuous warning signals are often mimicked by other species, which then gain protection from predators. How closely two mimetic species resemble one another depends upon the visual perception of the signal receiver. However, most studies of mimetic coloration have been conducted using only the human visual system, which differs greatly from that of most animals. To better understand mimicry, we should study mimetic visual signals through the eyes of the intended receiver. Here, we use avian visual models to test predictions of putative Batesian mimicry in two Amazonian butterflies, *Mimoides pausanias* and *Heliconius sara*. We calculated Just Noticeable Differences (JNDs) and tetrahedral color volumes for 11 different patches: iridescent blue, yellow bars, red spots and black background. Several color patches were not visually discriminable for both avian visual systems (UV/VIS and V/VIS), and visual discrimination (i.e. degree of mimicry) of color patches depended upon the avian visual system. These two butterfly species are more mimetic when viewed by their likely avian predators, which have V/VIS vision. Therefore, this mimetic assemblage may have evolved to be more spectrally accurate in the non-UV wavelengths which their avian predators are able to see. However, while many color patches of the two species were modeled to be difficult to discriminate, most color patches were not perfect matches regardless of visual system, and several patches were very poor mimics. Through this study we demonstrate the importance of testing putative mimetic assemblages using known predator perceptual models and lay a foundation for behavioral studies to further test mimicry in *H. sara* and *M. pausanius*.

Introduction

The three players of Batesian mimicry are involved in an evolutionary arms race: the palatable mimic is under selection to resemble the model to avoid predator recognition, the unpalatable model is under selection to appear different from the mimic, and the signal receiver (i.e. the predator) is under selection to improve discrimination between the model and mimic (Bates, 1862; Dawkins & Krebs, 1979). Mimetic resemblance is dependent upon the sensory ecology and physiology of the signal receiver (Stevens, 2013). Much previous research on mimicry has relied on our human perception and not the perception of the ecologically relevant signal receiver (e.g. Lindström, Alatalo & Mappes, 1997). Colorful mimetic signals have evolved in the context of visually guided predators, and these predators may differ greatly in their visual capabilities. Are individuals that appear similar to humans also mimetic in the eyes of their predators, and do predators differ in their ability to discriminate between mimics?

Recently there have been several tests of mimicry involving predator perception. Through an exhaustive study of reef fish mimicry, Cheney & Marshall (2009) found that individuals with a greater number of photoreceptors were better able to discriminate between mimics. Further work on mimicry in salamanders (Kraemer & Adams, 2014), orchids (Papadopulos *et al.*, 2013), avian brood parasites (Langmore *et al.*, 2011; Stoddard, 2012) and butterflies (Bybee *et al.*, 2012; Stoddard, 2012; Llaurens, Joron & Théry, 2014) has shown the importance and specificity of predator perception in the evolution of mimetic assemblages. Collectively, these studies demonstrate that the effectiveness of mimicry is dependent upon the visual system of the predator. However, most studies have only used one predator (Langmore *et al.*, 2011; Stoddard, 2012; Papadopulos *et al.*, 2013) or predators with very different visual systems (Kraemer & Adams, 2014). Cheney & Marshall (2009) examined how mimetic individuals are perceived by different predators with similar visual systems, but in a marine setting, making comparisons to terrestrial systems difficult (Lythgoe,

1979). Few studies have tested how differences in disparate predator perception affect mimicry signals.

In terrestrial ecosystems, avian predators are an important selective pressure on visual mimicry complexes due to birds' sensitive color vision and high visual acuity (Stoddard & Stevens, 2010). Birds are tetrachromatic, possessing four different photoreceptors. The three photoreceptors tuned to the visible spectrum (VIS) are conserved across bird species, and bird visual systems are classified by the sensitivity of the fourth photoreceptor. There are two categories: UV/VIS (ultraviolet sensitive) and V/VIS (violet sensitive) (Vorobyev & Osorio, 1998; Hart *et al.*, 2000; Hart & Hunt, 2007). The UV/VIS system is common in non-flycatcher and non-corvid Passeriformes, while the V/VIS system is found in flycatchers and most non-passerines (Hart *et al.*, 2000). Birds with different visual systems will likely differ in their ability to distinguish between species in a mimetic pair, especially if the species' coloration has an ultraviolet component; therefore, it is important to test mimicry with the appropriate avian visual system.

Neotropical butterflies (Lepidoptera) are an excellent system for studying mimicry. Indeed, the biologists who first described defensive mimicry, H. W. Bates and F. Müller, derived their hypotheses from observations of butterflies in South America (Bates, 1862; Müller, 1879). The Neotropics are known for diverse and complex mimicry systems of Lepidoptera, which primarily focus on unpalatable species in the subfamilies Heliconiinae, Ithomiinae and Danainae (DeVries, 1987; Mallet & Gilbert, 1995). Here, we study a sexually dimorphic butterfly, *Mimoides pausanias*, in which females are similarly sized and colored to *Heliconius sara*; in eastern Ecuador both species are black, yellow and blue (Fig. 1). The aposematic *H. sara* has cyanogenic glycosides, and birds will avoid attacking *H. sara* in aviaries (Chai, 1986). There are no explicit tests of the palatability of *M. pausanias*, but there are no known unpalatable species of *Mimoides* and the putatively mimetic color is restricted to females, most likely rendering this a Batesian relationship (De'Abrera, 1981). Furthermore, both species occur in the same gap habitats and fly at the same height to collect nectar from similar flowers (*Lantana spp*, *Salvia* spp; pers. obs), again rendering them likely to be mimetic to predators.

The main avian predators of *Heliconius* butterflies are tyrant flycatchers (Tyrannidae) and jacamars (Galbulidae), neither of which have ultraviolet sensitivity (Pinheiro, 1996, 2013; Hart, 2001). Observations of predation on *Heliconius* are rare and we are unaware of instances in which birds with the UV/VIS system have attacked *Heliconius*. Therefore, to be effective mimics *M. pausanias* and *H. sara* may not need to match in UV coloration. Here, we first test the hypothesis that *H. sara* and *M. pausanias* are mimetic by measuring the coloration of each species with spectrometry and then using visual models of UV/VIS and V/VIS birds to determine whether these colors are distinguishable to birds. We further hypothesize that these two species of butterflies will be more mimetic to their avian predators, which have the V/VIS visual system, than to avian species with the UV/VIS, which are not likely predators of these tropical butterflies. This work not only tests if there is a *H. sara* mimetic assemblage, but also if mimetic assemblages have been selected to match the visual sensitivities of their predators.

