

# Effect of melanism of alpine *Colias nastes* butterflies (Lepidoptera: Pieridae) on activity and predation<sup>1</sup>

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**Abstract**—Melanism in the sulphur butterfly *Colias nastes* Boisduval was quantified and related to their ability to remain active under cool or cloudy conditions and to their risk of predation. Darker individuals were more active, moved greater distances, and disappeared from the population at a greater rate. Disappearance of darker individuals was not due to greater predation. Light-coloured individuals suffered greater predation than dark individuals, but only at higher elevations. The broad geographical pattern of increased melanism in Arctic and alpine *Colias* butterflies is likely a response to the consistent pattern of cooler temperatures in these habitats rather than local variation in predation pressure.

**Résumé**—L'importance du mélanisme chez des coliaïdes verdâtres, *Colias nastes* Boisduval, a pu être mise en relation respectivement avec leur capacité de demeurer actifs dans des conditions fraîches ou nuageuses et avec leur risque de prédation. Les individus plus foncés sont plus actifs, ils se déplacent sur de plus grandes distances et ils disparaissent de la population à un rythme plus élevé. La disparition des individus plus foncés n'est pas due à une prédation accrue. Les individus plus clairs subissent une prédation plus grande que les individus plus foncés, mais seulement aux altitudes plus élevées. Les grands patrons géographiques d'augmentation du mélanisme chez les papillons *Colias* arctiques et alpins sont vraisemblablement des réactions aux patrons uniformes de températures plus froides dans ces habitats plutôt qu'à la variation locale de la pression de prédation.

[Traduit par la Rédaction]

## Introduction

Arctic and alpine ectotherms, including insects, are faced with an environment characterized by cold mean temperatures and cold extreme temperatures, the effects of which are ameliorated by physiological, morphological, and behavioural adaptations (Ring and Tesar 1981). Physiological adaptations include those that provide protection against freezing (Ring 1981), enable biochemical reactions at relatively cold temperatures (Hochachka and Somero 1973; Stiles 1979; Heinrich 1981), or help raise body temperature to levels permitting normal activity. For many insects, body temperatures must be comparable to those of endotherms (as high as the mid to high 30s (°C)) for flight and hence for flight-related behaviours such as feeding, courting, mating, and

oviposition. This is particularly so for species whose resources are widely dispersed and must be accessed by flight. Insects achieve high body temperatures at low ambient temperatures by a variety of means including shivering (Heinrich 1981), behaviours such as solar basking, and morphological characteristics such as increased "hairiness" and darkening of colour (melanism). Although not all ectotherms show melanism at high latitudes or altitudes, its frequency suggests not only that cold temperatures are a major constraint for Arctic and alpine insects, but also that melanism is a relatively common solution for dealing with this constraint (Mani 1968; Ring and Tesar 1981; True 2003).

Although it has frequently been suggested that melanism is an adaptation for increased absorption of solar radiation, it is also considered

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an adaptation for crypsis (*e.g.*, reducing rates of predation on dark-coloured backgrounds (Kettlewell 1973)), for increased structural strength of the cuticle (Chapman 1982), and for blocking out ultraviolet radiation (Mani 1968). Other factors being equal, the darker of two insects will heat up more in sunlight. This pattern has been demonstrated for several species of *Colias* sulphur butterflies (Watt 1968; Kingsolver 1983*b*; Ellers and Boggs 2004*a*), but this is perhaps not surprising given that any object will absorb more light and hence become warmer when its color is darker. It is important to demonstrate that this effect is beneficial in some way; for instance, that darker individuals are indeed more active in cooler environments relative to lighter conspecifics. Although the geographic pattern of increased melanism with increased altitude or latitude has been documented among populations at different sites (Watt 1968; Roland 1978; Ellers and Boggs 2004*a*), the advantages conferred to melanistic individuals should be demonstrable within sites where there is sufficient variation in both morphology and microclimate. In this paper, I review evidence that variation in melanism within a population of *Colias nastes* Boisduval butterflies is related to variation in flight activity in response to fine-scale variation in temperature and solar radiation, and I present data indicating that differential predation may also affect patterns of melanism along elevation gradients.

