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Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration

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ABSTRACT

Hypothesis: Structural coloration (iridescent ornamentation) should vary more greatly among individuals and with age than pigment coloration. Structural coloration requires nano-scale precision and might thus more honestly signal male age and condition than pigment-based colours.

Organism: A natural population of the orange sulphur butterfly, *Colias eurytheme*. *C. eurytheme* females prefer young males, choosing them on the basis of an unknown signal.

Site: Cultivated alfalfa (*Medicago sativa*) fields near Avondale, Arizona, August–September 2004.

Methods: I randomly sampled 70 'pristine' males and an additional 98 males in five age classes. I then measured key reflectance characteristics (brightness, hue and chroma) of the dorsal structural ultraviolet markings and of the coincident pigmentary yellowish-orange.

Conclusions: Consistent with the sexual selection hypothesis, structural reflectance parameters are more phenotypically variable. Structural reflectance also fades more with age. However, the higher phenotypic variance of structural colour renders it a less precise indicator of male age than pigment colour.

Keywords: age, *Colias eurytheme*, iridescence, Lepidoptera, mate choice, sexual selection.

INTRODUCTION

The ability to generate colour via structural means is widespread in nature and responsible for some of the most visually brilliant ornamentation presently known (Vukusic and Sambles, 2003). Structural colour effects grade in complexity from simple scattering (i.e. Rayleigh/ Tyndall scattering) to the complex multi-layer interference effects responsible for many iridescent butterfly wing colours (e.g. Vukusic *et al.*, 1999, 2000). Recent investigations in butterflies have revealed startling and hitherto unknown optical mechanisms, such as limited view colour flicker, and wavelength-specific polarization plane conversion [for a description of

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these mechanisms, see Vukusic *et al.* (2000, 2001)]. In many cases, structural colour mechanisms demand nano-structural surface arrays of high architectural complexity and precision, which has prompted evolutionary biologists to question their adaptive significance (Andersson, 1999). Given that structural colours are often elaborated in males, the leading hypothesis is that this trait is sexually selected (Brunton and Majerus, 1995; Kemp *et al.*, 2005). Mate choice experiments in butterflies (Silberglied and Taylor, 1978; Sweeney *et al.*, 2003) and birds (Johnsen *et al.*, 1998) support this hypothesis, although the ultimate advantages of using dedicated nano-structural coloration – in terms of signal design and content – are not yet fully understood.

One hypothesis for the evolution of structural colour badges is that they are relatively difficult to produce and therefore provide honest information to potential mates regarding condition, genetic viability or likely direct benefits. Evidence is mounting that structural ornamentation in birds may honestly signal male phenotypic condition (Keyser and Hill, 1999, 2000; Johnsen *et al.*, 2003). The necessarily high and uniform nanometre-scale precision required in the construction of this trait may predispose it as a magnifier of viability differences among genotypes (Andersson, 1999). In holometabolous insects, such as butterflies, constituent structural arrays are constructed during metamorphosis (Ghiradella, 1974) and are destined to degrade thereafter. Structural ornamentation in this group may therefore carry information pertaining to breeding value and/or larval-derived condition, but it also has important potential as an honest indicator of adult age. This is of likely importance in butterflies for two reasons. First, sperm quality may decline with age in male insects (Siva-Jothy 2000), and thus older males may be less likely to produce fertile ejaculates. Second, male butterflies transfer at mating nutritious nuptial gifts, known as spermatophores, from which females extract and utilize nutrients to increase their reproductive output (Boggs and Gilbert, 1979; Rutowski *et al.*, 1987). Given that the quantity and/or quality of these ejaculates decreases irrevocably with each successive mating (Svard and Wiklund, 1986), older males are (on average) likely to produce less nutritious ejaculates, and to take longer to deliver them (Rutowski and Gilchrist, 1986) because they are (on average) more likely to have mated previously or recently.

