THE ADAPTIVE VALUE OF MELANISM
IN ALPINE COLIAS BUTTERFLIES
(LEPIDOPTERA:PIERIDAE)

by

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required standard

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2 October 1981

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ABSTRACT

Many insect populations become darker at high elevations and high latitudes. Despite absence of empirical evidence, it is commonly believed that melanism allows more efficient basking by insects in sunlight, thereby raising body temperature and increasing activity. Variation in melanism within a single population of alpine Colias sulphur butterflies (Lepidoptera:Pieridae) allowed determination of the advantage, in cold environments, for this characteristic. Alternative hypotheses relating the effect of melanism to fecundity, predation, diel activity, and seasonal survivorship were tested by field and laboratory observation and experiment. At low temperature, melanistic females are more fecund. A balancing advantage for light coloured females does not exist at high temperatures. Dark individuals suffer lower predation rates at high altitude than do light individuals; the opposite is not true at low elevation. Duration of diel activity is markedly extended for darker butterflies under cool conditions, but only slightly for light individuals during warm sunny periods. Melanistic individuals are able to prolong the duration of activity for feeding, mate location, oviposition and escape from predation under cold conditions. This appears to the prime benefit for melanism in this population. This is the first demonstration, in a natural population, of the benefit of alpine melanism in insects.
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GENERAL INTRODUCTION

All phenotypic characters are influenced by variation in the genetic and environmental background of individuals. Phenotypic variation frequently occurs in a geographical pattern (Heslop-Harrison 1964), which correlates well with an environmental parameter. In this situation, it is tempting to propose an adaptive function to that character, and a selective mechanism associated with the environment. However, the actual demonstration of adaptive value of characters is rare although speculations are rampant. Frequent correlation of observed phenotypic characters and environmental gradients has resulted in the establishment of a variety of geographic rules (Glogers Rule, Bergmans Rule, Allens Rule). The usefulness of these rules is questionable in light of the lack of evidence of any mechanism by which they result, and for the existence of numerous exceptions to each (Mayr 1956).

The best demonstrations of the adaptive value of phenotypic characters have come from the study of polymorphisms (eg. Cain and Sheppard 1950; Kettlewell 1955,1956). Less work has been carried out on continuous characters (eg. Clausen et.al. 1940), and almost none on the adaptive value of characters which show no variation (Silberglied et.al. 1980). To show that a phenotypic character is truly adaptive requires the demonstration that it increase the "fitness" of individuals which possess the character relative to individuals which do not. Fitness is often defined as the total number of offspring surviving to reproductive age (Ehrlich et.al. 1974). At least
one of the two components of fitness: survivorship and fecundity (rate of reproduction), would therefore have to be shown to be affected by variation in phenotype.

The increased darkening of animals with an increase in altitude and latitude has been observed in many invertebrates (Walsingham 1885; Leussler 1935; Downes 1965; Watt 1968, 1969; Douglas and Grula 1978). Since this frequently occurs in ectotherms, workers have proposed that melanism is an adaptation increasing the absorption of solar radiation (Casey 1981). Little or no empirical evidence has been provided to show that melanism in cold environments affects the fitness of the individuals concerned.

My study is an attempt to determine the ecological importance of melanism in arctic and alpine sulphur butterflies of the genus *Colias* Fabricius (Lepidoptera: Pieridae), specifically *Colias nastes* Boisduval (Fig. 1) and *Colias meadii* Edwards. Melanism in this genus is in part genetically controlled, and is modulated by the effects of photoperiod and temperature (Hoffman 1978). If melanism is a character which benefits these insects in cold environments, it should be demonstrated to affect some parameter of fitness.

Kettlewell (1973), states four possible functions of melanism:

1. ultraviolet filtration.
2. aposemasis.
3. crypsis.
4. heat absorbance.
Figure 1. *Colias nastes* specimens showing variation of melanism at Marmot Creek Research Basin, Kananaskis, Alberta.
Factors affecting the degree of melanism and the possible effects which melanism has on Colias are shown in Figure 2. Fitness is determined by both survivorship and fecundity. Melanism can affect these by a variety mechanisms. If melanism is an adaptation to increase the degree of crypsis, it should reduce predation and increase survival. Melanism, as an adaptation for heat absorbance, can act to increase fecundity either indirectly by increasing the activity (feeding, mate locating, ovipositing) of the insect, or directly by increasing the rate at which biochemical processes occur. Since temperature has a strong impact on insect life span (Gilbert et al. 1976), melanism could also have the effect of reducing survivorship.

Heat absorbance has been the common explanation for melanism in arctic and alpine organisms (Leuusler 1935; Freeman 1958; Downes 1965; Casey 1981). That it is such a common belief, in light of almost total absence of evidence of its adaptive value, made this the primary focus of my thesis. I considered two components: 1. the effect of heat absorbance on insect activity (Chapter One), and 2. the direct effect of heat absorbance on rate of egg maturation (Chapter Two).

Crypsis is the best documented selective advantage of melanism in insects. The possibility of differential predation acting to cause the observed clines in Colias butterflies is addressed in Chapter Three.
Figure 2. Possible mechanisms by which melanism could act as an advantage for arctic and alpine Colias butterflies.
MELANISM AND THE DURATION OF DIEL ACTIVITY

INTRODUCTION

One of the difficulties faced by ectotherms in cold environments is the effect of cold temperature in reducing locomotion. Arctic and alpine insects have evolved characteristics which allow sustained activity in these thermally rigourous habitats. Adaptation to cold environments can be achieved behaviourally by basking (Clench 1966; Kevan and Shorthouse 1970; Casey 1981), physiologically through shivering (Heinrich 1971, 1975), and morphologically through structural and metabolic adjustment such as melanism (Douglas and Grula 1978; Watt 1968, 1969), or pubescence (Stiles 1979), and enzyme adaptation (Hochachka and Somero 1973).

Melanism, or the darkening of coloration, has long been noted as a common characteristic of many tundra insects (Walsingham 1885). Several hypotheses have been put forward to explain the greater incidence of melanism among arctic and alpine ectotherms (Mani 1968). These include the increased absorption of visible light to raise body temperature, increased screening of ultraviolet radiation at high elevation, and decreased water loss through the cuticle. Of these hypotheses, thermoregulation is perhaps the most often suggested. Dark coloured insects are able to raise their body temperature by absorbing more visible radiation (Gates 1980).