## Methods

Colour variation and behaviour of *C. nastes* butterflies were studied over a 660 m (2000 ft) altitudinal gradient between 2200 m and 2860 m at Mount Allen, Kananaskis, Alberta. Meadows here are characterized by dry alpine meadow vegetation. At this site, there is marked variation in the degree of melanism among individuals (Fig. 1*a*). Melanism in *Colias* butterflies is expressed as a change in the frequencies of black and yellow scales on the wings. Given this pattern, the degree of melanism of *Colias* butterflies can be readily quantified by counting the numbers of black and yellow scales in a fixed region of the wing. I counted a total of 200 black and yellow scales per individual within a single 16 mm<sup>2</sup> field of view of a dissecting microscope. This was done in the basal part of the discal cell of the ventral hind wing, that part of the wing lying immediately over the body during lateral basking by *Colias* butterflies.

The number of black scales out of 200 was used as a measure of the degree of melanism.

## Weather

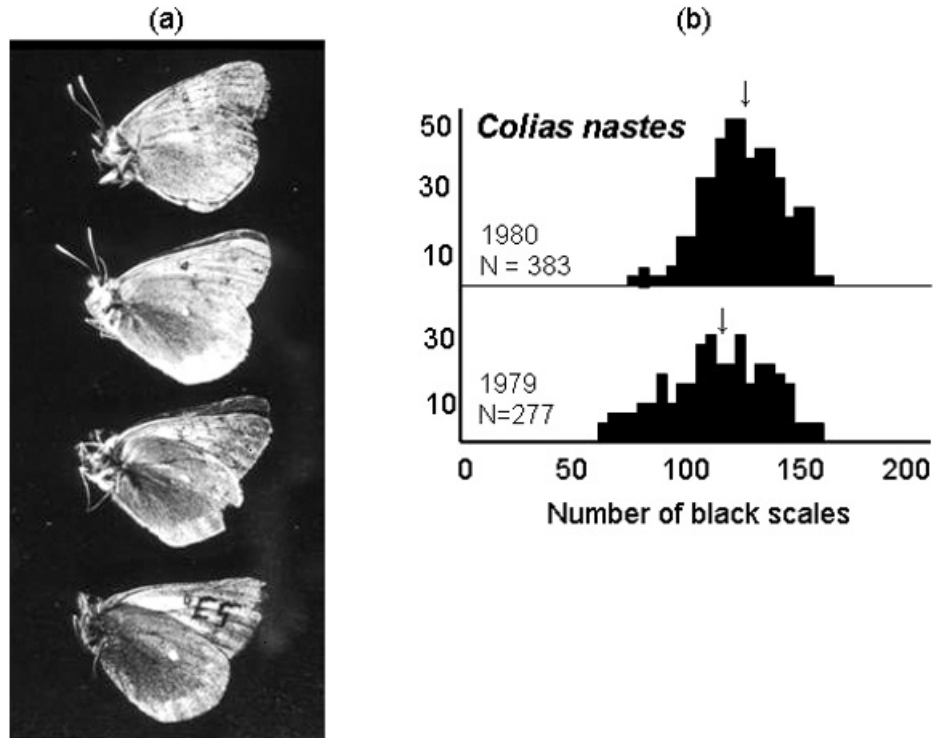
Temperatures in alpine environments decrease by about 1 °C for every 100 m increase in elevation (Geiger 1965). At Mount Allen, the 660 m of elevation within the alpine zone would provide an average gradient of about 6 to 7 °C on most days. To estimate this gradient within each day, a recording thermograph within a Stevenson Screen was set up at both the top and the bottom of the alpine zone, such that the ambient temperature could be interpolated (assuming a linear gradient) for any elevation at any time on any day during the study. Solar radiation was recorded with a solar pyrometer at each butterfly capture site along the gradient so that the amount of incoming solar radiation at the time of each capture of a flying butterfly would be known.

