

Iridescent hindwing patches in the Pipevine Swallowtail: differences in dorsal and ventral surfaces relate to signal function and context

Ronald L. Rutowski*, Alexandra C. Nahm† and Joseph M. Macedonia‡

School of Life Sciences, Arizona State University, Tempe, Arizona 85287-4501, USA

Summary

1. Iridescent colour signals are directional but, like diffusely reflected colours, vary within and among species in ways that may be adaptations to specific types of receivers in specific light environments.
2. The hindwings of pipevine swallowtail butterflies exhibit brilliant blue and iridescent colour patches on the ventral surface in both sexes and on the dorsal wing surface in males. Evidence suggests that the ventral iridescent blue is a component of the warning coloration of this distasteful species, while the dorsal blue iridescent wing area is a sexual signal. Given differences in the function and ecological context of signal production, we analysed reflectance spectra from the iridescent blue areas of both field-caught and laboratory-reared animals to test several predictions about the iridescent colour patches on these wing surfaces.
3. The ventral blue patches in the warning coloration of males and females should be most visible early and late in the day, due to wing orientation relative to sun angle. We therefore predicted that these iridescent colour patches would be brighter and of longer wavelengths than the male dorsal blue patch displayed during midday courtships. The prediction about reflectance intensity was supported but the prediction about hue was not.
4. We predicted that the sexually selected dorsal hindwing iridescence of males would be more variable among individuals and condition dependent than the naturally selected ventral iridescent colour patches. To assess variation and condition dependence, laboratory-reared and field-captured individuals were compared. The prediction about variation was not supported, but only the dorsal wing surfaces showed evidence of being condition dependent.
5. We investigated whether development of dorsal and ventral blue iridescence was coupled by determining correlations in colour properties between the wing surfaces. Our finding of positive correlations indicated a potential developmental constraint in the evolution of colour differences between the two wing surfaces.
6. Results of this study suggest that some properties of iridescent coloration on the hindwing of the pipevine swallowtail (especially intensity) may have been fine-tuned by evolution in response to prevailing ambient light conditions and viewing perspectives that differ among types of signal receivers.

Key-words: butterflies, iridescence, signal ecology, sexual signal, warning coloration

Introduction

The design features of communication signals are expected to covary with their function and the behavioural and

ecological circumstances under which they are presented (Bradbury & Vehrencamp 1998). For example, honest signalling theory suggests that signals used to assess the quality of potential mates will be more variable among individuals and condition-dependent than signals, such as warning signals, that are used in naturally selected predator–prey interactions (Andersson 1994; Cotton *et al.* 2004; Dale 2006; Ruxton, Sherratt & Speed 2004). Also, for reflected light signals, the available ambient illumination should favour signals whose properties enhance transmission and

*Correspondence author. E-mail: r.rutowski@asu.edu

†Present address: Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, Maryland 21250, USA.

‡Present address: Department of Biology, Florida Southern College, Lakeland, Florida 33801, USA.

reception (Hailman 1977; Endler 1993). For example, reflectors used to produce signals given under conditions of dim illumination should reflect a greater proportion of incident light than those used to produce signals given when light is plentiful. Lastly, and especially for iridescent signals, the relative positions and behaviour of sender, receiver, and light source should interact to maximize the visibility of the iridescent signal to intended receivers (Poulton 1890; Rutowski *et al.* 2007; Doucet & Meadows 2009).

The pipevine swallowtail butterfly, *Battus philenor* (Linnaeus), provides an excellent opportunity for evaluating these predictions within a single species. A field of brilliant iridescent blue coloration is found on both the dorsal and ventral surfaces of the male hindwing (Fig. 1). As in other butterflies (Oliver, Robertson & Monteiro 2009) the function and signaling context of these two wing surfaces are arguably very different. The colour pattern on the ventral hindwings of both sexes (orange and cream spots, as well as blue iridescent patches) acts as an aposematic signal that advertises distastefulness to potential predators (Brower 1958; Brower & Brower 1962; Codella & Lederhouse 1990). Their distastefulness stems from the larval sequestration of aristolochic acids in the foodplant (Fordyce, Marion & Shapiro 2005). While the relative roles of the ventral pattern elements have not been experimentally parsed, the potential importance of blue patches to avian or other predators is indicated by the fact that it is a conspicuous component of the coloration of species (at least four) that mimic *B. philenor* (Brower 1958). Previous work suggests that ventral hindwing coloration is an important signal when butterflies are perched late and early in the day with their wings closed (Wourms & Wasserman 1985; Lederhouse, Codella & Cowell 1987). At these times, ambient illumination is expected to be coming from the sun at a low angle and,

therefore, of low intensity and biased in spectral composition toward the long-wavelengths (Nordtug & Melø 1988; Endler 1993). Strong stabilizing selection on colour pattern in the context of aposematism is expected to produce a signal of relatively low variability among individuals (Ruxton, Sherratt & Speed 2004) and high reflectance to enhance conspicuousness and recognizability (Théry, Pincebourde & Feer 2008).

In contrast, the dorsal iridescent area of the hindwing is brightest in males, which, based on studies of other butterflies, suggests a role in mate choice (Kemp 2007, 2008a) or in intersexual interactions at territories (Lederhouse 1982). During aerial courtship manoeuvres in *B. philenor* (Rutowski, Alcock & Carey 1989; D. Papaj, pers. comm.), the male positions himself below the female and flies up in front of her repeatedly, clearly displaying his dorsal wing surfaces to her. Moreover, both intra- and intersexual interactions are most likely to occur in the middle of the day when ambient illumination is brightest and from a high sun angle (Rutowski, Alcock & Carey 1989). So, if the dorsal iridescent area is a sexually selected trait we predict it to be more variable among males and more condition-dependent than the ventral iridescent field. These are patterns that have been observed in the colour signals of other butterflies (Kemp 2006, 2008b; Kemp & Macedonia 2006; Kemp & Rutowski 2007), and the ability to discriminate colours is well established in swallowtail butterflies (Kelber & Pfaff 1999; Kinoshita, Shimada & Arikawa 1999; Takeuchi, Arikawa & Kinoshita 2006) including *B. philenor* (e.g. Snell-Rood & Papaj 2006).