Materials and methods

Specimen collection and preparation

In June 2014, we collected four female *H. sara* and four female *M. pausanias* individuals near Tena, Ecuador (1°06′28″ S, 77°45′45″W). Four *M. pausanias* female individuals were

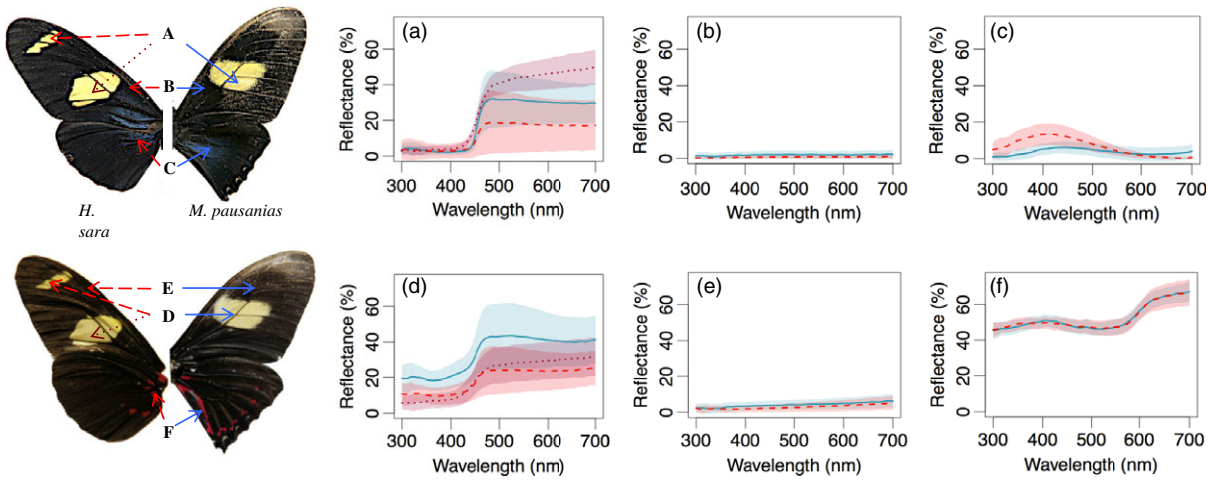

Figure 1 Dorsal and ventral wing patch reflectance for females of *H. sara* and *M. pausanias* for select patches. The left wings represent *H. sara*, whereas the right wings represent *M. pausanias*. Panels (a) and (d) are the dorsal and ventral yellow coloration, respectively, (b) and (e) are dorsal and ventral black, (c) is the iridescent blue on the dorsal hindwing and (f) is the red on the ventral hindwing. The blue line represents the average spectrum for *M. pausanias*, whereas the dashed red line represents the average for *H. sara*. The colored shading shows the 95 percent confidence interval for each species. In panels (a) and (d), the dotted red lines represent the proximal yellow patch of *H. sara*.

caught due to logistical constraints, to reduce population disturbances, and in certain locales it is suspected that only female *M. pausanias* mimic *H. sara* (DeVries, 1987). A recent study shows that four individuals are sufficient to test for the differences in spectral reflectance between species if each individual patch is measured repeatedly (Dalrymple *et al.*, 2015). Butterflies were collected with aerial nets and transported to the lab in glassine envelopes. Individuals were then euthanized by freezing, and each individual's wings were mounted for measurement on black cardstock with Scotch Photo Mount (3M, St. Paul, MN, USA).

Reflectance measurements

Once butterfly wings were mounted, we measured the spectral reflectance of each differently colored patch on both the dorsal and ventral surface of each wing, including the yellow patches of the forewing, black on both the forewing and hindwing, and the iridescent blue patches on the forewing and hindwing (Fig. 1, Table 1). All patches were measured at three separate points where wing wear was minimal (see Supporting Information for photographs of wings). Dorsal measures were taken from the right wing and ventral measures were taken from the left. Except for the iridescent blue patches, all patches were diffusely reflecting, enabling us to use a bifurcated reflectance probe connected to an Ocean Optics USB 2000 spectroradiometer (Dunedin, FL, USA). We first standardized the reflectance measurements with a white standard (Spectralon standard, Ocean Optics, Dunedin, FL, USA) and dark standard in which we occluded any light reaching the spectroradiometer. The reflectance probe was then held perpendicular to the wing surface and reflectance spectra were gathered with SpectraSuite software (Ocean Optics, Dunedin, FL, USA).

Hue and brightness of iridescent coloration depends upon the angle of illumination and observation (Meadows *et al.*, 2011). Therefore, iridescent reflectance must be measured under settings that control both illumination and viewing angle. We placed the mounted wing on the stage of a light table, set illumination angle and viewing angle to 60°, and then adjusted the viewing angle until the iridescent patch was maximally reflected (Meadows *et al.*, 2011).

Light environment measurements

The light environment in which a color is viewed can affect a viewer's perception of that color (Endler, 1990; Stevens, 2013). We were unable to collect light environment measurements from the habitats in Ecuador in which we collected these animals. Previous research on tropical light environments has demonstrated that they do not differ drastically between different rainforests (Endler, 1993). Thus, we measured irradiance and background spectra during mid-day in May 2014 in

Table 1 *P*-values for Just Noticeable Difference (JND) comparisons for chromatic contrasts between *H. sara* and *M. pausanias*

