

Stress-mediated covariance between nano-structural architecture and ultraviolet butterfly coloration

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Summary

1. Structural coloration is a striking component of sexual ornamentation, and may function as a signal of mate quality. Although the proximate optical mechanisms are often well defined, we know much less about the morphological basis for intraspecific variation in structural colour.

2. Males of the butterfly *Colias eurytheme* L. possess a thin-film interference array on their dorsal wing scales that generates a bright and iridescent ultraviolet (UV) signal. This signal is used in mate choice.

3. Using scanning electron microscopy, we investigated the covariance between nano-structural architecture and UV reflectance in samples that were variously subject to either nutrient stress (using a larval host-plant manipulation), or thermal stress (using transient heat and cold shocks during the pupal period). We employed these two stressors to mimic natural stressful processes and to accentuate the variance in UV signal characteristics.

4. Two primary structure–reflectance relationships were apparent. First, UV brightness increased with the density of scale ridges that bear the interference reflectors. Second, the breadth of above-wing angles for viewing the UV covaried with a measure of thin-film angular orientation. These relationships were, however, either limited to, or stronger among, males of the nutrient stress sample.

5. Our results are consistent with a causal effect of developmental stress on nano-structural architecture and henceforth UV reflectance, but also suggest that the proximate basis for signal variation may be intimately linked to the nature of prevailing stressors.

Key-words: butterflies, *Colias*, honest signalling, iridescence, structural colour

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Introduction

Structural coloration is a widespread and striking phenomenon in which colour is generated via an interaction between incident light and the micro- or nano-scale architecture of a surface layer (Parker 1998; Vukusic & Sambles 2003). Colours produced in this manner are brighter and more deeply saturated than those typically arising from pigments, and often possess additional unique visual properties, such as polarization, iridescence and angle-dependent visibility. Structural colour is responsible for such visual delights as the sparkle of peacock trains, the ‘metallic’ lustre of beetle elytra (Parker *et al.* 1998), and the iridescence of tropical butterfly wings (Vukusic *et al.* 1999). Research into the biological occurrence and operation of structural

colour is ongoing and, particularly in the case of butterflies, is continuing to reveal novel and surprisingly complex optical mechanisms (Vukusic *et al.* 2001).

Structural coloration has long been appreciated as an integral feature of biological signalling systems (Newton 1704), but its adaptive function has only recently been questioned (Fitzstephens & Getty 2000; Sweeney *et al.* 2003). Given that these colours are frequently male-limited, researchers have mostly addressed the possibility that they evolved or became exaggerated due to female mating preferences. This research has progressed on broad fronts, including investigations into the phenotypic variance, heritability and condition-dependence of structural ornamentation, as well as behavioural investigations into the significance of this trait in courtship (Brunton & Majerus 1995; Keyser & Hill 1999; Sweeney *et al.* 2003). One largely overlooked area, however, concerns the underlying proximate basis for intraspecific variation in reflectance properties. Whereas exemplar structural architectures and their optical functions are well