Here we present a study of the blue iridescence of *B. philenor* and how it varies between the wing surfaces, between the sexes, and between individuals reared in the laboratory and collected in the field. We begin by demonstrating that the blue reflectance from the hindwing of *B. philenor* exhibits the hue

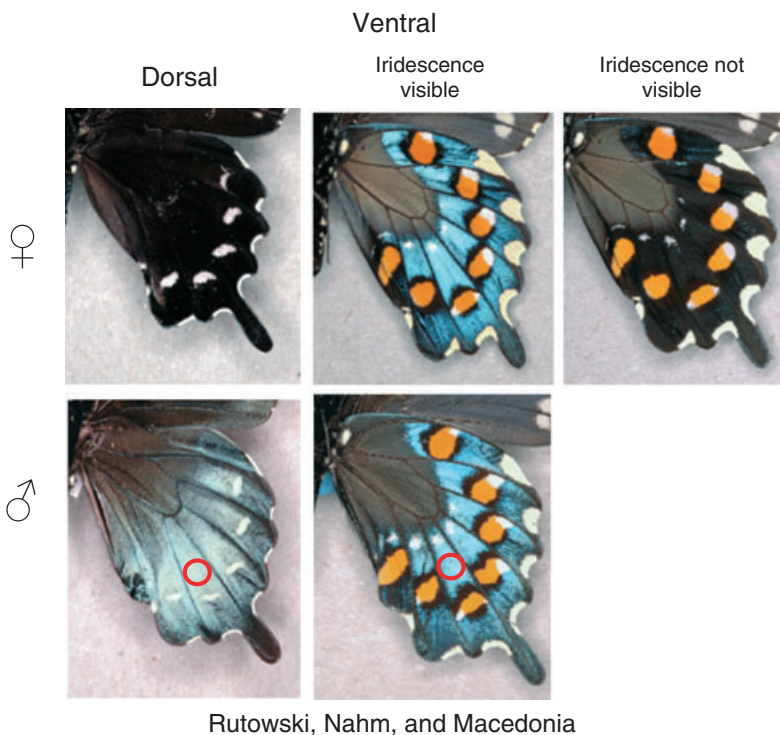


Fig. 1. Photographs of hindwing surfaces of *B. philenor*. Red circles on male wings show the location and approximate size of the areas from which spectral measurements were taken for both males and females. An image is included for the female wing as an example of how the ventral hindwing appears when the light source is positioned so that the iridescence was not visible to the camera.

shift with changes in angle of incidence and viewing angle that is the defining characteristic of iridescence (Simon 1971). We also assess the extent to which correlations exist within individuals between dorsal and ventral coloration. We then test several predictions that are drawn from the arguments above about differences among *B. philenor* hindwing surfaces that concern the properties of the presumptive iridescent signals.

1. The intensity of the ventral iridescent reflection will be greater than that of the dorsal iridescent reflection. This is predicted because of the lower light intensities under which the ventral signal is used.

2. The hue of the ventral iridescent patch will be at longer wavelengths than that of the dorsal iridescent patch. This is predicted due to the greater intensity of reddish light that will illuminate the aposematic ventral hindwing early and late in the day.

3. Under context-appropriate directions of solar illumination, the angle over which the iridescent reflection of the ventral surface is visible will be greater than that for the dorsal surface. This is predicted because the direction of approach of predators, most likely birds, toward perching butterflies is expected to be more variable than the direction from which females view male dorsal iridescent areas during courtship.

4. The colour of the ventral iridescence will be less variable among males than that of the dorsal iridescence. This is predicted if the ventral and dorsal iridescent patches are naturally and sexually selected, respectively.

5. The properties of the ventral iridescence will be less dependent on rearing conditions than those of the dorsal iridescence. This is predicted if the dorsal iridescent patch is a sexual signal and the ventral iridescent patch is a predator-directed signal.

Materials and methods

PROVENANCE OF SPECIMENS

All specimens were from a population of *B. philenor* that thrives near the confluence of Mesquite Wash and Sycamore Creek in the Mazatzal Mountains, Arizona (33° 43' 50" N, 111° 30' 50" W). Field-caught individuals were collected from late April to early June. We collected 50 males easily but females were more difficult to find and capture, so our sample of females was limited to 12 individuals. We also reared 50 males and 50 females in the lab. We collected eggs and early instar larvae in the field, and eggs from females brought into the lab that were provided with food plant for oviposition. Larvae were fed *ad libitum* on cuttings of the local larval food plant, *Aristolochia watsonii*. Larvae were reared to adulthood in a walk-in environmental chamber, programmed for 14 h of light at 30 °C and 10 h of dark at 24 °C. Relative humidity was a constant 55%.

SPECTRAL ANALYSES OF IRIDESCENT WING AREAS

In preparation for spectral measurements, both hindwings of each butterfly were removed from the thorax and mounted on black card stock with spray adhesive. Each individual's left hindwing was

mounted ventral side up and its right hindwing dorsal side up. Reflectance spectra were taken using an Ocean Optics USB 2000 spectrometer (Dunedin, FL, USA) with two separate optical fibres, one for delivering incident illumination from an Ocean Optics PX-2 xenon light source and the other for collecting light that was passed to the spectrophotometer. Both fibres were outfitted with a collimating lens such that the lens gathered light from a spot 2 mm in diameter from the centre of a larger illuminated spot about 5 mm in diameter. To measure the shift in hue with angle of incidence, the angle between the optical axes of the light beam and collector was set at 30°, 60°, 90°, and 120° which corresponds to angles of incidence of 15°, 30°, 45°, and 60°. We made all other measurements with a 60° angle between the optical axes of the light beam and collector (Fig. 2). The hindwing base (attachment point to the body) was directed at the collector's azimuth, and the hindwing tail was directed toward the light beam azimuth. Each wing was placed on a stage that could be tilted toward or away from the collector and then was tilted until the reflectance was maximized. Red circles in Fig. 1 show the specific wing areas from which spectral data were collected. Reflectance spectra relative to a Teflon tape white standard (which was laid on the wing) were gathered and analysed over the wavelengths of 300–700 nm.