Here I investigate the potential relative information content of dedicated nano-structural versus pigmentary ornamentation in the polyandrous butterfly *Colias eurytheme*. Males of this species utilize a highly specific and repeated nano-structural array on their dorsal wing surfaces that constructively interferes with light wavelengths in the 300–450 nm range (Ghiradella, 1974; Silberglied and Taylor, 1978). These ultraviolet (UV) reflecting structures occur on the uppermost surface of individual wing scales and are situated above low-wavelength absorbing pteridine pigments, which occur in granules within the scale lumen (Ghiradella, 1974). The result is specular and highly spectrally pure UV reflectance that almost perfectly overlays a 'background' of pigmentary yellow-orange (see below; also Fig. 1). These two coincident colour elements (the structural UV and the pigmentary yellowish-orange) may each vary independently among individuals; however, the UV reflectance has been implicated in female mate choice, whereas the yellowish-orange has not [refer to the detailed experimental work of Silberglied and Taylor (1978)]. Thus, the *C. eurytheme* dorsal wing offers a system in which structural and pigmentary colours occur on the same area of the wing and represent putative sexual and non-sexual traits, respectively.

I set out to evaluate two specific predictions. First, sexual ornaments are expected to be more phenotypically variable than equivalent non-sexual traits (Schluter and Price, 1993; Badyaev, 2004); hence, I predicted greater variance in structural UV reflectance characteristics. Second, female *C. eurytheme* are known to mate preferentially with younger males, and

it is thought that by doing so they may experience lower copulation durations (Rutowski and Gilchrist, 1986) and obtain higher quality ejaculates (Rutowski, 1985; see Discussion). I predicted that, if the cue for this choice is structural UV, then UV reflectance characteristics should degrade faster and more reliably than pigment yellowish-orange with increasing age under field conditions.

MATERIALS AND METHODS

Free-flying adult butterflies were sampled haphazardly from cultivated alfalfa (*Medicago sativa*, the larval foodplant of *C. eurytheme*) fields near Avondale, Arizona, between 27 August and 13 September 2004. From a total of several hundred samples, I selected 70 males with no visible wing wear (i.e. 'pristine' specimens) and a further 98 males in five evenly spaced wing wear classes to represent the breadth of age variation in this population. Wing wear is commonly used as a surrogate measure of age in butterflies (e.g. Rutowski, 1985; Karlsson, 1994; Kemp, 2000) and other insects (e.g. Plaistow and Siva-Jothy, 1996), and I used standard classification criteria based upon the degree of visible fading (i.e. loss of wing scales) of the outer melanic dorsal band (visible in Fig. 1) and damage to the wing margins:

1 = No visible fading or damage to wing margins
2 = Limited visible fading (< 20% scale loss), with one or two discrete 'chips' in the margins
3 = Some visible fading (20–40% scale loss), with several discrete chips in the margins
4 = Appreciable fading (40–60% scale loss), with multiple areas of damage to the wing margins including a slight 'tattered' appearance
5 = Extreme fading (> 60% scale loss) and 'tattered' wing margins

In reality, wing wear is a measure of adult activity, which will by necessity covary with age. Captured butterflies were killed, their forewings removed and mounted on black card using spray adhesive (3M brand), then pressed flat for 24 h.

I captured reflectance spectra for both forewings using an Ocean Optics USB-2000 spectrophotometer (25 averaged spectra, 125 ms integration time), with pulsed illumination provided at 90° (to the horizontal) by a PX-2 xenon light source. Figure 1 outlines the measurement set-up. The structurally derived UV portion of the reflectance curve is dependent upon the angle of the wing relative to the light source and receiver (i.e. the structural reflectance is 'limited view'). The relative angle conducive to peak reflectance varies slightly among individuals (D.J. Kemp, unpublished data) and therefore it is necessary to remove this source of variation when measuring each specimen's UV reflectance. I therefore rotated each sample wing surface within a 5–6° range (indicated in Fig. 1 by the symbol γ), and, while watching the reflectance curves on a computer screen in real time, recorded the spectrum at the orientation where the UV curve was highest. Given that the yellowish-orange coloration is not angle-dependent in its appearance, I captured another scan of each wing with the sample situated flat (the reflectance curves presented later are a composite of these two scans). Reflectance in all cases was expressed as a proportion of that obtained from a magnesium oxide white standard, which is assumed to provide 100% reflectance across the 300–700 nm range. The PX-2 light source was allowed 90 min 'warm-up' time, and re-calibrated against the white standard after every fifth measurement. Drift (from 100% reflectance of the standard) between calibrations was less than 5% in all cases and usually in the order of 2–3%.