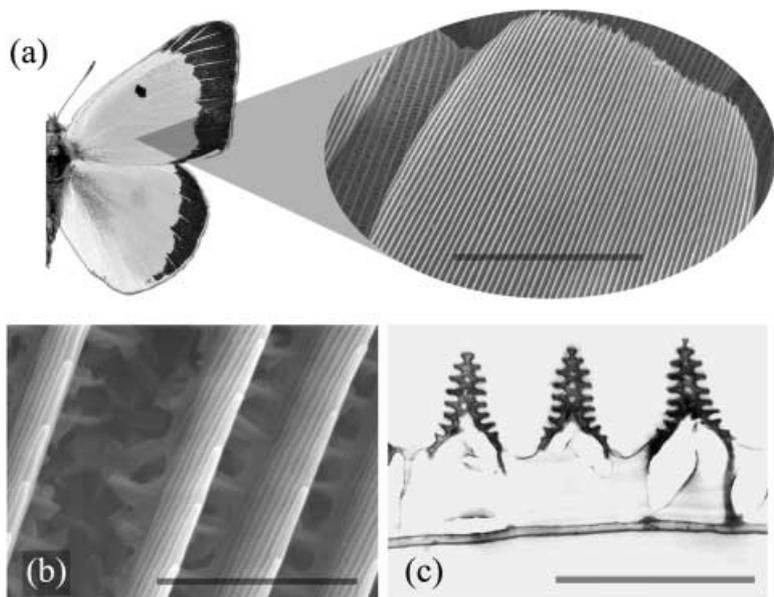


Fig. 1. (a) The dorsal *Colias eurytheme* wing surface featuring a $\times 1000$ SEM of a single scale (taken from the approximate position of wing where we sampled UV reflectance). Longitudinal scale ridges are apparent in the magnified image, which has been rotated 70° anticlockwise for illustrative purposes; scale bar = $30\ \mu\text{m}$. (b) $\times 18\ 000$ SEM of the same wing scale showing the ridges in close-up. The lamellae, which act as the thin-film mirrors, can be seen running along the lateral faces of each ridge and terminating at intervals along the ridge top; scale bar = $1.6\ \mu\text{m}$. (c) $\times 18\ 000$ TEM of a cross-section through the scale indicating the (horizontal) lamellae borne on the (vertical) scale ridges; scale bar = $1.5\ \mu\text{m}$. This image was obtained via the standard methodology outlined by Vukusic *et al.* (1999).

defined, very little is known about how these architectures typically vary among individuals within a population (but cf. Fitzstephens & Getty 2000; Shawkey *et al.* 2003). From an evolutionary perspective, and particularly from the perspective of evaluating sexual selection-based hypotheses, it would be useful to know the proximate basis for variation in constituent nano-structures, and how this affects signal variation.

In this study we investigate the relationship between nano-structure and UV reflectance in the butterfly *Colias eurytheme* L. Males of this species possess multi-layer interference reflectors on the uppermost surface of their dorsal wing scales (Fig. 1), which produce a bright ultraviolet (UV) signal centred around $350\ \text{nm}$ (Fig. 2). Unlike the diffuse reflectance of other structural colours (Stavenga *et al.* 2004), the UV in *Colias* is apparent from a limited range of viewing angles. Conspecific females prefer males that possess bright UV (Silberglied & Taylor 1978), although little is presently known of the adaptive value of this preference. We propose that the circumstances under which the wing structures are assembled predispose this trait as a potential indicator of several aspects of male quality. There is evidence that the UV signal is more phenotypically variable (Kemp 2006), and disproportionately affected by developmental stress (more so than pigmentary colour, unpublished data), which is consistent with it serving as an informative sexual trait (Badyaev 2004). Here we address the signal hypothesis via a study

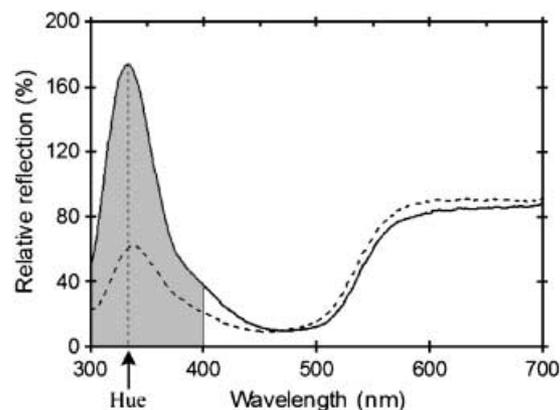


Fig. 2. Sample *Colias eurytheme* reflectance curves: spectra from the highest (solid line) and lowest (dashed line) UV-reflective individuals in the nutrient stress sample. We calculated UV brightness as mean reflectance in the $300\text{--}400\text{-nm}$ range (shaded area on brightest curve), and hue as the wavelength corresponding to peak reflectance (as indicated). UV reflectance amplitudes can exceed 100% because they are measured relative to an MgO standard.

of how UV reflectance is underpinned by structural variation among butterflies subjected to two different developmental stressors.

