



## Female iridescent colour ornamentation in a butterfly that displays mutual ornamentation: is it a sexual signal?



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In species wherein males display elaborate sexually selected traits or ornaments, conspecific females may also express these traits in full or partial form. This is called mutual ornamentation, and the co-occurrence of such traits in males and females remains poorly understood. In many sulphur butterflies (subfamily Coliadinae), males have a brilliant ultraviolet (UV) iridescence on their dorsal wing surfaces that functions as a sexual signal in courtship. In some of these sulphurs, such as the large grass yellow, *Eurema hecabe*, females also display dorsal iridescent patches, albeit smaller, restricted to the forewings and less bright than the male's, but the reasons for its occurrence in females are unknown. Here we present a study testing two functional hypotheses for the female UV-reflecting patch: an antiharassment hypothesis and a male mate choice hypothesis. The daily activity pattern of this species suggests that males are most likely to harass or choose among females from midday on. Observations made at this time of day on the characteristics of females related to male courtship duration suggest that males may preferentially court females with a large UV patch. Experiments with colour-manipulated models also suggest that males court with equal intensity females with and without a basal UV patch. Taken together the results provide no support for the antiharassment hypothesis. Because support for the male mate choice hypothesis was relatively weak and because of the limited potential for selection on female coloration due to male choice in these butterflies, the nonfunctional sexual correlation hypothesis remains a viable explanation for the female ornament.

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Elaborate male ornaments occur in many species of animals and are well documented to have evolved in the explicit sexually selected contexts of male–male competition, female mate choice, or both (Andersson, 1994; Clutton-Brock, 2007). In some of these species, females may display some level of development or expression of the male ornament. For example, in stalk-eyed flies in which the stalks that bear eyes in males and are important in male–male interactions and female choice, females also bear their eyes on stalks (Chapman, Pomiankowski, & Fowler, 2005; Wilkinson & Dodson, 1997). This case and others like it are referred to as mutual ornamentation (Kraaijeveld, Kraaijeveld-Smit, & Komdeur, 2007). While in many cases of mutual ornamentation the evolutionary processes driving the expression and elaboration of the ornament in males is clear, what is not clear is why the

ornament appears in females even in a relatively less developed state.

There are two major classes of hypotheses to explain female possession of an ornament found in conspecific males (Houde, 2001; Kraaijeveld et al., 2007). First, females may display the trait as an incidental result of genetic correlations between the sexes and produce no direct benefit for the female. That is, strong selection for the ornament in males may have led to highly penetrating genes that incidentally get expressed during development in the female. Second, females that display the ornament may accrue some fitness benefit through its effect on male behaviour. This could happen in one or more ways. For one, males may choose mates from among available females based on the development of the ornament. In this case, ornament expression in a female may be indicative of her potential quality as a mate and so increase her attractiveness to males and thereby her mating success (e.g. Amundsen & Forsgren, 2001; Cotton, Cotton, Small, & Pomiankowski, 2014; Nordeide, Kekäläinen, Janhunen, & Kortet, 2013). For another, females may express the trait to appear male-like and, thus, unattractive to males, thereby reducing male

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courtship attempts and persistence and the time and energy costs that they may impose on females. Finally, females and males may display the ornament because it functions as a social signal in nonmating interactions. To date, there have been relatively few systems that permit a multipronged approach to addressing these hypotheses for the expression of an ornament in females. In particular, systems have been few and far between that permit evaluation of genetic as well as behavioural hypotheses (Kraaijeveld et al., 2007).

In the sulphur butterflies (family Pieridae, subfamily Coliadinae), males often display a brilliant iridescent ultraviolet (UV) reflection from virtually all yellow or orange areas on their dorsal wing surface. Several studies suggest that the male's iridescent coloration is used by females in mate choice (Kemp, 2008a; Papke, Kemp, & Rutowski, 2007; Silberglied & Taylor, 1977) and by males to distinguish females from other males (Rutowski, 1977). Interestingly, in some of the species in which males have iridescent patches, females also display iridescent patches but they are typically smaller, less bright and restricted to the base of their dorsal forewings (Common & Waterhouse, 1982; Kemp, 2008b; Rutowski, Macedonia, Kemp, & Taylor-Taft, 2007; White, Macedonia, Birch, Dawes, & Kemp, 2012). In these species, the iridescence of both males and females is produced by the same multilayer interference mechanism on the wing scales (Rutowski et al., 2007; White et al., 2012).