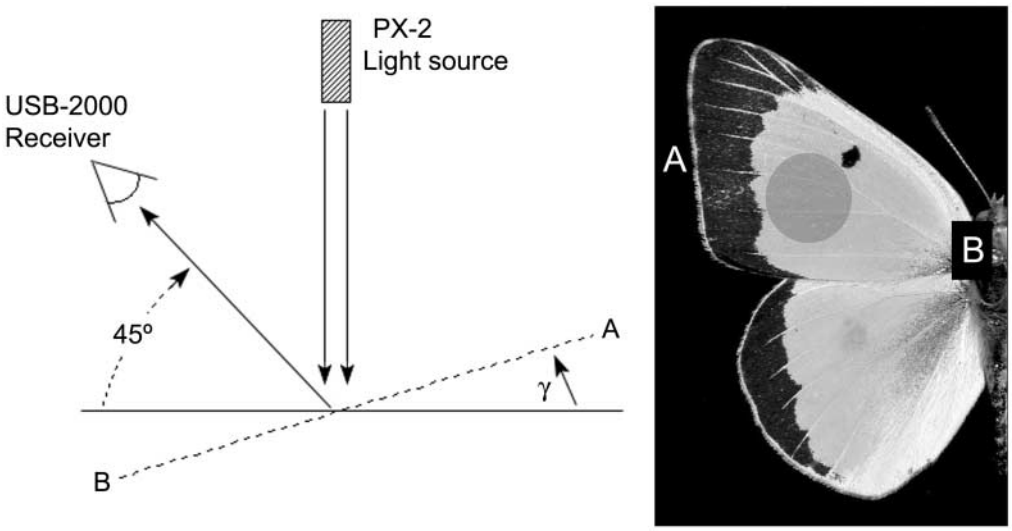

Fig. 1. Diagrammatic representation of the measurement set-up (left-hand panel) used to capture reflectance spectra from the dorsal male *C. eurytheme* forewing (right-hand panel). The spectrometer probe was angled at 45° relative to the horizontal, and focused to capture light from an approximately circular 5 mm area (depicted as the shaded circular area of male forewing). The section AB indicates the placement of the wing, which was rotated a fraction (γ) to locate each specimen's angle of maximum reflectance (as explained in the text). The 'light' region of these wings, which appears yellowish-orange to humans, is the region almost perfectly overlaid by the structural UV reflectance.

The standard dorsal male *Colias* reflectance curve (Fig. 2) exhibits a parabolic reflectance peak in the 300–450 nm range, low mid-wavelength (i.e. 450–550 nm) reflectance, and a rapid transition to high and uniform long wavelength (> 550 nm) reflectance. The UV peak derives from the presence of the specific nano-scale architecture, while the second two features collectively result from a combination of broad wavelength (i.e. 'white') light scattered off lower wing scale structures and the presence of short wavelength (i.e. < 550 nm) absorbing pteridine pigments (Watt, 1964). I summarized specimen reflectance curves in terms of seven colour parameters (symbols in parentheses correspond to those indicated in Fig. 2):

1. UV peak – the peak percent reflectance at the top of the UV 'curve' (α).
2. Long-wave peak – the peak percent long wavelength reflectance (β).
3. Mid-wave nadir – the lowest percent reflectance of the short human visual wavelength 'valley' (δ).
4. UV hue – the wavelength value corresponding to the UV peak (ε).
5. Yellowish-orange hue – the wavelength value at the halfway point (χ) between β and δ (ϕ).
6. UV chroma – the summed brightness values in a 20 nm range around the peak (b), divided by the summed brightness values of two 20 nm ranges either side of this range ($a_1 + a_2$).
7. Yellowish-orange chroma – the summed brightness values of the 50 nm range $\phi + 50$ (d) divided by the summed values of the range $\phi - 50$ (c).