The angle over which a wing surface's iridescence was visible was quantified for three individuals by positioning the light source normal to the wing surface and then moving the collector to selected positions in the hemisphere over the wing surface. The collector positions at which spectral samples were gathered lay along four lines of elevation starting at azimuth 0°, 45°, 90°, and 135°, respectively, relative to a line running from the centre of the wing to the wing base. Along each line of elevation a reflectance spectrum was taken every 15°, except at elevation 0° (the equator; wing surface not visible) and elevation 90° (directly overhead; light source and collector could not be positioned in the same position). For each collector position, maximum reflectance was derived from the spectrum and averaged over the three individuals. These means were plotted on a polar plot and lines of equivalent maximum percent reflectance drawn by visual interpolation.

COLOUR PARAMETERS

Because the reflectance spectra of iridescent wing surfaces were unimodal, we extracted the following colour parameters to describe and analyse the properties of the wing reflectance (Montgomery 2006).

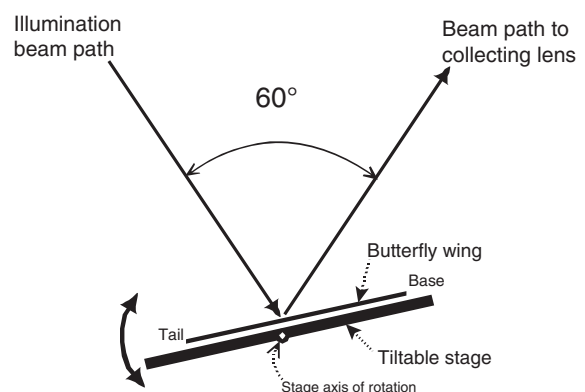


Fig. 2. Schematic diagram of the arrangement of the light source, collector, butterfly wing, and stage for measuring the iridescent reflectance.

1. Intensity: The largest percent of reflected light relative to the white standard in the spectrum between 300 and 700 nm.
2. Hue: The wavelength between 300–700 nm at which the percent reflectance was greatest. Note that because of the general flatness of spectra obtained from female dorsal hindwing surfaces, the hue values for this surface varied in ways that reduced their merit for describing coloration. Hence, hue values for the female dorsal hindwing were not included in these analyses.
3. Chroma: The sum of all reflectances measured at 1 nm intervals in the 100 nm-wide segment of the spectrum centred on the hue, divided by the sum of all reflectances in the total wavelength range (300–700 nm). For the female dorsal wing surface, chroma was calculated in the same way but using the average hue for the ventral female hindwing.

BODY SIZE AND CONDITION MEASURES

For laboratory-reared individuals, wet body mass was measured at approximately 24 h post eclosion when the meconium had been ejected. Dry body mass for males only was measured after the wings had been removed from the body and the body then lyophilized for 24 h. Both body masses were measured to the nearest milligram using an analytical balance (GR-202, A & D Engineering, Inc., Milpitas, CA, USA). Forewing length was measured to the nearest 0.1 mm using dial callipers from wing tip to the point where the costal vein inserts into the thorax.

For field-caught individuals, wing wear was used as a proxy for age and was scored on a 5 point scale with one being given to fresh individuals showing little scale loss or wing tattering and five being given to individuals with extensive scale loss and large pieces of the wings missing (Kemp 2006).

We used two measures of male condition, both of which follow from the premise that individuals with a high body mass relative to their linear dimensions are in good condition (see Schulte-Hostedde *et al.* 2005; but for a recent critique, Schamber, Esler & Flint 2009). One measure used was the ratio of body mass to forewing length for both dry and wet masses; the other described each individual's condition as the reduced major axis (RMA) residual from the regression between the logarithm of forewing length and the logarithm of body mass, done for both dry and wet masses.

STATISTICAL ANALYSIS

Statistical procedures (e.g. ANOVA) were run using SPSS version 15.1 (SPSS, Inc., Chicago, IL, USA). Statistical inferences were made at the 0.05 level of significance. Reduced major axis slope and intercept were calculated using an RMA web application (Bohonak & van der Linde 2004).

Results

HINDWING IRIDESCENT PATCHES: HUE SHIFT WITH CHANGES IN ANGLE OF INCIDENCE

The blue reflectance of the male dorsal, male ventral, and female ventral hindwings of *B. philenor* is iridescent, as indicated by the decrease in peak wavelength with increasing angles of incidence and complementary increases in viewing angle (Fig. 3).

IRIDESCENT AREA OF VENTRAL HINDWING

The intensity, hue, and chroma of the ventral iridescent reflectance are summarized for both sexes and for lab-reared and field-caught adults in Table 1. We ran an ANOVA on the three ventral hindwing colour parameters with source and sex as fixed factors (Table 2). There was no significant difference between the sexes in intensity, but the intensity of the lab-reared animals' ventral iridescent reflection was consistently greater than that of the field-collected animals. With respect to hue, females were greener (greater hue value) and less chromatic than males, and field-collected animals of both sexes were greener and less chromatic than lab-reared animals. These results indicate that there are sexual differences in the hue and chroma of ventral coloration and that the different rearing conditions (lab vs. field) produce consistent differences in all three colour parameters.

The differences in coloration between field-collected and laboratory-reared animals could be due to age as indicated by wing wear and in fact, for the ventral surface of the sample of field-caught males, wing wear was negatively correlated with intensity ($r = -0.311$, $P < 0.05$) and chroma ($r = -0.628$, $P < 0.05$) and positively correlated with hue ($r = 0.566$, $P < 0.05$; more worn individuals were greener; 49 d.f. for all). These changes in colour with wing wear are consistent with the direction of differences observed between field-captured animals in general and the less worn laboratory-reared animals.

