Multimodal signalling: structural ultraviolet reflectance predicts male mating success better than pheromones in the butterfly *Colias eurytheme* L. (Pieridae)

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In sexual selection, multimodal signals elicit mate choice when more than one sensory modality is activated. However, determining the relative use of each signal is difficult because it requires a comprehensive understanding of the mating system and how this system works under natural conditions. We examined the role of structural ultraviolet (UV) reflectance and pheromones in the butterfly *Colias eurytheme*. Both traits are important in mediating interspecific interactions and pheromones have been implicated in intraspecific mate choice. UV reflectance, which arises from the presence of a multilayer thin-film interference array, has potential as an honest indicator of male condition, viability and/or age. We investigated the relevance of these signal traits to courtship success by releasing virgin females in the path of free-flying males until each female had rejected and accepted at least one male. This design facilitated a within-subjects (females) analysis of mate choice, thus controlling for potentially confounding variation in intrinsic female receptivity. Principal component analysis indicated that variation across males in UV brightness and pheromones was essentially orthogonal. Females preferred younger males (as subjectively adjudged by wing wear), and while age covaried with UV brightness and almost all pheromone descriptors, UV brightness emerged as the best and most general predictor of male mating success. Our results suggest that this trait serves as an important intraspecific sexual signal in *C. eurytheme*, and they provide the clearest evidence to date regarding the functional relevance of structural coloration to female mate choice in butterflies. We discuss the preferential use of one secondary sexual characteristic (UV reflectance) over another (pheromones) with regard to evolutionary strategies.

When mate choice occurs, females use one or more male secondary sexual traits in making their decisions. Traditionally, studies of mate choice have assessed the influence of a single trait on mate choice. This approach, however, does not reflect the richness of sensory input that an animal is receiving, nor does it address the evolution of multiple traits under complex selection regimes. Relying on multiple sources of information was once thought to be an evolutionarily unstable strategy for females engaged in mate choice, but recent studies suggest that mate choice may generate and maintain multiple secondary sexual traits in a wide diversity of animals (Andersson 1994; Endler 1995; Backwell & Passmore 1996; Omland 1996; Marchetti 1998; Møller et al. 1998; Hill et al. 1999; Hankison & Morris 2003; McLennan 2003). Multiple signals can occur in a single sensory modality (e.g. chirp rate and carrier frequency in field crickets; Scheuber et al. 2003), or in multiple modalities (e.g. foreleg tuft size and vibration patterns in spiders; Hebets & Uetz 1999). The use of multiple signals is proposed to be adaptive for at least two reasons, either because they

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may act (1) as multiple messages about different male qualities or (2) as back-up signals for a more accurate assessment of a single male quality (Candolin 2003).

A recurrent question about multiple male courtship signals focuses on the relative importance of each signal as a determinant of male attractiveness and mating success. For example, in birds, is male song as important as male coloration in mate selection by females? Answers to such questions help us to understand not only the types of traits that are most important to females but also the strength and nature of selection pressures acting on those signals, thereby informing our understanding of the evolution of female preferences. We looked at a pierid butterfly, the orange sulphur, *Colias eurytheme* L., because males have a visual cue (structural ultraviolet (UV) reflecting scales) and an olfactory cue (lipid-based cuticular hydrocarbons acting as pheromones) (Taylor 1973; Grula et al. 1980; Rutowski 1980), both of which have been shown independently to be important in mate choice (Taylor 1973; Silberglied & Taylor 1978; Grula et al. 1980; Rutowski 1980, 1985). Previous studies suggest that pheromones may be more important in mediating intraspecific female choice while UV signals may be more important in avoidance of interspecific copulation (Silberglied & Taylor 1973), but to date, none of these studies have made an attempt to evaluate which of these two signals are more closely associated with male mating success in the field.

Pheromones are thought to be more important in mediating close range male−female interactions among day-flying Lepidoptera (Vane-Wright & Boppre 1993). In *C. eurytheme*, certain pheromones, notably *n*-heptacosane (C_{27}), 13-methyl hepatcosane (13-MeC_{27}) and *n*-nonacosane (C_{29}), must be present in order for mate acceptance by female *C. eurytheme* (Grula et al. 1980; Rutowski 1980). Males release these pheromones when they brush their wings against the female's antennae. Individual males vary in both the quantities and relative proportions of the three component chemicals (Sappington & Taylor 1990a, b) and pheromone characteristics of field-caught males in copula differ from those of nonmating males (Sappington & Taylor 1990c).