## Behaviour

*Colias nastes* butterflies were captured, marked with an identifying number on the distal part of the forewing using a permanent marker, and released along the altitudinal gradient. Mark–recapture was done over a period of 4 to 5 weeks from late July to the end of August. Prior to release, the degree of melanism of each newly captured individual was estimated as described above. Temperature and solar radiation at the time each individual was flying were determined by recording the date and time of each capture and comparing these with the recorded weather data. The locations of subsequent captures of the same individual within the gridded meadow allowed an estimate of the maximum distance moved between initial and final capture.

Ambient temperature and solar radiation were plotted for each time of capture of flying butterflies. By dividing the population of butterflies into two halves — individuals with a number of black scales greater than (dark half) or less than (light half) the median number — it is possible to determine whether darker individuals have a lower threshold for activity, the hypothesis being that melanism allows more activity at lower temperatures. This lower threshold was estimated by fitting the 95% equal frequency ellipse (Sokal and Rohlf 1969) around the scatter of points for each respective group (dark and light) and would include 95% of the observations of flight activity for each

**Fig. 1.** (a) Examples of variation in colouration of *Colias nastes* butterflies from Mount Allen, Alberta, and (b) the frequency distribution of melanism in the same population in 1979 and 1980, based on the number of scales in the ventral discal cell (out of 200) that are black, as opposed to being yellow. Arrows indicate median values splitting the population into “dark” and “light” halves (from Roland 1982).



group. By using the lower left quadrant of that ellipse, I estimated the lower threshold for the combination of solar radiation and temperature. The numbers of butterflies in each of the two groups exhibiting flight above and below the calculated threshold for the light-coloured group were used to compare relative activity. Counts of light and dark butterflies active above and below this threshold were compared using a *G* test for interaction.

### Predation

Two studies were done to determine whether melanistic individuals experienced lower predation at the higher-elevation capture sites. The first was an experiment on ground predation, and the second was a comparison of the incidence of avian beak marks among elevations.

Predation on dark and light butterflies on the ground was assessed as follows. I placed individuals of each colour group on the ground at two elevations (2480 m and 2750 m) at dusk, when flight by these butterflies is impossible. I then returned to the sites at dawn the next day, prior to

the initiation of flight. Dark and light butterflies were recorded either as being present and intact or as having had their wings removed and the body taken away. In the latter case, the wings were still present at the release site as evidence that the butterfly had definitely been depredated. No butterflies had simply disappeared. A comparable experiment during the day was not done because butterflies would simply fly off within seconds of being placed on the ground.

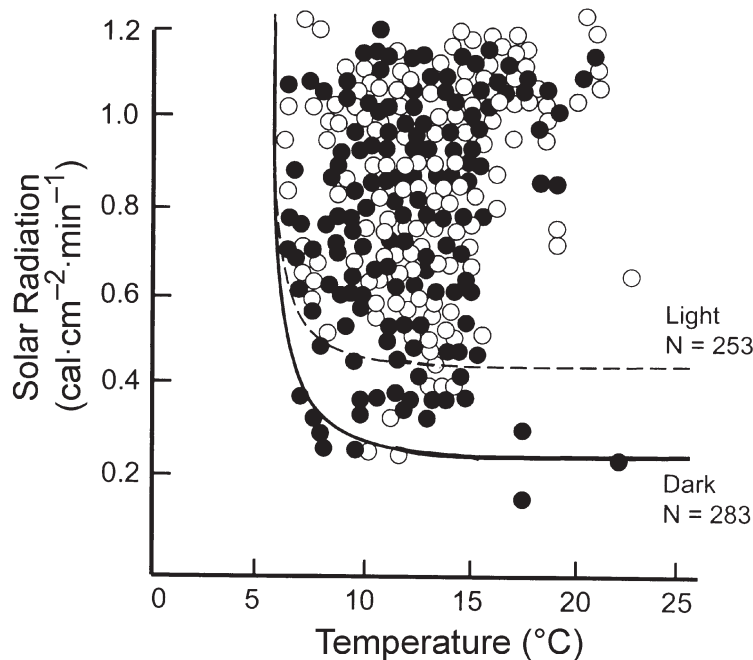
The presence or absence of beak marks (symmetrical V-shaped cuts on both left and right wings) was recorded during the course of the mark-recapture studies. The frequency of beak marks was compared among four elevation intervals (2400–2500 m, 2500–2600 m, 2600–2700 m, and 2700–2800 m) for the two colour groups using a *G* test for interaction.