IRIDESCENT AREA OF DORSAL HINDWING

The intensity, hue, and chroma of iridescent areas of the dorsal hindwing are summarized for both sexes and for lab-reared and field-collected individuals in Table 1. Again, we ran an ANOVA with sex and source as fixed factors (Table 2). The dorsal iridescent reflection of males is significantly more intense and more chromatic than that of females, and the iridescent reflection of laboratory-raised animals is brighter than that of field-caught animals. The dorsal iridescent areas are no more chromatic in field-captured than in laboratory-reared animals. These results show that there are sexual differences in the intensity and chroma of dorsal hindwing

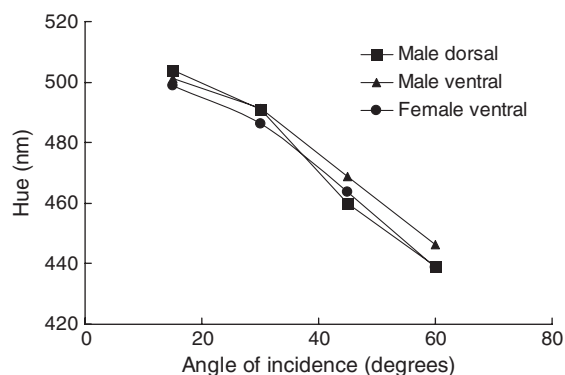


Fig. 3. Change in hue with angle of incident illumination for the ventral (males and females) and dorsal (males only) iridescence. Sample size is five males and five females.

Table 1. A summary of the colour parameters for both laboratory-reared and field-caught males and females. Colour parameters were calculated as described in the text. Statistics given are mean \pm standard deviation (minimum–maximum). Sample sizes are: 50 for lab males, 50 for lab females, 50 for field males, and 12 for field females

| Source | Surface | Sex | Intensity | Hue (nm) | Chroma |
|---------|---------|----------------------------|------------------------------|---------------------------------|---------------------------------|
| Lab | Dorsal | Male | 26.9 \pm 6.9 (11.2–50) | 490 \pm 15.8 (452–531) | 0.416 \pm 0.014 (0.384–0.441) |
| | | Female | 9.17 \pm 2.56 (2.21–16) | N/A | 0.296 \pm 0.028 (0.238–0.372) |
| Field | Ventral | Male | 86.3 \pm 22.4 (38.3–134.7) | 486 \pm 17.8 (458–531) | 0.439 \pm 0.015 (0.41–0.47) |
| | | Female | 82.8 \pm 18.2 (50.3–126.6) | 500 \pm 20.8 (457–569) | 0.423 \pm 0.012 (0.406–0.463) |
| | Dorsal | Male | 30.4 \pm 11.6 (12.2–79.2) | 499 \pm 23.1 (458–571) | 0.408 \pm 0.028 (0.323–0.459) |
| | | Female | 18.1 \pm 9.76 (7.91–44.6) | N/A | 0.314 \pm 0.048 (0.236–0.395) |
| Ventral | Male | 66.9 \pm 20.5 (29.2–102) | 504 \pm 25.9 (461–563) | 0.412 \pm 0.029 (0.337–0.453) | |
| | Female | 67.6 \pm 24.2 (32.2–110) | 514 \pm 28.8 (478–560) | 0.401 \pm 0.033 (0.327–0.437) | |

Table 2. The results of an ANOVA on the colour parameters for the hindwing surfaces with sex (male, female) and source (laboratory, field) as fixed factors

| Surface | Effect | Colour parameter [F-ratio (<i>P</i> -value)] | | |
|---------|---------------------|---|--|--|
| | | Intensity | Hue | Chroma |
| Ventral | Sex | 0.12 (0.73) | 7.75 (< 0.001) (male < female) | 6.43 (0.012) |
| | Source | 19.4 (< 0.001) (lab < field) | 15.5 (< 0.001) (lab > field) | 44.03 (< 0.001) (lab < field) |
| | Sex \times Source | 0.283 (0.6) | 0.394 (0.531) | 0.08 (0.778) |
| Dorsal | Sex | 95.3 (< 0.001) (male > female) | 112.2 (< 0.001) (male < female) | 197.4 (< 0.001) (male > female) |
| | Source | 16.3 (< 0.001) (lab < field) | 0.482 (0.489) | 0.232 (0.631) |
| | Sex \times Source | 3.26 (0.073) | 0.126 (0.723) | 1.47 (0.227) |

The direction of significant effects (in bold) is indicated. See Table 1 for sample sizes.

iridescent blue which are consistent with subjective observations, and that rearing conditions produce some consistent differences in intensity but not chroma.

Interestingly, for the dorsal surface of the field-caught males, wing wear was not accompanied by any changes in colour parameters (intensity, $r = 0.198$; hue, $r = 0.226$; chroma, $r = -0.173$; for all, $P > 0.05$, 49 d.f.). This suggests that the dorsal wing surface is affected less by wing wear than the ventral surface and that some observed differences in dorsal coloration might be due to differences in rearing conditions alone.

DIFFERENCES BETWEEN WING SURFACES IN MALES

For males alone we ran an ANOVA evaluating the effects of wing surface (dorsal, ventral) and source (lab, field) as fixed

factors (Table 3). The ventral iridescent patch was, as expected, significantly more intense than that of the dorsal wing surface. The chroma of the iridescent signals was also greater for the ventral than the dorsal wing surface. However, contrary to expectation there was no significant difference in hue between the male dorsal and ventral iridescent signals. As in the analysis of only the males' ventral surfaces, the dorsal iridescent areas of lab-reared males had a greater intensity and chroma and lower hue than the field-collected males. The single significant interaction term indicated that the differences in chroma between lab-raised and field-captured males were greatest for the ventral wing surface.