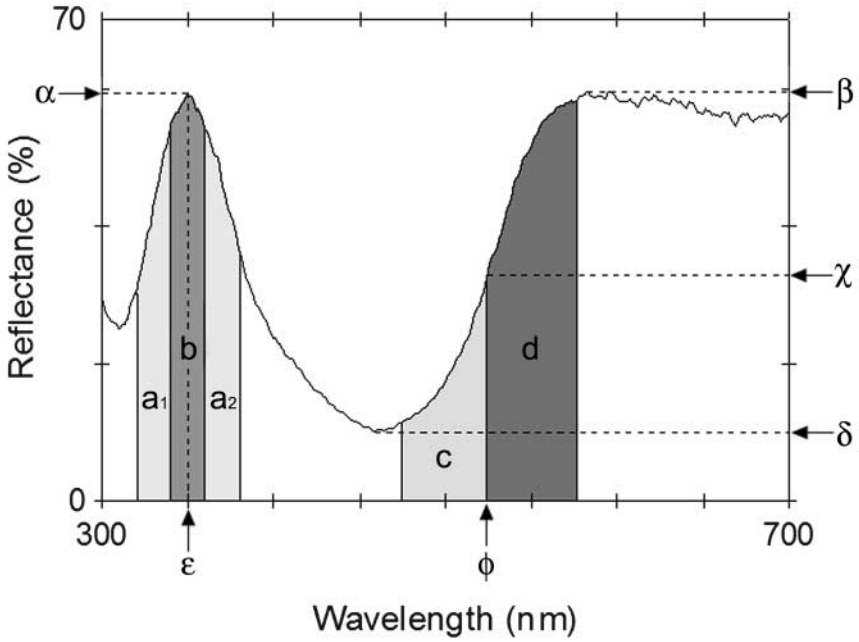

Fig. 2. Sample dorsal male *C. eurytheme* reflectance curve indicating the parameters measured for UV peak (α), UV hue (ε), UV chroma ($b/[a_1 + a_2]$), mid-wave nadir (δ), long-wave peak (β), and yellowish-orange hue (ϕ) and chroma (d/c).

Since it is not presently understood how potential receivers (e.g. female *C. eurytheme*) integrate the relative spectral information, the summary of reflectance characteristics via these parameters appears most reasonable. The fact that the three brightness measures (1, 2 and 3 above) are all proportions relative to white standard reflectance, and the two hue measurements (4 and 5) are in equivalent nanometre units, also eliminates the potentially confounding effects of scale in comparisons within these groups of parameters. Chroma measures are arbitrary and those obtained for the UV and yellowish-orange are not directly cross-comparable; hence, I report chroma values and regressions for age effects, but refrain from making among-colour component contrasts of chroma. None of the seven parameters departed from normality (Kolmogorov-Smirnov $d < 0.91$, $P > 0.20$).

I planned two specific comparisons with respect to the age-based change in brightness measures: first, a comparison of UV peak (α) with mid-wave nadir (δ), and second, a comparison of UV peak (α) and long-wave peak (β). Planned contrasts among coefficients were made using a generalized linear/non-linear modelling approach, in which parameter type was entered as a factor and age as a covariate. Here I specified a log-link function to allow for non-linear effects of the independent variable (wing wear) upon reflectance parameters. I carried out a similar analysis using the two hue variables. Differences in the response to ageing among focal parameter pairs (as above) would be evidenced by a significant parameter × wing wear interaction, which I evaluated using a maximum-likelihood approach. I also conducted a multinomial ordinal regression model of age, in which the best fitting model was selected based upon best subsets minimization of Akaike's information criterion (AIC). The AIC is an information theoretic derivative of the

log-likelihood function that better facilitates direct comparison between candidate models containing varying numbers of parameters (Burnham and Anderson, 2002). In keeping with the multimodel information theoretic approach (Burnham and Anderson, 2002), I report results from the two most highly informative models.

Finally, I used absolute residuals from the univariate least squares regressions to explore, using analysis of covariance (ANCOVA), whether this 'residual' variance varied with age, and whether it varied among colour elements (i.e. structural UV versus pigment yellowish-orange). Age (wing wear class) was the covariate in this analysis, colour element was the fixed factor, and the absolute residuals served as the dependent variable. Because the residuals were converted to their absolute value, this is an analysis of whether the amount of variation around each mean (i.e. the size of the 'spread') differed with age and/or among colour elements, irrespective of the location of each mean for each age class/colour element combination. The variance due to mean differences across age classes and between colour elements is removed from this analysis.

