

Functional ecological implications of intraspecific differences in wing melanization in *Colias* butterflies

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Variation in the degree of insect wing melanin affects thermoregulation, and is expected to be adapted to local environmental conditions, for example over an elevational gradient. The effects of melanization on flight activity and egg maturation rate were assessed in the closely related butterflies *Colias philodice eriphyle* and *C. eurytheme* using experimental manipulation of wing darkness and transplant experiments between high and low elevation sites. Experimental manipulation of wing darkness in *C. p. eriphyle* demonstrated that light males had reduced flight activity at high elevations, and darkened males had reduced flight activity at low elevations. In contrast, the transplant experiments revealed asymmetrical adaptation for male *C. p. eriphyle*. At high elevations darker, high-elevation males had higher flight activity than lighter, low-elevation males, but there was no difference between the two groups at low elevation. For females, melanization had no effect on flight activity. However, an increase in female *C. eurytheme* wing darkness led to a significantly higher egg maturation rate at cold ambient temperatures, which may increase female reproductive output under natural conditions. Therefore, dispersers moving down in elevation may be more successful than those moving up. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 82, 79–87.

ADDITIONAL KEYWORDS: egg maturation – elevational gradient – flight activity – thermoregulation – wing colour.

INTRODUCTION

Melanin plays important roles in insect ecology, including defence against parasites (Siva-Jothy, 2000), mate signalling (Wiernasz, 1989; Wiernasz, 1995; Ellers & Boggs, 2002, 2003), protection against ultraviolet radiation (Gunn, 1998; Zellmer, 1995) and thermoregulation (e.g. Watt, 1968; Roland, 1982; Kingsolver, 1987; De Jong, Gussekloo & Brakefield, 1996; Ottenheim *et al.*, 1999). The role of melanin in thermoregulation has received particular attention. Insects are ectothermic, yet those species that have been studied to date show a narrow thermal optimum for fitness-related activity such as flight. The optimum is often above ambient temperature (Watt, 1968;

Ashby, 1998; van Dyck & Matthysen, 1998). Many species use behaviour, such as solar basking or avoidance, to achieve body temperatures that are optimal for activity. Melanization of the wings or body increases solar absorption and allows dark-coloured insects to raise their body temperature to the thermal optimum for activity more effectively than can light-coloured insects under cool conditions (Watt, 1968; Roland, 1982; Guppy, 1986a; Kingsolver, 1987; De Jong *et al.*, 1996; van Dyck, Matthysen & Dhondt, 1997; Ottenheim *et al.*, 1999). Conversely, lighter morphs can be at an advantage in environments in which overheating is likely (Gibert *et al.*, 1998; Ottenheim *et al.*, 1999). Body or wing melanization is thus part of the interface between the ambient environment and the body temperature actually achieved by the insect.

Given this role in thermoregulation, the degree of melanization is expected to differ among populations

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that experience different environmental conditions, and the fitness of individuals in a local population should be higher than that of dispersers from other environments. The adaptive significance of variation in melanization is most easily studied across elevational or latitudinal gradients. Melanization generally increases in populations with increasing elevation or latitude (Downes, 1965; Mani, 1968; Watt, 1968; Brakefield, 1984; Guppy, 1986b; Stewart & Lees, 1996). Reciprocal transplant experiments between habitats allow a comparison of the fitness of locally adapted individuals and dispersers from other populations. Alternatively, experimental manipulation of wing colour can be applied to determine the effect of wing melanization on fitness.

Butterflies of the genus *Colias* are excellent examples of alpine melanization, as they show elevational clines in melanization levels both within and between species (Watt, 1968; Kingsolver, 1983a; Ellers & Boggs, 2002). A previously developed thermal model, incorporating wing absorptivity as well as several morphological and behavioural adaptations, accurately describes body temperatures for *Colias* across elevations (Kingsolver, 1983a). Achieving a body temperature within a narrow target range is a necessary condition for voluntary flight, which is essential for feeding, mating and egg laying. However, in *Colias*, males were estimated to fly in the field for only 50% of the total time that body temperature allows flight (Kingsolver, 1983a). For females this estimate is even lower (32.5%; Kingsolver, 1983b). These observations raise the question whether there may be additional fitness advantages to maintaining body temperature within the activity optimum. These could include not only ability to respond rapidly to predators, but also support of other physiological processes, including nutrient uptake and processing, or egg maturation.