One of the pierid butterflies that displays mutual iridescent ornamentation is the large grass yellow, *Eurema hecabe*, and this species shows special promise as a system for testing hypotheses for the female iridescence. This species is widespread and occurs from Africa through Asia to Australia (Braby, 2000). In tropical Australia, this species is common during the late wet season and early dry season, which permits behavioural tests of potential signal function, and it is easily reared in the laboratory (Kemp, 2008b), which permits the genetic dissection of features of the iridescence wing colour in both males and females. Studies of both sorts have already been done with males and their iridescent ornaments. Behavioural experiments have shown that females preferentially mate with males with bright iridescence (Kemp, 2008a). In addition, rearing experiments have shown that the brightness of the male's iridescence is condition dependent, as would be expected of a sexually selected indicator trait (Kemp, 2008b). Finally, in those same rearing experiments, female iridescence was also condition dependent (Kemp, 2008b). Interestingly, these data allowed a preliminary and unpublished genetic analyses that failed to reveal a significant genetic correlation between males and females in the brightness of the iridescent patch reflectance. While this demonstrates the potential to pursue such questions in this species, more certain conclusions await a dedicated study.

This potential lack of genetic ties in ornament brightness between males and females compelled an investigation into the potential for the female iridescent patch to function as an adaptive signal. Because there is no known social system (e.g. night-time aggregations, social foraging, communal breeding, etc.) reported in these butterflies beyond the mating system, there is no context for the iridescence to evolve as a social signal. This leaves two potential behavioural hypotheses for the signal's function. First, males may discriminate among females based on the iridescence, which leads to the prediction that male courtship persistence will positively correlate with characteristics of the female's iridescence. There is evidence for male mate preferences among conspecific females in *E. hecabe* (Takanashi, Hiroki, Satoh, & Obara, 1997) and other species of pierids (Rutowski, 1982, 1985). These studies agree that males court most intensely younger (less worn) and, for *E. hecabe* and *Pieris protodice* at least, larger females. However, whether such preferences in butterflies will lead to significant

variation in female mating success and thereby selection on female coloration is a point of disagreement in the literature (Finkbeiner, Briscoe, & Reed, 2014; Kemp & Macedonia, 2006; Kunte, 2009).

Second, females may benefit from displaying UV iridescent patches and thereby reducing the potential costs of harassment by males. In pierids, females typically mate within hours or even minutes after eclosion and then enter a period of being unreceptive to male mating attempts (e.g. Rutowski, 1978; Rutowski, Long, Marshall, & Vetter, 1981a). During this time, they feed and oviposit, which means that male harassment could impose significant time and energy costs on females, especially at high male population densities (Kemp & Rutowski, 2011). Male harassment might be curtailed by displaying a patch of UV iridescence and looking somewhat like males, which are avoided by other males (Rutowski, 1977; Silberglied & Taylor, 1978). This hypothesis leads to the prediction that female iridescence should reduce the duration of male courtship attempts.

Our general aim in this study was to test these functional hypotheses for iridescent wing patches in *E. hecabe* females. We had four specific aims that we addressed in sequence.

- (1) Male courtship persistence patterns vary with time of day in sulphurs (Marshall, 1982). Therefore, we first evaluated the daily pattern of changes in male courtship persistence in *E. hecabe* in the field to indicate when during a day mate choice and harassment of females by males were likely to occur.
- (2) As an additional part of our assessment of the daily pattern of male behaviour we also evaluated the mating histories of females found in copula at different times of the day to evaluate whether the mating status of females that males are likely to encounter changes with time of day.
- (3) During the period of the day when males are most persistent in courtship there is still a good deal of variation in how persistent males are. We thus examined whether this variation is related to the characteristics of focal females during this period of high male persistence. Again, females that are the target of an intense courtship effort should, according to the mate choice hypothesis, be those with bright or large iridescent patches and, according to the antiharassment hypothesis, be those with dull or small iridescent patches.
- (4) We tested whether aspects of female coloration influence how intensely females are courted by males by presenting males with models created from the wings of males and females, and then experimentally manipulating their coloration.