BUTTERFLY STRUCTURAL COLOUR

Butterflies exhibit a range and complexity of structural colour-producing mechanisms unrivalled in the animal kingdom (Vukusic & Sambles 2003). Perhaps the best studied example is the system of lamella-bearing vertical scale ridges exhibited by *Colias* (Ghiradella 1974), *Eurema* (Ghiradella *et al.* 1972), *Morpho* (Vukusic *et al.* 1999), and numerous other genera. This fine-scale surface architecture (Fig. 1) functions as a quarter-wave interference device by presenting (to incident light) a series of alternating lucent (air) and dense (cuticle) layers. The dense layers – the ‘lamellae’ visible in Fig. 1(c) – are the structural elements responsible for constructive interference, and thus technically represent a multiple series of optical thin films. Butterfly wing structures have featured in physics textbooks (Halliday *et al.* 1997), and their optical function is well defined. Very little is known, however, about how they vary among (and within) individuals, and what contributes to variation in the visual signal. Given that the architectural demands of these structural arrays are likely to be stringent (Ghiradella 1974), any within-scale variation in material density (thus refractive index), width or positioning of the nano-scale thin films should reduce salient signal characteristics such as peak brightness. Maximum obtainable brightness should also be determined, up to a point, by the density and spatial coverage of thin-film layering across the wing surface (the density of the ‘ridges’ and/or the number of ‘lamellae’ visible in Fig. 1a,b).

The wing scale structures causing butterfly structural reflectance are assembled, along with the rest of the adult phenotype, during metamorphosis (Ghiradella

1974); hence they are constructed over a limited time period and from a limited pool of larval-derived resources. This suggests two broad and non-mutually exclusive ways in which their development may be compromised by environmental perturbations. First, as the sculptured wing surfaces are composed of cuticle (procuticle with an outer layer of epicuticle; Ghiradella 1998) – the same material that constitutes the bulk of the adult superstructure – the structures may be adversely affected by resource limitation. Variation in the ability or opportunity to acquire larval resources may limit the available building blocks of ridge–lamellar arrays and thus lead to fewer or shorter ridges, and thus fewer lamellae. Alternatively (or additionally), resource limitation could affect the materials used during surface structure development, such as actin protein that plays a role in scale buckling (Ghiradella 1974, 1998). In both cases, we would expect to see reductions in (at least) UV brightness. Second, an individual's ability to construct a precise structural array may be limited by extrinsic factors during pupal development. Insect metabolic rates are strongly temperature-dependent; hence thermal fluctuations may particularly stress a male's ability to achieve the necessary nano-scale precision across all wing surface structures.

Here we searched for covariance between wing structures and UV reflectance among male *C. eurytheme* reared in two separate experiments: one in which larvae were subject to controlled variation in host-plant quality (thus varying the ability for nutrient acquisition); and another in which pupae were subjected to varying degrees of thermal stress (thus varying their developmental stability). The experiments were performed as part of a larger study into the environmental condition dependence and quantitative genetic architecture of UV reflectance. We predicted that UV reflectance parameters should be more likely to covary with (1) the density of thin-film structures (ridges and lamellae) among individuals of the nutrient stress sample; and (2) the consistency of architectural precision among individuals of the thermal stress sample. These predictions are borne from the putatively different nature and developmental consequences of stress between the two experiments (as outlined above).

Materials and methods

STUDY SPECIES

Colias eurytheme is a polyandrous pierid butterfly that utilizes the widely cultivated legume alfalfa (*Medicago sativa* L.) as a larval host plant. Males of this species locate females using a non-aggressive patrolling tactic, and make sexual discriminations using dorsal UV coloration. Populations often exist in high densities, and although females are repeatedly approached by courting males, they mate only two to three times throughout their lifetime (i.e. they choose among potential mates). This species has been studied extensively, and

detailed descriptions of its mating biology are given elsewhere (Silberglied & Taylor 1973, 1978).

REARING PROTOCOLS

Animals used here were subsampled from two large ($N > 1000$ individuals), split-family rearing experiments in which offspring (of 38 and 29 full-sibling families, respectively) were randomly distributed among environmental treatments. In the nutrient stress experiment, larvae in the 'high'-quality group were supplied with the qualitatively most succulent re-growth of alfalfa, whereas 'low'-quality group individuals were fed the toughest foliage available from mature, flowering plants. In the thermal stress experiment, all larvae were reared under high-quality conditions and then, on pupation, placed under one of three thermal treatments: (1) control – constant 28 °C; (2) heat shock – constant 28 °C with 1 h of every 4 h at 38 °C; and (3) heat/cold shock – constant 28 °C with 1 h of every 4 h at 38 °C followed by 1 h at 23 °C. Rearing protocols were otherwise identical, with larvae reared in semi-conical plastic containers under a dual temperature regime of 30 °C (day) and 25 °C (night) ± 1 °C (as were pupae in the nutrient stress experiment). Larvae were derived from laboratory-reared females that were mated with free-flying males in Chandler, AZ, USA (33°16' N, 111°48' W). Host plant was also collected from this site. On eclosion, adults were killed and their forewings were removed, pressed flat for 48 h and then mounted on card using spray adhesive (3M) applied to the ventral surface.