Patch	Visual model	JND	# JNDs > 1	*P*, mean JND > 1	# JNDs > 3	*P*, mean JND > 3	*W*, peafowl JND < blue tit JND	*P*, peafowl JND < blue tit JND
DFW-Blue	Blue Tit (UV)	14.59 (5.47)	16	0.00017	16	0.00017	115	1
	Peafowl (V)	11.70 (5.56)	16	0.00017	15	0.00285		
DFW-Black	Blue Tit (UV)	8.12 (5.39)	16	0.00017	12	**0.42247**	90	0.87786
	Peafowl (V)	4.66 (3.29)	14	0.02299	10	**1**		
DFW-Distal Yellow	Blue Tit (UV)	6.92 (3.23)	15	0.00285	14	0.02299	30	**0.00048**
	Peafowl (V)	2.80 (1.23)	15	0.00285	5	**1**		
DFW-Proximal Yellow	Blue Tit (UV)	3.55 (2.40)	15	0.00285	7	**1**	100	1
	Peafowl (V)	2.15 (1.03)	13	**0.11699**	3	**1**		
DHW-Black	Blue Tit (UV)	12.23 (6.42)	16	0.00017	15	0.00285	123	1
	Peafowl (V)	9.95 (6.53)	16	0.00017	14	0.02299		
DHW-Blue	Blue Tit (UV)	21.31 (11.06)	16	0.00017	16	0.00017	120	1
	Peafowl (V)	16.96 (9.66)	16	0.00017	16	0.00017		
VFW-Black	Blue Tit (UV)	3.98 (2.05)	15	0.00285	9	**1**	115	1
	Peafowl (V)	3.30 (1.72)	14	0.02299	7	**1**		
VFW-Distal Yellow	Blue Tit (UV)	5.21 (3.21)	15	0.00285	11	**1**	63	0.07459
	Peafowl (V)	2.42 (1.30)	14	0.02299	4	**1**		
VFW-Proximal Yellow	Blue Tit (UV)	6.40 (2.81)	16	0.00017	14	0.02299	44	**0.00583**
	Peafowl (V)	3.11 (1.16)	15	0.00285	9	**1**		
VHW-Black	Blue Tit (UV)	11.50 (4.78)	16	0.00017	15	0.00285	47	**0.00922**
	Peafowl (V)	5.72 (2.70)	16	0.00017	14	0.02299		
VHW-Red	Blue Tit (UV)	0.71 (0.43)	4	**1**	0	**1**	140	1
	Peafowl (V)	0.66 (0.39)	3	**1**	0	**1**		

Mean JNDs are given for each patch under each visual system, with standard deviations in parentheses. The patch names are represented by the location (D for dorsal, V for ventral, FW for forewing, and HW for hindwing) and the color. The number of JNDs greater than 1 and 3 are shown with Bonferroni-corrected *P*-values for sign tests examining whether the mean JND is significantly greater than 1 or 3. Bolded values indicate that the JND for that patch are not significantly different from 1 or 3. The final columns present the test statistic, *W*, and Bonferroni-corrected *P*-values for one-tailed Mann–Whitney tests of whether the mean JND under the peafowl model is less than the mean JND under the blue tit model, with significant *P*-values in bold.

lowland tropical rainforest in Soberania National Park, Panama (9.1167°N, 79.7000°W), which is similar to other rainforest irradiance (Endler, 1993).

Heliconius sara and *M. pausanias* both occur in disturbed rainforest and are frequently found in bright, open forest gaps (DeVries, 1987). We therefore measured the light environment of large gaps, which are characterized by no or little vegetative cover. We measured irradiance using a cosine-corrected irradiance probe, a USB 2000 Ocean Optics spectroradiometer and SpectraSuite software (Ocean Optics, Dunedin, FL, USA). We also characterized the spectral properties of the background against which these butterflies occur by measuring background radiance. Each radiance spectra were collected under optimal integration time using SpectraSuite software and a collimating radiance lens connected to an Ocean Optics USB 2000 spectroradiometer via an optic fiber (Ocean Optics, Dunedin, FL, USA).

Data processing and visual models

All data processing and analysis was performed using the *pavo* package version 0.5-1 (Maia *et al.*, 2013) implemented in R version 3.1.2 (R Core Team, 2013). To determine how well avian predators might discriminate the wing colors of *M. pausanias* and *H. sara*, we calculated the Just Noticeable Differences (JNDs) of each of the eleven wing patches we measured. JNDs quantify the discriminability of two colors, with JNDs less than one being physiologically indistinguishable by the viewer due to the large signal to noise ratio within the photoreceptor (Vorobyev & Osorio, 1998; Osorio & Vorobyev, 2005). Two colors with a JND above one will be seen as different colors of stationary objects when side by side in bright light. In more natural settings, two colors with a JND of three or less are unlikely to be seen as different (Siddiqi *et al.*, 2004; Langmore *et al.*, 2011). Furthermore, coloration is perceived by both chromatic differences (e.g. short wavelengths vs. long wavelengths) and by achromatic differences (e.g. gray vs. black). Therefore, we performed both chromatic and achromatic JND comparisons.

Within each color patch, the three reflectance measures were averaged and smoothed using *pavo* (Maia *et al.*, 2013). We then generated quantum catches of these colors with the von Kries transformation (Vorobyev & Osorio, 1998). Using the environmental measurements from Panama, we included tropical irradiance and tropical background vegetation in the visual models. Finally, we calculated chromatic and achromatic JNDs under two different models of bird vision (Vorobyev & Osorio, 1998; Hart, 2001). We used the visual system of the blue tit *Parus caerulus* as a model for UV/VIS (ultraviolet sensitive) vision (Hart *et al.*, 2000), and that of the peafowl *Pavo cristatus* as a model for V/VIS (non-UV sensitive) vision (Hart, 2002). Therefore, the lambda max values for the spectral sensitivities were 371, 448, 503 and 563 for the UV/VIS (blue tit) visual system and 421, 457, 505 and 563 for the V/VIS (peafowl) visual system (Hart, 2001). For the achromatic visual models the double cones were used with lambda max of 503