Here we report experiments assessing the effects of wing darkness on fitness indices under different environmental conditions in two closely related *Colias* species. First, it is demonstrated that varying wing darkness alone can affect body temperature, through experiments in which one wing was artificially darkened, while the corresponding wing on the other side was not. Secondly, the direct effects of wing darkness on flight activity are assessed through manipulation experiments comparing flight activity of butterflies with artificially increased wing darkness and natural colouring under different environmental conditions. Thirdly, we turn to the whole-organism level, using reciprocal transplant experiments to document flight activity of locally adapted individuals relative to individuals from other elevations. Finally, the effect of wing darkness on egg maturation rates is assessed, as an alternative fitness effect of melanization.

METHODS

SPECIES AND STUDY SITES

In the Rocky Mountains of Colorado, USA, *Colias philodice eriphyle* Edwards occurs in alfalfa fields or on native legumes in meadow habitat at elevations of 1000–3000 m a.s.l. Six sites were used in three elevation categories: very low elevation (Paonia, 1840 m), low elevation (Tomichi Creek, 2380 m; Elk Meadows, 2420 m), and high elevation (Brush Creek, 2810 m; Gothic, 2880 m; Willow Creek, 2920 m). Additionally, for one experiment, butterflies were collected at low elevation [Gold Basin, 2350 m; although in terms of butterfly wing melanization, this site was more similar to the very-low-elevation site (Ellers & Boggs, 2002)] and intermediate elevation (Jack's Cabin, 2615 m). All sites are native meadow habitat, although the very-low- and low-elevation sites also contain some feral alfalfa (*Medicago sativa*). All sites are located in Gunnison County, Colorado, USA, with the exception of Paonia, which is in Delta County.

Colias eurytheme Boisduval and *C. p. eriphyle* are not only very closely related (Pollock *et al.*, 1998), they also have the same ecology and thermal biology (cf. Watt, 1968), e.g. both are lateral baskers. In the Central Valley of California, *C. eurytheme* occurs in alfalfa fields for much of the year. *C. eurytheme* used here were caught in alfalfa fields near Tracy, California, USA.

THERMOCOUPLE MEASUREMENTS

The effect of wing darkness on the internal temperature in the thorax and abdomen was assessed using eight freshly killed male *C. p. eriphyle*. Wing colour was manipulated by colouring the basal part of the ventral hind wing with a black Sharpie marker. While this coloration may not produce exactly the same spectral reflectance properties as melanin, it does provide a good approximation to the thermal effects of increased melanization (Kingsolver, 1987). Only the left wing of each male was manipulated, which allowed us to measure body temperature under both natural and experimentally altered wing colour on the same individual by exposing either the natural or altered wing to solar radiation. Thus, paired comparisons could be made, which avoid the confounding effects of individual differences in body size, insulation or other factors.

The experiment was carried out between 10.00 and 12.00 hours on 27 and 28 July 2000, at high elevation (Gothic) under full sun. Males were placed with their wings perpendicular to the sun on a wide-mesh screen 20 cm above the ground, which was at the top of the vegetation and at the same height as natural basking sites. Thorax and abdomen temperatures were

recorded every 10 s for 2 min using a 0.12-mm implanted copper/constantan wire thermocouple, and then averaged per individual per wing side.

FLIGHT ACTIVITY WITH MANIPULATED WING DARKNESS

This experiment compared the flight activity of *C. p. eriphyle* males with artificially increased wing darkness to naturally coloured males at different elevations. It addressed the effect of wing darkness *per se* because all males originated from a single population and the experimental manipulation of wing colour assured that, on average, males with dark wings did not differ from light-coloured males in any other morphological or physiological thermal traits. Females were not included here because the flight activity experiment with transplanted individuals showed very low flight activity for females. The experiment was performed three times at high elevation (Gothic; 3 July, 1 August and 4 August 2000) and once at low elevation (Tomichi Creek; 26 August 2000). The males used at high elevation on 3 July were taken from the very-low-elevation site ($N = 8$), and on 1 and 4 August 2000 from a low-elevation site (Gold Basin; $N = 9$ and $N = 14$, respectively). The males used at low elevation came from the mid-elevation Jack's Cabin site ($N = 8$).