RESULTS

Pristine specimens

Left and right wing values for each reflectance parameter were highly correlated (Table 1). Given that these represent two independent measurements taken for each individual, the correlations indicate high repeatability for the estimation of each individual's structural UV and pigment yellowish-orange ornamentation. I subsequently averaged the left and right wing values for each individual. Among pristine specimens, the phenotypic variance in UV peak (Table 1) was greater than that of both longer wavelength spectral parameters (Levene's test, mid-wave nadir: $F_{1,138} = 84.6$, $P < 0.001$; long-wave peak: $F_{1,138} = 62.0$, $P < 0.001$). Phenotypic variance in UV hue was also greater than that of visible hue (Levene's test, $F_{1,138} = 25.87$, $P < 0.001$). These results collectively support the prediction of greater phenotypic variance in structural UV in freshly emerged adult males.

Variously aged specimens

Analyses of variously aged specimens (Tables 1, 2) indicated that all spectral parameters except UV hue co-varied significantly with wing wear. However, a comparison of age-based changes among UV and yellowish-orange hue proved non-significant (likelihood ratio test of colour component × wing wear interaction: $\chi^2_1 = 0.74$, $P = 0.38$); hence these reflectance characteristics changed indistinguishably with age. Among brightness measures (which are all measured on the same percentage reflectance scale), the age-based reduction in UV peak was greater than the increase in mid-wave nadir (likelihood ratio test of interaction effect: $\chi^2_1 = 10.4$, $P < 0.005$) and the reduction in long-wave peak ($\chi^2_1 = 13.4$, $P < 0.0005$). Hence, the UV peak appears to decline more rapidly with age than the two longer wave brightness parameters (Fig. 3). However, among the univariate regressions (Table 2), long-wave peak exhibited the largest coefficient of determination ($R^2 = 0.64$), and the next most informative parameter was yellowish-orange chroma ($R^2 = 0.52$). This result is opposite to that predicted, and means that if any one variable were to be used as a basis for deriving the most accurate prediction of male age (wing wear), then this should be long-wave peak.

Table 1. Summary of statistics for (a) pristine specimens and (b) variously aged samples

	(a) Pristine specimens ($n = 70$)				(b) Variously aged samples ($n = 98$)					
					Age class means					
Parameter	Mean	σ	Range	Repeatability (r)	1	2	3	4	5	Repeatability (r)
UV peak (α, %)	66.7	11.2	41.0–100.0	0.924	64.5	56.4	52.5	43.8	38.1	0.865
Mid-wave nadir (δ, %)	9.15	1.58	6.57–13.5	0.929	9.08	10.1	9.46	11.5	11.3	0.874
Long-wave peak (β, %)	58.9	2.90	51.7–68.8	0.900	60.4	55.0	52.0	49.1	45.8	0.812
UV hue (ε, nm)	341	10	318–360	0.890	342	341	335	342	339	0.948
Yellow-orange hue (ϕ, nm)	533	5	523–542	0.925	533	531	531	527	526	0.957
UV chroma ($b/[a_1 + a_2]$)	1.28	0.06	1.15–1.45	0.673	1.29	1.28	1.27	1.22	1.22	0.800
Yellow-orange chroma (d/c)	2.59	0.25	2.00–3.04	0.957	2.62	2.31	2.30	2.02	1.93	0.926

Note: The correlation (Pearson's r) between left and right wing measurements is presented here as repeatability, because it represents the repeatability of two independent measurements obtained for each individual. Symbols in parentheses next to each parameter correspond to those of Fig. 2.

Table 2. Summary of regression results for variously aged samples

	(b) Regression across age classes						
Parameter	MS_{effect}	$MS_{residual}$	$F_{1,96}$	P	R^2	Slope ± 1.S.E.	t_{96}
UV peak (α, %)	8284.9	116.5	71.1	<0.001	0.42	−6.52 ± 1.52	8.4
Mid-wave nadir (δ, %)	65.6	4.0	16.2	<0.001	0.14	0.58 ± 0.28	4.0
Long-wave peak (β, %)	2403.9	14.1	170.9	<0.001	0.64	−3.52 ± 0.53	13.1
UV hue (ε, nm)	58.3	88.1	0.66	0.418	0.01	−0.55 ± 0.67	0.8
Yellow-orange hue (ϕ, nm)	601.7	37.6	16.0	<0.001	0.14	−1.76 ± 0.44	4.0
UV chroma ($b/[a_1 + a_2]$)	0.070	0.003	23.6	<0.001	0.20	−0.02 ± 0.004	4.8
Yellow-orange chroma (d/c)	5.42	0.052	102.6	<0.001	0.52	−0.17 ± 0.02	10.1

Note: Symbols in parentheses next to each parameter correspond to those of Fig. 2.