Pierid butterflies of the genus Colias Fabricius are an often cited example of increased melanism with higher altitude
and latitude (Leussler 1935; Freeman 1965; Watt 1968, 1969; Kettlewell 1973). This clinal variation is expressed both within and between species (Watt 1968; Roland 1978). Control of the amount of melanin has both genetic and environmental components. Larvae and pupae exposed to cool temperatures during their development, result in darker adults than those reared under warm conditions. Temperature plays an important role in determining the ultimate coloration until three weeks prior to eclosion of the adult (Hoffman 1978). Experiments by Ward Watt and Richard Hoffman (Ward Watt pers. comm.) have shown, however, that in lowland and alpine Colias, dark and light strains can be selected which resist the environmental influences determining melanism.

To maximize heat gain from the sun, Colias perform lateral basking (Clench 1966; Kevan and Shorthouse 1970). The insect orients its body perpendicular to the sun and, with the wings folded over the back, tilts the wings such that incoming solar radiation is effectively perpendicular to the ventral wing surface (Watt 1968). The greatest expression of melanism is in the basal portion of the hind wing surface. During basking, this part of the wing lies directly over the thorax and abdomen of the butterfly. Heat gain by the melanized integument can then be transferred to the body of the insect primarily by conduction, and reradiation (Wasserthal 1975). Dark butterflies are more efficient at absorbing solar radiation to raise body temperature above ambient temperature. Basking behaviour comprises a large portion of the activity of these insects.
Heat seeking behaviour (orientation perpendicular to the sun), predominates when body temperature is below 30 to 35°C. (Watt 1968). Body temperature must exceed 30°C for butterflies to initiate voluntary flight. Heat avoidance behaviour (orientation parallel to the sunlight), predominates when body temperatures are above 38°C. Watt (1968) proposes that dark individuals have an advantage in gaining heat at cold temperatures; light individuals have an advantage in avoiding heat stress at high temperature, and high radiant load. This advantage for lighter individuals would be particularly important in alpine environments where, owing to intense insolation, and a lack of shade, temperatures near the ground can periodically be very high, often exceeding 40°C (Mani 1968). Taken together, these implicate ambient temperature and solar radiation as important constraints on daily and seasonal activity, and ultimate success of the insect.

Kettlewell (1973), in his book *The Evolution of Melanism*, states that "Watt has ... for the first time shown, by a series of finely controlled experiments on day-flying Lepidoptera, the selective advantages of pigmentation in regard to temperature control". Although Watt demonstrated that pigmentation affects temperature control, and that dark and light butterflies have the same threshold body temperature for the initiation of flight, no demonstration of differential activity by dark and light members of a population has been demonstrated in the field. Greater activity of melanic versus non-melanic two-spot
ladybird beetles *Adalia bipunctata* (Coccinellidae) has been demonstrated for individuals which were cooled and then allowed to warm up under an artificial light source (Benham et al. 1974). Results obtained from finely controlled experiments on a few individuals may have little relevance in a population exposed to extremely variable weather. In the field, *Heodus virgaureae* (Lycaenidae) is only capable of flight above a certain threshold combination of sunlight and ambient temperature (Douwes 1976). There is no indication of any colour variability in this species which might affect observed activity. The implied increase in activity of darker individuals in cool climates is based on little or no empirical evidence. The intent of this chapter, is to determine whether, under field conditions, dark *Colias* are capable of initiating flight earlier and maintaining greater diel or seasonal activity relative to light *Colias*.

Flight is a necessary prerequisite to feeding, mate location, oviposition and escape from predators. In a population which shows variability in the degree of melanism of its members, a detectable difference in duration of activity and survival of dark and light individuals should be demonstrable. The following hypotheses were tested: 1. Dark *Colias* should be observed to initiate flight earlier than lighter coloured butterflies. 2. A threshold combination of temperature and sunlight exists, below which light butterflies cannot remain active, and a similar but lower threshold occurs for dark butterflies. 3. Dark individuals are able to remain
active longer during the season, and therefore show longer residence than light individuals.
MATERIALS AND METHODS

Species

The major part of this study was carried out on the arctic sulphur butterfly *Colias nastes* Boisduval. Additional data were collected on Mead's sulphur butterfly *Colias meadii* Edwards.

*Colias nastes*, is a holarctic species typical of tundra environments. In North America, *C. nastes* ranges from the southern Arctic Islands to treeline. In the Rocky Mountains, it occurs as far south as the Canada-United States border (Hovanitz 1950).

*Colias meadii*, is also a tundra inhabitant, restricted to alpine areas of the Rocky Mountains from Colorado to central British Columbia and Alberta. The range of *C. meadii* overlaps *C. nastes* from approximately the Canada - U.S.A. border to central British Columbia and Alberta (Hovanitz 1950). Where they are sympatric, *C. meadii* is generally found at lower elevations than *C. nastes*. At all locations, adults fly from the beginning of July to the end of August (Hovanitz 1951).

Study Area

This study was conducted at Marmot Creek Research Basin, Kananaskis Valley, Alberta (50° 50' N, 115° 11' W), during the summers of 1979 and 1980. At this site, *C. nastes* spp. *streckeri* Grum-Grshmailo, ranges from 2350 to 2800 metres elevation, in dry alpine meadows and ridges. *C. meadii* spp.
elis Strecker, is found closer to treeline, from 2200 to 2550 metres. The study area consisted of a lower east facing cirque (2350 to 2500 metres) and a long flat south facing ridge which ascends from the cirque to an elevation of 2800 metres. Large meadows of grass and mountain avens (Dryas integrifolia M. Vahl.) typify the major part of the study area. Larval host plants, Astragalus alpinus L., Oxytropis podocarpa A. Gray, and Hedysarum sulphurescens Rydberg abound throughout these meadows. Total area of the study site is approximately ten hectares.