Wild-caught males were brought into the laboratory and fed honey water. The wing melanization of half of the males was manipulated by colouring the basal part of the ventral hind wing with a black Sharpie marker on both wings. The next day the males were put outside at one of the experimental sites in individual wide-mesh cages of $25 \times 25 \times 40$ cm. *Colias* exhibit normal behaviour in such cages (Tabashnik *et al.*, 1981). The cages had an open bottom and were placed over vegetation (*c.* 5–10 cm high), allowing the butterfly to seek shade if desired. The ambient air temperature inside the cages was measured every 5 min and did not differ from ambient temperature outside the cages. Experiments were carried out only under sunny conditions between 08.50 and 12.00 hours (Mountain Daylight Time) and lasted 30–60 min. All experiments were performed in the morning because wind is often stronger in the afternoon, increasing the variance in butterfly activity and hence introducing error to the data.

The cages were observed by two observers, and spaced 40 cm apart. Cages with a manipulated individual were spatially alternated with cages with an unmanipulated individual. At each site, all cages were observed simultaneously and thus all individuals experienced identical weather conditions, enabling a pairwise comparison of flight activity of manipulated and unmanipulated insects. Individual flight activity was scored using the one-zero sampling method (Altmann, 1974; Martin & Bateson, 1986). In this method,

the occurrence (scored as one, regardless of how many times it occurred) or non-occurrence (scored as zero) of behaviours are recorded for specified time intervals. This method is very useful if observing large numbers of individuals simultaneously, even though it compromises on the details of the behaviour. Flight activity was scored over 30-s intervals for a total of 30–60 min. Flight activity was defined as flying and wing-flicking (repeatedly opening and closing of the wings with or without walking) and averaged over all individuals per treatment at each 30-s interval.

As a control for the effects of marking, four males from high elevation (Gothic) were marked with a yellow Sharpie marker on both hind wings, which did not alter the colour. They were otherwise treated the same as those marked with a black marker. Yellow-marked individuals were tested at high elevation (Gothic, 3 July) against unmanipulated males from the same site.

FLIGHT ACTIVITY EXPERIMENT WITH TRANSPLANTED INDIVIDUALS

This experiment compared the flight activity of *C. p. eriphyle* that were transplanted between elevations and across sites. Populations at these elevations differ significantly in degree of wing melanization (Ellers & Boggs, 2002), and likely in other morphological or physiological traits important in transforming climate conditions into flight activity. Our data thus address whole-organism behavioural responses.

The experiment was repeated twice, and is a reciprocal transplant design. The first round used males and females that originated from low elevation (Elk Meadows; $N = 6$ males, 5 females) and high elevation (Willow Creek; $N = 6$ males, 5 females), testing flight activity at the low-elevation (Tomichi Creek) and high-elevation (Gothic) sites (17 and 20 June 2000, respectively). In order to test even more extreme differences in melanization, the second round used males and females from the very-low-elevation (Paonia; $n = 7$ males, 5 females) and high-elevation (Brush Creek; $n = 7$ males, 3 females) sites, testing flight activity at the very-low-elevation (Paonia) and high-elevation (Gothic) sites (27 and 28 June 2000, respectively). Paonia, Tomichi Creek and Gothic were chosen as experimental sites because of their suitability for the experimental setup and absence of disturbance by humans during the experiment. The elevation of these sites was also well-matched with the sites from which the butterflies originated, allowing us to explore flight activity in different sites at similar elevations.

The experiment was performed using the same caging and observation methodology described above ('Flight activity with manipulated wing darkness'). Observations were started between 09.00 and 10.50

hours (Mountain Daylight Time), only when the weather was sunny, and lasted for 30–60 min. On one day, individuals from both elevations were tested at the low-elevation experimental site. On the other day, the experiment was repeated with the same individuals at the high-elevation experimental site. Flight activity was averaged separately for males and for females from each source population for each 30-s time interval. Because all butterflies from both populations were in the same location at the same time and subject to identical weather conditions, the behavioural observations can be analysed as paired data.