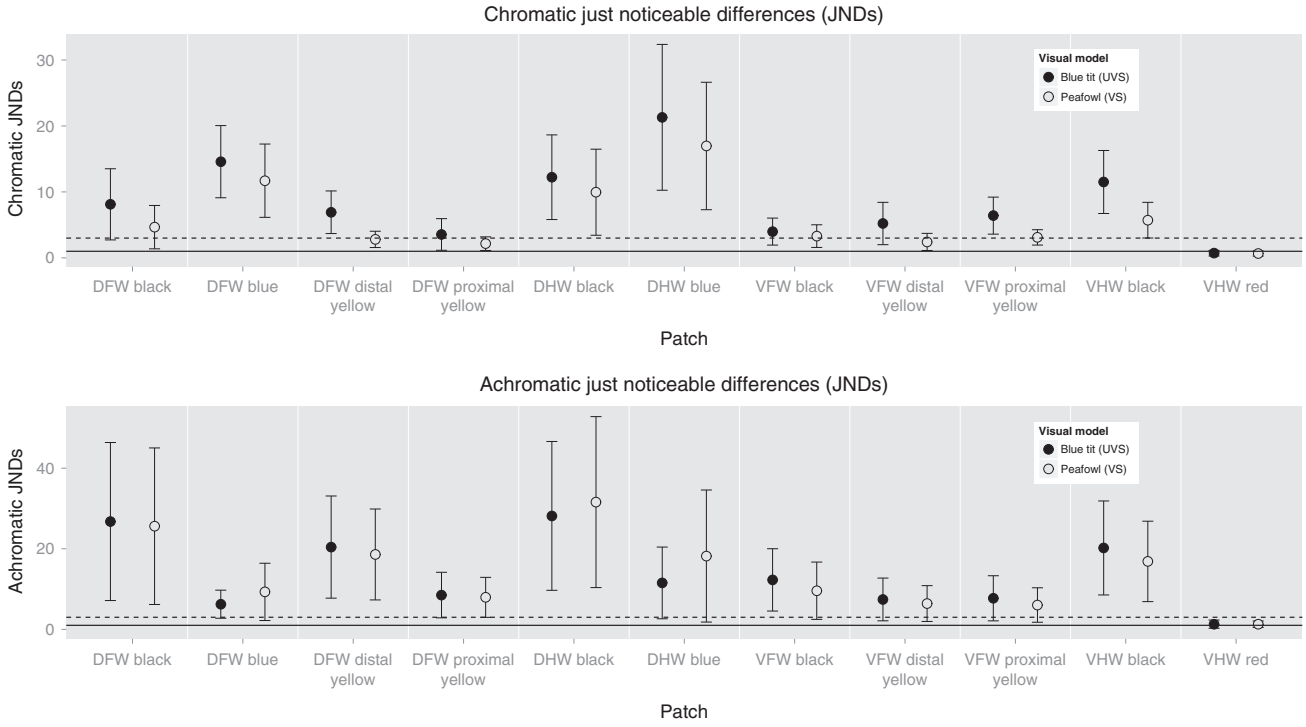

Figure 2 Chromatic and achromatic Just Noticeable Differences (JNDs) between *H. sara* and *M. pausanias* for both avian visual systems at 11 different color patches. The patch names are represented by the location (D for dorsal, V for ventral, FW for forewing, and HW for hindwing) and the color. Circles mark the mean JND for each patch, and the error bars show the standard deviation for each mean.

for the blue tit and 504 for the peafowl (Hart, 2001). The cone abundances were set to 1:1.9:2.68:2.7 for the blue tit model and 1:1.9:2.2:2.1 for the pea fowl (Hart, 2001, 2002. We only included neural noise, not quantum noise. For further details of the models, see the R code in Supporting Information.

Although the two species we used as the visual models do not occur in the tropics, avian visual systems are conserved and both models are reliable approximations of the visual sensitivities of Neotropical UV/VIS and V/VIS birds (Hart, 2001). We calculated all 16 possible pairwise JNDs between the four individuals of each species, and then found the mean JND for each color patch. The JNDs for each patch were idiosyncratically distributed, often highly skewed, and not normal. For these reasons, we used nonparametric sign tests. Because JNDs are threshold measures, differences are only biologically relevant when they are greater than the chosen threshold. Therefore, we used one-tailed tests to determine whether the mean JND was greater than 1 or 3. We also hypothesized that the blue tit visual model would be better able to distinguish between color patches, as *Heliconius* color patterns can have a UV component. To test this, we used one-tailed Mann–Whitney tests to determine whether the mean JND under the blue tit model was greater than the mean JND under the peafowl model. For all tests we examined 11 patches and used Bonferroni correction to adjust *P*-values to account for multiple testing.

We further tested the color match for each analogous patch between these species by comparing color volumes within avian tetrahedral color space. Color volumes encompass the variation in the color patch within avian perceptual color space (Stoddard & Prum, 2011). If two volumes are near and/or overlap, they are very similar if not identical as seen by the receiver (Stoddard & Prum, 2011). For this analysis, we did not average reflectance spectra within a patch, and instead used all 12 measures per species for each patch (3 measures × 4 individuals) to characterize the full color space occupied by each patch. We used *pavo* functions to plot convex hulls of the color space for each species and calculate the volume of the overlap between these hulls (see Supporting Information for R code).

Results

Model-mimic color similarity

The discriminability of analogous color patches of *H. sara* and *M. pausanias* varied greatly. The models suggest that several of the color patches would not be easily discriminable between the two species both chromatically and achromatically when seen by both avian visual systems. The chromatic JNDs were not significantly greater than one for the ventral hindwing red patch and the dorsal forewing proximal yellow patch for the V/VIS system (Sign test, *P*-value = 1 for red, Sign test, *P*-value = .011 for yellow) and not greater than one for the ventral hindwing red patch for the UV/VIS system (sign test, *P*-value = 1; Table 1). The achromatic JNDs were not significantly greater than one for only the ventral hindwing red patch for both visual systems (sign test, *P*-value = 1 for BT; sign test, *P*-value = 1 for PF; Table 1; Fig. 2).

Many patches had mean chromatic JNDs not significantly greater than 3, and thus would be difficult for birds to distinguish in natural lighting conditions. The UV/VIS system would have difficulties discriminating between the two species for the proximal yellow and black patches on the dorsal forewing (sign test, *P*-value = 1; sign test, *P*-value = 0.422; respectively; Table 1; Fig. 2), and the distal black and yellow on the ventral forewing (sign test, *P*-value = 1; sign test, *P*-value = 1; respectively; Table 1; Fig. 2). The V/VIS system would be unlikely to discriminate between all patches on the dorsal forewing except for the iridescent blue patch (see Table 1 for *P*-values). The V/VIS system would also be unlikely to differentiate between the two species for all patches on the ventral forewing. Seven of the 11 patches would be difficult for the V/VIS to distinguish, while only five of the 11 patches would be difficult for the UV/VIS (Fig. 2).

These difficulties in distinguishing color patches also extended to the achromatic component of bird vision, as many patches had mean achromatic JNDs not significantly greater than 3. The UV/VIS system would struggle to distinguish between the two species for the yellow patches on the dorsal forewing and ventral forewing (sign test, $P = 0.12$; sign test, $P = 0.42$; respectively, Fig. 2; Table 2). The V/VIS system would have even more difficulties distinguishing achromatic differences under non-ideal lighting for both iridescent patches, all yellow patches, and the black patch on the ventral forewing (see Table 2 for *P*-values; Fig. 2). The V/VIS would have difficulty discerning between seven of the 11 patches, whereas the UV/VIS would have difficulty with three of the 11 patches (Fig. 2). Furthermore, JND analysis of within-in species comparisons reveals great variation (Supporting Information Table S1), showing that some individuals of the same species are more discriminable than two individuals from the two different species.

