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Potential direct fitness consequences of ornament-based mate choice in a butterfly

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Abstract Female mate choice has been shown to provide direct mating benefits in several animal groups. In butterflies, for which there are increasing reports of fine-scale color-based mate choice, the evolutionary benefits that accrue from such mating biases, if any, are largely unknown. We addressed this issue in the butterfly *Colias eurytheme*, a species in which females choose mates on the basis of iridescent ultraviolet (UV) wing ornamentation and in which males donate reproductively beneficial nuptial gifts. In the first experiment, we assessed the mass of gifts donated to 77 virgin females by males sampled directly from a field encounter site. Despite large variance in the male adult phenotype and ejaculate, no single aspect of dorsal wing coloration, including UV brightness, chroma, or hue, was related to ejaculate mass. There was, however, an interesting interaction between the effects of male body size and copula duration upon ejaculate mass, with size scaling positively with ejaculate mass among males involved in shorter copulations (those lasting <70 min) but negatively among males in longer copulations. In the second experiment, we assessed the lifetime fecundity, fertility, and longevity of 85 females mated under similar circumstances to free-flying wild males. Although several wing color parameters proved subtly informative in more sophisticated multivariable models, no model predicted more than about 20% of the variation in any single female fitness parameter. The duration of copulation, which ranged from 35 min to over 16 h and which carries putative costs for females, was, again, only very weakly predicted by male wing color parameters (i.e., R^2=0.089). Given the overall minor predictive power of male wing coloration in general and of UV brightness in particular, our results do not strongly support the hypothesis that female *C. eurytheme* prefer bright UV males to obtain direct benefits or to minimize the costs associated with lengthy copulations.

Keywords Coloration · Lepidoptera · Ornamentation · Sexual selection · Spermatophore · Ultraviolet

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Introduction

The females of many animals, particularly birds, fish and insects, possess mating biases that favor males bearing conspicuous visual ornamentation (Andersson 1994). Efforts to understand the evolution of these biases have focused on the benefits that females might receive from being choosy. Potential benefits have been grouped as either 'direct,' such as nutritious nuptial gifts or parental care, or 'indirect,' such as the genes for attractive or high-quality offspring (Andersson 1994). Whereas empirical support for direct fitness benefits arising from ornament-based female choice has accumulated across several animal groups, principally birds (e.g., Linville et al. 1998; Préault et al. 2005; Senar et al. 2002; Velando et al. 2005), key empirical support for indirect benefits has proven more challenging to garner (Andersson and Simmons 2006). Direct and indirect

benefits are unlikely to operate independently (Iyengar and Eisner 1999; Wedell 2006), but this data asymmetry has nonetheless fueled speculation among evolutionary biologists regarding the relative importance of each in shaping the evolution of mating biases and exaggerated sexual ornamentation (e.g., Kirkpatrick 1996).

In many insect species, males provide direct mating benefits in the form of nutritious material provided at the time of copulation (Boggs 1995; Vahed 1998). This material, which may be transferred either internally (Boggs and Gilbert 1979) or externally (Thornhill 1976; Gwynne 1984), may contribute to female longevity (Wedell 1996; Karlsson 1998) and/or subsequent reproductive success (Gwynne 1984; Fox et al. 1995a). Theory suggests that, provided the requisite variation that exists among males, females should choose among potential mates in ways that maximize their likely nutritional benefit from copulation. However, because there is likely to be conflict between the sexes, the presence of such biases would favor males that advertise above their potential level of material investment, wherever possible, which would lead to a breakdown of the signaling system. Selection should, therefore, ultimately lead to the evolution of signals that present honest or 'cheat-proof' indicators of future mating investment (Zahavi 1975). Investigations into the signaling of direct benefits in insects have examined the ways in which preferred male traits, such as body size and age, could honestly signal nutrient contribution (see, e.g., Fox et al. 1995a, b; Bussière et al. 2005). However, although relatively well studied in vertebrate systems (especially birds; see the above literature examples), few studies have investigated color-based male sexual ornaments as signals of direct mating benefits in insects.

In this study, we address the potential for ornamental color-based signaling of paternal investment in butterflies. Several features of butterfly mating systems suggest opportunities for the evolution of such a signaling system. First, color-based female mate choice appears to be relatively widespread within this group, and females are capable of relatively fine-scale discriminations of wing pattern and brightness (Costanzo and Monteiro 2007; Kemp 2007). Second, male butterfly ejaculates contain nutrients that, in some species, are incorporated into female eggs (Boggs and Gilbert 1979) and boost subsequent fecundity (Oberhauser 1997; Karlsson 1998). Third, the size of the male ejaculate (as well as the time required for its production) is variable and may relate to prior mating history, age, body size, and larval diet (Rutowski et al. 1987; Cook and Wedell 1996; Lauwers and Van Dyck 2006). Several studies indicate that females mated with non-virgin males are subjected to longer copulations and suffer reduced reproductive output, perhaps as a consequence of having received a smaller nutrient contribution (see the review of Torres-Vila and Jennions 2005). Last, there are several candidate mechanisms by which variation in male wing color could be linked to phenotypic mate quality. Given that male color arises from nutritionally expensive pigments and/or surface nanostructures (see below), it could honestly indicate the quality of the juvenile environment and, hence, the adult energy budget (Talloen et al. 2004; Kemp and Rutowski 2007). Male color also fades with age (Kemp 2006), which may promote covariance with prior mating history in species in which males accumulate matings throughout their lifetime. Male color, therefore, has the potential to indicate both the male's 'starting' nutritional condition (which is likely to covary at least to some extent with his potential for nuptial investment) and lifetime changes in nuptial investment potential (and copulation duration) as a result of having made past investments.