## METHODS

### *Study Populations and Locales*

The field work was done from February to May in 2008 with two populations of *E. hecabe* near the Cairns campus of James Cook University, in North Queensland, Australia. One population inhabited a pair of vacant lots in a subdivision near Trinity Beach (16°47'7"S, 145°41'49"E), which was adjacent to a large mowed area and dense stands of *Aeschynomene indica*, a larval food plant for *E. hecabe*. The other population was in a clearing between a forest and the northern edge of the university campus in Smithfield (16°48'60"S, 145°41'18"E). All individuals in these populations were of the wet (or summer) season form (Kobayashi, Hiroki, & Kato, 2001). At this site an alternative larval food plant, *Sesbania cannabina*, was abundant. In both locations, the most commonly used nectar source was snake weed (*Stachytarpheta* spp.).

### Courtship Persistence Patterns

We assessed temporal patterns of courtship persistence at the Trinity Beach site. We observed male–female interactions on two different days in 2008 (1 March, 0920–1050 hours, 71 interactions; 1 April, 0854–1128 hours, 70 interactions) and timed their duration from when the male arrived within 30 mm of the female to when the male departed for the last time from the vicinity of the female.

Copulating pairs were readily found throughout the day. We collected these mating pairs on 7 days between 17 March and 1 April in 2008 and placed each in a vial that was then placed in the shade in the field. Once the pair separated we assessed the mating history of the female in each pair by dissecting out her bursa copulatrix under distilled water and assessing the number and condition of spermatophores in the bursa. Condition was scored following procedures in Rutowski (1981b).

### Male Courtship Persistence and Female Phenotype

Once we determined that males showed the most variation in courtship persistence and courted for the longest periods in the afternoon, we initiated a study of the effect of female coloration on male courtship persistence. In the early afternoon (approximately 1200–1500 hours) we observed courtship interactions between free-flying males and females. We timed encounters from when a male arrived within approximately 30 mm of a female and initiated courtship behaviour until the terminations of each interaction as evident by the male flying away. These data were collected on 7 days between 1 March and 4 April in 2008. Females that were courted in these interactions were captured where possible, then promptly transported to the laboratory and freeze-killed at  $-20^{\circ}\text{C}$ . We subsequently measured their forewing length, using dial callipers, from the wing tip to the point of insertion into the thorax, which is a strong correlate of overall body size in this species (Kemp, 2008b).

We then removed the females' forewings for two measurements of dorsal coloration. The first involved quantifying the spectral reflectance of the iridescent markings situated in the proximal forewing region. Reflectance spectra were collected using the technique described and illustrated in Kemp (2008b). This was done with an Ocean Optics USB2000 spectrophotometer and a pulsed Ocean Optics PX2 xenon light source. The illuminating light and collecting light was transmitted via 400  $\mu\text{m}$  diameter optical fibres. Spectral capture was defined by 10 sequentially averaged scans of 100 ms integration time and with a boxcar smoothing interval of 20 nm. From the collected spectra, we extracted several colour parameters (hue, intensity and yellow chroma) as described in Kemp (2008b). For analysis, we used the average of colour parameters from the UV patches on the left and right forewing of each specimen.

The second series of measures focused on the areal size of the iridescent patch on each female's dorsal forewing. Dorsal wing surfaces were illuminated with a UV light source (UVP Blak-Ray B-100) and photographed with an Olympus C3040 Zoom digital camera fitted with a UV pass filter. In all photographic sessions, the camera was situated directly above the wing surface with illuminating light coming from a  $45^{\circ}$  angle of incidence and from the direction of the base of the wing (part inserted into thorax). This arrangement visualized the largest area of UV reflectance from the dorsal forewing. A ruler was included in each image for calibration during image analysis. We opened the images in ImageJ (<https://imagej.nih.gov/ij/>), and after calibration, we outlined the iridescent patch with the wand function to obtain the areal size of the patch. Again, the average of the areas for the left and right forewing were used for analysis.

We used stepwise logistic regression to evaluate whether female forewing length (a surrogate for body size) or various aspects of the iridescent patch, including UV intensity, UV hue, yellow chroma, patch area and forewing length, best predicted interaction duration. For this analysis we scored each interaction duration as brief ( $<10$  s) or long ( $\geq 10$  s) for two reasons. First the distribution of observed interaction durations was very much skewed with a high peak of durations in the  $<5$  s range and with a long (up to several minutes) and highly variable tail of durations  $>10$  s (see Fig. 2). Second, some interaction durations were not precisely timed with the stopwatch. Some brief interactions clearly occurred but began and ended before the stopwatch was started, while for some lengthy interactions, the stopwatch was stopped when it was mistakenly thought that the interaction had ended. These could still be clearly categorized as brief or long per the stated criteria.