EGG MATURATION EXPERIMENT

A total of 42 *C. eurytheme* females were collected from alfalfa fields near Tracy in California's Central Valley on 23 July and 29 August 2001. In addition, eight first-generation greenhouse-reared females were used. We used *C. eurytheme* for this experiment due to ease of availability and rearing. Females were fed honey water twice a day and kept with vetch (*Vicia americana*) for oviposition under L:D 16:8 and 27°C:15°C. Given that the egg load of females from the field is unknown, the number of mature eggs at the start of the experiment was minimized by allowing females to lay eggs during the day and to be kept cool during the night to reduce the rate of new egg maturation. After each day the number of eggs laid was counted. Females entered the experiment the day after laying more than 100 eggs or the day after laying a decreasing number of eggs on two consecutive days. In total, 34 females were used in the experiment (27 wild-caught and 7 greenhouse-reared) and they were assigned randomly to one of three treatments: black ($N = 13$), yellow ($N = 14$), or control ($N = 7$). Wing darkness was manipulated as described in the thermocouple experiment, with one wing darkened using a black Sharpie, and one wing unmanipulated. In the morning, females were immobilized on a white-paper background using hairclips and placed for 5 h under a 60 watt incandescent lamp (25 cm height) in a temperature-controlled chamber at 14.5°C. Females in the black treatment were placed with their (black) manipulated wing towards the lamp, and females in the yellow treatment were placed with their (yellow) unmanipulated wing towards the lamp. These 27 females were then frozen for further analysis. The seven females assigned to the control treatment were frozen before the start of the experiment, allowing the number of mature eggs present in the ovaries at the start of the experiment to be counted. All females were dissected using dissection needles to tear open the abdomen. Careful preparation allowed the unfolding of the ovaries and counting of the eggs. The bursa copulatrix was opened to count the number of spermatophores.

As spermatophores contain nutrients, which may increase egg production, the number of spermatophores was included as a variable in the analysis. For all females, the following variables were recorded: number of mature eggs in the ovaries, number of immature eggs in the ovaries, total number of eggs laid, number of eggs laid on the day before entering the experiment, number of spermatophores, and wing wear (on a scale from 1 to 5 that estimates scale loss and wing fraying).

It was verified that the experimental design raised body temperature by recording body temperatures with implanted copper/constantan thermocouples in the abdomens of two other previously killed *Colias*, placed in the same experimental set-up and with the same black Sharpie colouring on one hind wing. After 5 min, four temperature measurements were taken: two measurements with the manipulated wing up and two measurements with the unmanipulated wing up.

RESULTS

FLIGHT ACTIVITY EXPERIMENT WITH MANIPULATED WING DARKNESS

Thermocouple measurements showed that manipulating wing darkness significantly increased male thorax and abdomen temperatures. Thoracic and abdominal temperature was significantly higher when the wing with experimentally increased melanization was exposed to the sun (Table 1).

At the high-elevation site, three trials on different days compared the flight activity of males from low- and very-low-elevation sites with unmanipulated vs. artificially darkened hind wings. In all cases, the incidence of flight activity was higher in the group with artificially darkened wings, although the difference was significant on only two of the days (Fig. 1; Wilcoxon signed rank test: 3 July: $z = 1.46$, $P = 0.143$; 1 August: $z = 4.10$, $P < 0.001$; 4 August: $z = 2.79$, $P = 0.005$). In contrast, in the trial at the low-elevation site, unmanipulated males from mid-elevation sites had significantly higher flight activity than the males with artificially darkened hind wings (Fig. 1; Wilcoxon signed rank test: $z = 3.572$, $P < 0.001$).

Table 1. Mean abdominal and thoracic body temperature (\pm SE) of dead *Colias philodice eriphyle* males with natural and experimentally darkened hind wing colour

	<i>N</i>	Natural	Darkened	<i>t</i> -value
Thorax	8	38.6 \pm 1.0	41.1 \pm 0.9	2.46*
Abdomen	7	39.7 \pm 1.5	43.1 \pm 1.2	5.28**