We used the orange sulfur butterfly, *Colias eurytheme*, to assess whether male wing color is related to the mass of their ejaculate, the duration of copulation, and/or their partner's subsequent reproductive fitness (i.e., fecundity, fertility, and longevity). *C. eurytheme* is an extensively studied polyandrous species (see below) that exemplifies a 'gift-giving' mating system in which male-derived nutrients contribute to female reproduction (Rutowski et al. 1987). Females of this species also choose mates based upon male-limited wing ornamentation, the phenotypic expression of which depends upon juvenile diet quality (Kemp and Rutowski 2007) and age (Kemp 2006). Our goal was to see whether, under natural field conditions, females could reliably use male wing color to predict the direct mating benefits (and/or the absence of costs, such as a lengthy copulation) that they might receive upon mating.

Wing coloration, mate choice, and reproductive biology in *C. eurytheme*

C. eurytheme is a small- to medium-sized butterfly whose wings appear yellowish orange to human observers because of the presence of a suite of pteridine pigments (Watt 1964). Males (but not females) also exhibit bright, iridescent ultraviolet (UV) reflectance across the majority of their dorsal wing surface. This coloration arises from an intricate nanoscale surface architecture that functions as a quarter-wave interference mirror for incident UV light wavelengths (Ghiradella 1974). Male UV markings function as a sexual signal (Silberglied and Taylor 1978; Papke et al. 2007), and female mating preferences have been correlated with increased UV brightness (Papke et al. 2007). Females are also known to mate preferentially with younger mates (Rutowski 1985), which, because of the covariance between age and UV brightness (Kemp 2006), could exist as either a cause or a consequence of the preference for

brighter UV markings. Because males with extensive or recent mating histories produce smaller ejaculates (Rutowski et al. 1987), females may prefer UV bright males to increase their chances of receiving a large ejaculate from a younger, potentially virgin individual. Non-virgin males may also subject females to considerably longer copulations, which, because such males may not even begin to transfer material until many hours into the copulation, has been interpreted as copulatory mate guarding (refer to the detailed work of Rutowski and Gilchrist 1986). These longer copulations could be costly to females for a host of reasons, including the possibility of increased exposure to toxic seminal proteins, sexually transmitted pathogens, or predation risk (e.g., Wing 1988), or simply because of the opportunity cost of lost oviposition time. Recent empirical research in *C. eurytheme* has also demonstrated that the expression of male wing coloration (especially the iridescent UV component) is dependent upon the nutritional quality of their larval environment (Kemp and Rutowski 2007). This has the potential consequence that, regardless of prior mating history, brighter UV males may provide, on average, larger or higher quality ejaculates. Hence, there are several proximate bases for predicting that male UV brightness, in particular, could be linked with ejaculate size and/or quality, and subsequent female reproductive fitness in this species

Materials and methods

Rearing and experimental protocols

We reared 156 virgin females of *C. eurytheme*, the offspring of eight field-caught females (from Avondale, AZ, USA) under a dual temperature/photoperiod regime of 14:10 L/D and 28:20°C. Standard rearing protocols were used (see, e.g., Kemp and Rutowski 2007), with the offspring cultured on field-collected cuttings of Alfalfa (*Medicago sativa*). Once emerged, adult females were given unique labels on their ventral hindwing with Sharpie® brand marker pens, and the length of their right forewing was measured as a surrogate for body size to the nearest 0.01 mm using digital calipers. Individuals were then placed in small, sealed plastic canisters, and stored in a refrigerator until use (no more than 5 days later).

From 15–25 July 2006, these virgin females were mated to free-flying males in cultivated alfalfa fields in Scottsdale, AZ, USA. This process involved releasing each female into the flight path of an actively mate-locating (patrolling) male, who, once having detected the female, then actively pursued copulation. Females were generally coy, and sometimes repeat presentations were necessary to achieve a successful copulation. In these cases, we endeavored as best as possible to persist with presenting each female to the same male, such that the male partner could be considered as being randomly 'assigned' (randomly with respect to the initial choice of the male). However, in perhaps 30% of cases and for various reasons, females had to be presented to two or several males, which posed a slight opportunity for the expression of female and/or male mating biases.