Field Methods

Differences in melanism are due to a change in relative frequency of black and yellow scales covering the wing. Dark individuals have a greater proportion of black scales to yellow scale. In the field, the number of black and yellow scales in the basal portion of the ventral hind wing surface was counted with the aid of a dissecting microscope equipped with an ocular scale. To facilitate counting, the live specimen, with wings folded over its back, was placed in a glassine envelope. The insect was positioned such that the basal part of the Cu₂ cell was exposed through a small hole in one side of the envelope. The lines on the ocular scale were used as pointers. The number of black scales in 200 sample points was counted.
Body Temperature Measurement

It was important to determine whether my method of quantifying melanism allowed discrimination of large enough colour differences which could result in differences in body temperature. Differences in melanism between species causes a marked difference in heat gain when exposed to sunlight (Watt 1968). To compare temperatures of *Colias nastes* specimens, a series of copper-constantan thermocouples (32 gauge), and a Doran Mini Thermocouple Potentiometer Model E4221 were used. So that a series of butterflies of different melanism could be monitored simultaneously, five specimen-thermocouples and one calibration thermocouple were each connected in parallel through a six-way single pole switch before connecting to the potentiometer. Each of the six thermocouples was connected in series with a cold-junction reference thermocouple in an ice bath. Only dead dry specimens in the normal basking position were used. The difference in results which would be obtained using live or freshly killed specimens would be due to the greater heat capacitance of a fluid-filled body as compared to a dried one. This would affect the rate of heat gain, but not the stabilized body temperature. Previous studies have shown that the use of dead or live specimens makes little difference on heat gain (Watt 1968; Wasserthal 1975). Evaporative cooling plays a very minor part in determining body temperature in these butterflies (Watt 1968). The dried abdomen of each specimen was removed, and a false abdomen was made of Lepage's Bondfast glue around the thermocouple, and attached between the
wings in the normal position of the abdomen. Butterflies were all positioned such that their ventral hindwing surface was perpendicular to the sunlight. Measurements were made on two series of five butterflies on two different days. From sunrise until solar noon, "body" temperature was recorded for each specimen every half hour. Simultaneous measurement of ambient temperature and solar radiation were also made.

Four tests were carried out to determine activity of dark and light individuals:

(1). Initiation of diel flight activity.
(2). Maintenance of activity over a range of weather conditions.
(3). Duration of seasonal activity.
(4). Survivorship of dark and light morphs during the season.

Initiation of Flight

Since heating rates of dark and light individuals differ (Watt 1968), the time at which flight is initiated in the morning might in part be determined by colour. To test this, specimens were collected, kept overnight in glassine envelopes, and prior to sunrise, were set out in normal basking position. The degree of darkening of each individual was known, and the order in which they flew off was recorded. It was predicted that dark individuals should fly off earlier than light ones.
Maintenance of Activity

Butterflies were sampled from all elevations over which the species ranged. When an individual was sighted, its behaviour was recorded as: basking, flying, feeding, courting, or ovipositing. Vertical basking, a heat avoidance behaviour (Watt 1968), was also recorded, but was very rarely observed. Individuals were then pursued and captured by handnet. It should be noted that Colias butterflies, especially C. nastes are extremely cryptic when resting on the ground or vegetation, and are generally detectable only when flying. All behaviours recorded therefore, were of individuals which were flying when first observed.

Continuous records were kept of ambient temperature and amount of solar radiation. Two Stevenson Screens were set up: one at treeline (2350 m), and a second at the top of the study area (2750 m). Each screen contained a recording hygrothermograph. Daytime temperatures consistently differed by two to five degrees Centigrade between the two sites, and so, interpolation between them allowed determination of the ambient temperature at any elevation for any point in time. Solar radiation was recorded at each sampling sub-site with a Weather Measure Model R401 mechanical pyranograph. Since a considerable lag (approximately five minutes) occurs in this instrument, those parts of the recording charts with large and rapid fluctuations in solar radiation were not used. This was done because of the resulting difficulty in pinpointing the exact values of solar radiation at the time of capture of the insect.
Graph values of instantaneous solar radiation intensity were converted to gm.cal/cm²/minute.

Additional data for each specimen included: time of day, elevation, and sex. For each capture of an individual, therefore, its sex, degree of melanism, ambient temperature, amount of solar radiation, and behaviour were known.

Seasonal Activity

Since temperature and the amount of sunlight decrease over the adult flight season, I predicted that a greater proportion of the population would be dark later in the summer. A simple test of the seasonal duration of activity was to compare the degree of melanism of captured individuals to the date on which they were captured. Field work spanned the entire flight season of the butterflies (early July to the end of August).

Survivorship

Captured individuals were permanently marked with an identifying number on the ventral surface of the fore wing with a fine point permanent marker. The hind wing covered the number when the butterfly was at rest, so it would not be obvious to predators when the insect was on the ground. In flight, the ventral wing surface would not be easily seen by aerial predators. Quantification of melanism was done at a central site on the study area from which all specimens were released at first capture. This allowed easier calculation of distances
moved between the initial and subsequent capture. Recaptured butterflies were released from the point of capture.
RESULTS

In 1979, 272 captures of 229 Colias nastes and 146 captures of 125 Colias meadii were made over the seven week flight period (July 11 to August 28). Recapture rates for the species were: 18.8% for C. nastes, and 16.8% for C. meadii.

In 1980, the flight season was slightly longer (July 5 to August 26), and weather was generally cooler. Only C. nastes was studied in this year. A total of 566 captures of 433 individuals was made. Recapture rate was 30.7%, with some individuals recaptured up to six times. The greater number of recaptures was primarily due to a more intense sampling program.

Colias nastes is darker in colour (higher black scale count) than C. meadii (Fig. 3). In 1980, the population of C. nastes was darker than the 1979 population (Fig. 3a and b). The primary difference in melanism between the two years was a decrease in the number of lighter coloured individuals (Chi-square goodness of fit, p<.001). Little difference in the proportions of darker individuals occurred between years. In both years, a wide range of melanism was exhibited by this population.

During larval development, cool temperatures cause darker coloration in the resultant adults (Hoffman 1978); this could explain the decreased number of light individuals observed in 1980. Since it was suspected that cool temperatures might also affect the ability of lighter butterflies to fly, the observed increased melanism in 1980 might simply reflect a reduced
Figure 3. Frequency distribution of black scale counts from the quantification of melanism of wing colour. High counts represent dark individuals, low counts represent light individuals. Median scale counts are indicated by arrows. (a). *C. nastes* 1980; (b). *C. nastes* 1979; (c). *C. meadii* 1979.
C. nastes
1980
N=383

C. nastes
1979
N=277

C. meadii
1979
N=113

# Black Scales
capture of light adults. To test this, histograms of black scale counts for 1979 and 1980 were recalculated from individuals caught on warm sunny days when all butterflies should be able to fly. Lighter butterflies were still underrepresented ($p < .005$). Therefore, a real difference in coloration does occur between years, indicating a strong environmental component affecting observed melanism of the population.