### Model Presentations

We explored how the presence and size of the UV forewing patches on females affected the behaviour of males by presenting models to males in the field (12 April – 8 May 2008). The models were constructed from the wings of dead butterflies that had been air dried for several days after being removed from the body of males and females. We constructed the models using black cardboard and followed the design used in Rutowski (1981b) (Fig. 1a). We manipulated the coloration of the models using a non-UV-reflecting ink (yellow, Art Spectrum, Melbourne, Victoria, Australia) whose reflectance at wavelengths greater than 400 nm closely matched that of the male dorsal wing surface (Fig. 1e). The ink was diluted in ethanol and applied to the wing surface using a small paint brush. The effect was to obliterate the UV reflectance while leaving the non-UV reflections largely unaltered (Fig. 1d). We used UV photographs to confirm that we achieved the desired manipulations.

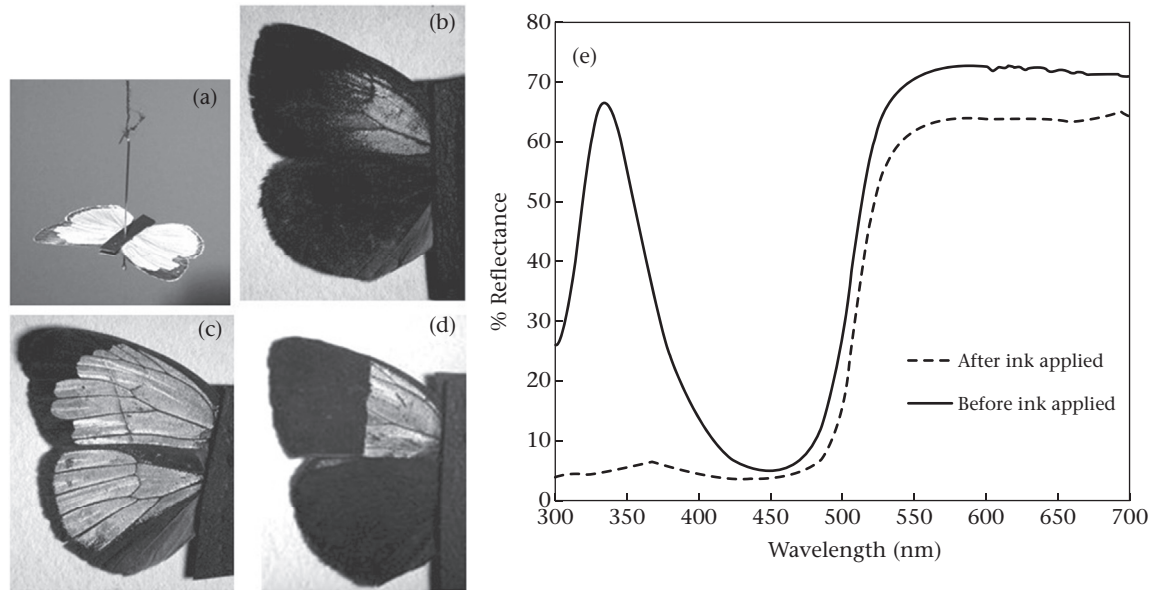
In total, we constructed and presented seven types of models, with at least four replicates of each type, to males: (1) Male wings with no alteration of coloration (Fig. 1c); (2) Female wings with no alteration of coloration (Fig. 1b); (3) Female wings with ink applied to the dorsal yellow area of the forewings; (4) Female wings with ink applied to the dorsal yellow area of the hindwings; (5) Male wings with ink applied to the dorsal yellow area of all wing surfaces; (6) Male wings with ink applied to the ventral surfaces of all wing surfaces; (7) Males wings with ink applied to the dorsal yellow surface of the hindwings and the distal dorsal yellow areas of the forewings (Fig. 1d).