In-copula pairs were stored at ambient temperature in gauze-covered and paper-lined round plastic containers (236-ml capacity). Copulation duration was measured to the nearest 5 min. All males, once separated, were immediately frozen for later measurement of body size and wing color parameters. One group of females, totaling $N=71$, were frozen for later dissections to determine the mass of the male-donated ejaculate. All frozen females were later dissected, and we weighed the fresh mass of their bursa copulatrix (containing the male spermatophore and accessory secretions) and the fresh mass of the spermatophore only. Spermatophores were then dried to a constant mass in an incubator at 60°C for 24 h then reweighed.

A second group of 85 freshly mated females, the offspring of four field-caught mothers, were transported live to the lab after copulation for subsequent assay of adult fitness traits. These females were fed until satiation with a 10% sucrose–water solution, then placed immediately into a gauze-topped 946-ml cylindrical plastic 'oviposition chamber.' Each chamber contained a sprig of the larval hostplant, *M. sativa*, and a cotton ball soaked with 10% sucrose–water solution for ad-libitum feeding. The chambers were kept in an incubator under the light and temperature conditions described above. We replaced the foodplant and sucrose–water feeder daily, and counted the eggs laid before placing them in an incubator at 30°C. The eggs were again counted on the second day to determine the rate of fertility (fertilized eggs change color from yellow to red after 24 h). For each female, we subsequently calculated adult lifespan (in days), lifetime fecundity (total number of eggs laid), and average percent fertility (fertile eggs/total eggs).

Spectrometry and color analyses

We quantified the coloration of the copulating males' dorsal wing surfaces using the beam method of spectrometry (as per Fig. 1 in Kemp 2006). We used an Ocean Optics USB-2000 spectrophotometer (25 averaged spectra, 125 ms integration time), with pulsed illumination provided by a PX-2 xenon light source. Our measurement set-up, which is illustrated in Kemp (2006), consisted of the light source positioned at 90° to the horizontal (normal to the plane of the sample) and the sensor positioned at 45° and focused to capture input from an approximately 3.5-mm circular area. We measured a standardized region of each specimen's left

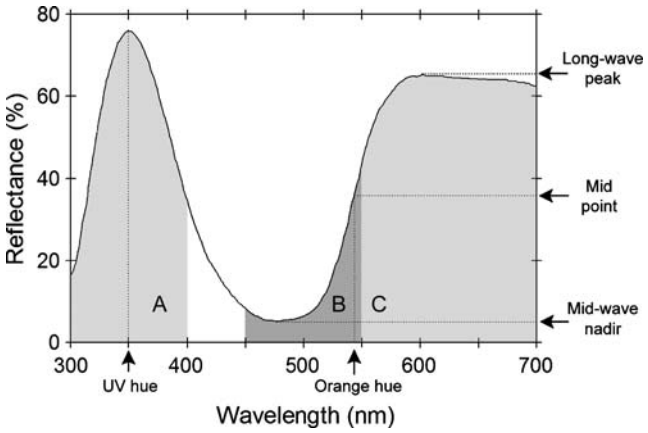

Fig. 1 Summary of the measured parameters for a representative *C. eurytheme* wing reflectance curve. *Shaded region A* UV brightness, *shaded region B* mid-wave brightness, and *shaded region C* long-wave brightness. *UV chroma* region *A*÷*B*, *orange chroma* region *C*÷*B*. UV and orange hue values are as indicated and described in the text

and right forewings, which were placed on matte black card and positioned horizontally, and so that their base pointed toward the collector's azimuth. Reflectance in all cases was expressed as a proportion of that obtained from a magnesium oxide white standard.

In addition to spectrometry, we used whole-wing video viewing to measure the angular-dependent visibility of each specimen's UV reflectance. Forewings were viewed using a video camera fitted with a Tiffen 18A visible light-absorbing filter, with light provided at 90° by a tungsten–halogen fiber-optic illuminator. This illuminator provides output adequate for our purposes at wavelengths greater than about 350 nm. An additional low pass filter was used to remove infrared (>700 nm) wavelengths; thus (with the Tiffen 18A filter and low-pass filters combined), the video CCD was exposed to light spanning at a range of 350–400 nm. Images were viewed in real time via a direct feed to a television monitor, and we used a universal stage to rotate wings around their proximal–distal 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, hereafter referred to as 'UV angular breadth.'

From the spectrometric data, we calculated a series of summary variables designed to capture the primary axes of variation in the male reflectance curve. Specific information on the visual sensitivities of female *C. eurytheme* is lacking, which precludes the use of sophisticated visual modeling. However, because physiological research suggests that *Colias* visual sensitivity spans a full spectral range from 300 to 700 nm (Post and Goldsmith 1969), we summarized the physical properties of reflectance spectra over this range. For simplicity we use the labels 'brightness,' 'hue,' and 'chroma' but recognize our calculated parameters would only approximate these color characteristics as potentially perceived by *Colias* butterflies. The summary variables (illustrated in Fig. 1) are

(1) UV brightness ($R_{300\text{-}400}$)—summed reflectance from 300 to 400 nm
(2) Mid-wave brightness ($R_{450\text{-}550}$)–summed reflectance from 450–550 nm;
(3) Long-wave brightness ($R_{550\text{-}700}$)—summed reflectance from 550–700 nm
(4) UV chroma—calculated as $(R_{300-400} - R_{450-550})/(R_{300-400} + R_{450-550})$
(5) Orange chroma—calculated as $(R_{550-700} - R_{450-550})/(R_{550-700} + R_{450-550})$
(6) UV hue—the wavelength corresponding to the maximum point of the UV curve
(7) Orange hue—the wavelength corresponding to the 50% midpoint of the mid-wave 'nadir' and the long-wave peak

Values calculated for each specimen's left and right forewing were strongly correlated (r>0.90) and were subsequently averaged.