**Body Temperature Measurements**

If we assume that body temperature is a function of ambient temperature, solar radiation and degree of melanism, then multiple regression analysis should provide a measure of the contribution each makes. Ambient temperature, solar radiation and melanism all affect body temperature significantly (Table I). An increase in the number of black scales, results in an increase in body temperature. Therefore, scale counts obtained from my quantification technique have a real meaning as a description of the degree of melanism within the species, and its effect on body temperature.

**Initiation of Flight**

Since the rank order of melanism is known for specimens set out in the morning, a Spearman's Rank correlation can be made with the order in which individuals flew off. No
Table I. Multiple regression of body temperature as a function of ambient temperature (°C), solar radiation (gm.cal/cm²/min), and degree of melanism (black scale count) for Colias nastes.

<table>
<thead>
<tr>
<th>variable</th>
<th>partial correlation</th>
<th>regression coefficient</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>air temp.</td>
<td>.395</td>
<td>.625</td>
<td>p&lt;.001</td>
</tr>
<tr>
<td>solar radiation</td>
<td>.508</td>
<td>.025</td>
<td>p&lt;.001</td>
</tr>
<tr>
<td>degree of melanism</td>
<td>.362</td>
<td>.047</td>
<td>p&lt;.001</td>
</tr>
</tbody>
</table>

total $r^2 = .798$, N=156

correlation between the two variables was found. There is no tendency for dark C. nastes to initiate flight earlier than light ones.

**Maintenance of Activity**

Melanism in these species is not polymorphic but is expressed as a continuum with normal distribution (Fig. 3). Initially, the median count of black scales was used to arbitrarily divide the population into a dark half (less than the median number of black scales), and a light half (greater than or equal to the median number of black scales).

By plotting captures of flying individuals from the two colour groups on axes of air temperature and solar radiation, lower limits of observed flight can be determined. Data for C. nastes were combined for both years for the comparison of lower threshold activity curves for dark and light butterflies. When combined, the median count was 125.5 black scales. The
sample was therefore divided into a dark half (126 or more, N=283), and a light half (125 or fewer, N=253). These captures were then plotted on axes of solar radiation and temperature (Fig. 4). The lower limit of temperature and solar radiation at which each group was able to fly was determined by excluding the extreme 2.5% of the points. In this way, some of the variance in estimates of colour and measurement of weather is eliminated. Resultant limit lines were therefore defined as the threshold combination of solar radiation and temperature below which members of that group were not observed to be active.

A large differential between the limits of flight for dark and light Colias exists (Fig. 4). To test whether the threshold lines are significantly different for the dark and light individuals, the number of captures above and below the higher threshold were counted for the dark and light half of the sample, and a 2 x 2 contingency table was made (Table II). Plots of radiation-temperature for other behaviours were similarly made (Fig. 5) and threshold differences between dark and light groups compared for each (Table II).

Melanism apparently affects the ability of the insects to fly and to feed. Once the butterflies are flying, the degree of melanism may not affect their ability to carry out other activities, although sample sizes for oviposition and courting are small.

Each behaviour appears to have a distinct threshold below which it cannot be undertaken (Figs. 5a-d). This difference among behaviours however, could simply reflect smaller sample
Figure 4. Solar radiation and air temperature at which dark (●) and light (○) C. nastes were observed flying. Lower limit lines for flight by the two groups are indicated. Dark and light groups are defined in the text.
Figure 5. Solar radiation and air temperature at time of observation of various behaviours for dark (●) and light (○) C. nastes. Lower limit lines for each group are indicated.
Table II. Comparison of distributions of radiation-temperature observations for various activities by dark and light Colias nastes.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Melanism*</th>
<th>Above Light Threshold</th>
<th>Below Light Threshold</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flying</td>
<td>dark</td>
<td>263</td>
<td>20</td>
<td>.0088</td>
</tr>
<tr>
<td></td>
<td>light</td>
<td>247</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td>dark</td>
<td>59</td>
<td>8</td>
<td>.0394</td>
</tr>
<tr>
<td></td>
<td>light</td>
<td>69</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Courting</td>
<td>dark</td>
<td>7</td>
<td>3</td>
<td>.1999</td>
</tr>
<tr>
<td></td>
<td>light</td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ovipositing</td>
<td>dark</td>
<td>11</td>
<td>0</td>
<td>.0578</td>
</tr>
<tr>
<td></td>
<td>light</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Vertical basking</td>
<td>dark</td>
<td>0</td>
<td>1</td>
<td>.5000</td>
</tr>
<tr>
<td></td>
<td>light</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* division into dark and light based on population median black scale count.
** Fishers Exact Probability Test, one-tailed.

The advantage of melanism in cold environments, in terms of its effect on activity, is only on the duration of flight.

To determine whether flight differences hold true on a finer scale, the total sample was divided into five melanism size resulting in a higher threshold. Distribution of radiation-temperature observations during flight were therefore compared to the distributions for other behaviours to determine whether apparent differences in the respective thresholds are an artifact of sample size. Ambient conditions for flight did not differ significantly from those for feeding, although they did differ significantly from those for other activities (Table III).
Table III. Comparison of distributions of radiation-temperature observations for flight and other behaviours.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Above Threshold</th>
<th>Below Threshold</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding</td>
<td>135</td>
<td>3</td>
<td>.12</td>
</tr>
<tr>
<td>Flying</td>
<td>510</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Courting</td>
<td>23</td>
<td>0</td>
<td>.0087</td>
</tr>
<tr>
<td>Flying</td>
<td>434</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Ovipositing</td>
<td>18</td>
<td>1</td>
<td>.0053</td>
</tr>
<tr>
<td>Flying</td>
<td>357</td>
<td>179</td>
<td></td>
</tr>
<tr>
<td>Vertical basking</td>
<td>2</td>
<td>0</td>
<td>.013</td>
</tr>
<tr>
<td>Flying</td>
<td>61</td>
<td>475</td>
<td></td>
</tr>
</tbody>
</table>

* threshold for each pair, is that for the first activity
** Fishers Exact Probability Test, One-tailed.