* $P < 0.05$; ** $P < 0.01$

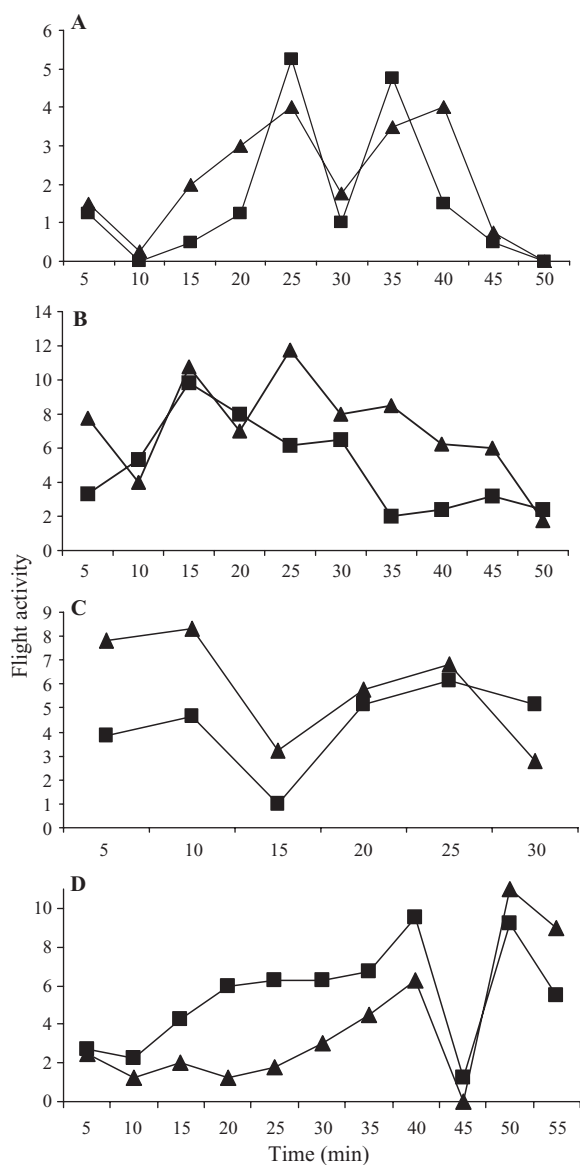


Figure 1. Difference in flight activity of *Colias philodice eriphyle* males with unmanipulated (squares) and experimentally darkened (triangles) hind wing colour on three days (A, 3 July 2000; B, 1 August 2000; C, 4 August 2000) at a high-elevation site and one day (D, 26 August 2000) at a low-elevation site. For graphical purposes the observations are pooled into 5-min time blocks. See Methods for male origin and observational details.

During the flight activity experiments, air temperature measured inside the cages varied between 23°C and 30°C at the low elevation site, and between 22°C and 32°C at the high elevation site. Therefore, males encountered a similar range of temperatures at all sites. In all four experiments there was no significant correlation between the air temperature and the

amount of flight activity, either for darkened or control males, indicating that our result was robust for the range of temperatures encountered (3 July: $r = 0.49$ and $r = 0.47$; 1 August: $r = -0.44$ and $r = -0.62$; 4 August: $r = 0.22$ and $r = 0.21$; 26 August: $r = 0.46$ and $r = 0.51$ for darkened and control males, respectively, all $P > 0.05$).

Marking male hind wings with a yellow marker had no effect on activity level relative to that of unmanipulated males ($z = 0.382$, $P = 0.703$). Thus, the application of ink to the wings could not account for the changes in flight activity of males with darkened wings.

TRANSPLANT EXPERIMENT

Activity levels differed significantly between elevations and populations in the transplant experiments. At high elevations, flight activity of males from high-elevation populations was greater than that of males transplanted from lower elevations (Fig. 2, Table 2). This was consistent with the results from the experimental manipulation experiment. However, against expectations, at low elevations there was no difference in flight activity between resident and transplanted males (Fig. 2, Table 2).

For females there was no difference in flight activity at low elevation, but low-elevation females showed greater flight activity than did high-elevation females in one of the two high elevation experiments (Fig. 3, Table 2).

EGG MATURATION EXPERIMENT

At the start of the egg maturation experiment, females had on average only 4.75 (± 0.77 SD) mature eggs in their ovaries. After the experiment, females subjected to the black treatment had a significantly greater number of mature eggs than females in the yellow treatment (Mann–Whitney U -test: $Z = -2.236$, $P = 0.023$, Fig. 4). On average, females in the yellow treatment matured 23.7 eggs during the experiment, whereas females in the black treatment matured 56.9 eggs. Abdominal temperatures measured on other individuals showed that the black treatment led to an increase in mean abdominal temperature of 3.3°C (± 0.43 SD, $N = 2$) compared to the yellow treatment.