Male age assessment

We used established protocols (i.e., Kemp 2006) to assess the age of males in this study. These assessments involved scoring the extent of visible scale loss and damage to wing margins in terms of five discrete categories (refer to Kemp 2006 for a description). We made separate assessments based upon the dorsal and ventral wing surfaces, which were highly correlated (Spearman R=0.815, N=156, p< 0.0001) and were averaged for subsequent analyses.

Statistical analyses

Our analyses were designed primarily to assess whether female *C. eurytheme* could use the existing natural levels of variation in male wing coloration to predict, in advance, the likely direct benefits (e.g., material contributions) and/or costs (e.g., exposure to male seminal toxins or increased risk of predation) they may receive from specific matings. We investigated bivariate relationships (e.g., between copulation duration and male body size) using Pearson's product–moment correlations, and we used standard t tests and analysis of variance to assess group differences among parametric variables. For multivariate analyses of continuous dependent variables, such as spermatophore mass, lifetime fecundity, and copulation duration, we adopted a maximum likelihood-based model fitting approach using the generalized linear/nonlinear modeling function of Statistica (v7.0). This is a 'data-based' approach to selecting the model that best accounts for the observed variation in the variable(s) of

interest. In line with 'information theoretic' approaches to the analysis of non-experimental data (Burnham and Anderson 2002), we evaluated model fit using Aikaike's Information Criterion (AIC). The AIC is equivalent to $-L_q+2q$, where L_q is the maximized log-likelihood and q is the number of variables in the model. The use of this criterion is an improvement upon simply using the log-likelihood value to compare among candidate models because it adjusts for varying numbers of parameters. An information–theoretic approach is strongly recommended for observational data because traditional approaches (e.g., analysis of variance) are often theoretically unjustified and perform poorly in simulations (refer to Burnham and Anderson 2002 for an in-depth treatment of this issue).

In each case, we conducted a best subsets analysis and selected the most parsimonious multivariate model as the one that minimized the AIC value. Once the best fitting model was identified, we assessed its overall statistical significance, and the significance of individual model parameters using log-likelihood and Wald tests, respectively. We also calculated semi-partial r values as estimates of the size and direction of individual effects in multivariable models. Variables included as initial predictors in the best subsets analyses of male ejaculate material, and female fitness parameters included male winglength, UV brightness, mid-wave brightness, long-wave brightness, UV chroma, orange chroma, UV hue, orange hue, and UV angular breadth. Female winglength was also included in the analyses of female fitness components, along with family as a random factor to account for potential genetic differences in laboratory egg-laying (see also Rutowski et al. 1987). On the basis of large observed variation in copulation duration, we also explored the relevance of this variable to male ejaculate characteristics, female fitness measures, and as a predictive covariate in multivariable models relating male coloration to these parameters.

All male ejaculate and female fitness parameters were distributed approximately normally except for percent female fertility, which was subsequently normalized using the log transformation. Means are quoted throughout with 95% confidence intervals, unless otherwise stated.

Results

Male coloration

Male color parameters exhibited reasonable variance, and all, except for UV hue, were significantly correlated with the color-independent estimate of adult age (i.e., wing-wear; Table 1). All significant age relationships except for mid-wave brightness were negative, thus, indicating that, with advancing wing wear (↔age), individuals display duller, less chromatic, and more angularly restricted UV, coupled with duller, less chromatic and more yellowish long-wave reflectance.

Experiment 1: male ejaculatory investment

In this experiment we obtained 71 successful copulations involving reared virgin females, which lasted for an average of 123.1±37.3 min (range=37–1,015 min). Dissected (fresh) female bursas weighed 5.80±0.57 mg (range= 1.36–11.02 min). Freshly dissected spermatophores weighed 3.85±0.40 mg (range=1.11–7.50 min), which represents approximately 66% of bursa mass, whereas dried spermatophores weighed 1.40±0.17 mg (range= 0.28–3.16 min). These three measures of the male ejaculate covaried extremely tightly ($0.954<r<0.982$), so, to limit redundancies in the data and analyses, we described ejaculate mass as a single principal component (PC) that explained 97.8% of the initial variance. This PC loaded

Table 1 Summary statistics for male wing color parameters in each of the two experiments, and the correlations between wing color parameters and visually assessed wing-wear (a measure of adult age)