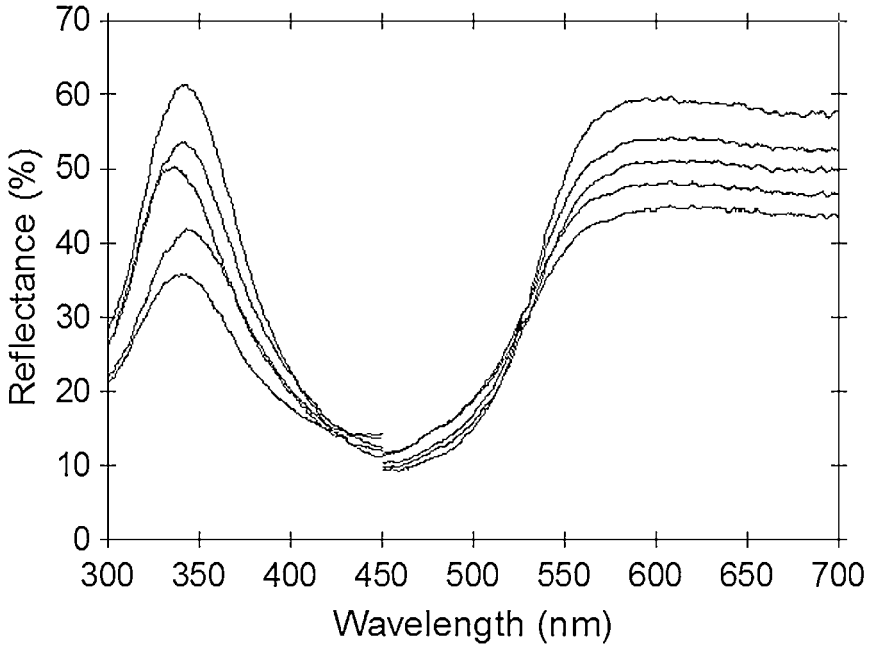

Fig. 3. Reflectance spectra averaged across members of each *C. eurytheme* wing wear class (age classes increase from top to bottom). The curves become disjointed slightly at 450 nm because they are composites of two separate scans [one with the wing rotated to find the maximum UV peak (shown from 300 to 450 nm), and the other with the wing situated flat to measure the non angle-dependent reflectance from 450 to 700 nm; see Methods].

I then conducted a multinomial logistic regression to predict age on the basis of all spectral variables. The highly significant most informative model (AIC = 219.6, $G_4 = 111.6$, $P < 0.001$) included long-wave peak (Wald = 23.0, $P < 0.001$), yellowish-orange chroma (Wald = 6.6, $P < 0.01$), yellowish-orange hue (Wald = 4.3, $P < 0.05$) and UV chroma

(Wald = 2.7, $P = 0.10$). That UV chroma did not have a significant effect in this model suggests that it should be treated with caution. Accordingly, the next most informative model (AIC = 220.4, $G_3 = 108.7$, $P < 0.001$) included all variables as above except UV chroma. The relative prevalence of yellowish-orange parameters in these models again suggests, contrary to prediction, that age (wing wear) would be better predicted on the basis of variation in the pigment colour component.