Groups with equal range of black scale counts within each. Groups were plotted on axes of radiation and air temperature and their flight threshold lines drawn as described previously. If the total sampling time is used to measure the "typical" weather for the study area, then an estimate of the proportion of the flight season which has combinations of radiation and ambient temperature lying above the five limit lines can be made. Values of temperature and solar radiation for each of the two years, were taken from the recording charts for every ten-minute period and plotted (Figs. 6a and b). The number of these observations lying above the respective limit lines was totalled. This sum was used to calculate the proportion of the total number of ten-minute observations which were suitable for flight. For this population, I estimated the amount of time
Figure 6. Lower limit lines of flight by five melanism groups of *C. nastes*. Lines are drawn over a scattergram of solar radiation and air temperature for the entire flight period in (a) 1979, (b) 1980. (melanism groups defined in text.)
Solar Radiation
(gm·cal cm² min)
that groups of increasing amounts of melanism would be able to fly. In both 1979 and 1980, the amount of time during which activity is impossible is substantial (Table IV).

<table>
<thead>
<tr>
<th># of Black Scales</th>
<th>1979</th>
<th>1980</th>
</tr>
</thead>
<tbody>
<tr>
<td>146-165</td>
<td>87.9</td>
<td>87.0</td>
</tr>
<tr>
<td>126-145</td>
<td>81.8</td>
<td>80.3</td>
</tr>
<tr>
<td>106-125</td>
<td>76.9</td>
<td>76.4</td>
</tr>
<tr>
<td>86-105</td>
<td>70.5</td>
<td>69.2</td>
</tr>
<tr>
<td>66-85</td>
<td>64.3</td>
<td>57.1</td>
</tr>
</tbody>
</table>

Those butterflies most affected in a cold year (1980), are lightest in colour (Table IV).

Only two instances of vertical basking (heat avoidance) were observed. There was no way therefore, to test whether a differential threshold for this behaviour existed between dark and light butterflies. Flight by dark butterflies may be reduced or cease when temperatures and intensity of solar radiation are high because they must avoid over heating by shade seeking and vertical basking (Watt 1968). Light individuals would be able to prolong flight during these hot periods. No decrease in the proportion of dark individuals occurs at noon on "cool" days (ambient temperature less than 12°C at 1.5 m above ground surface) (Fig. 7a). On "hot" days, the proportion of dark butterflies in our samples decreased markedly during the warmest part of the day (Fig. 7b).
Figure 7. Daytime change in the proportion of dark individuals on (a) "cool" days (less than 12°C), and (b) "hot" days (greater than 12 °C).
"Cool" days

Proportion Dark in Colour

Mountain Standard Time

"Hot" days
Seasonal Activity

Throughout the flight season, there is high variability in melanism for the population. There is little tendency for individuals captured late in the season to be darker in colour (Fig. 8). In 1979, there was a significant increase in melanism through the season, but this does not correlate with a cooling in temperature (Fig. 8). A general cooling during the later part of 1980 (Fig. 8) is not reflected in an increase in darkening of these butterflies. Therefore, I feel that melanism plays little importance in prolonging flight late in the season by compensating for a reduction in ambient temperature.

Survivorship

Comparison of survival in each year between dark and light butterflies, required that the population be arbitrarily divided into two groups using the median black scale count as described previously. Rather than using the median black scale count for C. nastes from both years as in calculating thresholds, the median count for each year was used to split the respective samples (1979: 120 black scales, 1980: 128 black scales). Survivorship estimates were therefore calculated for each half of the population for each year.

The number of days between the first and last capture of a butterfly is a conservative estimate of the survivorship of that animal. It must have been alive and resident prior to first capture, and was still alive and resident after the last
Figure 8. Seasonal change in mean melanism (number of black scales) and weekly mean daytime temperature in 1979 and 1980.
capture. When the number of animals surviving a given number of
days are totalled and plotted, an estimate of the loss rate of
animals can be made. What is often termed a survivorship curve
is in fact a residence curve (Ehrlich 1961) since loss of
animals from the population can be due to both mortality and
emigration. Residence curves, although affected by both
parameters, cannot separate them.

A negative exponential loss rate has been suggested to
describe the shape of the residence curves (Andrzejewski and
Wierzbowska 1961). This technique requires the calculation of a
mean residence time (t) for the population by summing the
number of butterfly-days and dividing by the total number of
individuals. The inverse of this value (\( P_0 \)) is the number of
individuals lost from the population per unit time (per day).
Given the number of individuals initially caught (\( N_o \)), the
expected number surviving (N) at any time t would be described
by the equation:

\[
\hat{N}_t = N_o \cdot e^{-P_0 \cdot t}
\]

Plotted on a log scale, \( \hat{N}_t \) will therefore have a linear
relation to t. The expected straight line should describe the
observed data. In 1980, the observed distribution of loss of
butterflies from the population fits the expected exponential
decay if the group of individuals caught only once is
eliminated. Inclusion of this group results in a poor fit of
observed to expected. Animals which are caught only once may be
individuals moving through the population (Andrzejewski and
Wierzbowska 1961), and therefore can be used as an indication
of the dispersal component of the population.

In 1980, *C. nastes* had a higher initial loss of dark individuals than lighter ones (Fig. 9), and steeper exponential decay (Kolmogorov-Smirnov Two-Sample Test, \( p < .05 \)). Dark individuals are therefore lost from the population at a faster rate than lighter conspecifics. A similar difference occurred in the 1979 *C. nastes* population (Fig. 10, \( p < .10 \)) and *Colias meadii* population (Fig. 11, \( p < .05 \)). Dark individuals are therefore subject to greater mortality or have a higher rate of emigration. From the comparison of the duration of flight activity of *Colias* butterflies (Fig. 4), we know that dark individuals are active a substantially greater amount of time than light individuals. Mean daily distance travelled between sequential captures was also greater for darker individuals (Table V).

**Table V.** Mean daily distance moved between sequential captures of dark and light *Colias nastes* butterflies.

<table>
<thead>
<tr>
<th>Colour</th>
<th>N</th>
<th>Mean distance (m/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dark</td>
<td>61</td>
<td>147.2</td>
</tr>
<tr>
<td>light</td>
<td>89</td>
<td>109.3</td>
</tr>
</tbody>
</table>

\( p < .037 \), Mann-Whitney U one-tailed test

All of these observations suggest that dark individuals fly more frequently and for a longer duration, resulting in higher emigration from the population.