Structural reflectance is widespread among animals (e.g. Brunton 1998; Macedonia et al. 2000; Kodric-Brown & Johnson 2002; Eaton & Lanyon 2003; Parker et al. 2003; Sweeney et al. 2003; Vukusic et al. 2004; Kemp et al. 2005) and has been implicated in female mate choice decisions in birds and fish (Andersson & Amundsen 1997). Many butterflies also have extremely bright structural patterning on their dorsal wing surfaces and this signal has been shown to mediate species recognition (Brunton & Majerus 1995). In general, structural coloration arises from an interaction between incident light and the micro- or nanoscale surface architecture, and in butterflies, may result from one or several optical mechanisms, such as constructive interference, scattering and diffraction (Vukusic & Sambles 2003). In *C. eurytheme*, visual cues primarily involve UV reflectance of nanostructures on the dorsal wing surface (Silberglied & Taylor 1978). Males reflect UV light (300−400 nm) from the dorsal wing surface of all four wings (Silberglied & Taylor 1973). This highly

directional reflectance results from a complex array of multilayer thin films that constructively interfere with light in UV wavelengths only. Silberglied & Taylor (1978) showed that female *C. eurytheme* use the presence of UV reflectance to distinguish conspecific males from the UV-absorbing males of a closely related species, *C. philodice*. Peak UV brightness also varies greatly among male *C. eurytheme* (Kemp 2006), suggesting that variation in UV reflectance could be used by females in making intraspecific mate choice decisions.

We evaluated the relative importance of naturally occurring interindividual variation in pheromones and UV reflectance in the mating system of *C. eurytheme*. By use of a within-subjects experimental design, we demonstrate that female mating decisions are best explained by variation in male UV brightness and less so by pheromone characteristics. We also provide the first evidence for the role of bright structural coloration for female mate choice in butterflies.

METHODS

General

We reared virgin females on alfalfa (*Medicago sativa*) from eggs laid by female *C. eurytheme* collected in Chandler, Arizona, U.S.A. in May 1998. Females used in the experiment were kept at 4°C for up to 5 days posteclosion, and were marked with a red felt-tipped pen on both ventral hindwings for identification before release in the field. Female, but not male, *C. eurytheme* are dimorphic: most are orange-yellow, some are white (alba). We only used orange-yellow phenotype females because alba females cannot be unequivocally identified as *C. eurytheme* and are known to show different preferences for male pheromones (Sappington & Taylor 1990c).

Mating Trials

We performed mating trials by releasing females individually in front of flying males. We considered a courtship to begin when the male arrived within 0.5 m of the female, and to end when the male left or the pair mated, and we timed each courtship to the nearest second using a digital stopwatch. Unsuccessful males were captured immediately and mated males were gently separated from the female within 30 s of the beginning of copulation to prevent transfer of seminal fluids and a spermatophore that would render the female unreceptive. All females included in this study were recaptured between subsequent trials. If a female escaped during the trials, her data were not included. This procedure was repeated until each female had mated with and rejected (see below) at least one male. All trials were run between 0800 and 1200 hours. We used a total of 48 females.

A male was scored as rejected if he did not mate and the female performed one or several of the following behaviour patterns: (1) wings spread posture: the female alights, spreads her wings open and curls her abdomen dorsally, thereby preventing or delaying mating (Obara 1964); (2) aerial avoidance: the female flies horizontally away from

the male; (3) ascending flight: the female flies upward until the male stops pursuit (Rutowski 1985). We excluded trials in which the male approached and started courting the female but left before a clear rejection by the female.

Accepted and rejected males were freeze-killed and stored at $-10°C$ for later measurements of UV reflectance, pheromones, body size and age. Pheromones have been previously shown to be unaffected by such storage (Grula et al. 1980). We tested to see whether UV was similarly unaffected by measuring UV reflectance before and after storage on 12 butterflies. There was no difference in UV reflectance for these animals (paired t test: $t = 0.116$, $P = 0.910$). Forewing length was measured from apex to insertion as a surrogate measure of body size. Age was estimated for males on a relative scale from 1 to 9 (Table 1).

