

Colour pattern evolution in butterflies: a phylogenetic analysis of structural ultraviolet and melanic markings in North American sulphurs

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ABSTRACT

The males of many butterflies are more brightly coloured than conspecific females and this sexual dichromatism often extends into ultraviolet (UV) wavelengths. Sexual selection, acting either in the context of intra- or intersexual interactions, is thought responsible for the relative brightness of the male visage; however, this hypothesis has been difficult to test experimentally. In this study, we undertake an indirect evaluation of this idea by analysing the phylogenetic distribution of two elements of male colour pattern – UV reflectance and melanic markings – in North American sulphur butterflies. Only the UV element, which derives from nanometre-scale surface structures and is extremely bright and spectrally pure, is expected to be of sexually selected origin. We therefore predicted, based upon prior theoretical treatments, that UV colour pattern should exhibit a polyphyletic distribution typical of a sexually selected trait, whereas melanic patterns should not. We charted the distribution of each colour element across the dorsal hind- and fore-wing surfaces of 26 species (spanning four genera), and summarized the resulting intraspecific variation in colour pattern using principal components analysis. Analysis of among- versus within-sister species group variance confirmed, as predicted, a largely polyphyletic distribution for UV but not melanic patterns. We also found that variation in fore-wing UV patterning could be considered orthogonal of hind-wing variation. These findings shed interesting light on the evolution of structural coloration in this group and add strength to the idea that brilliant male coloration is principally of sexually selected origin.

Keywords: Lepidoptera, mate choice, sexual selection, visual signalling.

INTRODUCTION

In many species of butterflies, males are more brightly coloured than females, and this is true not only in wavelengths that humans can see but also ultraviolet (UV) wavelengths (Silberglied, 1979, 1984). The observation that males typically sport a more brilliant and precisely

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patterned coloration has led many to conclude that their visage is primarily a product of sexual selection acting in the context of intra- or intersexual interactions (Darwin, 1874; Silberglied and Taylor 1973; Silberglied, 1984; Lederhouse and Scriber, 1996). This hypothesis has proven difficult to test, however, because many butterflies do not adapt well to the cage environments necessary for experimental analysis of female preferences, because large numbers of hand-reared virgin females are required and for several additional reasons (as expanded upon by Silberglied, 1984). Moreover, because many butterflies show such brief courtships, some, beginning with A.R. Wallace (1889), and as recently as Wiklund (2003), have discounted this hypothesis for bright male coloration in butterflies as unlikely. Nonetheless, there are several published experimental results that suggest females in some species do discriminate among potential mates on the basis of colour (Silberglied and Taylor, 1978; Rutowski, 1985; Wiernasz and Kingsolver, 1992).

More recently, Brunton and Majerus (1995) and Brunton (1998) have taken a less direct approach to testing the idea that sexual selection has played a role in the evolution of bright coloration in butterflies. Their focus was on the iridescent UV reflection present on the male's dorsal wing surfaces in many species of sulphur butterflies – this reflectance has been implicated as a signal in both intra- and intersexual interactions (Rutowski, 1977; Silberglied and Taylor, 1978). Using comparative methods, Brunton and Majerus (1995) and Brunton (1998) investigated (1) whether patterns of variation in male UV were more indicative of intra-specific versus interspecific signalling, and (2) whether phylogenetic patterns in the presence/absence of structural UV in European *Colias* support a sexual signalling hypothesis. Both studies support the hypothesis that male-limited structural UV coloration has evolved in a sexual signalling context, and may have a role in intraspecific mate choice; however, further independent investigation of these hypotheses would be useful.