Finally, I saved the absolute residuals of each of the above univariate regressions, and entered these into an ANCOVA to assess whether the variance (average departure from each mean) changed among wing wear classes (covariate) and/or colour elements (fixed factor). In terms of hue, there was a significant effect due to colour element (i.e. UV hue versus yellowish-orange hue; fixed effect: $F_{1,192} = 6.07$, $P < 0.05$), but no effect due to wing wear (covariate: $F_{1,192} = 2.6$, $P = 0.11$) and no interaction ($F_{1,192} = 0.04$, $P = 0.82$). Inter-individual variation in UV hue (mean absolute residual = 7.56 ± 0.48% [which represents the average absolute departure from the mean of each age class]) was equally greater than yellowish-orange hue across all age classes (mean = 4.48 ± 0.48%). In terms of brightness, there was a significant effect due to the type of parameter (i.e. UV peak versus mid-wave nadir versus long-wave peak; fixed effect: $F_{2,288} = 40.8$, $P < 0.0001$), but no effect due to wing wear (covariate: $F_{1,288} = 2.5$, $P = 0.11$) and no interaction ($F_{2,288} = 0.18$, $P = 0.83$). Inter-individual variation in the UV peak (mean absolute residual = 9.0 ± 1.1%) was greater than both the visual nadir (mean = 1.4 ± 0.3%; planned contrast: $F_{1,288} = 214.7$, $P < 0.0001$) and the visual peak (mean = 2.9 ± 0.4%; planned contrast: $F_{1,288} = 138.0$, $P < 0.0001$). This replicates the Levene's test findings among pristine males for increased relative variance in structural UV, but suggests further that the magnitudes of variances and their relative differences will be maintained throughout the functional adult lifetime of these butterflies. Figure 4 illustrates this by showing the greater spread of UV peak values around the fitted age regression line (Fig. 4a), relative to the two pigment-based colour descriptors (Fig. 4b), across all age classes.

DISCUSSION

Relative to non-sexual morphological traits, sexually selected ornaments are predicted to exhibit greater evolutionary lability (Iwasa and Pomiankowski, 1995) and heightened levels of phenotypic variance (Schluter and Price, 1993; Pomiankowski and Møller, 1995; Badyaev, 2004). Higher phenotypic variation may result from enhanced functional or developmental integration of the trait with organismal processes, and from condition-dependent or facultative trait expression (Rowe and Houle, 1996; Badyaev, 2004). Inherently variable traits may be favoured on perceptual grounds as visual 'magnifiers' of mate quality (Endler, 1992; for an empirical example, see David *et al.*, 2000). Structural coloration, such as the ultraviolet of *C. eurytheme*, has unique potential to serve as a 'magnifying' visual signal [refer to the detailed arguments of Fitzpatrick (1998) and Andersson (1999)], mainly due to the high architectural precision required in building the constituent nanometre-scale structures (Ghiradella, 1974). Here I demonstrate heightened variance in the structural component of male *C. eurytheme* dorsal coloration. One candidate explanation for this effect is that UV reflectance was measured with reduced accuracy; however, the uniformly high left and right wing repeatabilities for all traits (UV and yellowish-orange; Table 1) suggest that this is not the case. I therefore conclude that structural UV magnifies inter-individual visual differences among male *C. eurytheme*, a result consistent with the hypothesis that this trait serves a special sexual