	Experiment 1 (N=71), male ejaculate experiment			Experiment 2 (N=85), female fitness experiment			Correlation with wing-wear
	Mean	95% CI	Range	Mean	95% CI	Range	
UV brightness (%)	40.2	37.9–42.5	17.5–68.2	41.0	38.9–43.1	15.1–66.3	$r=-0.533$ ($p<0.001$)
Mid-wave brightness (%)	17.9	17.2–18.5	12.5–24.0	16.9	16.2–17.7	11.8–39.6	$r=0.496$ ($p<0.001$)
Long-wave brightness (%)	53.0	51.9–54.2	38.9–62.6	53.7	52.5–54.8	36.1–70.8	$r=-0.766$ ($p<0.001$)
UV chroma	0.364	0.328–0.400	-0.061–0.608	0.401	0.365–0.437	-0.446–0.650	$r=-0.606$ ($p<0.001$)
Orange chroma	0.494	0.476–0.511	0.343–0.636	0.521	0.503–0.539	0.283–0.657	$r=-0.741$ ($p<0.001$)
UV hue (nm)	337	335–339	317–358	338	336–340	312–380	$r=-0.134$ ($p=0.088$)
Orange hue (nm)	534	532–535	503–542	536	535–537	521–543	$r=-0.550$ ($p<0.001$)
UV angular breadth (°)	31.8	30.4–33.3	15–43	31.4	30.1–32.6	13–43	$r=-0.201$ ($p<0.05$)

Refer to Fig. 1 for definition of the color parameters.

almost perfectly with fresh bursa mass (r=0.984) and both fresh (r=0.993) and dry (r=0.989) spermatophore mass. Copulation duration was negatively related to male ejaculate mass (r=−0.319, N=71, p<0.01). There was no evidence that males invested differentially based upon female body size (relationship between female winglength and male ejaculate mass, r=−0.088, N=71, p=0.465).

The most parsimonious multivariate model of ejaculate mass included male winglength as the sole predictor, but this model proved not to be statistically significant (AIC=203.14; G_1=3.02, p=0.082) and only explained 4.3% of variation in ejaculate mass (i.e., R^2=0.043). With copula duration included as a covariate, the best-fitting model of ejaculate mass (AIC=197.03; G_2=11.13, p<0.005; R^2=0.152) included UV brightness (Wald=3.43, p=0.064; semi-partial r=−0.214), along with copulation duration (Wald=7.25, p<0.01; semi-partial r=−0.367). The negative semi-partial r value for UV brightness indicates that brighter males produced smaller ejaculates on average, although it should be noted that this parameter proved to be a non-significant predictor in the model.

Because the lack of a positive relationship between male body size and ejaculate mass (refer to Table 2) contradicts previous reports for *C. eurytheme* (Rutowski and Gilchrist 1986), we further explored the nature of this relationship in two subsets of the data, divided by a cut-off in copula duration of 70 min. This cut-off was used in Rutowski and Gilchrist's (1986) study of mating biology to distinguish between matings involving putative virgin versus non-virgin males. Interestingly, whereas our data for copulations lasting less than 70 min indicate positive but non-significant covariance between male body size and ejaculate size (r=0.337, N=31, p=0.063), data for copulations lasting greater than 70 mm indicate that large males donated smaller spermatophores (r=−0.388, N=40, p=0.013; Fig. 3). The nature of the covariance between male UV brightness and ejaculate mass was, however, unchanged across this 70-min cut-off point and, notably, negative but non-significant in both cases (copulations lasting <70 min, r=−0.285, N=31, p=0.120; copulations lasting >70 min: r=−0.125, N=40, p=0.444).

Experiment 2: female fitness correlates

The 85 copulations obtained in this experiment lasted for 100.86±24.93 min (range=35–608 min). Three of these experimental females proved to be infertile and laid, on average, only 122 lifetime eggs (compared to the average of 692 for all other females; t_{83}=3.67, p<0.0005), which suggests they received no viable sperm. The incidence of these cases could not be explained by male body size or any male wing color variable, or any linear combination of these variables (best subsets logistic regression, G<2.40, p>0.493), and they were excluded from further analyses.

Females generally started laying eggs the second day after mating (i.e., after they had been in the lab for one whole day). Average daily egg numbers declined linearly after about day 10, whereas fertility declined in a roughly exponential fashion (Fig. 2). Lifetime fecundity did not vary among the four full sibling families ($F_{3,78}$=1.066, p=0.368), but families differed significantly in longevity ($F_{3,78}$=3.148, p=0.030) and lifetime fertility ($F_{3,78}$=2.912, p=0.040). Female body size (winglength) was not related to longevity (r=−0.038, n=82, p=0.735), lifetime fecundity (r=0.183, n=82, p=0.100), or percent lifetime fertility (r=−0.197, n=82, p=0.076). Despite the large variation in copulation duration, this parameter was not related to subsequent female longevity (r=−0.107, N=82, p=0.337), lifetime fecundity (r=−0.065, N=82, p=0.563), or percent fertility (r=0.004, N=82, p=0.974). Average female lon-

Table 2 Bivariate relationships between male traits and male ejaculate mass (experiment 1), components of female post-mating fitness (experiment 2), and copulation duration