The study by Brunton (1998) of European *Colias* tested the prediction that if UV reflectance has evolved as a sexual signal, then this trait should exhibit a polyphyletic distribution. This was based on the reasoning that, relative to non-sexual morphology, sexual traits should be subject to rapid and changing rates of evolution (Iwasa and Pomiankowski, 1995). A putatively sexually selected trait, such as UV reflectance, would be expected to appear and disappear relatively frequently through evolutionary time, thus leading to a contemporary distribution that appears polyphyletic. Brunton's (1998) analysis found support for this prediction, and hence for the general structural UV sexual signalling hypothesis in butterflies. However, several limitations are apparent. First, the analysis was limited to a single genus (*Colias*) of relatively limited geographic distribution (Europe). Second, other known non-sexual traits (which may have served as 'controls') were not analysed simultaneously. Hence, we do not know to what extent other traits may also exhibit the predicted polyphyletic distribution. Lastly, Brunton's (1998) analysis was limited to the presence/absence of structural UV, thus glossing over more subtle interspecific variation in the pattern of dorsal reflectance.

In this study, we set out to expand the study of Brunton (1998) to a broader investigation of UV reflectance patterns, relative to another (putatively non-sexual, or not strongly sexual) element of visual appearance – melanic markings – in five North American Pierid genera. Our goal was to evaluate the prediction that only UV patterning should exhibit a polyphyletic distribution indicative of a sexually selected evolutionary origin (Iwasa and Pomiankowski, 1995). In analysing colour pattern, our data also shed some light on evolutionary and/or developmental influences upon UV trait expression across different dorsal wing regions.

METHODS

Colour patterns in Pierid butterflies

Due to the relative simplicity of their dorsal colour scheme, butterflies in the family Pieridae (the ‘whites’ and ‘yellows’) present an excellent model group for the investigation of colour pattern evolution (e.g. Watt, 1964, 1968; Silberglied and Taylor, 1978). Variation in the visual appearance of Pierids can be ascribed to four primary sources: (1) UV derived from surface nanostructures, (2) browns and black derived from melanin pigmentation (Ellers and Boggs, 2003), (3) yellows and oranges derived from pteridine pigmentation (Watt, 1964), and (4) white, derived from pigment or simple surface scattering. In this study, we investigate the phylogenetic distribution of variation in patterning due to the first two elements (UV and melanin). Melanic markings are not suspected to function strongly as a sexual signal in this group (e.g. Silberglied and Taylor, 1978), and so provide a control for the investigation of this hypothesis with respect to structural UV. Both colour elements exhibit significant interspecific variation in absolute presence/absence and, where present, in their pattern of distribution across the dorsal wing surface.

Colour pattern visualization and quantification

We assessed species colour pattern using high-quality images presented in faunistic works (Howe, 1975; Scott, 1986; Tilden and Smith, 1986) and using pinned specimens. Ultraviolet colour patterns in sample specimens were visualized using a Sanyo VCD 2950 video camera fitted with a 49-mm low-pass UV transmitting filter. Samples were illuminated with high-intensity light in the wavelength range 300–700 nm (derived from a fibre-optic illuminator coupled with an infrared-blocking filter).

Butterfly wing surfaces are bisected by a characteristic and family-specific network of veins (radiating outward from the point of thoracic insertion), thus creating a series of discrete wing regions known as ‘cells’ (Scott, 1986). We used 14 cells (6 fore-wing and 8 hind-wing; Fig. 1) as designated wing regions in which to score the presence/absence of two specific colour elements: (1) structural UV reflectance and (2) melanic (black) markings. Each colour element was deemed ‘present’ if we adjudged it to extend across more than 5% (as visually estimated) of cell area. For each colour element we therefore derived 14 binary (two-state) variables, each representing the presence/absence of that element in a particular cell.

Statistical analysis

We carried out a formal phylogenetic analysis by dividing 26 species into nine sister species groups (on the basis of an available molecular phylogeny; see Fig. 2) (Brunton, 1998; Pollock *et al.*, 1998), and testing for significant within- versus between-groups homogeneity in colour pattern. Principal components analysis (PCA) was first used to derive summary variables for each colour pattern element (structural UV and melanic black). We entered the 14 binary (presence/absence) variables into a PCA and extracted the components with eigenvalues greater than one. Principal components analysis is robust to deviations from normality of independent variables (Tabachnick and Fidell, 1996). Varimax rotations were used to increase interpretability of the extracted components. We subsequently entered the