There was no significant difference in the total number of eggs laid, the number of eggs laid on the day before entering the experiment, physiological age (estimated as the number of immature eggs in the ovaries), wing wear, or number of spermatophores between the two treatments (Mann–Whitney U -test); therefore the increase in egg maturation rate could not be explained by these variables.

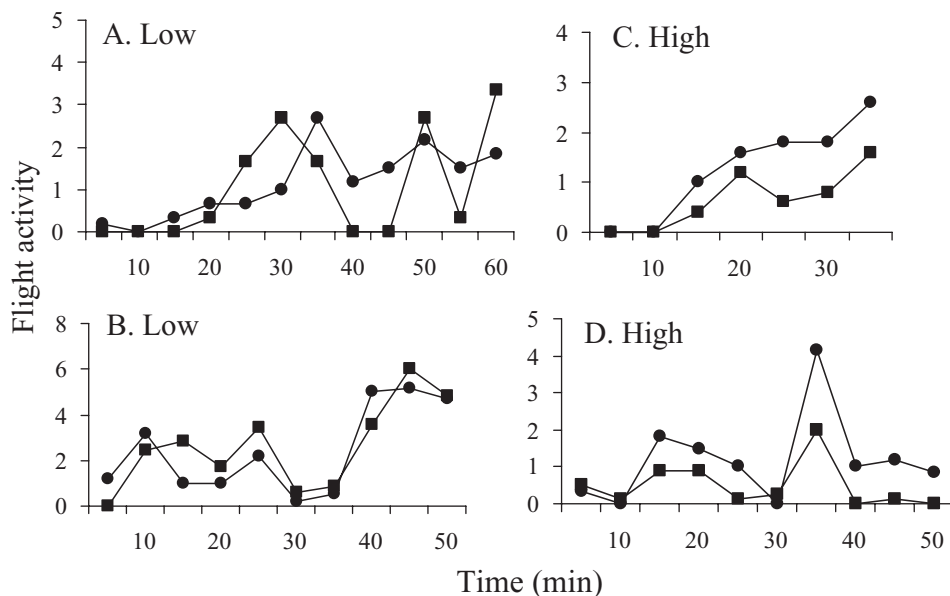


Figure 2. Difference in flight activity of *C. p. eriphyle* males originating from low elevation (squares) and from high elevation (discs) in transplant experiments performed at low and high elevation at four dates (A, 20 June; B, 26 June; C, 17 June; D, 28 June). For graphical purposes the observations are pooled into 5-min time blocks. See Methods for male origin and observational details.

Table 2. *P*-values for the differences in flight activity levels between *C. p. eriphyle* originating from low- and high-elevation populations, assessed together in trials at low- or high-elevation sites. Tests are Wilcoxon signed rank tests comparing flight incidence between groups for each 30-second period (see Methods)

Comparison: sites of origin		Trial site	
		Low elevation	High elevation
Low vs. high elevation, males	Expt 1	NS	0.042
	Expt 2	NS	0.001
Low vs. high elevation, females	Expt 1	NS	0.042
	Expt 2	NS	NS

DISCUSSION

Variation in the degree of melanization at different elevations was expected to be adapted to local environmental conditions, and thus to increase fitness of individuals of the resident population compared to dispersers from other elevations. Our results indicate that the relationship between melanization and fitness-related traits is complex. The results show that experimentally increasing hind wing darkness *per se* increases body temperature when the wings are exposed to solar radiation, independently of other morphological characters, such as thoracic fur thickness or body size. Although experimentally darkening wings affected flight activity at both low and high elevations, in transplant experiments there was an

asymmetrical effect of melanization on flight activity. Low-elevation males had a reduced flight activity when transplanted to higher elevations but not vice versa. In females variation in melanization was of minor importance for flight activity in the transplant experiments. This is consistent with known differences in flight patterns of males and females (Kingsolver, 1983b). However, darkening the hind wing increased egg maturation rate at cold temperatures, which can have an important effect on female reproductive output.