Observed differences are not sex biased. Flight thresholds
Figure 9. 1980 residence curves for dark (●) and light (○) C. nastes. Lines are the negative exponential loss rate as calculated with the exclusion of those animals caught only once (Andrzejewski and Wiersbowa 1960).
Colias nastes
1980
Figure 10. 1979 residence curves for dark (●) and light (○) C. nastes. Lines as in Figure 9.
Colias nastes
1979

# Individuals

# Days in Residence
Figure 11. 1979 residence curves for dark (●) and light (○) C. meadii. Lines as in Figure 9.
Colias meadii
1979

Number of Individuals

Number of Days in Residence
Figure 12. Flight thresholds for male and female *Colias nastes* as a function of solar radiation and air temperature.
Figure 13. 1980 residence curves for male (●) and female (◆) C. nastes. Lines as in Figure 9.
are similar (Fig. 12); residence curves are not significantly different (Fig. 13); and mean dispersal distances do not differ between sexes (Table VI).

<table>
<thead>
<tr>
<th>Table VI. Mean daily distance moved between sequential captures of male and female Colias nastes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
</tbody>
</table>

p<.764, Mann-Whitney U two-tailed test

Although not statistically different, it is of note that female residence time was less than that for males (Fig. 13), but dispersal distance was shorter (Table VI). This supports similar data for Colias alexandra Edwards in Colorado (Watt et al. 1977). Greater loss of females with less movement implicates higher mortality among females than among males.

If dark butterflies are leaving the population more rapidly because of higher body temperatures, it would be expected that greater loss of all individuals would occur at higher ambient temperatures by virtue of the relationship between air and body temperature (Table I). For the entire C. nastes population, a steeper residence curve occurred in 1979 (Fig. 14, p>.05), which was the warmer of the two years (Figs. 6 and 8). Experiments and observations carried out to test the possibility of crypsis and differential predation of dark and light C. nastes are discussed in Chapter three.
Figure 14. Residence curves for entire Colias nastes population in 1979 (■) and 1980 (●). Lines as in Figure 9.
Colias nastes

# Individuals

# Days in Residence

1979

1980
DISCUSSION

That dark insects in sunlight heat up more quickly than light insects seems trivial since any dark object will do so relative to one lighter in colour. The primary shortcoming of previous studies on the adaptive value of melanism for arctic and alpine insects, has been the failure to extrapolate the carefully measured body temperature differences to the activity and ultimate success of the insect in the natural environment. Extreme spatial and temporal variability in both temperature and sunlight in tundra environments (Mani 1968) could well result in no effective difference among individuals in the supposed advantages which higher body temperatures convey to Colias butterflies. Differences may be so subtle as to not be measurable in a highly variable environment. If, however, a clear and substantial advantage relating variation in a character to the survivorship and/or fecundity of the organism can be demonstrated (despite large environmental variability), a strong conclusion regarding the adaptive value of that character can be made.

Thermocouple experiments have shown that the degree of melanism within this species has a highly significant effect in determining stabilized body temperature (Table I). Melanism also affects the rate of heat gain (Watt 1968), but the advantage of being melanistic is not due to the increase in the rate of heating of the insect since there was no indication that melanistic C. nastes voluntarily flew earlier in the morning than did lighter individuals.
A body temperature in excess of 30°C is required by Colias butterflies before flight is initiated. From Watt's (1968, 1969) data, the time difference between a dark and light butterfly reaching this critical temperature, is only 10 to 30 seconds. The only way therefore, that a significant correlation would be expected when comparing the darkness of colour to the order in which they flew off, is if they flew the instant they were capable of doing so. Even if this did occur, the implied advantages of longer flight (increased feeding, mate location, oviposition and escape from predation) would be negligible. For many flying insects, the temperature threshold between non-flight and flight for a population is usually very narrow (Taylor 1963). Differential heating rates resulting from variability in melanism appear to have little effect in broadening the time differential for insects to reach this threshold.

In sunlight, dark individuals not only heat up more quickly, but stabilize at a higher body temperature. The stabilized temperature difference between dark and light individuals can be as much as four degrees Centigrade (Watt 1968, 1969; Douglas and Grula 1978). Although absolute body temperatures vary with different ambient temperatures, a difference between morphs always occurs provided the butterflies are exposed to sunlight. Under certain combinations of ambient temperature and sunlight, the stabilized temperature of dark butterflies would lie above the necessary flight threshold, lighter ones would lie below it. In such situations,
only the darker members of a population would be observed flying.

Under these conditions, large segments of the population are incapable of feeding, locating mates, ovipositing, or escaping from predators. We know that although other activities have different temperature-radiation thresholds, melanism only affects the insects' ability to fly. Therefore, when considering the advantage of melanism for diel activity, it is only of consequence to consider its effect on flight. If flight is not possible during a substantial part of the short adult season, melanistic individuals possess a clear advantage.

During the flight season of *C. nastes* (July and August), mean daily solar radiation at Marmot Creek is .74 gm.cal/cm²/min, a level at which all butterflies should be able to fly. At the arctic IBP site, Devon Island N. W. T. (75° 30' N, 84° 40' W), which lies immediately north of the extreme range of *C. nastes* (Hovanitz 1950), mean solar radiation during the flight season is always below .4 to .5 gm.cal/cm²/min (Courtin and Labine 1977). Under these conditions, Figure 6 would predict that only butterflies with black scale counts greater than 125 would be able to fly. A small sample (7) of *C. nastes* from the Boothia Penninsula (70° N, 94° W), revealed a mean black scale count of 153, with a range of 124-176.

This begs the question of why all *Colias* are not dark; why there should be such a wide range in observed melanism especially at low latitudes. Watt (1968) suggested that light *Colias* are able to avoid heat stress since heat avoidance
behaviour predominated at body temperatures in excess of 38°C. At high altitudes, in low latitudes, intense insolation and little shade certainly result in very high temperatures near the ground, often exceeding 40°C (Mani 1968). Watt (1968) anecdotically observed that on calm sunny days in the alpine near Jasper, Alberta, *C. nastes* showed decreased activity during midday. This he attributed to increased heat avoidance behaviour such as shade seeking and vertical basking. Kettlewell (1973) noted that during "heat waves" in Britain, samples of *Biston betularia* contained higher proportions of the light *typica* morph, and decrease in the proportion of the dark *carbonaria* morph. *Colias philodice eurytheme* in California, show peaks in daily activity at solar noon during the fall, but marked suppression of activity from 12:00 to 16:00 during the summer (Leigh and Smith 1959). This was attributed to the need to avoid heat stress. Under hot conditions, differential survival or fecundity as a result of differential flight time would therefore convey an advantage to light individuals.

To maintain the cryptic green colour of the butterfly, a certain proportion of yellow scales to black scales must be maintained. If the insect were entirely black, they would be more highly visible while flying (Kettlewell 1973).