REFLECTANCE SPECTROMETRY AND VIDEO VIEWING

We captured reflectance spectra from a standardized region of card-mounted left and right forewings using an Ocean Optics USB-2000 spectrophotometer (25 averaged spectra, 125 ms integration time), with pulsed illumination provided at 90° (to the horizontal plane) by an Ocean Optics PX-2 xenon light source. The collector probe was situated at 45° and focused (Ocean Optics 74-UV lens) to capture light from a circular 2-mm area (Fig. 1a). The wing was positioned horizontally so that its base pointed toward the collector's azimuth. Structural UV in *Colias* is dependent on the orientation of the wing relative to the light source and receiver, so we rotated each wing several degrees to find the orientation where the amplitude of the UV curve was maximal. Relative reflection was expressed as the percentage of that obtained from a magnesium oxide white standard. We calculated UV brightness as the mean 300–400-nm reflection amplitudes, and UV hue as the wavelength corresponding to peak reflection (Fig. 2). Variation in brightness (peak amplitude) among spectra correlates highly with variation in measures of chroma (because curves with higher peak amplitudes also become more 'narrow'), hence here we used brightness only.

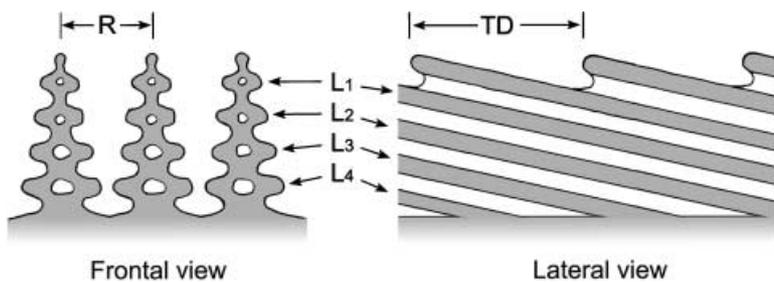


Fig. 3. Schematic diagram of ridge–lamellar structures indicating the morphological measurements made. The frontal view shows a cross-section through three ridges, each carrying four lamellae, and the lateral view is that of a single ridge face. R = ridge spacing; L1–L4, individual lamellae; TD = distance between successive lamellar terminations. In the lateral view, note how each lamella inserts into the scale base at an angle, which causes them to terminate at intervals along the ridge top (also visible in Fig. 1b). Diagram not drawn to scale.

Whole-wing UV reflectance was viewed with a video camera fitted with a Tiffen 18A visible light-absorbing filter, with light provided at 90° by a tungsten–halogen fibre-optic illuminator filtered to remove infra-red wavelengths (the video was therefore exposed to reflected light spanning 350–400 nm). Images were viewed in real time on a television monitor. We used a single-axis goniometer to rotate wings around their long axis and measure the angle spanning the point at which UV reflectance first became visible to the point at which the last of the reflectance was extinguished. This is a measure of the angular spread of UV reflectance from the wing surface, which we refer to hereafter as ‘angular breadth’.

SPECIMEN PROVENANCE

We maximized our ability to detect covariance by subsampling individuals (from the two larger experiments) on the basis of divergence in reflectance characteristics. In each case we selected four groups of individuals, respectively, comprising those with the 10 (nutrient stress experiment) or 12 (thermal stress experiment) highest and lowest values for each of two UV reflectance parameters: brightness and angular breadth. Individuals were sampled randomly with respect to treatment. The nutrient stress sample included 20 individuals in each host-plant treatment, whereas the thermal stress sample comprised 17 control group, 13 heat shock and 16 heat/cold shock males.

ELECTRON MICROSCOPY

We obtained SEMs using a Hitachi S-3200N scanning electron microscope set to a 25 kV accelerating voltage, 6 mm working distance and zero stub tilt, with samples cold-sputter prior-coated with either 4 or 7 nm gold. Here we sampled, for each individual, three replicate pieces of wing from the approximate location of spectrometric capture. We placed scale bars on the images that were verified at the full range of magnifi-

cation settings against optical diffraction gratings (the periodicities of which were determined to the nearest 2 nm using optical diffraction experiments).