## Results

### Activity

The dark half of the *C. nastes* population at Mount Allen was active over a greater range of

**Fig. 2.** Solar radiation and ambient temperature recorded at each time of capture of both dark and light *Colias nastes* butterflies at Mount Allen, Alberta, 1980. Lines were fitted as described in the text (from Roland 1982).



temperatures and solar radiation (more lower values) than was the light half of the population (Fig. 2). There was little difference in the lower threshold for temperature between the two groups (*i.e.*, low ambient temperature affected both groups equally), but there was a large difference in the effect of solar radiation on activity of the two groups, with 7% of the dark-coloured group being active below the threshold for the light-coloured group but only 2.3% of the light-coloured group being active below that threshold ( $P < 0.01$ ). In effect, members of the dark group were able to make use of solar radiation to increase their body temperature and remained active when the lighter individuals were not able to do so. This pattern has been observed for both *C. nastes* and the congener *C. meadii* Edwards at this site (Roland 1982).

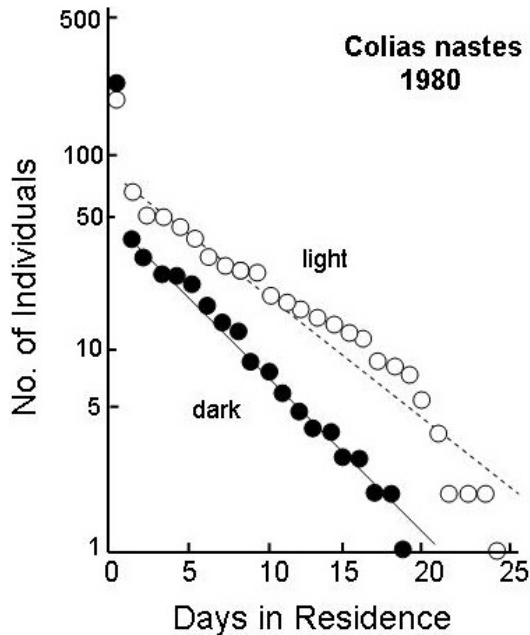
The mark-recapture studies also indicated that dark individuals were more active. Losses from the population were due to both mortality and emigration. The rate of loss of individuals from the dark half of the population was greater than that from the light half, suggesting that the darker group was moving more and, as a result, emigrating at a faster rate (Fig. 3) (but see section on predation below). That greater losses of dark individuals were due to increased movement is supported by the fact that the mean distance

moved was greater for the dark half of the population (147.2 m) than for the light half (109.3 m,  $P = 0.037$ , Mann-Whitney  $U$  test, one-tailed (Roland 1982)). Similar patterns are apparent when loss rates in 1979 and 1980 are compared; the loss rate was faster in the warmer of the two years (Roland 1982), indicating greater movement and hence greater emigration. Because activities such as feeding, courting, and oviposition by these butterflies require flight, it is expected that the darker half of the population would be able to do more of each under cooler conditions, relative to the lighter half of the population.

#### Predation

Butterflies on the ground from dusk to dawn suffered high rates of predation by an unknown predator (Fig. 4a). Predation rates differed between the two elevations, with 47% mortality per night at the higher elevation but only 12% at the lower elevation (Fig. 4b;  $G = 10.18$ ,  $df = 1$ ,  $P = 0.0014$ ). There was a significant interaction between elevation and colour group ( $G = 14.06$ ,  $df = 2$ ,  $P = 0.001$ ; Fig. 4b), with similar predation rates between groups at the lower elevation but higher predation on the light-coloured group at the upper elevation (Fig. 4b). Whatever the ground predator is at the upper elevation, it

**Fig. 3.** Minimum number of marked *Colias nastes* butterflies in residence for each time interval based on mark–recapture data for both the dark and the light half of the population at Mount Allen in 1980. Lines are the negative exponential loss rates estimated by linear regression (from Roland 1982).



seems to be more successful on the lighter *Colias* butterflies. Lower predation on darker individuals suggests that the more rapid loss of darker *C. nastes* from the population, based on mark–recapture data (Fig. 3), did not result from greater predation on darker individuals. The identity of the predator is not known but could be small mammals or ants.