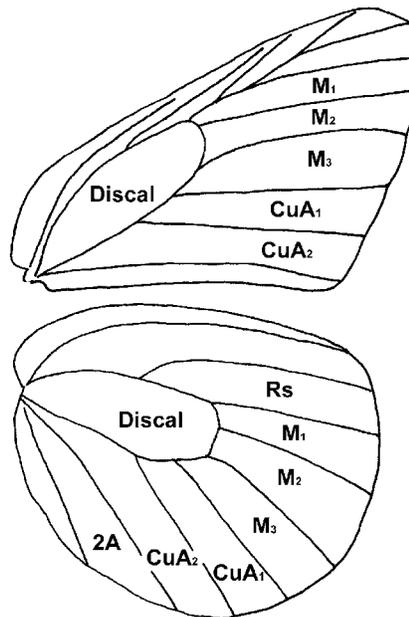


Fig. 1. Wing venation of a Coliadine, showing the six fore-wing and eight hind-wing cells used in the classification of colour pattern.

principal components into a parametric multivariate analysis of variance to test for within-sister groups variance in each colour pattern element. In this analysis, a significant effect would be consistent with a monophyletic trait distribution; hence, an evolutionary hypothesis for UV reflectance pattern as a sexually selected trait does not predict such an effect. We supplemented this analysis by summing the total number of cells scored for each colour element and comparing these values across sister groups using univariate analysis of variance (ANOVA).

In all the analyses of variance, we inspected the residuals for signs of deviation from normality (Tabachnick and Fidell, 1996) and applied the non-parametric alternative (i.e. Kruskal-Wallis test) where appropriate.

RESULTS

More species were UV reflectant (19 of 29) than were not, and all nine sister groups contained at least one UV reflectant species (Fig. 2). Slightly more species ($n = 22$) exhibited dorsal melanic markings, but these were distributed across only seven sister groups. Based upon the molecular phylogeny (and assuming the presence of each colour element as the ancestral state), UV reflectance has been independently lost a total of eight times, whereas melanic markings have been lost only twice (i.e. *Phoebis* and *Anteos*).

We extracted two principal components to explain the observed interspecific variation in UV reflectance pattern. These components collectively explained 81.2% of variance given by the 14 wing cell variables, and broadly corresponded to a fore-wing (UV_PC2) and hind-wing (UV_PC1) component (Table 1). Our analysis of melanic wing pattern yielded three components that explained 88.3% of the available variance. The first of these com-