Using $\times 1000$ magnification SEMs (Fig. 1a), we counted the number of ridges spanning two independent 30- μm lengths (one length for each of two wing samples), each placed in the centre of a scale and running orthogonal to the principal ridge axis. The two replicate measures were correlated (both samples: Spearman's $r_s = 0.65–0.81$, $N = 40$, $P < 0.00001$); thus we averaged them, divided by 30, and used the reciprocal as a measure of the mean distance (μm) between successive ridges (ridge spacing, RS in Fig. 3). Second, we counted the number of lamellae per ridge (L1–L4, Fig. 3) from $\times 8000$ or $\times 12\,000$ magnification images. This parameter varied little among different ridges and images for each individual; where variation was evident it was at most one lamella, and we then took the maximum. Last, we used $\times 5000$ images to measure the distances between progressive lamellar terminations along the tops of six to eight adjacent ridges (TD , Fig. 3). This resulted in 80–100 measurements, of which we then calculated the mean and coefficient of variation (CV , calculated as σ^2/\bar{x}) to give a scale-independent measure of architectural consistency. The mean distance between lamellar terminations along the ridge top will represent the angle of insertion (or angular orientation) of successive lamellae, relative to the horizontal scale axis (Fig. 3). Increasing CV s of termination distances may indicate increasing variation in lamellar spacing, orientation and/or thickness, all of which would be expected to reduce the brightness and/or spectral purity of UV reflectance. We checked repeatabilities by randomly halving the pool of measurements for each individual and comparing them. Whereas mean termination distance was highly repeatable (both samples: $r_s = 0.86–0.92$, $N = 38–44$, $P < 0.00001$), CV appeared so only in the thermal stress sample (nutrient stress sample: $r_s = 0.29$, $N = 38$, $P = 0.073$; thermal stress sample: $r_s = 0.60$, $N = 44$, $P < 0.00005$). We therefore restrict our analysis of this parameter to the latter sample.

STATISTICAL TREATMENTS

We investigated the covariance between morphology and UV reflectance by constructing a model for each reflectance parameter (UV brightness, hue and angular breadth) based on the most informative linear combination of morphological predictors. This approach is preferable to a series of univariate contrasts because it allows for the simultaneous assessment of all morphological effects in unison. We also simultaneously analysed data from the nutrient stress and thermal stress experiments, which (by evaluation of interaction terms) allowed us to test formally whether the nature of detected relationships between morphology and reflectance was consistent across samples.

In each case we selected the most parsimonious model from all possible subsets as the one that minimized

Akaike's information criterion (AIC). The AIC is an information-theoretic metric that is most appropriate for observational data (Burnham & Anderson 2002). Although some of the reflectance data were sampled non-randomly, the distribution of our data set (with data from both samples pooled) did not deviate markedly from normality, and we obtained best fits using a normal distribution and log-link function to allow for non-linear effects of predictor variables. We evaluated the significance of multivariate models and constituent model parameters using non-parametric likelihood-ratio tests and Wald statistics, respectively. We used semi-partial correlation coefficients to estimate the size of constituent effects in multivariate models, and Pearson's *r* to estimate bivariate effects. Additional non-parametric statistics are used throughout, with all analyses conducted using STATISTICA ver. 7.0.

Results

STRUCTURAL REFLECTANCE CHARACTERISTICS

The structural component of the male *Colias* dorsal reflectance curve consists of a single UV peak that varies among individuals most markedly in maximum amplitude (Fig. 2) and thus in brightness. Our samples exhibited high interindividual variance in this parameter, as well as in angular breadth, but less variance in hue (Table 1). Angular breadth and UV brightness covaried positively among individuals of the nutrient stress sample ($r_s = 0.34$, $N = 40$, $P < 0.05$) but not the thermal stress sample ($r_s = -0.03$, $N = 46$, $P = 0.85$). Hue was strongly correlated with angular viewing breadth (both samples: $r_s = 0.48-0.54$, $P < 0.005$) but not UV brightness ($r_s = -0.13-0.03$, $P > 0.39$). There were significant effects of experimental treatment on both UV brightness (nutrient stress sample, Mann-Whitney $U = 102$, $P < 0.01$; thermal stress sample, Kruskal-Wallis rank ANOVA, $H_{2,46} = 6.12$, $P < 0.05$) and angular breadth (nutrient stress sample, $U = 81$, $P < 0.005$; thermal stress sample, $H_{2,46} = 15.63$, $P < 0.0005$),

but not hue (nutrient stress sample, $U = 199$, $P = 0.98$; thermal stress sample, $H_{2,46} = 2.92$, $P = 0.23$). All significant effects were in the direction of decreased UV reflectance values with increasing developmental stress.