These models were suspended by a thread from the end of a 1 m long stick and dangled in front of free-flying males in the field between 1115 and 1330 hours when there was full sun (distinct shadows cast). We made every effort during the interactions to position the models in front of and below the male, which is usual position of the female during the initial phases of courtship. Males' courtship responses were timed with a hand-held digital stopwatch to the nearest 0.1 s and were deemed to have begun when a male altered flight path and came within 3 cm of the model and to have ended when the male flew off more than 10 cm from model and did not return. Each replicate model was presented to no more than 10 males.

### Statistics

Statistical analyses were done with the 'Data Analysis' add-in of Microsoft Excel, with SPSS 15 (SPSS Inc., Chicago, IL, U.S.A.), and with Statistica v.7.1 (StataCorp, College Station, TX, U.S.A.). Inferences were made throughout at the 0.05 level of significance.

Analysis of the responses to models began with the natural log transformation of all measured response durations to normalize



**Figure 1.** (a) An example of a model made from *E. hecabe* wings that was presented to males in the field. (b) The appearance in UV imaging of dorsal wing surfaces of a model made from the wings of *E. hecabe* female. (c) The same for a model made from male wings. (d) The same for a model made from male wings in which the yellow area of the ventral hindwing and the distal yellow area of the dorsal hindwing had been painted with a yellow and non-UV-reflecting ink. (e) The spectral reflectance of the UV-reflecting surface of dorsal male hindwing before and after being painted with the ink.

their distribution. The analysis then proceeded in two stages that are detailed in the [Results](#), involving general linear models (GLM) followed by the appraisal of specific planned (a priori) contrasts. We chose a hierarchical approach to the analysis to emphasize the a priori logic behind our choice of the various treatments, as outlined in the [Results](#).

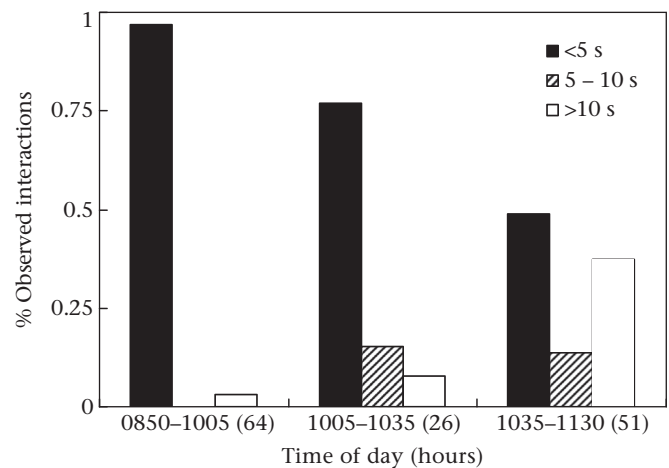
#### Ethical Note

Much of this research used noninvasive observational techniques in the field. Where necessary, individuals were euthanized by freezing as soon as possible after collection in the field so that we could assess phenotype and reproductive state. This research adhered to the ASAB/ABS Guidelines for the use of animals in research, and all relevant guidelines of Australia and the United States of America.

## RESULTS

### Daily Pattern of Male Courtship Persistence

Qualitative observations suggested that during the late wet season (March–April) and early dry season (May–June) in north Queensland, *E. hecabe* becomes active around 0800–0900 hours and are active foraging, interacting and ovipositing until about 1500–1600 hours. At both study sites activity in the afternoon was curtailed by an increase in the intensity of the trade winds such that these small butterflies took refuge and perched in dense vegetation. Our observations also suggested a typical daily pattern of variation in male courtship persistence. In the morning before about 1030 or 1100 hours, males interacted with females but courted them for only 1–2 s, whereas after 1100 hours, lengthy interactions of more than 10 s and often up to as much as several minutes were common. Data on interaction durations at different periods in the morning collected on 2 days ([Fig. 2](#)) confirmed the impression of a dramatic late morning shift to greater courtship persistence among males ( $\chi^2 = 39.5$ ,  $P = 0.0001$ ). This suggests that females are most



**Figure 2.** Change in male courtship persistence with time of day over two mornings in 2008 (1 March, 1 April). Numbers in parentheses indicate the total number of observations for each time period over both days.

likely to be subjected to harassment or mate discrimination by males in late morning and early afternoon.