	Experiment 1 (N=71)	Experiment 2 (N=82)			Both (N=153)
	Ejaculate mass	Female longevity	Lifetime fecundity	Percent fertility	Copulation duration
Male winglength	−0.210	0.090	−0.054	0.051	0.039
UV brightness	−0.129	0.109	0.132	−0.019	−0.210
Mid-wave brightness	0.002	-0.025	0.143	0.065	0.281
Long-wave brightness	0.033	0.051	−0.062	−0.016	−0.150
UV chroma	−0.101	0.065	−0.026	−0.006	−0.285
Orange chroma	0.013	0.043	−0.164	−0.011	−0.298
UV hue	0.022	0.069	0.126	0.011	0.048
Orange hue	0.044	0.030	−0.247	−0.146	−0.238
UV angular breadth	−0.100	0.056	0.024	−0.246	−0.017

Pearson's product–moment correlations are given here purely as an estimate of bivariate effect size; formal statistical contrasts (construction of most parsimonious predictive models) are undertaken as outlined in the results. Male ejaculate mass is a principal component representing three strongly covarying measures of the male ejaculate (see text).

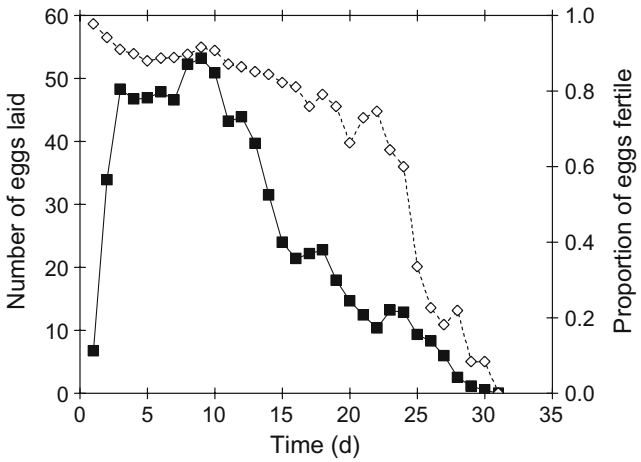

Fig. 2 Average female fecundity (number of eggs laid, *left Y-axis* and *closed squares*) and fertility (percent fertility, *right Y-axis* and *open diamonds*) as a function of time since mating

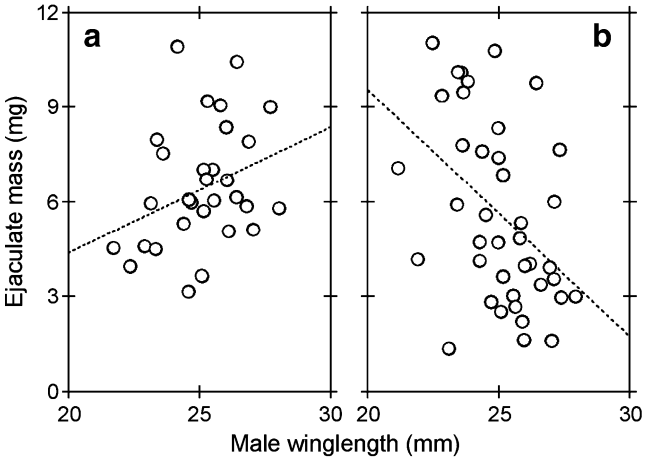

Fig. 3 The relationship between male winglength and the mass of their ejaculate, grouped by **a** copulations lasting less than 70 min and **b** copulations lasting greater than 70 min. We show ejaculate mass here to facilitate direct comparison with prior work (i.e., Fig. 3 of Rutowski and Gilchrist 1986); these relationships are virtually identical if the male ejaculate PC is instead used as the dependent variable

gevity, lifetime fecundity, and fertility are summarized in Table 3.

The strongest bivariate covariances between male traits and female fitness measures were those between (a) orange hue and lifetime fecundity, and (b) UV angular breadth and fertility (Table 2). The most parsimonious multivariate model of female longevity (AIC=497.5, G_5=14.55, p=0.012) included family (Wald=4.181, p=0.040), orange chroma (Wald=5.280, p=0.022, semi-partial r=0.216), and orange hue (Wald=2.696, p=0.101, semi-partial r=−0.155). This model explained 15.8% of the variance in female longevity (a model containing only family explained 10.8%). The most parsimonious model of lifetime female fecundity (AIC=1140.72, G_3=15.62, p<0.005, R^2=0.168) included orange hue (Wald=14.98, p<0.0005, semi-partial r= −0.366), UV brightness (Wald=10.35, p<0.005, semi-partial r=0.295), and UV hue (Wald=2.97, p=0.085, semi-partial r=0.163). Finally, the most parsimonious model of female fertility (AIC=207.50, G_5=17.56, p<0.005) included family (Wald=11.08, p=0.026), UV angular breadth (Wald=8.93, p<0.005, semi-partial r=−0.319), and UV hue (Wald=2.05, p=0.152, semi-partial r=0.150). This model explained 20.4% of the variance in fertility, which is approximately 10% more than a model only containing 'family' (i.e., R^2=0.101). Including copula duration as a covariate in these analyses did not affect the most parsimonious solutions.