Coefficients of variation for the intensity, hue, and chroma of the dorsal wing surface were 0.257, 0.00324, and 0.033, respectively, while those for the ventral wing surface were

Table 3. The results of an ANOVA on the colour parameters for the *B. philenor* male hindwing with surface (dorsal, ventral) and source (lab, field) as fixed factors

| Colour parameter [F-ratio (<i>P</i> -value)] | | | |
|---|--|--|---|
| Effect | Intensity | Hue | Chroma |
| Surface | 426 (< 0.001) (dorsal < ventral) | 0.148 (0.701) | 9.213 (0.003) (dorsal < ventral) |
| Source | 12.5 (< 0.001) (lab > field) | 22.8 (< 0.001) (lab < field) | 38.0 (< 0.001) (lab > field) |
| Source \times Surface | 18.4 (< 0.001) | 1.41 (0.237) | 6.06 (0.015) |

The direction of significant effects (in bold) is indicated. See Table 1 for sample sizes.

0.26, 0.036, and 0.035, respectively. The patterns evident in these results are that the difference between wing surfaces are small and those for the dorsal wing surface were slightly smaller, 11% or less, than those for the ventral wing surface which is contrary to prediction.

For all colour parameters and for lab-reared males, the value for the dorsal wing surface was correlated with that for the ventral wing surface (Fig. 4).

VISIBILITY OF BLUE IRIDESCENCE FROM DIFFERENT VIEWING ANGLES

Figure 5 shows how the intensity of the ventral and dorsal iridescent reflections changes with viewer position in the hemisphere over the wing surface when the light source is at the apex of the hemisphere. We consider this light source position to approximate that of the solar orb (1) when the ventral iridescent patch of a butterfly might be seen by a predator when it approaches a butterfly at its overnight perch with the wings closed very late or very early in the day (low sun angle), and (2) when the dorsal iridescent patch of a male butterfly is displayed during courtship when he is positioned with his wings open below a female around the middle of the day (high sun angle). Although the maximum intensity of the ventral iridescent reflection is much greater than that of the dorsal surface, the overall pattern in the two plots is very similar.

DIFFERENCES IN BODY SIZE AND CONDITION

Body size characteristics for laboratory-reared and field-caught animals of both sexes are summarized in Table 4. An ANOVA was carried out on both forewing length and wet body mass with sex and source (lab or field) as fixed factors. For both measures of body size, lab-raised animals were much larger than field-collected animals (mass: $P < 0.001$; forewing

length; $P < 0.001$). Females had a larger forewing length than males ($P = 0.003$) but the sexes did not differ in body mass ($P = 0.766$) among lab-reared or field-captured animals (sex \times source: $P = 0.235$). However, the sex difference in forewing length was larger in lab-raised than in field-caught animals (sex \times source: $P = 0.05$). Combined with results reported above, lab-rearing produced animals that were larger, and whose iridescent patches were more intense, blue, and chromatic than those of field-caught animals. Not all of these differences were explained by the fact that some animals in the field sample displayed substantial wing wear.

To assess the impact of condition on male coloration, we evaluated how intensity, average brightness from 300–700 nm, hue, and chroma of the iridescent signals varied with two indices of condition in the lab-reared animals. These measures included (1) the ratio of male body mass to forewing length (calculated for both wet and dry body mass), and (2) the RMA residuals calculated from the regression of logarithm of male body mass against logarithm of forewing length (calculated for both wet and dry body mass). Average dorsal brightness for males was significantly positively correlated with the ratio of wet body mass to forewing length ($r = 0.286$, $P < 0.05$), but no other colour parameter (including average brightness) for either wing surface was significantly positively correlated with either of these condition indices (for all, $r < 0.279$, $P > 0.05$).

Discussion

The directionally reflecting blue coloration on both hindwing surfaces in *B. philenor* meets the definition of iridescence, as indicated by the observed shift in hue with changes in the angle of incident illumination. At this time the precise mechanisms responsible for producing the iridescent colours in this species are not known, although they are known for others

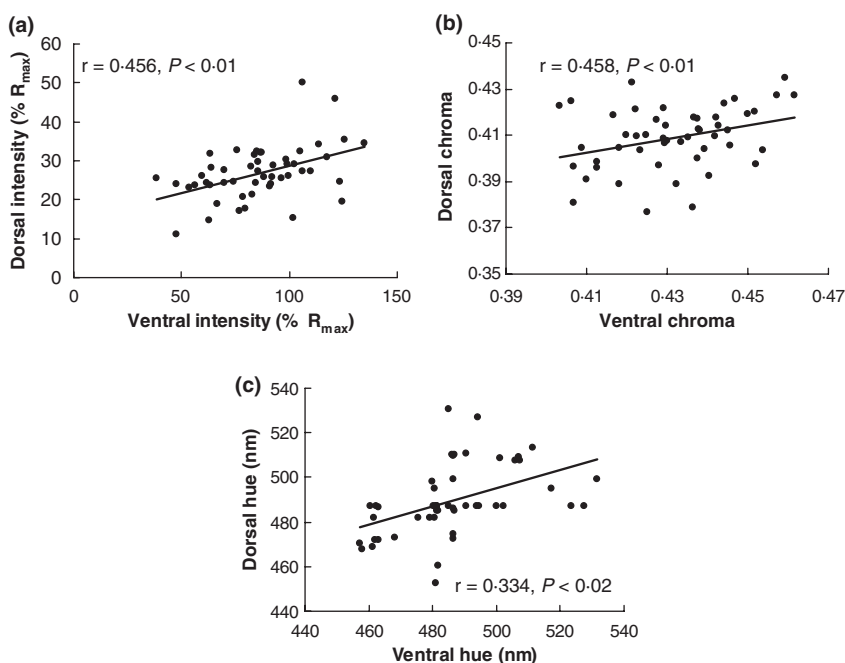


Fig. 4. The relationship between (a) intensity, (b) hue, and (c) chroma of the dorsal and ventral hindwings blue patches for 50 lab-reared males.