Differences between visual systems

The UV/VIS and V/VIS visual systems were quite similar in their ability to distinguish achromatic differences in wing color between the mimetic pair: there were no color patches for which the mean achromatic JND of UV/VIS system was significantly greater than the V/VIS system (one-tailed Mann–Whitney test, see Table 2 for *P*-values; Fig. 2). However, the UV/VIS system was better able to distinguish between the species for three color patches: the dorsal forewing distal yellow (Mann–Whitney, *P*-value < 0.001); the ventral forewing proximal yellow (Mann–Whitney, *P*-value = 0.006); and the ventral hindwing black (Mann–Whitney, *P*-value = 0.009; see table 1 for all patches). These color patches had more variation in their UV spectra, such that UV-sensitive birds could distinguish between the species more readily than birds without UV vision.

Differences in color space volume

The color volumes of each patch comprised a very small area within tetrahedral color space and several patches overlapped in tetrahedral color space for the two species under both visual

Table 2 *P*-values for Just Noticeable Difference (JND) comparisons for achromatic contrasts between *H. sara* and *M. pausanias*

Patch	Visual model	JND	# JNDs > 1	*P*, mean JND > 1	# JNDs > 3	*P*, mean JND > 3	*W*, peafowl JND < blue tit JND	*P*, peafowl JND < blue tit JND
DFW-Blue	Blue Tit (UV)	6.24 (3.49)	16	0.00017	16	0.00017	168	1
	Peafowl (V)	9.30 (7.11)	15	0.00285	12	**0.42247**		
DFW-Black	Blue Tit (UV)	26.76 (19.61)	16	0.00017	16	0.00017	148	1
	Peafowl (V)	25.60 (19.43)	16	0.00017	16	0.00017		
DFW-Distal Yellow	Blue Tit (UV)	20.42 (12.68)	16	0.00017	14	0.02299	149	1
	Peafowl (V)	18.59 (11.28)	14	0.02299	14	0.02299		
DFW-Proximal Yellow	Blue Tit (UV)	8.51 (5.65)	14	0.02299	13	**0.11699**	148	1
	Peafowl (V)	7.94 (4.96)	16	0.00017	12	**0.42247**		
DHW-Black	Blue Tit (UV)	28.16 (18.47)	16	0.00017	16	0.00017	172	1
	Peafowl (V)	31.58 (21.22)	16	0.00017	16	0.00017		
DHW-Blue	Blue Tit (UV)	11.52 (8.90)	16	0.00017	15	0.00285	169	1
	Peafowl (V)	18.19 (16.39)	15	0.00285	13	**0.11699**		
VFW-Black	Blue Tit (UV)	12.28 (7.74)	16	0.00017	16	0.00017	122	1
	Peafowl (V)	9.59 (7.11)	15	0.00285	13	**0.11699**		
VFW-Distal Yellow	Blue Tit (UV)	7.43 (5.30)	14	0.02299	12	**0.42247**	136	1
	Peafowl (V)	6.40 (4.45)	16	0.00017	12	**0.42247**		
VFW-Proximal Yellow	Blue Tit (UV)	7.71 (5.60)	14	0.02299	14	0.02299	125	1
	Peafowl (V)	6.04 (4.28)	15	0.00285	11	**1**		
VHW- Black	Blue Tit (UV)	20.20 (11.66)	15	0.00285	14	0.02299	133	1
	Peafowl (V)	16.86 (9.98)	15	0.00285	15	0.00285		
VHW-Red	Blue Tit (UV)	1.29 (1.02)	8	**1**	1	**1**	155	1
	Peafowl (V)	1.28 (0.80)	9	**1**	1	**1**		

Mean JNDs are given for each patch under each visual system, with standard deviations in parentheses. The patch names are represented by the location (D for dorsal, V for ventral, FW for forewing, and HW for hindwing) and the color. The number of JNDs greater than 1 and 3 are shown with Bonferroni-corrected *P*-values for sign tests examining whether the mean JND is significantly greater than 1 or 3. Bolded values indicate that the JND for that patch are not significantly different from 1 or 3. The final columns present the test statistic, *W*, and Bonferroni-corrected *P*-values for one-tailed Mann–Whitney tests of whether the mean JND under the peafowl model is less than the mean JND under the blue tit model, with significant *P*-values in bold.

models (Table 3, Fig. 3). The dorsal and ventral forewing yellow patches had high percentage overlap as did the dorsal hindwing black (Table 3, Fig. 3.). Several color patches did not have any overlap between the two species, including the red patches, which had a JND of less than one. However, the non-overlapping color patches were close to one another in color space (see Fig. 3 for select patches).

Discussion

Differences between avian visual systems

The perception and classification of visual mimics is crucial to understanding mimicry and predator avoidance strategies. We used visual models to test several predictions of a mimicry assemblage from different predators' perspectives. The discriminability of the colors of these two species varied greatly between color patches and was dependent upon the visual system of the bird species viewing them. Furthermore, these species were more similar when viewed by the V/VIS system of their presumptive predators and were more discriminable by birds with UV vision.

Female *M. pausanias* have likely evolved to mimic only the non-UV reflectance of the unpalatable *Heliconius* model because the avian predators with which it has evolved only see the visible spectrum, rendering mimicry in the UV spectrum unnecessary. The findings here support previous research on *Heliconius* mimicry in which the V/VIS system is poor at discriminating between mimics (Bybee *et al.*, 2012; Llaurens *et al.*, 2014). Bybee *et al.* (2012) investigated the perceptual differences in the yellow patch in *Heliconius* butterflies and closely related genera to find that *Heliconius* butterflies were the best at distinguishing between yellow patches, while birds were inept. Llaurens *et al.* (2014) tested the mimetic resemblance of tiger patterned *Heliconius* butterflies to *Melinaea* species and found that the V/VIS system was the least likely to discriminate between mimetic species, whereas *Heliconius* individuals were able to discriminate between mimics.