Pheromone Measurements

Pheromone measurement methods were based on those of Sappington & Taylor (1990a). We removed all four wings from each male and used three of these to determine pheromone information so as to maximize detection of pheromones, while reserving the final forewing for use in detection of UV reflectance. We placed two hindwings and one forewing in a vial for 10 min with enough hexane to completely cover the wings. The hexane was then filtered using a syringe-driven filter unit with a 0.20-mm pore size PTFE membrane to remove scales and other debris from the solution. The filtered hexane was evaporated under a gentle stream of nitrogen, leaving a residue of hydrocarbons, which were resuspended in 99 µl of hexane. This solution was transferred to a 150-µl glass insert that was placed inside a 1.8-ml autosampler vial capped with a PTFE/silicone septum. An internal standard with a ratio of 1 mg of octacosane suspended in 1 ml of hexane was added, so that the final volume was 100 µl of hexane.

We injected $1-2$ µl of each sample into a Varian gas chromatograph/mass spectrometer equipped with a flame ionizing detector using an autosampler and hydrogen as the carrier gas. A nonpolar, SE-54 30-m-long bonded-hose vitreous silica capillary column was used. The

column had an inner diameter of 0.32 mm and a film thickness of 0.25 mm. The detector was set at 250°C. The column temperature began at 50°C and was held at this temperature for 2 min, after which it was increased by 10°C/min until it reached 275°C, then held at this temperature for 2 min. Total programme length was 28 min. We determined the amount and ratios of heptacosane (C_{27}), 13-methyl heptacosane (13-MeC_{27}) and nonacosane (C_{29}) by comparing areas under the peak relative to the internal standard.

UV Reflectance Measurements

We measured UV reflectance from the remaining forewing using three techniques: (1) reflectance spectrometry using an integrating sphere; (2) directional reflectance spectrometry; (3) whole-wing UV image capture. All three of these techniques have been used by other investigators (e.g. Macedonia 2001; Acorn 2002; Raguso & Willis 2005), and while all three techniques have been shown to be valid at measuring UV reflectance accurately, no single method has been widely accepted as best.

We first placed each forewing on a piece of black felt of known reflectance, and measured brightness in the range of $350-400$ nm using an integrating sphere attached to a spectrophotometer (Spectral Instruments model 440, Tucson, Arizona, U.S.A.). The integrating sphere had a port diameter of 13 mm, and illumination was provided by a tungsten light source, which emitted wavelengths no shorter than 350 nm.

Second, we captured a reflectance spectrum from a circular 5-mm (central) area of wing using an Ocean Optics USB-2000 spectrometer (25 averaged spectra, 125 ms integration time; EI Dorado Hills, California, U.S.A.). Pulsed illumination was provided at 90° to the horizontal using a PX-2 xenon light source, and the collector probe was situated at 45°. Given that the iridescent UV reflectance is dependent upon specific positions of incident light source and receiver, and the relative angle conducive to peak brightness varies slightly among individuals (Kemp 2006), we rotated each sample within a range of $5-6°$ to locate the best angle for peak brightness to be captured. Relative reflection, in this case, in the range of $300-400$ nm, was expressed as a proportion of that obtained from a MgO white standard. This method of UV colour measurement has proven highly repeatable among individuals of *C. eurytheme* (Kemp 2006).

Last, we captured an image of whole-wing UV reflectance using a video camera fitted with a Tiffen 18A visible light-absorbing filter, a Takumar lens (55 mm, f 1.8), and light provided at 90° by a tungsten-halogen fibre-optic illuminator filtered to remove infrared wavelengths. Spectrophotometric measurements made between 250 and 800 nm indicated that with this combination of light source, filters and lens, the wavelength of the most intense light reaching the camera's light-sensitive element is between 370 and 385 nm. In this wavelength range, $20-40\%$ (at 380 nm) of the light from the light source is received by the camera. On either side of this peak, the

Table 1. Relative condition score and corresponding description for field-caught males

Condition score*	Description of condition
1	No scale loss
2	Small amount of scale loss
3	Some scale loss, slight tattering
4	Some scale loss, moderate tattering
5	Moderate scale loss, moderate tattering
6	Moderate scale loss, distinct tattering
7	Considerable scale loss, conspicuous tattering
8	Considerate scale loss, severe tattering
9	Few scales left, severe tattering

*Used as an estimate of age in this study.

percentage of light received drops to less than 1% below 350 nm and to 2% or less above 400 nm. UV light is clearly the primary contributor to the images we made with this system.