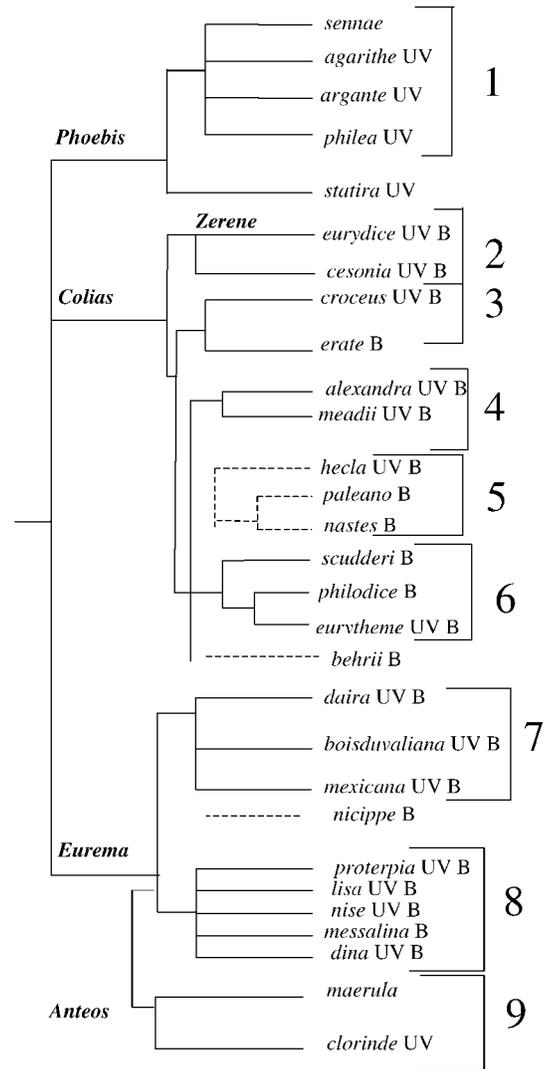


Fig. 2. Study species, groupings and phylogeny, based upon the molecular work of Pollock *et al.* (1998) and Brunton (1998). The letters 'UV' and 'B' to the right of specimen names indicate the presence of ultraviolet and melanic dorsal markings, respectively. Large-type numbers represent the sister species groupings used in the phylogenetic analysis. *Phoebis statira*, *Colias behrii* and *Eurema nicippe* were not placed in groupings due to their ambiguous phylogenetic position.

ponents (M_PC1) contributed most to the solution (explaining 64.2% of the variance) and was correlated with the majority of cells across both wings (Table 1).

As predicted by the UV sexual signalling hypothesis, analysis of between-sister groups variance was clearly significant in the case of melanic markings ($F_{24,44} = 4.96$, $P < 0.00005$) but not UV reflectance ($F_{16,32} = 1.627$, $P = 0.118$). This suggests a monophyletic distribution for the former and a polyphyletic distribution for the latter colour element. This finding

Table 1. Summary of principal components analyses (component loadings and eigenvalues) for UV and melanic wing markings

	Cell	UV reflectance		Melanic markings		
		UV_PC1	UV_PC2	M_PC1	M_PC2	M_PC3
Fore-wing	M ₁	0.682	0.623	0.919	0.124	0.158
	M ₂	0.682	0.623	0.919	0.124	0.158
	M ₃	0.380	0.814	0.919	0.124	0.158
	CuA ₁	0.261	0.926	0.947	0.158	0.154
	CuA ₂	0.353	0.865	0.947	0.158	0.154
	Discal	0.191	0.949	0.315	0.102	0.829
Hind-wing	RS	0.809	0.235	0.959	0.178	0.017
	M ₁	0.891	0.195	0.959	0.178	0.017
	M ₂	0.875	0.296	0.959	0.178	0.017
	M ₃	0.835	0.415	0.959	0.178	0.017
	CuA ₁	0.852	0.377	0.882	0.242	0.071
	CuA ₂	0.733	0.334	0.243	0.916	0.119
	2A	0.626	0.239	0.192	0.913	-0.096
	Discal	0.799	0.507	0.113	0.469	-0.591
Eigenvalue		6.512	4.86	8.990	2.184	1.189
% Variance		0.465	0.347	0.642	0.156	0.085

Note: Cell identification symbols correspond to those given in Fig. 1. The ‘% Variance’ row gives the proportion of total variance explained by each principal component. Highlighted loading values are >0.70.

was replicated in a simpler analysis of the total absolute number of melanic ($H_{8,26} = 15.85$, $P = 0.044$) and UV reflective (Kruskal-Wallis ANOVA: $H_{8,26} = 7.72$, $P = 0.460$) wing cells.

DISCUSSION

The findings of this study are in excellent accord with those presented by Brunton (1998) in her original analysis of the phylogenetic occurrence of structural UV reflectance in European *Colias*. This accordance across studies shows that the polyphyletic distribution of structural UV extends to higher taxonomic levels than the single genus *Colias*. Moreover, we expanded Brunton’s (1998) approach by analysing variation in UV reflectance patterns (rather than simple presence/absence), and testing – then subsequently rejecting – the prediction of polyphyly for a putatively non-sexual colour trait (melanic markings). These results fit nicely with prediction from the sexual signalling hypothesis for the evolution of structural UV (Iwasa and Pomiankowski, 1995; Brunton, 1998). Evidence is therefore building that structural UV reflectance in butterflies does have special significance to intersexual signalling processes, as it may have in birds (Keyser and Hill, 1999, 2000; Eaton and Lanyon, 2003). At this stage, it is difficult to ascribe any specific signalling role, although Brunton and Majerus’ (1995) analysis suggests that this trait may be more significant to intraspecific (i.e. mate choice or male–male signalling) rather than interspecific (i.e. species recognition) communication.