The frequency of beak marks on *C. nastes* butterflies was similar at all four elevations (Fig. 5;  $G = 4.44$ ,  $df = 3$ ,  $P = 0.218$ ) and was similar between the two colour groups (Fig. 5;  $G = 0.00$ ,  $df = 1$ ,  $P = 1.00$ ). There was, however, a marginally significant trend for a higher frequency of beak marks on dark-coloured *C. nastes* at the highest of the four elevations (Fig. 5;  $G = 8.98$ ,  $df = 4$ ,  $P = 0.06$ ). Given the nature of beak marks, however, it is unclear whether this higher frequency of marks on dark butterflies reflects a higher predation rate on this group or, perhaps more accurately, a higher rate of successful escape (lower predation). At any rate, the frequency of beak marks does not provide strong evidence that the more rapid loss of dark individuals from the population, based on mark–recapture studies (Fig. 3), was due to

greater mortality of dark individuals, but it is consistent with the greater activity of dark morphs and their greater chance of being attacked by birds (and escaping).

## Discussion

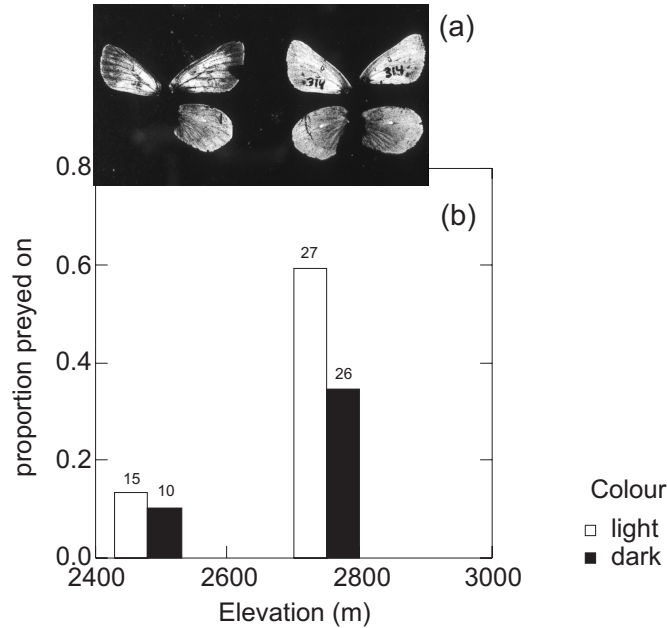
Many invertebrates, including butterflies and other insects, exhibit melanism in cooler environments such as Arctic and alpine habitats. The challenge is to demonstrate that this actually conveys an advantage for these ectotherms in carrying out their normal activities. I was able to demonstrate that within a population of *C. nastes*, dark individuals are indeed more active under cool conditions than light individuals. These patterns have been demonstrated for other species of *Colias* such as *C. meadii* (Roland 1981; Kingsolver 1983a), *C. philodice eriphyle* Edwards (Kingsolver 1983b; Ellers and Boggs 2004a), and *C. alexandra* Edwards (Kingsolver 1983b).

Why are not all *Colias* in the population melanistic? Why are there any light individuals at all? There is some evidence to suggest that heat stress at the hottest times of day may be a detriment to the more melanistic individuals (Kingsolver and Watt 1983) and that lighter individuals remain more active when temperatures are high. This would be particularly true in the alpine zone, where there is little shade and ground temperatures at noon can easily exceed 40 to 45 °C. There may be an upper temperature boundary above which only the lighter individuals are active (Fig. 2). There is indeed evidence that dark individuals are less active around noon (Roland 1982; Ellers and Boggs 2004a). The advantages of melanism would be balanced by the greater amount of time that dark individuals would spend avoiding heat, particularly at lower elevations (Kingsolver and Watt 1983). If beak marks are an indication of rates of mortality (not just of attack), then there may be an advantage for the lighter individuals at the higher elevations but no advantage at lower elevations. Such a pattern would produce a gradient of melanism opposite to that typically seen. This contradiction suggests, therefore, that beak marks are in fact more accurately a measure of successful escape; the advantage for dark individuals at high elevations would be consistent with the observed gradients of melanism.