As with our directional spectrometric measurements (above), we rotated each sample using a single-axis goniometer and captured the image from the angle at which UV reflectance was subjectively maximal. We also included the same white paper 'standard' in each image. Using Adobe Photoshop, we then converted each digital image to greyscale and calculated the mean brightness of the UV-reflectant wing area using the lasso tool to trace the area and the histogram function to give mean luminosity (on a scale of 0–256). We similarly calculated the brightness of the paper standard and used these measures to adjust among images for minor variations in lighting conditions. Repeat measurements of a random 20 samples indicated high repeatabilities of this whole-wing UV brightness measure (Pearson's correlation: $r_{18} = 0.899$, $P < 0.0001$).

Statistical Analyses

We used principal component analysis to initially condense variables describing pheromones (C_{27}, 13-MeC_{27} and C_{29}), pheromone ratios (C_{27}:13-MeC_{27}, C_{27}:C_{29} and 13-MeC_{27}:C_{29}) and UV reflectance (as above) to fewer orthogonal predictors. We explored the relevance of individual principal components to courtship outcome using paired t tests (with data paired by female), and we used best subsets multiple logistic regression to construct the most informative model of mate choice based upon minimization of Akaike's information criterion (AIC). The AIC is an information theoretic derivative of the log-likelihood function that better facilitates comparison among models containing varying numbers of predictor variables (Burnham & Anderson 2002). We used Wald tests to evaluate the strength and statistical significance of individual predictors. Because the data compare two males (one accepted and one rejected) for each female, we emphasized a within-subjects (i.e. females) analysis by randomly assigning one male from each pair as the 'focal' male and creating a new dependent variable, coded 0 = focal male rejected and 1 = focal male mated. Predictor variables were derived by subtracting the nonfocal male's parameter value from the focal male's value.

Our experiment was designed to facilitate a within-subjects contrast of courtship outcome, and thus, is less suited to an analysis of courtship duration. The influences upon courtship duration are likely to vary depending upon outcome; hence, unsuccessful and successful courtships should be analysed separately. However, given that we lacked within-female replication of these cases, we were unable to conduct an analysis of male signals that also controlled for the potentially confounding effect of variation in intrinsic female receptivity.

All analyses were conducted using Statistica v7.1 (StatSoft, Tulsa, Oklahoma, U.S.A.), and means are accompanied throughout with 95% confidence intervals.

RESULTS

Successful courtships lasted significantly longer on average (21.2 ± 4.8 s) than unsuccessful courtships (12.7 ± 3.39 s; paired t test: $t_{47} = 2.93$, $P < 0.01$; Cohen's $d = 0.598$). Males that were accepted by females were younger (as classified using wing wear) than rejected males (logistic regression: $G_1 = 12.4$, $N = 48$, $P < 0.0005$; mean age difference (accepted – rejected): Cohen's $d = -1.46$), and all potential signal trait variables except for C_{27} covaried with age (Table 2).

Evaluation of the mean signal characteristics grouped by courtship outcome (Table 2) suggests that UV brightness variables, the pheromone nonacosane (C_{29}) and the C_{27}:C_{29} pheromone ratio are factors in courtship outcome. The principal component analysis condensed the 10 predictors into five orthogonal components, which collectively explained 88.2% of the available variance. Evaluation of component loadings (Table 3) suggests that three of the components (PC1, PC2 and PC5) each represent pheromones and pheromone ratios, one component represents UV brightness (PC3), and the remaining component represents body size (PC4). The apparent segregation of size, UV and pheromone variables across components suggests that these traits vary somewhat independently among free-flying *C. eurytheme* males. PC3 contains all three UV brightness measurements, so it appears that there is no difference in the usefulness of the three measurement types used in this study.