Melanism provides little or no benefit in prolonging seasonal activity. *Colias nastes* and *Colias meadii* over-winter as third instar larvae (Ae 1958). *Colias meadii* has a developmental threshold of 5°C, and at 27°C, takes 12 days to reach third instar (Ae 1958). A mean August temperature at
Marmot Creek of 8.8°C would result in larvae requiring at least five times as long (nine weeks) to reach the over-wintering stage. Whether or not a melanistic butterfly can remain active an extra week in the autumn seems irrelevant if its offspring are not able to reach third instar by the middle of October when temperatures never rise above the developmental threshold of 5°C (Water Surveys of Canada 1977).

I initially hypothesized that dark Colias might persist longer during the flight season and therefore show a lower rate of loss of individuals from the population. Residence curves indicate that darker individuals are in fact lost from the population at a greater rate. This is the case for C. nastes in both years, and for the congener C. meadii, suggesting it to be a general phenomenon. Differential loss by emigration or predation could result from the greater flight activity of darker butterflies for two reasons: (1) greater flight activity would increase distances moved by individuals, and therefore increase the likelihood of emigration from the sampling area; (2) greater flight activity would increase the likelihood of predation on the wing by avian predators (see Chapter 3). Data show that dark individuals are moving significantly further per day (Table V), implicating differential emigration as causing the greater loss of dark butterflies. This does not, however, rule out the possibility of differential mortality acting to increase or decrease survival of dark individuals relative to light ones. Life span of individuals with high body temperature (darker in colour) would be expected to be shorter than those
with lower body temperatures (Gilbert et al. 1976). The greater loss of dark individuals from the population might be the result of both higher emigration rate and shorter life span relative to light individuals.

Strong evidence has been presented here to support the hypothesis that melanism allows the increased activity of insects in cold environments. Flight appears to be the only activity which is influenced by the degree of melanism of the butterfly. Increased melanism of butterflies at this site, can result in an increase in flight activity of up to 55%. The assumption remains however, that increased flying time results in increased success by the individual. Certainly complete inability to fly would result in no offspring. Mate location by male Colias require continual patrolling of open areas (Scott 1975). The importance of flight is therefore paramount in determining the ability of males to locate mates. From the time of emergence, Colias females are continuously maturing eggs (Stern and Smith 1960). Eggs are oviposited singly, and flight is necessary for females to move from plant to plant. Females probably never oviposit all their eggs during their lifespan (Stern and Smith 1960), and so, any gain in flight time would result in increased fecundity. Nectar feeding by these butterflies requires flight between flowers (usually single-headed Composites: Haplopappus lyallii A. Gray and Erigeron aureus Greene). Reduced feeding by adult females decreases egg output (Stern and Smith 1960). If the amount of courting and copulation, oviposition, and egg maturation are proportional to
the amount of time flight is possible, then a marked difference in fecundity of dark and light *Colias* could be envisaged. The direct effect of a raised body temperature on fecundity is addressed in Chapter 3. The greater variability in microclimate of southern alpine areas as compared to arctic sites (Remmert and Wunderling 1969), might account for the ability of lighter coloured *Colias* to be successful members of these southern populations.

Differences in melanism between years are partly determined by the effect of temperature on larval development. Since 1979 was warm relative to 1980, two predictions can be made regarding the relative importance of the genetic and environmental effects determining the degree of melanism: (1). If melanism is primarily genetically determined, a warm year (1979) should provide an advantage to lighter individuals, and their light coloured offspring would therefore constitute a larger proportion of the population the following year (1980). (2). If melanism is primarily environmentally controlled, an advantage gained by light butterflies in a warm year (1979) would be masked by a darkening of their offspring in the following colder year (1980). Greater representation of dark individuals occurred in 1980 indicating a strong environmental component. Unfortunately, no unequivocal statement regarding the relative importance of the two determinants of melanism can be made since there is no "control" by which to compare these results. For example, if there was no genetic effect to increase representation of "light" genes, the melanism in the
1980 population melanism might have been even darker than observed. Since the relative importance of the genetic influence on melanism is not known, advantages demonstrated by lighter or darker individuals can only be accurately termed "advantages", not selective advantages.

This is the first field demonstration of an advantage for insects to be melanistic in cold environments. Carefully controlled thermocouple experiments have shown melanism to increase insect body temperature. These differences have not until now been extrapolated to the natural environment which shows large spatial and temporal variation in temperature and solar radiation. Melanism provides an obvious benefit to the insect by increasing its ability to maintain flight activity under cold conditions. Fecundity is highly dependent on flight, and the substantial increase in flight time during the short season would result in an increased potential number of offspring. Those butterflies whose flight time is curtailed most by cold weather are lightest in colour. Increase in latitude and altitude would cause more of the lighter individuals to be incapable of normal activity. Fecundity would be reduced most in the lightest members of the population. The adaptive value of melanism in cold environments for these butterflies probably results from an increased fecundity manifest through substantial differences in flight activity.
MELANISM AND FECUNDITY

INTRODUCTION

Fitness, or the relative success of an organism to produce offspring, is generally measured as the total number of offspring of that individual, surviving to reproductive age. The total number of progeny produced is determined by both the rate of production of young and the duration of the reproductive stage of the life cycle (Futuyma 1979). Fecundity of female insects can be affected directly by biotic factors such as amount of food (Dethier 1959), quality of food (Gilbert 1972), and number of matings (Benz 1970), and by abiotic factors such as temperature (Greenfield and Karandinos 1976), and humidity (Stern and Smith 1960). Behaviour, physiology, morphology, or phenology of insects indirectly affect fecundity by modulating the effect of these factors. It is these phenotypic characters upon which selection acts.

Body temperature of a female insect strongly affects the rate at which she is able to produce eggs (Uvarov 1931). Rate of production has a temperature threshold below which no eggs mature. Increased body temperature results in increased rate of egg output to some maximum. At even higher temperatures, fecundity decreases.

Arctic and alpine insects are strongly affected by cold temperature and short season of the environment in which they live. By definition, arctic and alpine habitats have a mean daily maximum temperature which never exceeds 10°C in any
month. A variety of mechanisms are used by insects to increase body temperature at low ambient temperature. These include basking behaviour (Clench 1966, Kevan and Shorthouse 1970), and morphological adaptation such as pubescence (Stiles 1979) and melanism (Watt 1968; Kettlewell 1973; Douglas and Grula 1978).