The mating history of females found in copulating pairs also varied with time of day. Of 23 females observed in copula before 1030 hours, 22 contained only a single spermatophore when the mating was complete, which suggests that they were virgins when the mating began. The other female had no spermatophore (i.e. was still a virgin). In contrast, of 30 females found in copula after 1245 hours, 12 (40%) contained two to four spermatophores, a significantly higher percentage than earlier in the day (chi-square test with Yate's correction:  $\chi^2 = 9.98$ ,  $P = 0.007$ ). Two or more spermatophores suggest that the females were previously mated when the mating in which they were found had begun. The increase in male courtship persistence then appears to coincide with a decline in the abundance of virgin females as the day proceeds.



### Characteristics of Females Correlated with Male Persistence

To examine how courtship persistence relates to female phenotype, we focused on interactions in the early afternoon when the potential for male courtship persistence and harassment is high. We observed a total of 82 interactions, and in 68 cases, the females were young (fresh wings, not worn, as occurs in older females). We focused the analysis on the interactions with these females to reduce the potential effects of female age. Of those 68 interactions 28 were categorized as brief (<10 s) and 40 as long (>10 s). Analysis with logistic regression produced only a single variable that was significantly and positively related to interaction duration, the average size of the UV patch on the female dorsal forewings ( $P = 0.022$ ). Males courted females with large patches longer than those with small patches. No other variable measured was found to be a significant predictor of interaction duration after the first step in the regression analysis (mean UV hue:  $P = 0.956$ ; mean UV intensity:  $P = 0.698$ ; mean yellow chroma:  $P = 0.712$ ; forewing length:  $P = 0.178$ ). However, UV patch size was positively correlated with patch yellow chroma, patch intensity and forewing length (all  $P < 0.001$ ), and negatively correlated with patch hue ( $P < 0.034$ ). Overall these results are consistent with the expectation from the male mate choice hypothesis for the function of the female UV patch.

### Colour Manipulation Experiments

A GLM on the log-transformed durations of the male responses to the seven model types (Table 1) revealed a highly significant effect of model treatment on male response duration ( $P < 0.00001$ ). Planned (a priori) contrasts revealed that males responded significantly longer to unaltered female wings than to unaltered male wings ( $P < 0.00001$ ). Two contrasts were not significant, which suggested that responses could be pooled among the treatments involved. Specifically, there was no difference in the response durations elicited by the model with unaltered male wings and the model with male wings inked only on the ventral wing surface ( $P > 0.452$ ). This result suggests that the ink had no effect on the responses to male wings with the dorsal coloration unaltered. Results from these two models were subsequently pooled under one treatment ('male dorsal unaltered'). Similarly, there was no difference in response durations elicited by the model with unaltered female wings and the model with female wings inked only on the dorsal hindwing ( $P > 0.3805$ ). This suggests that the ink applied to the female hindwing did not change females' attractiveness, and we subsequently pooled the results from these two models under one treatment ('female dorsal forewing unaltered').

**Table 1**  
Median courtship duration of *E. hecabe* males to models presented in the field in the afternoon

Model	N	Median male courtship duration (s) (range)	Statistical comparisons <sup>1</sup>
♀ unaltered	42	4.5 (1–400)	a
♂ unaltered	41	1.3 (0.8–7.7)	b
♀ no DFW UV	40	4.8 (1–146)	a
♀ no DHW UV	49	3.1 (1–104)	a
♀ no DW UV	49	5.3 (1–37.7)	a
♂ no DW UV	46	4.9 (1–89)	a
♂ V control	40	1.0 (1–13.3)	b
♂ FW UV only	38	1.45 (1–12.5)	b
♂ partial FW UV only	48	2.4 (1–15.6)	a, b

D: dorsal; V: ventral; W: all wings; FW: forewings; HW: hindwings. Means were calculated before log transformation. N is the number of responses summarized.

<sup>1</sup> Statistical comparisons show the results of planned GLM contrasts. All models with the same letter were not significantly different. See text for details on model features and statistical comparisons of response patterns.

After pooling responses in these ways, we ran a second GLM, which again revealed a significant treatment effect ( $P < 0.000001$ ). Planned contrasts were then examined to answer a series of questions.