Despite the artificial nature of the experimental darkening of the wing, our treatment successfully raised the body temperature of the butterflies in the laboratory as well as in the field. Experimental manipulation of wing colour with felt markers has been used

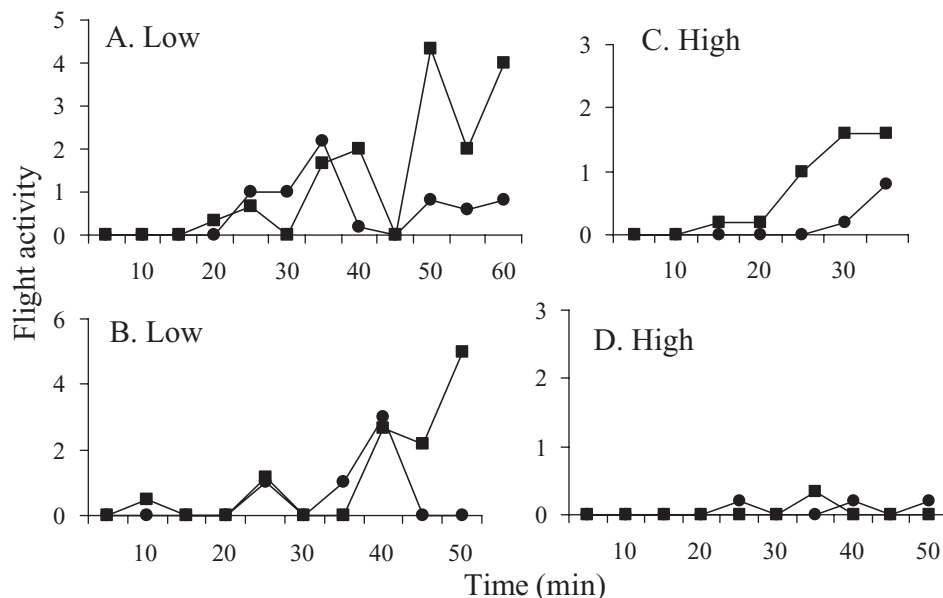


Figure 3. Difference in flight activity of *C. p. eriphyle* females originating from low elevation (squares) and from high elevation (discs) in transplant experiments performed at low and high elevation at four dates (A, 20 June; B, 26 June; C, 17 June; D, 28 June). For graphical purposes the observations are pooled into 5-min time blocks. See Methods for female origin and observational details.

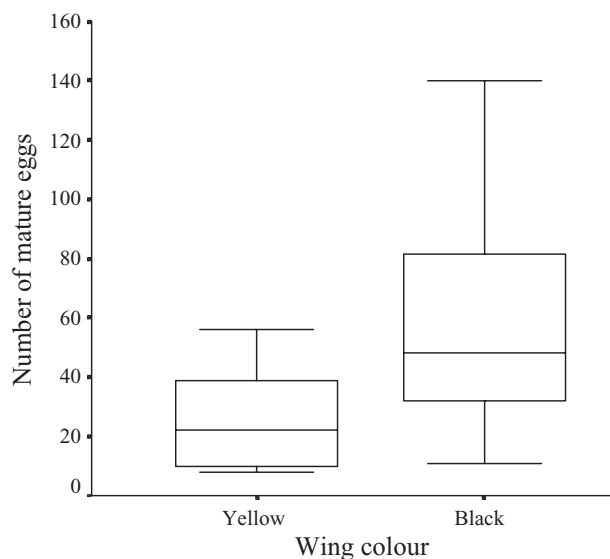


Figure 4. The number of mature eggs of *Colias eurytheme* females with natural (yellow) and experimentally darkened (black) hind wing colour. The boxplots show median, quartiles and extreme values for each treatment.

before in studies of mate choice (Wiernasz, 1989; Wiernasz, 1995) and of thermoregulation (Kingsolver, 1987; Berwaerts *et al.*, 2001). *Colias* wings consist of a mosaic of scales that are either yellow or black, with the percentage of black scales highest near the thorax

but gradually decreasing through rest of wing. Even the wings of the darkest naturally occurring forms still have yellow scales in the lower area of the wing. Therefore, our treatments may have overestimated the thermal effects of wing melanization. This is consistent with our data showing that the body temperatures of the dead individuals with manipulated wing colour were above the optimum temperature of 35–39°C (Watt, 1968; Watt, 1997); behaviour of live animals will, of course, affect the body temperatures actually achieved.