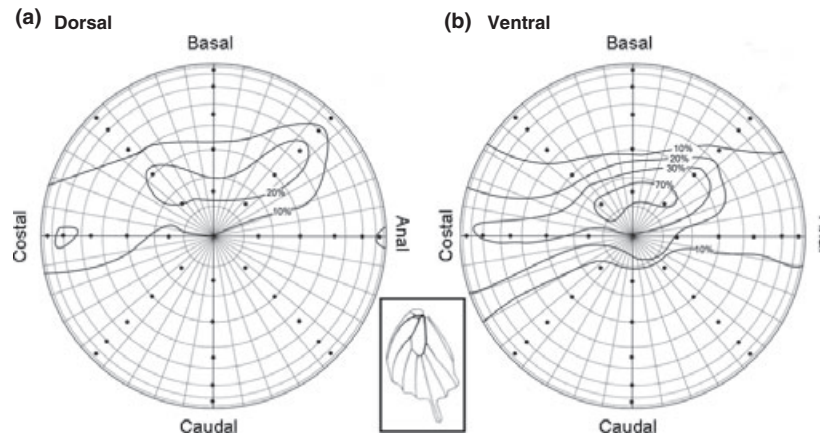


Fig. 5. Hemispherical plots showing how the intensity of the reflection from the hindwing iridescent patch changes as viewer position changes for (a) ventral and (b) dorsal surfaces. In each plot, the light source was at the apex of the hemisphere over the wing surface. Both azimuth (radial) and elevation (concentric) lines are at 10° intervals. Insert: A hindwing shown as oriented under the hemisphere when the measurements were made.

Table 4. Descriptive statistics for body size of both lab and field samples as measured by forewing length and body mass

| Measure | Source | Sex | Mean \pm SD | Min–Max | N |
|----------------------|--------|--------|-----------------|-----------|----|
| Forewing length (mm) | Lab | Male | 49.1 \pm 2.22 | 43.2–52.3 | 50 |
| | | Female | 51.8 \pm 1.92 | 46.8–55.4 | 50 |
| | Field | Male | 42.8 \pm 3.71 | 33.1–50 | 50 |
| | | Female | 43.5 \pm 3.41 | 33.9–46.9 | 12 |
| Wet body mass (mg) | Lab | Male | 457 \pm 79.8 | 332–711 | 50 |
| | | Female | 477 \pm 84 | 304–767 | 50 |
| | Field | Male | 188 \pm 55.7 | 84–314 | 50 |
| | | Female | 178 \pm 15.3 | 83–264 | 12 |

(for review, Kinoshita & Yoshioka 2005). The similarities in the properties of the reflection of ventral and dorsal iridescent signals, as well as in scale structure observed with scanning electron microscopy (unpublished observations), lead us to suspect that the reflectance mechanism is the same on both wing surfaces and in both sexes.

HINDWING COLORATION AND FUNCTION

Consistent with qualitative perceptions, we found that the iridescent reflection of the dorsal hindwing of males is much more intense than that of females and that the only sex difference on the ventral surfaces is that males are 9 to 14 nm 'bluer' than females. The magnitude of the difference is potentially detectable by a swallowtail butterfly (Takeuchi, Arikawa & Kinoshita 2006; Koshitaka *et al.* 2008), and we have planned experiments to determine what visual cues, if any, are used by males to distinguish females from other males. Krebs & West (1988) provide evidence that males of the tiger swallowtail (*Papilio glaucus*) do use visual cues in mate identification.

Several predictions outlined in the Introduction are reiterated here with comments on their status in light of the results.

1. The intensity of the ventral iridescent reflection will be greater than that of the dorsal iridescent reflection. This pre-

diction was supported; the intensity of the ventral iridescence in both males and females was on average more than twice that of the dorsal iridescence. This finding supports the hypothesis that the signals are tuned to ambient light conditions when they are displayed. Further understanding of the causes and implications of this difference will require additional experimentation. Several key questions warrant special attention. The first question concerns whether the relatively less intense dorsal wing surface functions adequately as an intraspecific sexual signal under midday illumination. Answering this question will need take into consideration that the male's wings are being flapped when the colour signal is being displayed to the female which will lead to temporal changes in intensity and chroma but probably not hue like the iridescent signals of other species (Rutowski 1977; Vukusic *et al.* 1999; Rutowski *et al.* 2007). The dark ground colour of the wing (probably due to melanin deposited in the iridescent and other scales (Nijhout 1991)) against which the iridescence will be viewed no doubt enhances the amplitude of these temporal changes in appearance as has been suggested for the ultraviolet iridescence of male sulphurs viewed against scales invested with ultraviolet-absorbing pterin pigments (Rutowski *et al.* 2005).

The second question is whether the extreme brightness of the ventral wing iridescence contributes to the aposematic signal presented to avian predators under low-sun-angle and indirect illumination. The third question addresses how the

blue iridescent patches interact with other colour patches, especially on the ventral hindwing to produce an effective signal. Contrasts in intensity and hue between for example the blue and orange patches (internal contrasts), may be important for the overall effectiveness of the aposematic coloration of this animal (see Endler & Théry 1996; Théry, Pincebourde & Feer 2008 for examples of internal contrast in sexual signals). Answering these questions will require behavioural study and inferences about perception that take into account actual illuminance levels at different times of the day and the colour processing systems of intended receivers in addition to the spectral properties of the various colour patches.

2. The hue of the ventral iridescent patch will peak at longer wavelengths than that of the dorsal iridescent patch. This prediction was not supported; there was no difference in hue between the male's dorsal and ventral iridescent patches. In contrast, Schultz *et al.* (2008) found interspecific differences in damselfly coloration that were as predicted by the chromatic features of environmental light conditions at the times of day when the different species were active. Their study took into account the colour spaces of potential receivers, which we do not do here. The failure to meet this prediction could be because (1) the differences in the hue of ambient illumination are not large enough to drive the evolution of differences in the reflectance properties of the iridescent patches on the two wing surfaces, or (2) the developmental processes that produce iridescent scales are coupled across the two wing surfaces in a way that has constrained differentiation of colour signals on each side (see below).

3. Under context-appropriate directions of solar illumination, the angle over which the iridescent reflection of the ventral surface is visible will be greater than that for the dorsal surface. This prediction was supported primarily because of differences in intensity between wing surfaces. The overall similarity in the angular spread of the iridescent reflections suggests to us that the basic scale arrangement does not differ dramatically between the two wing surfaces. However, because the ventral hindwing iridescence is so much brighter than the dorsal, when viewed by the same visual system under the same illumination the ventral iridescence will be perceivable over a larger angle.