VARIATION IN NANO-STRUCTURAL PARAMETERS

The ridge-lamellar architecture among our males consisted, on average, of seven to eight lamellae borne on ridges spaced roughly 970-980 nm apart, with lamellae terminating at 900-960 nm intervals along the ridge tops (Table 1). There was moderate interindividual variation in this architecture (CVs in Table 1). Structural variables were largely orthogonal, with only one significant bivariate correlation - that between lamellar termination distance and lamellae number for the nutrient stress sample ($r_s = 0.40$, $N = 39$, $P < 0.05$).

STRUCTURE-REFLECTANCE RELATIONSHIPS

We gained insights into the covariance between structural architecture and UV reflectance by constructing multivariate linear models of each reflectance parameter. Given that we only obtained reliable measurements of 'architectural consistency' (the CV of lamellar termination distances) for thermal shock males, we began by building models for this sample only, using all morphological predictors. Architectural consistency did not feature in any of these most parsimonious multivariate solutions and, as a univariate predictor, did not covary strongly with any reflectance parameter ($-0.16 > r > 0.05$, $N = 44$, $P > 0.28$). We therefore excluded this variable from further analysis and proceeded to analyse both samples simultaneously.

The most parsimonious multivariate model for UV brightness (AIC = 488.5; $G_3 = 22.2$, $N = 79$, $P < 0.001$) included ridge spacing (Wald = 21.0, $P < 0.001$, semi-partial $r = -0.43$); experiment (Wald = 4.9, $P < 0.05$, semi-partial $r = 0.23$); and number of lamellae (Wald = 3.55, $P = 0.059$, semi-partial $r = 0.18$). Thus brightness was most closely related to ridge spacing, brighter

Table 1. Descriptive statistics for UV reflectance parameters and morphological traits

Parameter	Nutrient stress sample				Thermal stress sample			
	<i>N</i>	Mean	CV (%)	Range	<i>N</i>	Mean	CV (%)	Range
UV brightness (%)	40	62.6	29.7	39.4-105.5	46	66.8	36.8	30.3-122.9
Angular breadth (°)	40	29.5	26.4	17.5-41.5	46	28.0	25.0	16.0-39.5
UV hue (nm)	40	340	2.4	325-356	46	351	3.6	328-387
Number of lamellae per ridge	39	7.8	9.9	6-9	46	7.3	15.0	4-9
Ridge spacing (nm)	40	973	7.4	828-1121	40	980	7.5	845-1132
Lamellar termination distances:								
Mean (nm)	39	905	11.4	738-1166	44	965	13.4	676-1371
CV	-	-	-	-	44	0.242	17.0	0.190-0.383

UV brightness = mean percentage reflectance 300-400 nm; angular breadth = above-wing viewing angle for UV; hue = wavelength corresponding to peak reflectance. Figure 3 illustrates the morphological measurements. CV = coefficient of variation, which proved repeatable only for the thermal stress sample of lamellar termination distances.