The fact that the greatest difference between these two mimetic species was in the UV spectrum is intriguing in the context of conspecific communication between butterflies. Several recent studies have found that butterflies mate assortatively and that UV reflectance may be crucial in this process (Jiggins, Estrada & Rodrigues, 2004; Finkbeiner, Briscoe & Reed, 2014). Furthermore, *Heliconius* species have two different UV-sensitive photoreceptors (Briscoe *et al.*, 2010), suggesting that ultraviolet patterns are important for *Heliconius*. It is likely that individuals within this mimetic complex use UV reflectance for conspecific interactions.

Table 3 Patch color volume overlap for the two mimetic species

Color patch	Visual model	*M. pausanias* volume	*H. sara* volume	Overlap volume	% Overlap
DFW-Blue	Blue Tit (UV)	0.00586	0.00068	0	0
	Peafowl (V)	0.00286	0.00028	0	0
DFW-Black	Blue Tit (UV)	0.00271	0.01210	0.00008	3.06%
	Peafowl (V)	0.00137	0.01598	1.480×10^{-06}	0.11%
DFW-Yellows	Blue Tit (UV)	0.00022	0.00148	0.00004	19.22%
	Peafowl (V)	0.00010	0.00145	0.00001	12.37%
DHW-Black	Blue Tit (UV)	0.01882	0.09849	0.00352	18.68%
	Peafowl (V)	0.01837	0.08868	0.00530	28.86%
DHW-Blue	Blue Tit (UV)	0.02026	0.00278	0	0
	Peafowl (V)	0.01020	0.00136	0	0
VFW-Black	Blue Tit (UV)	0.00076	0.00069	0	0
	Peafowl (V)	0.00009	0.00024	0.00001	11.20%
VFW-Yellows	Blue Tit (UV)	0.00042	0.00098	0.00003	6.36%
	Peafowl (V)	0.00015	0.00093	0.00004	27.87%
VHW-Black	Blue Tit (UV)	0.00109	0.00221	0	0
	Peafowl (V)	0.00031	0.00110	0	0
VHW-Red	Blue Tit (UV)	0.00001	0.00001	2.461×10^{-10}	0.003%
	Peafowl (V)	0.00001	0.00001	0	0

The values for each patch for *M. pausanias*, *H. sara* and the overlap volume are represented as a percentage of total tetrahedral color space. The patch names are represented by the location (D for dorsal, V for ventral, FW for forewing, and HW for hindwing) and the color. Percentage overlap is the quotient of the overlap volume divided by the smaller of the two volumes. Each patch volume is a very small area within tetrahedral color space. There are nine overlaps listed because the two yellow patches of H. sara were combined.

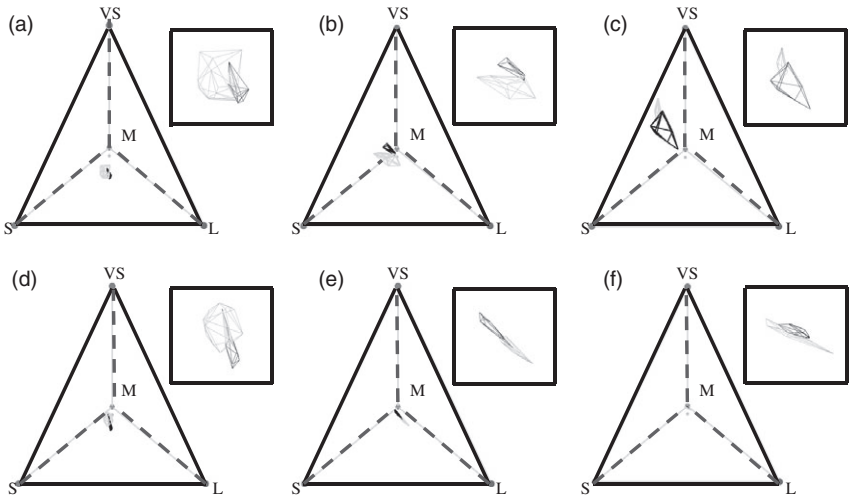

Figure 3 Avian tetrahedral color spaces and color volumes for the six patches in Fig. 1. All colorspaces are for peafowl (V/VIS) vision. The inlays are magnified images of the color volumes to show overlap between the two species. Light gray volumes are *H. sara* and black volumes are *M. pausanias*. (a) Dorsal yellow patch with both the proximal and distal yellow patches of *H. sara* being incorporated. (b) Dorsal black patch. (c) Dorsal hindwing iridescence. (d) Ventral yellow patches with both proximal and distal yellow patches of *H. sara* being incorporated. (e) Ventral black patch. (f) Ventral hindwing red patch.

Imperfect mimetic coloration

The finding that the coloration of several patches of *H. sara* and *M. pausanias* are difficult for predators with V/VIS visual systems to differentiate is perhaps not surprising, since the species' color resemblance is what prompted us to conduct this research. Our results demonstrate that most of the coloration of *H. sara* and *M. pausanias* is mimetic as seen by V/VIS birds under natural conditions, as many patches had JNDs not significantly greater than 3. These two species of butterfly are sympatric both spatially and temporally. Both species occupy disturbed rainforest habitats and are seen under variable light environments and against different backgrounds (Endler, 1993), rendering their mimetic coloration even more difficult to distinguish (Siddiqi *et al.*, 2004). The JNDs of one and three are estimates of true discriminability and tests with live predators and learning trials are needed to determine how mimetic these two species truly are in nature.

As revealed here, these two species are not perfect mimics. Most patches, while difficult to distinguish under natural lighting conditions, are discriminable by both avian visual systems under ideal conditions. Researchers previously expected that strong natural selection should drive mimics to achieve perfect resemblance (Fisher, 1930), but now there are many examples where mimics do not resemble their models perfectly (e.g. hover flies and bees: Edmunds, 2000; Penney *et al.*, 2012;