Individually, courtship outcome was related to PC3, the UV brightness component (paired t test: $t_{47} = 3.57$, $P < 0.001$; mean courtship difference (successful – unsuccessful): $d = 0.567$) and PC5, a pheromone ratio component ($t_{47} = 2.05$, $P < 0.05$, $d = 0.399$), but not to PC1, a second pheromone ratio component ($t_{47} = 0.78$, $P = 0.44$, $d = 0.162$), PC2, the C_{27} and C_{29} pheromone component ($t_{47} = 1.34$, $P = 0.19$, $d = 0.231$) or PC4, the body size component ($t_{47} = 0.67$, $P = 0.50$, $d = -0.125$). Of these five contrasts, PC3 was the only significant contrast (at $\alpha = 0.05$) once P values were adjusted using the step-up sequential Bonferroni correction (Rice 1989). The positive loadings of PC3 with UV brightness (Table 3) indicates that favoured males had brighter UV reflectance, and the negative loadings of PC5 with the ratios C_{27}:C_{29} and C_{27}:13-MeC_{27} suggests that favoured males had marginally smaller values for these ratios.

The most informative multiple logistic regression model of courtship outcome included PC3, UV brightness, and PC5, pheromone ratios (AIC = 56.5, $G_2 = 15.3$, $N = 48$, $P < 0.0005$); however, only PC3 (Wald's $W = 8.50$, $P < 0.005$) was a significant predictor (PC5: $W = 2.01$, $P = 0.156$). This model was only marginally more informative than a model including only PC3 (AIC = 57.1, $G_1 = 12.6$, $N = 48$, $P < 0.0005$), and one including PC3, PC2 and PC5 (AIC = 56.7, $G_3 = 17.1$, $N = 48$, $P < 0.001$). PC3 was again the only significant predictor in the latter multiple model (PC3: $W = 7.67$, $P < 0.01$; PC2: $W = 1.35$, $P = 0.92$; PC5: $W = 1.21$, $P = 0.27$). Therefore, PC3 (i.e. the UV brightness component) was clearly the most useful predictor of mate choice in this population of *C. eurytheme*. The average reflectance spectrum of accepted and rejected

Table 2. Summary of trait covariance with age (wing wear), trait means for rejected and accepted males and effect size (a measure of the difference between groups independent of sample size; Cohen 1988)

Trait	Age covariance (Spearman's r)	Rejected males ($N=48$)		Accepted males ($N=48$)		Effect size (Cohen's d)
		Mean$\pm$95% CI	Range	Mean$\pm$95% CI	Range	
Wing length (mm)	**−0.203**	23.4$\pm$0.54	18.7−27.5	23.3$\pm$0.40	20.7−26.5	0.072
UV (1) (% transmittance)	**−0.503**	11.5$\pm$0.70	5.4−15.3	13.0$\pm$0.88	8.5−19.1	**0.551**
UV (2) (% transmittance)	**−0.293**	37.6$\pm$4.1	8.8−67.4	43.4$\pm$2.8	25.4−65.1	0.486
UV (3) (mean luminosity)	**−0.419**	0.653$\pm$0.035	0.397−0.968	0.718$\pm$0.040	0.447−1.020	**0.503**
C_{27} (g)	0.030	1.33$\pm$0.25	0.24−4.18	1.58$\pm$0.52	0.40−10.69	0.182
C_{29} (g)	**−0.263**	2.29$\pm$0.32	0.63−8.33	3.47$\pm$1.02	1.05−18.68	0.459
13-MeC_{27} (g)	**−0.492**	3.21$\pm$0.85	0.32−16.13	3.68$\pm$0.83	0.35−13.69	0.165
C_{27}:C_{29}	**0.336**	0.582$\pm$0.083	0.289−1.750	0.476$\pm$0.051	0.053−1.068	0.450
C_{27}:13-MeC_{27}	**0.463**	0.753$\pm$0.256	0.102−4.046	0.611$\pm$0.207	0.119−4.011	0.180
13-MeC_{27}:C_{29}	**−0.421**	1.36$\pm$0.259	0.236−3.679	1.32$\pm$0.233	0.157−3.709	0.049

The three UV brightness measures correspond to measurements taken using (1) spectrometry with an integrating sphere, (2) spectrometry with 5-mm circular capture area and (3) digital image capture (see Methods). Highlighted Spearman r values were significant at $P < 0.05$ ($N = 96$), and highlighted d values were greater than 0.500, which is subjectively classified as a medium-sized difference in behavioural research (Cohen 1988).

males (Fig. 1) clearly differed in the UV wavelengths compared to the visible spectrum.