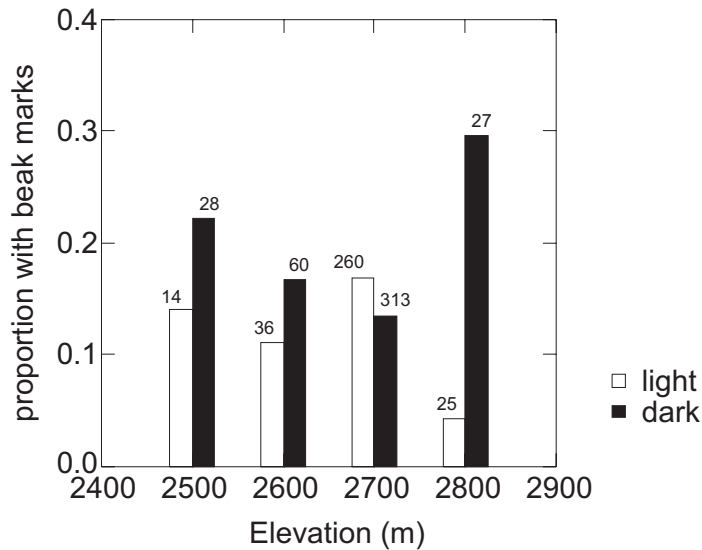
The advantage of melanism for alpine (and probably Arctic) *Colias* butterflies appears to



**Fig. 4.** Ground predation on *Colias nastes* butterflies in alpine tundra at Mount Allen: (a) wing remains at the end of one night's exposure and (b) the frequency of predation based on wing remains for the two colour groups placed at low (2480 m) and high (2750 m) elevations. Data are pooled totals from two different nights. Numbers above bars are butterfly sample sizes.



**Fig. 5.** Frequency of beak marks on two colour groups of *Colias nastes* butterflies over four elevation intervals (see text) in 1980 at Mount Allen. Numbers above bars are sample sizes.



be that it allows increased activity, thus ensuring sufficient time to feed, mate, mature eggs, and oviposit (as is the case for non-alpine or Arctic *Colias* species (Kingsolver 1983a; Ellers and Boggs 2004a)), without an increased risk of predation to offset these fitness gains.

Greater survival at high elevations could be an additional advantage for melanistic individuals, but only if the backgrounds on which they rest are also darker at high elevations, a pattern for which there is no evidence (Roland 1981), or if the ground predator is most abundant at high

elevations and is better able to see light-coloured individuals under conditions of reduced light (dusk and dawn). The greater frequency of beak marks on dark individuals at the highest elevation (Fig. 5) implies that dark individuals may be at greater risk, in part perhaps because they are more active and hence more evident to day predators.

Melanism is seen repeatedly among high-altitude and high-latitude ectotherms (Ring and Tesar 1981; True 2003). The pattern observed for many *Colias* butterfly species has been documented among sites and hence among populations (Watt 1968; Roland 1978; Ellers and Boggs 2004a) and is most strongly expressed in Arctic and alpine *Colias* species. There is little data, however, in support of such gradients of melanism across elevations within a single site. Movement within populations is likely sufficient to obscure the demonstrated advantages of melanism at high elevations. Because there is a genetic component to melanism in *Colias* butterflies (Ellers and Boggs 2004b), the gradients are more evident among populations (limited dispersal) than within populations (much dispersal). Data presented here for alpine *C. nastes* butterflies at a single site support the hypothesis that melanistic individuals do have the advantage of being more active under cooler conditions, although there may be an attendant negative consequence of a higher risk of predation. Whereas climate is consistently cooler with increased latitude and altitude, the relationship between predation and latitude or altitude would be much more variable. Selection for melanism through predation is likely a more local phenomenon; decreased predation could be an added advantage of melanism at some sites but is probably not the explanation for broad geographical patterns of increased melanism with increases in altitude and latitude.

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