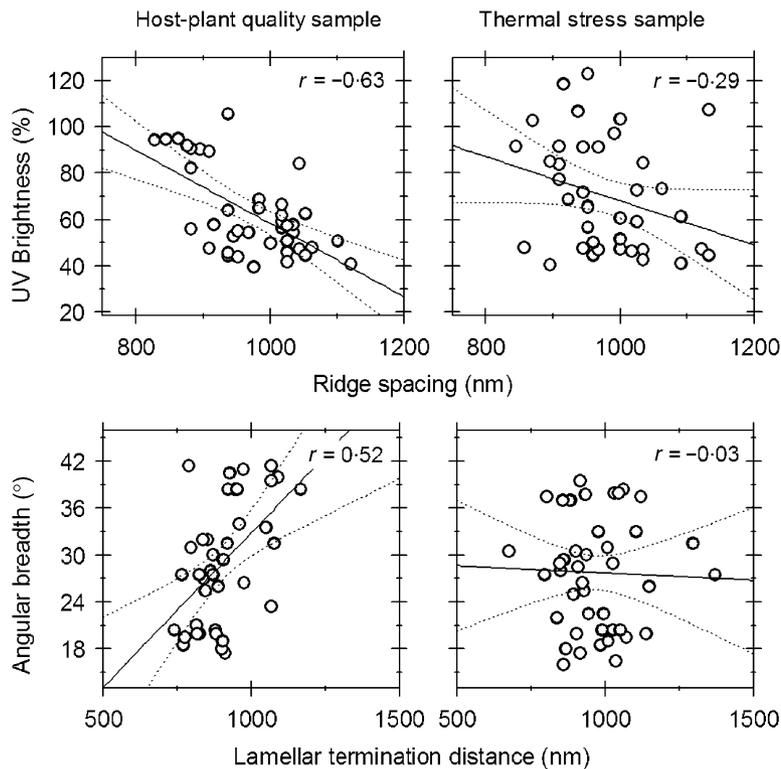


Fig. 4. The two largest covariances between nano-structural morphology and UV reflectance in *Colias eurytheme*, shown separately for each sample. Best-fitting lines are accompanied by 95% CI; Pearson's r is given as a measure of effect size. See Table 1 and Fig. 3 for description of parameters.

males tending to have more closely spaced ridges (Fig. 4). The absence of the ridge spacing–experiment interaction term indicates that this relationship was consistent across samples; that is, it appears as a fundamental relationship. However, the relative size of this effect was twice as large in the nutrient stress sample (Fig. 4). The experiment effect indicates that mean brightness varied among samples (Table 1), while the near significance of lamellar number in this multivariate model indicates a secondary positive effect due to increasing lamellae per ridge.

The most parsimonious model of angular breadth (AIC = 560.8, $G_4 = 17.5$, $N = 83$, $P < 0.005$) included lamellar termination distance (Wald = 7.18, $P < 0.01$, semi-partial $r = 0.28$); the experiment–termination distance interaction (Wald = 4.82, $P < 0.05$, semi-partial $r = 0.23$); experiment (Wald = 4.26, $P < 0.05$, $r = 0.22$); and number of lamellae (Wald = 4.65, $P < 0.05$, semi-partial $r = 0.19$). The salient feature here is the significant interactive effect involving lamellar termination distance, which results from the presence of strong positive covariance in the nutrient stress sample only (Fig. 4). Angular breadth was also positively (but less strongly) related to the number of lamellae per ridge.

The most parsimonious model of UV hue (AIC = 593.9; $G_1 = 16.5$, $N = 86$, $P < 0.001$) included experiment (Wald = 21.61, $P < 0.001$, semi-partial $r = 0.45$); hence we found no morphological basis for predicting hue variation.

Discussion

The brilliant UV iridescence of male *Colias* wings has long been understood as an optical interference effect arising from the nanoscale ridge–lamellar surface architecture featured in Fig. 1 (Silberglied & Taylor 1973; Ghiradella 1974). However, whereas early attention focused on the occurrence of this trait across species (Silberglied & Taylor 1973, 1978), more recent studies have revealed that substantial levels of intraspecific variation also exist (Brunton & Majerus 1995; Kemp, in press). Understanding the nature and causes of this variation should prove key to testing evolutionary hypotheses, such as the idea that these structural colours function adaptively as honest indicators of mate quality. Here we present evidence that variation in different components of the *Colias* UV signal (brightness and angular viewing breadth) each relate to different components of the wing scale nano-structure. Our results also suggest that the primary covariances vary to some extent between butterflies subject to different developmental stressors (nutrient limitation vs developmental instability). Given the strong effect of each stressor on UV reflectance, the most parsimonious explanation for these results is that the two stressors impacted on different aspects of the ridge–lamellar structure (see below). We focus our discussion on the presence and nature of these primary covariances and their implications for the ecology and evolution of iridescent ornamentation.