The trials with experimentally manipulated and unmanipulated wing colour isolated the effects of wing darkness on flight from other thermal adaptations. The transplant experiments, on the other hand, allowed us to test for flight activity differences at the whole organism level, simulating dispersal of individuals between elevations. The expectation was that the results would reflect local adaptation, and parallel our results for the manipulation experiment. However, in the transplant experiments for male *Colias* an asymmetrical response was found. While the high-elevation males showed significantly greater activity in high-elevation trials as expected, in neither of the low-elevation transplant experiments was the flight activity of the high-elevation males reduced relative to that of low-elevation males.

There are several possible explanations for this asymmetry in flight activity, in which high-elevation males did relatively well at both trial elevations. One

of the reasons high-elevation males are expected to have a poorer flight performance at low elevation is overheating (Watt, 1968; Roland, 1982; Kingsolver & Watt, 1984). In our experiments, if overheating occurred in high-elevation males at low elevation, they did not reduce their flight activity, in which case thermal stress may have led to detrimental effects on fitness (Kingsolver & Watt, 1983). Behaviours associated with maintaining body temperatures within the optimal range may have occurred on a time scale of less than 30 s, allowing flight activity to be observed within our observational time frame. However, a more likely explanation is that overheating did not occur because of thermal adaptations other than melanization, such as morphological or physiological adaptation to thermal environments, or higher thermal preference or tolerance. Such adaptation has been found in other species (Forsman, 2000; Dahlggaard, Hasson & Loeschke, 2001), and emphasizes the importance of experimental manipulation in studies on thermal adaptation.

The flight behaviour of females was different from that of males. Not only were there no consistent differences in the level of flight activity between high- and low-elevation females, but flight activity of females was also extremely low in general. Even though females are known to initiate flight at somewhat higher body temperatures than males (Watt, 1968), they are also more melanized than males from the same population (Ellers & Boggs, 2002). It thus seems unlikely that the sex difference in flight activity can be explained entirely by thermoregulatory constraints, especially at the warmer low-elevation sites. Rather, it suggests that necessity for flight may not be the only selection pressure on the degree of melanization.

Indeed, our egg maturation experiment showed that artificially melanized females had a higher egg maturation rate under cold ambient temperatures. Stern & Smith (1960) provided evidence for a unimodal relationship between egg-laying rate and ambient temperature in *C. eurytheme*, with a maximum around 32°C. This suggests that melanization accelerates egg maturation over a large range of ambient temperatures likely to be encountered by *Colias*. Rates of egg maturation will also influence whether the number of eggs a female lays per day is limited by egg load (egg limitation) or by oviposition opportunities (time limitation) (Ellers, Sevenster & Driessen, 2000). A field estimate of the average number of eggs laid per day for a related species parapatric to *C. philodice eriphyle* and that also oviposits on legumes is 48 eggs per day (*Colias alexandra*; Nielsen & Watt, 1998). There is no reason to believe that the number in *C. p. eriphyle* or *C. eurytheme* is dramatically different. Thus, many females may benefit from a higher egg maturation

rate than could be achieved without thermoregulation, in order to avoid egg limitation. Also, the short average adult life-span of *Colias* in the field (2–5 days; Watt, Han & Tabashnik, 1979) may select for more rapid egg maturation in young females, which would shorten the pre-reproductive adult period and increase the number of eggs actually laid.

Our results on effects of hind wing darkness on egg maturation rates could reasonably be expected to apply to other physiological processes such as sperm production rate, digestion or nutrient uptake. Sperm and ejaculate production rates may be especially critical for male *Colias*, which can have the opportunity to mate several times at unpredictable intervals over their relatively short life-spans.

In conclusion, our results suggest that gene exchange through dispersal may be more likely to flow down an elevational gradient rather than up it, due to asymmetrical effects on flight time and differences in egg maturation ability. However, other fitness consequences for dispersers such as mate selection may prevent successful gene flow between elevations (Ellers & Boggs, 2003). In sum, the adaptive significance of melanization depends on the behavioural and physiological adaptations of colour morphs and must be considered relative to the prevailing environmental conditions.

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