- (1) Are male wings less attractive because of their UV reflectance? Yes, dorsally painted male wings (i.e. male wings with iridescent UV reflectance removed) were courted by males for as long as females with unaltered dorsal forewings ( $P > 0.823$ ). This result also indicates that (a) the sexual difference in ventral wing colour is not a major factor in male discrimination of females from other males, (b) that the paint produced long-wavelength reflections that were as attractive to males as unpainted females and (c) that chemical cues that might be involved (Takanashi et al., 1997) were lost when the wings were air dried.
- (2) Does the dorsal UV reflectance on female wings make them less attractive? The anti-harassment hypothesis predicts a positive answer to this question, but in fact female forewings painted dorsally to remove their UV reflectance were treated like unaltered female wings with the UV patch intact ( $P > 0.405$ ). Hence, we found no evidence that the UV patch reduces female attractiveness.
- (3) Do males distinguish females from other males using the basal distribution of UV reflectance on the forewing? We presented males with models made from male wings that had the UV reflectance reduced to a patch on the forewing base (Fig. 1d). These models elicited a significantly greater response duration than unaltered male wings ( $P < 0.0063$ ) but a significantly reduced response duration than unaltered female wings ( $P < 0.0025$ ). This suggests that this manipulation produced models with an intermediate level of attractiveness.

## DISCUSSION

### Daily Changes in Male Courtship Persistence

Our results inform the possible explanations for female ornamentation in *E. hecabe*. We argue that the two main possible functional explanations for female forewing UV patches (anti-harassment and male choice) are most likely to be realized when male courtship persistence is at a peak. Daily changes in persistence patterns suggest this is most likely after 1100 hours. At this point and later in the day males may court females for up to several minutes. This shift is like that reported in two other sulphur butterflies, *Colias eurytheme* and *C. philodice* (Marshall, 1982).

We concur with Marshall (1982) that this is an adaptive shift in male courtship strategy driven by a change in the mating histories of females that are available for courting. Early in the morning there are many newly emerged virgin females. Our observations indicate that this is when virtually all eclosions occur in the laboratory. Even though virgin females discriminate among males based on their iridescent coloration (Kemp, 2008a), they alight and permit an attractive male to mate within seconds of his initiation of courtship. Hence, early in the morning, if a female does not quickly respond to a male's approach (i.e. by alighting and assuming a receptive posture with her abdomen tip protruding from between the closed hindwings), the male benefits from moving on in search of another female. As newly eclosed virgins enter copulation, each of which may last an hour or more, mate-seeking males will encounter previously mated females at an increasing rate as the morning progresses. In the morning, females in mating pairs were not previously mated, whereas in the afternoon a significantly higher proportion of females in mating pairs were previously mated.

Although previously mated *E. hecabe* females are most likely to be in an unreceptive state, as in other pierids (Obara, 1964a, 1964b; Rutowski, 1978; Sugawara, 1979), this unreceptive state might presumably be overcome with intense courtship effort by males. Hence, there is adaptive value in males being more persistent with individual females in the late morning and afternoon.

#### *Female Coloration and Male Behaviour*

Our observational and experimental studies of the characteristics of females that elicit prolonged male courtships provide no support for the prediction from the male harassment hypothesis that the UV patch on the female forewing deters male courtship attempts. Female wings with the UV patch removed were as attractive to males as those with this patch intact. Also, free-flying females with a relatively large UV patch were courted longer, not less, by males than females with a small patch. Hence, the male harassment hypothesis was not supported.

Our studies provide some support for the male mate choice hypothesis. First, the correlational results suggest that males courted females with larger UV patches on the forewing for longer times than those with smaller patches. Whether the size of the UV patch is the specific focus of male attention is brought into question by the result that patch size is correlated with measures of body size and the coloration of the patch. Nevertheless the results do suggest that the characteristics of the UV patch are of interest to males.

The results of the experiments in which UV reflectance was manipulated were mixed. First, dorsal UV reflectance in this species does affect male behaviour. The large and brilliant UV patches on the dorsal surfaces of all four wings of the male do deter courtship attempts of other males. Removal of those patches makes male wings as attractive to other males as female wings. However, the male choice hypothesis along with the results of the correlational study predicts that removing the UV patch from the female dorsal forewing, essentially reducing its area to zero, should make them less attractive to males. This did not happen, which suggests that a UV-reflecting patch restricted to the base of the dorsal forewing is a signal to males that that individual is a female.