DISCUSSION

Our results suggest three major findings. First, female *C. eurytheme* prefer to mate with relatively younger males (i.e. males with less wing wear). Second, almost all UV reflectance and pheromone traits covaried with age (Table 2) yet variation among these two trait groups was largely orthogonal and therefore uncorrelated (Table 3). Third, among potential signal traits, the brightness of UV reflectance was the strongest and most informative predictor of male courtship success. Females may also use some unmeasured trait that is correlated with UV reflectance instead of UV reflectance per se. However, based on our work and that of others, the brilliant structural UV coloration of males is likely to be important in female intraspecific mate choice in *C. eurytheme*. Given that previous work in these butterflies used experimental manipulations of the presence/absence of this trait (e.g. Silberglied & Taylor 1978; Sweeney et al. 2003), our results are especially informative because we conducted this research under field conditions using free-flying males with naturally occurring individual variation in UV brightness.

If females use UV reflectance as a means of determining relative age of males, then evolutionary theory suggests that they might be receiving a benefit from this choice (Andersson 1994). The benefits of female choice are considered to fall into two broad categories: material or direct benefits, and genetic or indirect benefits (Andersson 1994). In some animals, females choose males based on the material resources that they can provide, such as parental care or nuptial gifts, while in other cases, females are thought to derive genetic benefits, such as good, diverse or compatible genes. What might female *C. eurytheme* receive from mate choice? Possible genetic benefits from choice remain undocumented in this species; however, a material benefit has been identified. Male butterflies include proteins in the spermatophore that is passed to females during mating (Boggs & Gilbert 1979; Boggs & Watt 1981). Age in male butterflies is negatively correlated with the amount of protein available for packaging in the male's spermatophore because older males are more likely to have mated previously (Boggs & Watt 1981; Rutowski & Gilchrist 1986; Rutowski et al. 1987).

Table 3. Summary of results (component loadings and eigenvalues) for the principal component analysis (conducted on all raw data, $N = 96$)

Trait	PC1	PC2	PC3	PC4	PC5
Forewing length	−0.069	0.039	0.096	**0.984**	−0.036
UV (1)	−0.201	0.053	**0.827**	0.108	0.200
UV (2)	0.001	0.051	**0.799**	−0.094	0.124
UV (3)	−0.068	0.005	**0.880**	0.153	0.086
C_{27}	0.003	**0.711**	0.027	0.081	−0.642
C_{29}	0.067	**0.964**	0.049	0.004	0.041
13-MeC_{27}	**−0.729**	0.607	0.118	0.047	0.155
C_{27}:C_{29}	0.092	−0.090	−0.285	0.033	**−0.911**
C_{27}:13-MeC_{27}	0.403	0.085	−0.178	−0.0120	**−0.836**
13-MeC_{27}:C_{29}	**−0.915**	−0.185	0.129	0.071	0.238
Eigenvalue	1.593	1.860	2.252	1.027	2.087
Proportion of total	0.159	0.186	0.225	0.103	0.209

Loadings greater than 0.700 are highlighted for emphasis. UV variables correspond to those in Table 1.
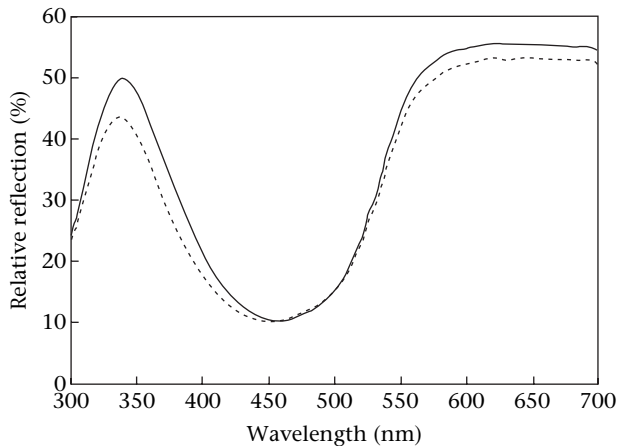
Figure 1. The mean reflectance spectra of accepted (solid line) versus rejected (dashed line) males, as measured using the 5-mm beam spectrometry method, UV2.

