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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.

Keywords: *Colias eurytheme* L.; mate choice; multimodal signalling; orange sulphur butterfly; sexual selection; UV reflectance and mating success

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