snakes: Kikuchi & Pfennig, 2012). This has led to several hypotheses explaining "imperfect mimicry": "eye-of-the-beholder", "jack-of-all-trades" and "relaxed selection" (Pfennig, 2012; Pfennig & Kikuchi, 2012). The eye-of-the-beholder hypothesis asserts that imprecise mimicry is due to artifacts of human perception (Cuthill & Bennett, 1993). We have negated this possibility through the use of visual models of predators, again demonstrating the benefits of testing mimicry by incorporating predator perception. The jack-of-all-trades hypothesis posits imperfect mimics are under selection pressures to resemble more than one unpalatable model. This may explain some of the variation we found in this mimetic pair, as there is anecdotal evidence that three other butterfly species, *Heliconius leucadia*, *Heliconius doris* and *Battus belus*, are involved with this mimetic assemblage (De'Abrera, 1981). The relaxed-selection hypothesis asserts that model species that are particularly abundant and well-defended will increase avoidance behaviors in predators, resulting in weaker selection for a perfect mimetic match. *H. sara* is abundant throughout the Neotropics and is protected by cyanogenic glycosides resulting in strong aversion by predators (Nahrstedt & Davis, 1980; Chai, 1986; Pinheiro, 1996) and perhaps there is weak selection for *M. pausanias* to improve its mimetic resemblance. Another possible explanation could be that *H. sara*, like all models in Batesian pairs, is under selection to "escape" from its mimic by evolving new colors patterns (Edmunds, 2000). Further research into the predation pressures on the mimetic coloration of all species involved with the *H. sara* and *M. pausanias* will enable a better understanding of the imperfect mimicry reported here.

Developmental constraints could also lead to imperfect mimicry. Studies of butterflies and vertebrates have revealed a convergent molecular basis for a variety of color pattern traits (Reed *et al.*, 2011; Kikuchi, Seymoure & Pfennig, 2014). Given this, it is possible that pigments in color patches of *M. pausanias* and *H. sara* that are indistinguishable (e.g. the ventral hindwing red patch) are produced by the same or similar molecular pathways while color patches that are easily distinguishable might be produced by different pathways that are developmentally constrained and unable to produce identical color phenotypes. For example *Heliconius* butterflies use 3-hydroxykynurenine as a yellow pigment, whereas *Mimoides* use papiliochrome pigments for yellow coloration (Nijhout, 1991; Koch *et al.*, 2000; Briscoe *et al.*, 2010). *M. pausanias* may be unable to perfectly mimic the yellow of *H. sara* due to constrained pigment production.

The data here reveal large variation in patch reflectance not just within species, but also within individual patches (see Supporting Information Table S1). This large intra-individual variation may further confuse predators and lead to predators avoiding a range of similar mimetic colors. The proximate mechanisms leading to the variation that we found here could be due to differences in condition dependence of the individual, and/or wing degradation due to age and wear on individual wings (Lehnert, 2010; Pegram, Nahm & Rutowski, 2013). Unfortunately, we had little control over wing wear for these wild-caught insects, although we did take precaution in our measurements to avoid damaged or worn areas of the wing.

Conclusion

Batesian mimicry requires mimics to resemble unprofitable models as perceived by natural predators. Differences between visual systems due to disparate spectral sensitivities are crucial for understanding visual signals. We show that two species of tropical butterflies from different families have mimetic coloration as seen by their predators with V/VIS-sensitive vision, but are more easily discriminable by birds with UV-sensitive vision. This leads us to conclude that *M. pausanias* and *H. sara* have evolved mimetic coloration for predators without UV-sensitive vision.

Acknowledgments

We are grateful to the Environmental Ministry of Ecuador for permission to collect butterflies (14-EXP-C3-FAU-DNB/MA). W.O. McMillan and the Smithsonian Tropical Research Institute were crucial for logistical support. We thank I. Aldas and the McMillan Lab for assistance with butterfly collecting, and R. Maia for help with *pavo*. R.L. Rutowski, K.J. McGraw, R. Simpson, the McGraw lab at Arizona State University, and two anonymous reviewers provided valuable comments on an earlier version of this manuscript.

References

Bates, H.W. (1862). Contributions to an insect fauna of the Amazon Valley (Lepidoptera: Heliconiidae). *Trans. Linn. Soc. Lond.* **23**, 495–556.

Briscoe, A.D., Bybee, S.M., Bernard, G.D., Yuan, F., Sison-Mangus, M.P., Reed, R.D., Warren, A.D., Llorente-Bousquets, J. & Chiao, C.C. (2010). Positive selection of a duplicated UV-sensitive visual pigment coincides with wing pigment evolution in *Heliconius* butterflies. *Proc. Natl Acad. Sci. USA* **107**, 3628–3633.

Bybee, S.M., Yuan, F., Ramstetter, M.D., Llorente-Bousquets, J., Reed, R.D., Osorio, D. & Briscoe, A. (2012). UV photoreceptors and UV-yellow wing pigments in *Heliconius* butterflies allow a color signal to serve both mimicry and intraspecific communication. *Am. Nat.* **179**, 38–51.

Chai, P. (1986). Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. *Biol. J. Linn. Soc.* **29**, 161–189.

Cheney, K.L. & Marshall, N.J. (2009). Mimicry in coral reef fish: how accurate is this deception in terms of color and luminance? *Behav. Ecol.* arp017.

Cuthill, I.C. & Bennett, A.T.D. (1993). Mimicry and the eye of the beholder. *Proc. R. Soc. Lond. B Biol. Sci.* **253**, 203–204.

Dalrymple, R.L., Hui, F., Flores-Moreno, H., Kemp, D.J. & Moles, A.T. (2015). Roses are red, violets are blue–so how much replication should you do? An assessment of variation in the colour of flowers and birds. *Biol. J. Linn. Soc.* **114**, 69–81.

Dawkins, R. & Krebs, J.R. (1979). Arms races between and within species. *Proc. R. Soc. Lond. B Biol. Sci.* **205**, 489–511.

De'Abrera, B. (1981). *The butterflies of the neotropical region*. Melbourne: Lansdowne Editions.

DeVries, P. (1987). *The Butterflies of Costa Rica and their natural history*. Princeton: Princeton University Press.

Edmunds, M. (2000). Why are there good and poor mimics? *Biol. J. Linn. Soc.* **70**, 459–466.

Endler, J.A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.* **41**, 315–352.

Endler, J.A. (1993). The color of light in forests and its implications. *Ecol. Monogr.* 2–27.

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.A. (1930). *The genetical theory of natural selection*. Oxford: Clarendon Press.

Hart, N.S. (2001). The visual ecology of avian photoreceptors. *Prog. Retin. Eye Res.* **20**, 675–703.

Hart, N.S. (2002). Vision in the peafowl (Aves: *Pavo cristatus*). *J. Exp. Biol.* **205**, 3925–3935.