Proteins are then sequestered in females' bodies and eggs, increasing the overall fitness of both the male and female. This method of transferring proteins is particularly important in the Lepidoptera because they are generally incapable of ingesting proteins as adults and must completely rely on reserves. Given that UV brightness is correlated with age (Table 2), female preferences for brighter males may indeed bring with it a material benefit.

If UV brightness is an informative cue for the material benefit of limited nutrients in the form of proteins, it should be an honest indicator of this benefit (Green 1991; Knapp & Kovach 1991). Ultraviolet reflectance influences mate choice in other taxa, including birds and fish (Hunt et al. 2001; Kevan et al. 2001). In grosbeaks, structural coloration may act as a reliable signal of male mate quality (Keyser & Hill 2000) because structural UV reflectance is costly to make and therefore honestly indicates phenotypic condition (Keyser & Hill 1999). Structural UV has the potential to be an honest indicator in *C. eurytheme* because the formation of the structural array requires fine-scale precision. Because butterfly scales undergo complete formation during the imago stage of development, structural UV scales cannot be replaced or repaired over the course of an adult's lifetime. However, the actual costs to producing UV-reflectant scales in *Colias* are not yet known. Future work should focus on the extent to which this trait carries additional information on the phenotypic and genetic mate quality of male *C. eurytheme*.

It has long been thought that visual cues mediate long-range mate location or identification in butterflies, whereas pheromones come into play when the male is in contact with the female and thus serve as short-range cues more likely to be used in assessing mate quality (Grula & Taylor 1980; Silberglied 1984; Vane-Wright & Boppre 1993). Our results do not specifically address the long- versus short-range function of visual cues because we short-circuited the usual sequence of mate-locating events by presenting virgin females within close proximity of mate-locating males. While our findings suggest that male colour pattern is an important predictor of the outcome of short-range male–female interactions, these results need to be reconciled with behavioural evidence for the last 30 years showing that pheromones in *C. eurytheme* are positively correlated with increased receptivity (Rutowski 1980) and ganglia activity (Grula et al. 1980). Previous studies have also shown that individual variation in male pheromones is used by females to distinguish between males (Grula et al. 1980). In addition, pheromones probably influenced the evolution of courtship structure, which consists of a male flying in front of a female and repeatedly touching his wings to her antennae. It is unknown whether pheromones are costly to produce, but since they are formed by lipids that are modified and then diverted to the cuticle, they cannot be used in other metabolic pathways. Whether or not pheromones are an honest indicator of age is as yet untested. However, we suggest that one pheromone (C_{27}) is unlikely to be an honest indicator of age because it does not diminish with age.

Our results suggest an intriguing evolutionary question: given that males produce two types of signals in distinct sensory modalities, each of which is presumably costly, why do females appear to rely more heavily on brightness of UV reflectance? There are two main hypotheses for the presence of multiple signals: (1) multiple messages, in which two or more signals give information about distinct qualities and (2) back-up signals, in which two or more signals give reinforcing information about the same quality (Candolin 2003). Our data do not permit a clear test of these hypotheses. However, because UV reflectance appeared to be more important to females, our results are most consistent with the second hypothesis, namely, in *C. eurytheme*, pheromones are acting as a back-up to UV reflectance.

An interesting finding of this study was that successful courtships lasted longer than unsuccessful courtships. This finding is counter to the simple expectation that suitable or attractive males should be accepted relatively quickly by females. However, the lack of information on the mechanics of courtship and the nature of female–male signalling processes makes this finding difficult to interpret. On the one hand, relatively longer successful courtships may indicate that the unsuccessful males in this study were simply less motivated to persist in protracted courtships and that they quit before a coy female could be persuaded to mate. Rutowski (1985) reported that successful courtships in *C. eurytheme* have a typical minimum duration and he suggested that males who court less than this minimum are unlikely to succeed in mating. On the other hand, females may have influenced the decisions of (ultimately unsuccessful) males by signalling early in the interaction their lack of intention to mate. Rejection postures, such as the 'extended abdomen' display of many pierids (e.g. Obara 1964) are known for butterflies. It is not known to what extent these behavioural signals, or indeed any additional visual and/or chemical female courtship signals, serve to influence the courtship motivation of male *C. eurytheme*. Antiaphrodisiac compounds have been reported to strongly modify male courtship behaviour in pierid butterflies (Andersson et al. 2000).

Acknowledgments

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