Part of the reason why we know so comparatively little about female mate choice in butterflies (at least with respect to male colour traits) is that the critical experiments are logistically very demanding (refer to Silberglied, 1984, for an expanded discussion). Large numbers of virgin females are required, experiments usually need to be conducted in flight cages, and the experimenter needs to be able to control (or at least reliably assess) the sexual receptivity and motivation of females. The most thorough investigation of male UV signalling in butterflies (Silberglied and Taylor, 1978) utilized 10,000 field-collected virgins and a series of large outdoor flight cages. This study showed that UV functions in species recognition (*C. eurytheme* females would not mate with UV minus [UV−] conspecific males), but it is not known whether females also make intraspecific discriminations based upon qualitative reflectance characteristics (i.e. intensity, hue or saturation). By contrast, studies of male butterfly mate choice are relatively easy to perform (Silberglied, 1984) and subsequently plentiful (Rutowski, 1977; Silberglied, 1984, and references therein; Knüttel and Fiedler, 2001; Fordyce *et al.*, 2002; Ellers and Boggs, 2003; Sweeney *et al.*, 2003). Various colour pattern elements have been implicated in male choice (generally assayed as a male approach to a dummy or tethered female), including structural reflectance (Sweeney *et al.*, 2003). However, since male butterflies are generally more brilliantly coloured than females (Darwin, 1874; Silberglied, 1984), and make greater use of structural coloration, studies into female preferences are ultimately required to fully understand the evolution of this exaggerated visual trait.

In addition to the evidence for intersexual signalling, several studies have demonstrated an intrasexual signalling role for structural UV – for example, in *Hypolimnas misippus* (Stride, 1956, 1957), *Eurema lisa* (Rutowski, 1977) and *Colias eurytheme* (Silberglied and Taylor, 1978). In most cases, the available evidence suggests that UV coloration inhibits the approach and courtship of actively mate-locating conspecific males. Thus, the trait also appears to function in intraspecific sex recognition. However, the strength of selection for efficient sex recognition based upon colour pattern is expected to be weaker than selection due to mate choice (where present), and there is no special reason to expect the expression of such signals across individuals to be condition dependent (as structural reflectance may well be) (e.g. Keyser and Hill, 2000; D.J. Kemp, unpublished data). Secondary sexual characters should often serve multiple functions (Berglund *et al.*, 1996), and we consider sex recognition as a logical secondary function for such an obvious visual trait. Male–male combat is a potentially ‘stronger’ intrasexually selected context, yet this cannot be responsible for variation in UV patterning seen across the butterflies studied here (nor any Pierid), since males are non-territorial and do not engage in contests. The only relevant study thus far (Rutowski, 1992) found no evidence for a role of male structural UV in deciding or mediating territorial contests in the Nymphalid *Hypolimnas bolina* (for an example dealing with non-structural coloration, see Lederhouse and Scriber (1996)).

One additional outcome of our analysis was that variation in UV pattern appeared largely orthogonal between the fore- and hind-wings, and that a single principal component was sufficient to describe the variation in each case (i.e. for each wing; Table 1). Hence, intraspecific variation in fore-wing UV patterning appears to be independent of hind-wing pattern variation. This could reflect a lack of intra-wing (relative to inter-wing) developmental specificity in the expression of structural UV reflectance, or, alternatively, the action of differing selection upon the signal characteristics of each wing. Melanic markings did not display a similar pattern of orthogonal among-wing variation, which could further reflect the differing selective roles of these colour pattern elements. Perhaps the main role for melanic wing markings in butterflies is thermoregulation (Watt, 1968), but most of the

markings we deal with here are too distant from the point of thoracic wing insertion to be of any use in conductive heat transfer. These markings may serve some sexual signalling function (i.e. via increasing intra-wing colour contrast), but the present analysis suggests this role is probably secondary to that putatively given by structural UV. Experimental documentation of female (rather than male) butterfly mate preferences would help shed further light upon this issue, and help further elucidate the *raison d'être* of structural coloration in butterflies.

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