The largest and most consistent relationship we observed was between ridge spacing and UV brightness. This morphological variable describes the spatial density of ridge/lamellar structures, hence it represents the relative proportion of wing scale area that is effectively covered by the alternating air–cuticle layering. With all else equal, a greater proportional coverage of thin-film mirrors should reflect more UV light at favourable orientations, thus furnishing greater peak brightness. This relationship was evident in both samples, which strengthens the case for this as a fundamental source of covariance, although the effect is notably stronger in the nutrient stress sample (Fig. 4). Longitudinal scale ridging is a ubiquitous feature of butterfly wing scales, iridescent or not, and *C. eurytheme* males have more closely spaced scale ridges than males of the non-iridescent sister species *Colias philodice* (see Fig. 2 of Silberglied & Taylor 1973). An architecture consisting of more closely spaced ridges probably demands more cuticular material (and is therefore more costly to produce), hence the presence of this covariance offers one proximate mechanism by which UV brightness could act as an honest indicator of male phenotypic condition.

The second primary relationship, that between lamellar termination distance and angular breadth, also makes intuitive sense in the light of our contemporary understanding of butterfly iridescence (Ghiradella *et al.* 1972; Vukusic *et al.* 2001). Given that successive lamellae run roughly parallel along the lateral ridge face, our

measure of lamellar termination distance effectively indicates the angle of 'insertion' of lamellae, relative to the plane of the scale (Fig. 3). Shallower angles of insertion cause lamellae to terminate at greater distances along the ridge top, and our results suggest this relates to a greater breadth of UV viewing angles. Lamellar tilting has been reported among other butterflies that possess these ridge–lamellar architectures (Ghiradella *et al.* 1972; Ghiradella 1998; Vukusic *et al.* 2001; Lawrence *et al.* 2002), and across species the limited-view nature of iridescence increases with the angle of lamellar tilt. In *Triodes magellanus*, for example, lamellae are oriented $\approx 54^\circ$ relative to the horizontal scale axis, and iridescence becomes visible only as one nears grazing incidence (Lawrence *et al.* 2002). Our reported covariance (that angular breadth increases with termination distance and so decreases with lamellar tilt; Fig. 4) is consistent with this comparative relationship. Angular breadth may be an adaptive feature of iridescent signals because it relates to the temporal duration during a wing-beat cycle in which the reflectance will be visible (Vukusic *et al.* 2001).

A notable feature of our results is that structure/reflectance covariances were stronger in the nutrient stress sample (Fig. 4). One explanation for this is that thermal stress impinged on other, optically important (but unmeasured) aspects of these ridge–lamellar structures, weakening the predictive power of our measured parameters. Given that insect metabolic rates are temperature-dependent, the transient heat and cold shocks that we administered should have led to inconsistent rates of nascent scale development. This motivated our (unsupported) prediction that UV reflectance in this sample should relate more strongly to the architectural precision of the nano-structural arrays. However, although we did not find such covariance, our index of architectural consistency (the CV of lamellar terminations) did not encompass all the potentially important ways in which this complex structural array could vary. Variation in the consistency of attributes such as lamellar width, orientation along the ridge face (Fig. 3), and material density (thus refractive index) could all affect the resulting UV signal. Whole-scale attributes, such as scale curvature, also probably determine the spread and intensity of UV reflectance. Characterization of these parameters, some of which will require transmission electron microscopy (Shawkey *et al.* 2003), may facilitate better prediction of UV reflectance in our samples. Accurate quantification of thin-film spacing may also help to predict intraindividual variations in hue, as it does for the iridescence of male damselflies (Fitzstephens & Getty 2000).

Finally, from a functional perspective, our results suggest that the proximate cause of UV variation among wild males will probably depend on differences among them in their exposure to specific developmental stressors. *Colibas eurytheme* breeds in cultivated alfalfa fields that are regularly mown, hence resource limitation is probably a prevalent stressor in these populations. The

covariances reported for the nutrient stress sample may often exist, particularly that between UV brightness and ridge spacing. Similarly, variation in the number of lamellae (per ridge) may not routinely and greatly affect UV reflectance in the wild, based on its secondary predictive role among our experimentally stressed populations. However, we cannot discount the emergence of these and other structure–reflectance relationships in populations subject to different stress regimes. It will also be interesting to see whether our observed covariances apply in other butterflies that possess these ridge–lamellar architectures (e.g. *Morpho*; Vukusic *et al.* 1999), and this appears to be an excellent arena for future investigation.

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