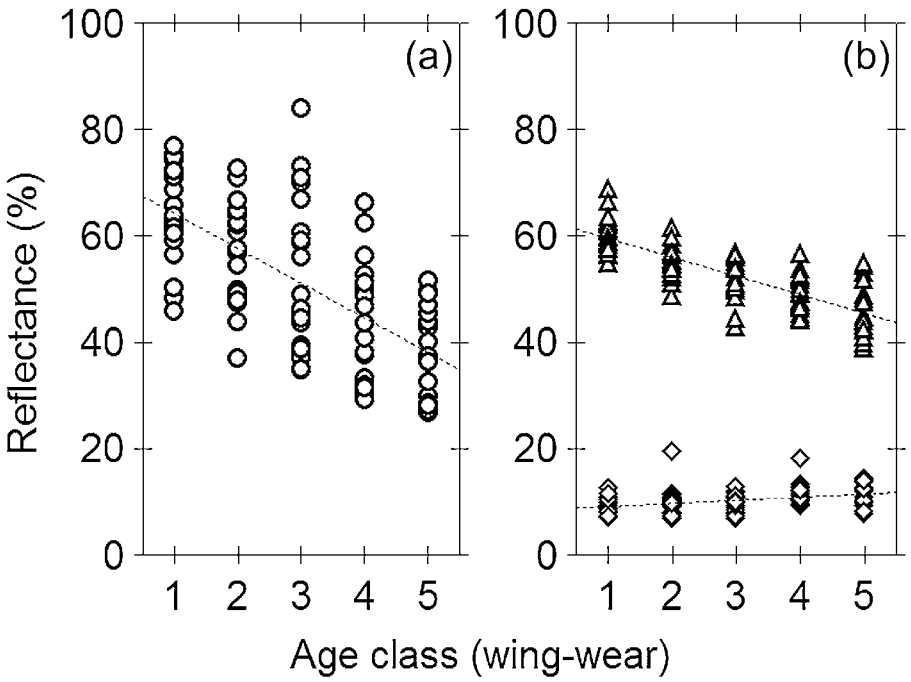

Fig. 4. Age (wing wear) based declines in the two studied classes of *C. eurytheme* brightness parameters: (a) peak UV reflectance (circles), and (b) mid-wave nadir (diamonds) and long-wave peak reflectance (triangles). Data points are reflectance values relative to a magnesium oxide white standard. The greater residual variance of the UV peak is evident as the greater spread of values around the fitted line across all age classes.

signalling function (Silberglied and Taylor, 1978). Evidence is mounting in the avian literature that male structural reflectance parameters are condition-dependent and phenotypically variable (Keyser and Hill, 1999, 2000; Johnsen *et al.*, 2003), and thus carry unique information pertaining to direct and (possibly) indirect mating benefits.

Recent theoretical treatments emphasize a life-history perspective upon sexual advertisement and the evolution of mate choice (e.g. Beck and Powell, 2000; Kokko *et al.*, 2002). Age is a key life-history variable that, among polyandrous insects, will indicate the likely 'direct' material resources on offer (Svard and Wiklund, 1986), and possibly also male fertility (Siva-Jothy, 2000). Research by Rutowski and Gilchrist (1986) indicated that older (more worn) male *C. eurytheme* also require longer copulation durations, and although the mass of ultimately transferred material was deemed equivalent (i.e. 5.39 ± 2.0 [worn] versus 5.84 ± 1.9 mg [fresh]), the statistical power of this contrast was a very low $1 - \beta = 0.16$ (reconstructed for a one-tailed comparison at $\alpha = 0.05$). Female *C. eurytheme* are known to mate preferentially with younger males (Rutowski, 1985); however, the present results suggest that solely age-based mate choice would be better visually mediated by the pteridine pigment components of male ornamentation. This is because although UV brightness erodes at a faster rate than pigmentary characteristics (Table 2; Fig. 4), the continued presence of higher 'residual' variance renders this trait a less accurate predictor of age (Table 2). In each case, the apparent visual degradation probably results from the loss of the individual wing scales that

house both the pteridine pigments and the multi-layer thin-film architecture responsible for structural reflectance (Ghiradella, 1974). Importantly, there are two distinct wing scale types in butterflies: the outermost 'cover' scales, which in *C. eurytheme* contain both pigments and UV-producing structures, and 'ground' scales, which underlie the cover scales and contain only pigments (Ghiradella, 1974). If the outermost scales are more readily lost over time, which appears logically probable, then this could explain the relatively more rapid degradation in the UV signal.

Concurrent laboratory-based research on *C. eurytheme* (D.J. Kemp and R.L. Rutowski, unpublished data) has indicated high additive genetic variance for male UV brightness, and greater environmental sensitivity of this trait (relative to the brightness and chroma of pigment yellowish-orange). This suggests that important information may be encoded in the components of phenotypic variance reported here. Structural UV may not offer the most accurate visual indicator of male age, but due to its high heritability and condition-dependent expression (D.J. Kemp and R.L. Rutowski, unpublished data), there is the possibility that this trait offers as an integrated indicator of male age, phenotypic condition and/or genetic quality. The magnitude of residual UV hue and brightness variation remains constant across age classes, so any such additional information contained within the 'residual' UV variance (pertaining to genetic identity and/or phenotypic condition) could combine additively with that due to age. Given that male *C. eurytheme* can distinguish the subtle variation in hue and brightness [i.e. the difference between greenish-yellow and yellowish-green of conspecific female versus male ventral hind wings (Silberglied and Taylor, 1978)], it is likely that they would be able to distinguish among the contemporaneously reported variation, at least in brightness (which ranged up to 59% in fresh samples and 26% across age classes). A female preference targeting 'bright' UV males may therefore select (on average) younger males in good phenotypic condition, with genes for producing bright UV reflectance. Proper evaluation of this idea will require identification of the physical cue used by females of this species to select younger mates, and determination of the extent of covariance between UV trait expression and evolutionarily relevant male characteristics under field conditions.

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