4. The colour of the ventral iridescence will be less variable among males than that of the dorsal iridescence. This prediction was not supported. Male and female wing surfaces were equally variable with respect to the three colour parameters evaluated. Heightened variation is expected for male signals that are used by females as indicators of genetic or material benefits that she might acquire from the male (Kemp 2006). However, signals of species identity are not necessarily expected to show such elevated variation relative to non-sexual signals (Dale 2006). Given that *B. philenor* is sympatric over much of its range with species that mimic its coloration (Brower 1958; Brower & Brower 1962), the dorsal iridescence may play a role in species recognition.

5. The properties of the ventral iridescence will be less dependent on rearing conditions than those of the dorsal

iridescence. As predicted, the dorsal iridescent coloration showed some evidence of condition dependence not found in the iridescent blue of the ventral wing surface. However, the evidence was not strong. Laboratory conditions may have been too invariable and salubrious (including stable temperature and humidity regimes as well as continuous access of larvae to high-quality food plant) to reveal the full effect of condition dependence. The patterns of variation in the coloration are also difficult to determine due to difficulties in sorting out the effects of age, rearing environment, and diet. Especially perplexing was that the blue iridescent patches of field-captured males were significantly more intense than that of lab-reared males. The reasons for this are unclear; the effects of manipulating larval food availability on male coloration are currently under investigation in our lab.

DORSAL-VENTRAL CORRELATIONS IN COLORATION: DEVELOPMENTAL CONSTRAINT OR FUNCTIONAL OVERLAP?

The ventral intensity, hue, and chroma are correlated with the respective colour parameters on the dorsal hindwing. Developmental coupling may lead to these correlations. That is, it may not be possible for the expression of genes for the structures that produce iridescent colour patches to be independently adjusted for the two wing surfaces. This seems unlikely given cases in butterflies of extreme differences between the dorsal and ventral wing colour pattern, e.g. butterflies in the genera *Delias* (Common & Waterhouse 1982) and *Memphis* (DeVries 1987). In contrast to *B. philenor*, however, these examples do not involve the expression of structural colours on both wing surfaces, which may impose some special and as yet unknown developmental constraints.

FINAL CONSIDERATIONS

The visibility of the iridescent colour patches in *B. philenor* depends greatly on the angle of view and of the incident illumination. The directionality of the iridescence leads to questions about the source of the light contributing to a visible iridescent reflection. In a trivial sense the light is coming from the reciprocal location of the viewer in the space above the wing surface. Previously we have argued that the solar orb is the most relevant light source contributing to iridescent signals (Rutowski *et al.* 2007). However, from considering the complexities of the behavioural and ecological contexts in which iridescent signals are produced by *B. philenor*, it is clear that more information is required to understand better how iridescent signals act and are perceived in nature. For example, it would be useful to know the solid angle of the irradiance contributing to the visible iridescence as well as the spectral properties of the light in that solid angle. This is a complex problem that is currently under consideration in our laboratory.