The male choice hypothesis also predicts that, if the iridescent patch is eliminated or restricted to the forewing base on a male's wing, this should increase that male's attractiveness. While males responded similarly to male wings with no UV reflectance and to female wings, male wings with the UV reflection limited to the base of the dorsal forewings were unattractive to males. However, response duration to male wings with UV reflectance restricted to the dorsal forewings did not differ significantly from that to female wings with the dorsal forewing UV reflectance intact. Overall, these results suggest that restricting males' UV reflectance to the forewing base leads other males to treat them like females. The effect of this manipulation may have been greater if we had controlled a potential confounding factor. In *E. hecabe*, a spot within the iridescent patch of a male is much brighter than that of a similarly sized spot within the female's iridescent patch (Rutowski et al., 2007; White et al., 2012), which may have led to males being less interested in male wings with basal UV reflectance than they would have been if the brightness of the reflection was lower.

Our conclusion is then, from the correlational study, that males find females with the basal UV reflection present more attractive and, from the model experiment, that males do court most strongly individuals with UV reflectance absent or restricted to the basal dorsal forewing. We suspect that the manipulation in the model experiment did not allow a clear test of whether there is a male preference for larger UV patches in females, especially with the variation in environmental conditions across the duration of the

experiment and the uncontrolled variation in female coloration in the intact wings.

If a male preference for females with basal UV reflectance is real, could this be sufficient to lead to selection for this ornamentation in females? At this point, it is not clear why males might be particularly attentive to female iridescent patch size, but this feature, if costly, could possibly be an indicator of body size or overall quality, female characteristics that could influence their reproductive value to males. The potential for such male preferences as a significant selection pressure in the evolution of female coloration in animals is an open question (Edward & Chapman, 2011). In butterflies specifically, some authors question this potential role of male mate choice (Aardema & Scriber, 2013; Kemp & Macedonia, 2006; Kunte, 2009) while others support it (Ellers & Boggs, 2003; Finkbeiner et al., 2014; Kronforst et al., 2006; Prudic, Jeon, Cao, & Monteiro, 2011). Answering this question for *E. hecabe* will need to await studies of the heritability of female coloration and the extent to which female reproductive success might be limited by access to males in the field.

Two prior studies with *E. hecabe* suggest that female fecundity might, under some circumstances, be limited by access to males. First, in a study done by caging *E. hecabe* virgin females with males, Hiroki and Obara (1997) found that a large proportion of females remained unmated for several days after eclosion, which could constrain time available for oviposition in these relatively short-lived animals. However, this could have been a result of interference in the unnaturally high density in the cage. Second, females of *E. hecabe* (Hiroki & Obara, 1998) and other pierid butterflies (Daniels, 2007; Rutowski, 1982), especially old virgin females and mated females that have not mated for some days, engage in courtship solicitation, that is, approaching and chasing males. The occurrence of this behaviour suggests there are times when availability or interest of males limits female fecundity. Further study is needed to get a clear idea of whether and how female coloration factors into their success at obtaining matings, but to us, the existing evidence is not strong.

We also conclude that the genetic correlation hypothesis remains viable, that is, the hypothesis that the strong selection on males to produce UV reflectance has led through genetic correlation to the expression of this trait in females. Our examination of the data set reported in Kemp (2008b) suggests that there is no genetic correlation for UV brightness in this species, but there has been no study of the genetics of UV patch size. Assessing genetic correlations for UV patch size will be difficult because of the very limited variation among males in the wing area that is UV reflecting.

When males and females share ornaments, there is a continuum among species in the extent to which female ornaments are equivalent in size and elaboration to those of males and therefore whether in females the investment in the ornaments and the costs incurred are equal to those of the males (Swierk & Langkilde, 2013). In those species in which the female ornament is only a rudimentary expression of the male ornament, such as in the butterfly studied here, perhaps the expectation is weak or nil that it will be an informative, costly or functional signal in sexual interactions (Watson & Simmons, 2010). Certainly, the results here bear out this prediction in that the evidence of a substantial behavioural response to the female ornament in the mate-seeking behaviour of this species is weak.

We agree with other recent suggestions (Edward & Chapman, 2011; Tobias, Montgomerie, & Lyon, 2012) that a more refined conceptual framework for describing and understanding female ornaments and mutual mate choice as well as more empirical results are still needed. Such a framework should ideally allow us to explain the selective and other circumstances that lead to inter-specific diversity in the nature and degree of mutual ornamentation.

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