Table 3 Descriptive statistics for female fitness variables (N=82)

	Mean	95% CI	Range
Female longevity (d)	18.40	17.27–19.53	6–31
Lifetime fecundity (eggs)	692.3	633.5–751.1	146–1254
First 5 d fecundity (eggs)	192.9	172.8–213.1	0–414
Fertility (%)	0.938	0.923–0.954	0.593–0.996

Copula duration

Virgin females in both experiments were released simultaneously in haphazard order, and so we pooled the data, with experiment included as a fixed factor, to analyze the duration of copulation with respect to male traits (to see if females could predict the likely duration of their copulation in advance). The most parsimonious multivariate model (AIC=−1149.30, G_2=15.58, p<0.0005) included mid-wave brightness (Wald=8.21, p<0.005, semi-partial r=0.213) and UV brightness (Wald=1.98, p=0.159; semi-partial r=−0.102). However, although significant, this model explained only 8.9% of the observed variation in copula duration.

Discussion

Can female butterflies predict the direct benefits and costs of mating with various members of the male population? We attempted to answer this question by examining whether the phenotypic attributes of free-flying male *C. eurytheme* relate to the mass of their subsequently donated ejaculate, the duration of copula, and/or to the potential fitness of their mates. Our first experiment indicated that, despite large variation in age-correlated male color parameters and in the mass of their ejaculate, the former may not accurately predict the latter. In the second experiment, in which we directly examined fitness-related traits in singly mated females, we found that several wing color parameters

proved subtly informative in multivariable predictive models. Female longevity was predicted by a linear combination of orange hue and chroma; fecundity was predicted by a combination of UV brightness, UV hue, and orange hue, whereas fertility was predicted by the angular breadth and hue of the male's UV reflectance. Finally, from a more explicit mating costs perspective, we found that copula duration was predicted by a combination of mid-wave and UV brightness (R^2=0.089). Although these results do support a link between male ornamentation and direct mating benefits/costs, the models were only weakly predictive (less than ~20%), which suggests that the underlying relationships may have limited biological significance. Furthermore, male UV brightness, which appears to be the focus of color-based mate choice in this species (Silberglied and Taylor 1978; Papke et al. 2007), did not emerge as an especially informative indicator of either ejaculate mass, copula duration, or female fitness. We discuss the full complexities of these data in light of the *Colias* mating system and prior work on butterflies in which males make nutrient contributions upon mating.

From what is already known about the reproductive ecology of polyandrous butterflies in general and of *C. eurytheme* in particular, we can construct a candidate evolutionary scenario for wing-color-based signaling of direct benefits in this species. This scenario is based explicitly on the following key conditions: First, in copulations involving virgin males, variation in ejaculate mass scales primarily with body size, and copulations last no more than about 70 min (Rutowski and Gilchrist 1986). Second, non-virgin males experience at least a temporary reduction in the amount of material they can donate and subject females to longer copulations (Hughes et al. 2000; Lauwers and Van Dyck 2006; Torres-Vila and Jennions 2005, and references therein). In *C. eurytheme*, copulations involving non-virgin males last as much as an order of magnitude longer and have negative effects on female survival and fecundity, relative to copulations involving putative virgins (Rutowski et al. 1987). Third, males accumulate matings with age, which means that on average, an older male would be more likely to be non-virgin, and to donate a smaller ejaculate in a longer-lasting copulation (Rutowski and Gilchrist 1986). Fourth, male wing color 'fades' irreversibly over time, with older males exhibiting less bright and less chromatic UV, brighter mid-wave, and duller long-wave reflectance (Kemp 2006; also Table 1 of this study). If all these conditions indeed hold, there would seem an opportunity for females in a mixed-age population to use male wing coloration to bias their selection of younger, potentially virgin males capable of donating larger ejaculates via shorter copulations. To the extent that male ejaculates contribute to female fitness (Boggs and Watt 1981) and/or that longer copulations are costly, such female choice would be adaptive. Our data indicate, however, that despite an order of magnitude variation in spermatophore mass (i.e., 0.28–3.16 mg) and copula duration (i.e., 35 min to >16 h), these parameters were largely independent of the age-related components of male wing coloration. This finding is interesting on two main counts; first, because it suggests the above model is either oversimplified or inaccurate and, second, because it contradicts the idea that female choice for UV brightness in *C. eurytheme* (Papke et al. 2007) functions to maximize the direct benefits females receive from their mates.