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References

- Andersson, M. (1994) *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Bohonak, A.J. & van der Linde, K. (2004) *RMA: Software for Reduced Major Axis Regression, Java Version*. Website: <http://www.kimvlinde.com/professional/rma.html>.
- Bradbury, J.W. & Vehrencamp, S.L. (1998) *Principles of Animal Communication*. Sinauer Associates Inc., Sunderland, MA.
- Brower, J.V.Z. (1958) Experimental studies of mimicry in some North American butterflies. II. *Battus philenor* and *Papilio troilus*, *P. polyxenes*, and *P. glaucus*. *Evolution*, **12**, 123–136.
- Brower, L.P. & Brower, J.V.Z. (1962) The relative abundance of model and mimic butterflies in natural populations of the *Battus philenor* mimicry complex. *Ecology*, **43**, 154–158.
- Codella, S.G. Jr & Lederhouse, R.C. (1990) The effect of wing orientation on aposematic signaling in the Pipevine Swallowtail butterfly, *Battus philenor*. *Animal Behaviour*, **40**, 404–406.
- Common, I.F.B. & Waterhouse, D.F. (1982) *Butterflies of Australia, Field Edition*. Angus and Robertson Publ., London.
- Cotton, S., Fowler, K. & Pomiankowski, A. (2004) Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis?. *Proc Royal Soc B*, **271**, 771–783.
- Dale, J. (2006) Intraspecific variation in bird colors. *Bird Coloration, Volume 2, Function and Evolution*, (eds G. E. Hill & K. J. McGraw), pp 36–86. Harvard University Press, Cambridge, MA.
- DeVries, P.J. (1987) *The Butterflies of Costa Rica: and Their Natural History: Papilionidae, Pieridae, and Nymphalidae*. Princeton University Press, Princeton, NJ.
- Doucet, S.M. & Meadows, M.G. (2009) Iridescence: a functional perspective. *Interface Focus, Supplement 2*, **6**, S115–S132.
- Endler, J. (1993) The color of light in forests and its implications. *Ecological Monographs*, **63**, 1–27.
- Endler, J.A. & Théry, M. (1996) Interacting effects of lek placement, display behavior, ambient light, and color patterns in three Neotropical forest-dwelling birds. *American Naturalist*, **148**, 421–452.
- Fordyce, J.A., Marion, Z.H. & Shapiro, A.M. (2005) Phenological variation in chemical defense of the Pipevine Swallowtail, *Battus philenor*. *Journal of Chemical Ecology*, **31**, 2835–2846.
- Hailman, J.P. (1977) *Optical Signals: Animal Communication and Light*. Indiana University Press, Bloomington.
- Kelber, A. & Pfaff, M. (1999) True colour vision in the Orchard Butterfly, *Papilio aegaeus*. *Naturwissenschaften*, **86**, 221–224.
- Kemp, D.J. (2006) Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. *Evolutionary Ecology Research*, **8**, 515–527.
- Kemp, D.J. (2007) Female butterflies prefer males bearing bright iridescent ornamentation. *Proceedings of the Royal Society B*, **274**, 1043–1047.
- Kemp, D.J. (2008a) Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). *Behavioral Ecology*, **19**, 1–8.
- Kemp, D.J. (2008b) Resource-mediated condition dependence in sexual dichromatic butterfly wing coloration. *Evolution*, **62**, 2346–2358.
- Kemp, D.J. & Macedonia, J.M. (2006) Structural ultraviolet ornamentation in the butterfly *Hypolimnas bolina* L. (Nymphalidae): visual, morphological and ecological properties. *Australian Journal of Zoology*, **54**, 235–244.
- Kemp, D.J. & Rutowski, R.L. (2007) Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. *Evolution*, **61**, 168–183.
- Kinoshita, M., Shimada, N. & Arikawa, K. (1999) Colour vision of the foraging swallowtail butterfly, *Papilio xuthus*. *Journal of Experimental Biology*, **202**, 95–102.
- Kinoshita, S. & Yoshioka, S., eds., (2005) *Structural Colors in Biological Systems: Principles and Applications*. Osaka University Press, Osaka, Japan.
- Koshitaka, H., Kinoshita, M., Vorobyev, M. & Arikawa, K. (2008) Tetrachromacy in a butterfly that has eight varieties of spectral receptors. *Proceedings of the Royal Society London B*, **275**, 947–955.
- Krebs, R.A. & West, D.A. (1988) Female mate preference and the evolution of female-limited Batesian mimicry. *Evolution*, **42**, 1101–1104.
- Lederhouse, R.C. (1982) Territorial defense and lek behavior of the black swallowtail butterfly, *Papilio polyxenes*. *Behavioral Ecology and Sociobiology*, **10**, 109–118.
- Lederhouse, R.C., Codella, S.G. & Cowell, P.J. (1987) Diurnal predation on roosting butterflies during inclement weather: a substantial source of mortality in the black Swallowtail, *Papilio polyxenes*. *Journal of the New York Entomological Society*, **95**, 310–319.
- Montgomery, R. (2006) Analyzing colors. *Bird Coloration, Volume 1, Mechanisms and Measurements* (eds G. E. Hill & K. J. McGraw), pp. 90–147. Harvard University Press, Cambridge, MA.
- Nijhout, H.F. (1991) *The Development and Evolution of Butterfly Wing Patterns*. Smithsonian Institution Press, Washington and London.
- Nordtug, T. & Melø, T.B. (1988) Diurnal variations in natural light conditions at summer time in arctic and subarctic areas in relation to light detection in insects. *Holarctic Ecology*, **11**, 202–209.
- Oliver, J., Robertson, K.A. & Monteiro, A. (2009) Accommodating natural and sexual selection in butterfly wing pattern evolution. *Proceedings of the Royal Society B*, **276**, 2369–2375.
- Poulton, E.B. (1890) *The Colors of Animals: Their Meaning and Use, Especially Considered in the Case of Insects*. D. Appleton and Co., New York.
- Rutowski, R.L. (1977) The use of visual cues in sexual and species discrimination by males of the small sulphur butterfly, *Eurema lisa* (Lepidoptera, Pieridae). *Journal of Comparative Physiology*, **115**, 61–74.
- Rutowski, R.L., Alcock, J. & Carey, M. (1989) Hilltopping in the pipevine swallowtail butterfly (*Battus philenor*). *Ethology*, **82**, 244–254.
- Rutowski, R.L., Macedonia, J.M., Morehouse, N. & Taylor-Taft, L. (2005) Pterin pigments amplify iridescent ultraviolet signal in males of the orange sulphur butterfly, *Colias eurytheme*. *Proceedings of the Royal Society B*, **272**, 2329–2335.
- Rutowski, R.L., Macedonia, J.M., Merry, J.W., Morehouse, N., Yturralde, K., Taylor-Taft, L., Gaalema, D., Kemp, D.J. & Papke, R. (2007) Iridescent ultraviolet signaling in the Orange Sulphur butterfly (*Colias eurytheme*): spatial, temporal, and spectral properties. *Biological Journal of the Linnean Society*, **90**, 349–364.
- Ruxton, G.D., Sherratt, T.N. & Speed, M.P. (2004) *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry*. Oxford University Press, New York.
- Schamber, J.L., Esler, D. & Flint, P.L. (2009) Evaluating the validity of using unverified indices of body condition. *Journal of Avian Biology*, **40**, 49–56.
- Schulte-Hostedde, A.I., Zinner, B., Millar, J.S. & Hickling, G. J. (2005) Restitution of mass-size residuals: validating body condition indices. *Ecology*, **86**, 155–163.
- Schultz, T.D., Anderson, C.N. & Symes, L.B. (2008) The conspicuousness of colour cues in male pond damselflies depends on ambient light and visual system. *Animal Behaviour*, **76**, 1357–1364.
- Simon, H. (1971) *The Splendor of Iridescence: Structural Colors in the Animal World*. Dodd, Mead, and Co., New York.
- Snell-Rood, E.C. & Papaj, D.R. (2006) Learning signals within sensory environments: Does host cue learning in butterflies depend on background? *Animal Biology*, **56**, 173–192.
- Takeuchi, Y., Arikawa, K. & Kinoshita, M. (2006) Color discrimination at the spatial resolution limit in a swallowtail butterfly, *Papilio xuthus*. *Journal of Experimental Biology*, **209**, 2873–2879.
- 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.
- Vukusic, P., Sambles, J.R., Lawrence, C.R. & Wootton, R.J. (1999) Quantified interference and diffraction in single *Morpho* butterfly scales. *Proceedings of the Royal Society B*, **266**, 1403–1411.
- Wourms, M.K. & Wasserman, F.E. (1985) Bird predation on Lepidoptera and the reliability of beak marks in determining predation pressure. *Journal of the Lepidopterists Society*, **39**, 239–261.

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