Hart, N.S. & Hunt, D.M. (2007). Avian visual pigments: characteristics, spectral tuning, and evolution. *Am. Nat.* **169**, S7–S26.

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.*). *J. Comp. Physiol. A.* **186**, 375–387.

Jiggins, C.D., Estrada, C. & Rodrigues, A. (2004). Mimicry and the evolution of premating isolation in *Heliconius melpomene* Linnaeus. *J. Evol. Biol.* **17**, 680–691.

Kikuchi, D.W. & Pfennig, D.W. (2012). A Batesian mimic and its model share color production mechanisms. *Curr. Zool.* **58**, 4.

Kikuchi, D.W., Seymoure, B.M. & Pfennig, D.W. (2014). Mimicry's palette: widespread use of conserved pigments in the aposematic signals of snakes. *Evol. Dev.* **16**, 61–67.

Koch, P.B., Behnecke, B. & ffrench-Constant, R.A. (2000). The molecular basis of melanism and mimicry in a swallowtail butterfly. *Curr. Biol.* **10**, 591–594.

Kraemer, A.C. & Adams, D.C. (2014). Predator perception of Batesian mimicry and conspicuousness in a salamander. *Evolution* **68**, 1197–1206.

Langmore, N.E., Stevens, M., Maurer, G., Heinsohn, R., Hall, M.L., Peters, A. & Kilner, R.A. (2011). Visual mimicry of host nestlings by cuckoos. *Proc. R. Soc. Lond. B Biol. Sci.* **278**, 2455–2463.

Lehnert, M.S. (2010). New protocol for measuring Lepidoptera wing damage. *J. Lepid. Soc.* **64**, 29–32.

Lindström, L., Alatalo, R.V. & Mappes, J. (1997). Imperfect Batesian mimicry – the effects of the frequency and the distastefulness of the model. *Proc. R. Soc. Lond. B Biol. Sci.* **264**, 149–153.

Llaurens, V., Joron, M. & Théry, M. (2014). Cryptic differences in colour among Müllerian mimics: how can the visual capacities of predators and prey shape the evolution of wing colours? *J. Evol. Biol.* **27**, 531–540.

Lythgoe, J.N. (1979). *Ecology of vision*. Oxford: Clarendon Press; Oxford University Press.

Maia, R., Eliason, C.M., Bitton, P.P., Doucet, S.M. & Shawkey, M.D. (2013). pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol. Evol.* **4**, 906–913.

Mallet, J. & Gilbert, L.E. (1995). Why are there so many mimicry rings? Correlations between habitat, behavior and mimicry in *Heliconius* butterflies. *Biol. J. Linn. Soc.* **55**, 159–180.

Meadows, M.G., Morehouse, N.M., Rutowski, R.L., Douglas, J.M. & McGraw, K.J. (2011). Quantifying iridescent coloration in animals: a method for improving repeatability. *Behav. Ecol. Sociobiol.* **65**, 1317–1327.

Müller, F.. (1879). Ituna and Thyridia: a remarkable case of mimicry in butterflies. *Proc. Entomol. Soc. Lond.* **1879**, xx–xxiv.

Nahrstedt, A. & Davis, R.H. (1980). The occurrence of the cyanoglucosides linamarin and lotaustralin, in Acraea and Heliconius butterflies. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **68**, 575–577.

Nihjout, F. (1991). *The development and evolution of butterfly wing patterns.* Washington, DC: Smithsonian Institution Press.

Osorio, D. & Vorobyev, M. (2005). Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. R. Soc. Lond. B Biol. Sci.* **272**, 1745–1752.

Papadopulos, A.S., Powell, M.P., Pupulin, F., Warner, J., Hawkins, J.A. & Salamin, N., *et al.* (2013). Convergent evolution of floral signals underlies the success of Neotropical orchids. *Proc. R. Soc. Lond. B Biol. Sci.* **280**, 20130960.

Pegram, K., Nahm, A.C. & Rutowski, R.L. (2013). Warning color changes in response to food deprivation in the Pipevine Swallowtail Butterfly, *Battus philenor*. *J. Insect Sci.* **13**, 110.

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**, 461–464.

Pfennig, D. (2012). Mimicry: ecology, evolution and development. *Curr. Zool.* **58**, 604–607.

Pfennig, D. & Kikuchi, D. (2012). Life imperfectly imitates life. *Nature* **483**, 410–411.

Pinheiro, C.E.G. (1996). Palatability and escaping ability in Neotropical butterflies: tests with wild kingbirds (*Tyrannus melancholicus*, Tyrannidae). *Biol. J. Linn. Soc.* **59**, 351–365.

Pinheiro, C.E.G. (2013). Jacamars (Aves, Galbulidae) as selective agents of mimicry in neotropical butterflies. *Rev. Bras. Ornitol.* **12**, 3.

R Core Team. (2013). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: http://www.R-project.org/.

Reed, R.D., Papa, R., Martin, A., Hines, H.M., Counterman, B.A. & Pardo-Diaz, C., *et al.* (2011). *Optix* drives the repeated convergent evolution of butterfly wing pattern mimicry. *Science* **333**, 1137–1141.

Siddiqi, A., Cronin, T.W., Loew, E.R., Vorobyev, M. & Summers, K. (2004). Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *J. Exp. Biol.* **207**, 2471–2485.

Stevens, M. (2013). *Sensory Ecology, Behaviour, & Evolution*. Oxford: Oxford University Press.

Stoddard, M.C. (2012). Mimicry and masquerade from the avian visual perspective. *Curr. Zool.* **58**, 630–648.

Stoddard, M.C. & Prum, R.O. (2011). How colorful are birds? Evolution of the avian plumage color gamut. *Behav. Ecol.* **22**, 1042–1052.

Stoddard, M.C. & Stevens, M. (2010). Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proc. R. Soc. Lond. B Biol. Sci.* rspb20092018.

Vorobyev, M. & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. Lond. B Biol. Sci.* **265**, 351–358.

Supporting Information

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

Figures S1–S8. Photographs of individual butterflies. The first photograph and the last three are the *M. Pausanias* (labeled with B_ or Bat_) individuals and 2–5 are *Heliconius sara* (labeled with *H. sara*).
Table S1. Results of the within-species JND comparisons
Data S1. R scripts: this file contains all data preparation and analysis, as implemented using *pavo*.
Data S2. Background spectra, illumination spectra, and photographs of all specimens used in the analysis.