A clearly simplistic feature of the above scenario is that it fails to consider potential dynamic feedbacks between mate choice, male attractiveness, ageing, mating history, and ejaculate provisioning that might affect the evolutionary stability of color-based direct benefits signaling. For example, if females prefer brightly colored males, then young males might quickly accumulate matings and, therefore, be more likely to have mated recently than their older and duller contemporaries. This would, in turn, erode the likely benefit of females to preferring brightly colored males in the first place. A similar argument pertains to the female's use of male body size as a cue of likely spermatophore size (which may explain why larger males did not provide heavier ejaculates in this study, especially in longer copulations; see below). Alternatively, if more attractive males are more active or otherwise sustain greater rates of wing-wear, then their coloration (thus attractiveness) could fade more rapidly with age and/or mating history than males that were less bright to begin with. The issue of any additional underlying female mating biases could also be relevant, that is, whether females prefer males bearing certain phenotypic characters for reasons unrelated to direct material benefits (e.g., as a result of sensory biases, selection for mate/species recognition or selection to obtain indirect benefits). Such biases, if present, could impinge upon the ability of attractive males to provide large ejaculates and/or achieve short copulations because these males would mate more frequently, on average, than their less attractive contemporaries. Consideration of these issues may help explain why our presently studied features of the male phenotype failed to predict likely direct mating benefits and, moreover, why direct benefits might not maintain the known patterns of female mate choice in this species. Clearly, we need more information regarding the actual mating histories and propensities of males under natural conditions to more fully understand the causes and consequences of these color-based female mate preferences.

Although the present design cannot directly address the dynamic nature of butterfly mating ecology, the data do offer several potentially interesting insights. First, an analysis of ejaculate mass using copula duration as a covariate (i.e., with copula duration statistically controlled)

indicated that males with brighter UV wing markings tended to donate slightly less material. Assuming that females did not more quickly ingest the ejaculates of brighter UV males during copulation itself, the most likely explanation here is that these males produced ejaculates at a lower rate. As a key symptom of a recent mating (Rutowski and Gilchrist 1986), this would accord with the putatively greater intrinsic attractiveness of bright UV males (Papke et al. 2007). Second, our analysis revealed an interesting interaction between the effects of male body size and copulation duration upon ejaculate mass (Fig. 3). In copulations lasting less than 70 min, male size scaled positively with ejaculate mass almost identically to previous reports for this species (compare Fig. 3a with Fig. 3 of Rutowski and Gilchrist 1986). This agrees with general expectations for insects with gift-giving mating systems (Fox et al. 1995b; Fedorka and Mousseau 2002; Czesak and Fox 2003; although see Bussière et al. 2005). In copulations lasting longer than 70 min, however, which probably involved non-virgin males (Rutowski and Gilchrist 1986), male size was negatively related to ejaculate mass (Fig. 3b). This result could again derive from a greater likelihood of larger individuals having more recent or more extensive mating histories. If so, we would also expect larger non-virgin males to subject females to longer copulations, which a post hoc analysis indeed revealed to be the case (relationship between male body size and the duration of copula among matings lasting >70 min, r=0.257, N=71, p <0.05).

Whereas the mass of the male ejaculate may influence female reproductive fitness, other aspects of ejaculate quality, such as nutritional value, may be of additional or principal importance (e.g., Bussière et al. 2005). The findings of our second experiment, in which female longevity, fecundity, and fertility were all weakly predicted by male wing coloration, could indicate the visual signaling of mass-independent aspects of ejaculate quality. However, in light of the relative weakness of the relationships (especially in bivariate terms; Table 1) and the subdued relevance of UV brightness as a predictor, it seems unlikely that UV-based female mate choice (Papke et al. 2007) functions principally in this context. Two potential caveats to this conclusion merit discussion. First, because precopulatory female choice was largely constrained in this experiment (see "Materials and methods"), there is the possibility that females allocated differentially to reproduction depending upon perceived mate quality (i.e., cryptic female choice; Eberhard 1996). However, the conditions under which this mechanism could obscure any fitness benefits of mating with bright UV males seem restrictive, given that females appear to favor these males when allowed to choose (Papke et al. 2007). The second caveat concerns the issue of larval- versus adult-derived nutrients in determining the resource budget of female butterflies. Females in our study were reared on high quality larval diets and had ad-libitum access to nectar throughout adulthood. Prior studies investigating the effects of male ejaculates on female insect fitness have revealed stronger effects when females were confronted with nutrient stress during one or more developmental stages (e.g., Wagner and Harper 2003; although see Bonduriansky et al. 2005). Reduced resource acquisition may elevate the importance of male-derived nutrients to reproduction (Leimar et al. 1994) and has been shown to affect female mate choice (Hunt et al. 2005). The presence of substantial body size variation in the field (see, e.g., data presented in Kemp and Rutowski 2007) also suggests that individuals may be variously exposed to nutrient stress under natural conditions. Given these considerations, it is possible that our benign experimental conditions obscured the otherwise potentially relevant effects of male ejaculate variation. This possibility stands for future evaluation.

Finally, our data barely touch upon the possibility that male color may also or more strongly indicate male genetic quality. Most of the studied male color parameters are heritable (Kemp and Rutowski 2007), and there is evidence for genetic-based mate choice in other insects (Jones et al. 1998; Iyengar and Eisner 1999; Tallamy et al. 2003), including butterflies (Wedell 2006). Given this, and the relatively unconvincing evidence for direct benefits signaling (this study), investigating the potential for the color-based signaling of genetic benefits might pose an interesting and potentially fruitful avenue for future empirical study in this species.

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