Among the sulphur butterflies of the genus *Colias* Fabricius, the tundra sulphur butterfly *Colias nastes* Boisduval, shows marked increase in darkening of its wing coloration with increase in both altitude and latitude. This variation is equally expressed in both sexes. In association with obvious prolonged basking behaviour (Kevan and Shorthouse 1970), it is thought that melanism in these butterflies is an adaptation acting to raise the insects body temperature (Watt 1968, 1969).

The effect which melanism has on fecundity, by virtue of its effect on body temperature, has not been tested. Melanism does affect the duration of flight activity and feeding by *Colias* butterflies (Chapter One). Melanism therefore, does indirectly affect fecundity by increasing imaginal feeding rate which raises fecundity (Stern and Smith 1960). Increased body temperature resulting from melanism could, however, be acting directly on the rate of egg maturation by generally increasing the rate of biochemical processes. Intensity of sunlight affects body temperature, and the magnitude of this effect is proportional to the degree of melanism of the insect (Chapter One). If melanism is an adaptation which increases fecundity in cold climates, a difference in fecundity related to difference
in degree of melanism should be demonstrable.

Body temperature greatly determines fecundity of *Colias philodice* Latreille (Stern and Smith 1960) (Fig. 15a). This relationship would be similar for all individuals. The curve of egg production against ambient temperature would in theory be shifted to the left for dark individuals, and to the right for lighter butterflies (Fig. 15b). Therefore, it would be predicted that at low ambient temperatures, dark females will produce more eggs than light females; the opposite would occur at higher ambient temperature.

Differences in flight activity may affect the likelihood of females being mated. Colour of female *Colias* can also affect attractiveness to males (Graham *et al.* 1980). Therefore, fecundity differences resulting from a difference in mating frequency could occur. If melanism increases the likelihood of mating in cold habitats, it would be predicted that dark females should mate more frequently by being more active.

After a short one to two day pre-oviposition period, female *Colias philodice* mature eggs throughout their lifespan and oviposit these eggs singly (Stern and Smith 1960). *C. nastes* appear to mature and oviposit eggs similarly. Lifespan can be as long as 25 days, but averages 6.8 days for females in the population I studied (Chapter One). If body temperature is consistently higher during the day in dark females, a marked difference in egg maturation rate should result. The intent of this chapter is to compare the rates of egg production of dark and light *Colias* females under the same
Figure 15. (a) Fecundity of *Colias philodice* as a function of body temperature. Oviposition rating is the percentage of the maximum rate. Adapted from Stern and Smith 1960. (b) Theoretical fecundity curve for dark and light *Colias* as a function of ambient temperature.
temperature and light intensity. My working hypotheses were:

1. Dark females under cold conditions mature and oviposit eggs more rapidly than lighter females.
2. This difference decreases as ambient temperature increases.
3. Dark females mate more frequently than lighter conspecifics.
METHODS AND MATERIALS

Female Colias nastes were collected in the field by handnet. Two collections were made each season: one at the start of the six-week flight season, and a second two to three weeks later. At the time of capture it was possible, in most cases, to determine whether females had mated. This was done by lightly squeezing the abdomen to feel the hard round spermatophore (Stern and Smith 1960). The degree of melanism of each female was quantified as described in Chapter One. One collection of Colias meadii females was made in 1979.

All oviposition experiments were carried out in controlled environment chambers. Each female was placed in a clear plastic cage (25cm X 20cm X 15cm) screened on one side. The legume, Astragalus alpinus, is known to be a primary host plant for Colias nastes (Ehrlich and Ehrlich 1961), and each female was provided with several upon which to oviposit. Since it was hypothesized that dark females were capable of maturing more eggs than light females under colder conditions, comparison of egg output was made under a range of temperatures (12-34°C). Maximum light intensity possible in these chambers was only 0.4 gm.cal/cm²/min (one third the value of sunlight at solar noon). Light intensity was therefore not varied among experiments. Growth chambers were cycled on a 14:10 hour cycle for both light and temperature. "Night" temperature was 5°C for all treatments regardless of "daytime" temperature.

Comparison of fecundity was made utilizing both natural colour variation and by manipulating the degree of darkness.
Manipulation was done by slightly darkening the basal portion of the ventral hindwing surfaces, on half of each group of butterflies, with a fine-point black permanent marker (Fig. 16). The undarkened half of each replicate was to receive a "control" treatment by application of yellow permanent marker. This was, however, not done since it produced a dark brown colour which would act similar to the black treatment in terms of its effect on raising body temperature. Both experimental and control groups had the same range of natural colour variation. Colour manipulation trials were run at 22°C only. Summary of experiments is presented in Table VII.

<table>
<thead>
<tr>
<th>Species</th>
<th># Females</th>
<th>Temp (°C)</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. nastes</td>
<td>12</td>
<td>12</td>
<td>natural</td>
</tr>
<tr>
<td>C. nastes</td>
<td>12</td>
<td>22</td>
<td>natural</td>
</tr>
<tr>
<td>C. meadii</td>
<td>12</td>
<td>22</td>
<td>natural</td>
</tr>
<tr>
<td>C. nastes</td>
<td>20</td>
<td>22</td>
<td>natural</td>
</tr>
<tr>
<td>C. nastes</td>
<td>21</td>
<td>22</td>
<td>manipulated</td>
</tr>
<tr>
<td>C. nastes</td>
<td>20</td>
<td>22</td>
<td>manipulated</td>
</tr>
<tr>
<td>C. nastes</td>
<td>20</td>
<td>34</td>
<td>natural</td>
</tr>
</tbody>
</table>

Every one to two days, the total number of eggs each female had oviposited was counted. In most cases, experiments were run for a 14 day period. Plants were only replaced if they
Figure 16. Colour manipulated and normal Colias nastes females used in fecundity experiments.
showed signs of deterioration. At the completion of each experiment, females were dissected. The presence and number of spermatophores in each female was recorded to confirm that mating had occurred. The number of chorionated eggs remaining in the female (unoviposited eggs), was also recorded. This was added to the number of oviposited eggs to provide the actual number of eggs matured. In this way, differences which were due to difficulty in actual oviposition could be determined, and any effect which plant size or quality might have on oviposition behaviour was reduced.
A great deal of variation in the daily oviposition rate occurred among females (coefficient of variation equalled 156% in the largest group). In subsequent analysis, attempts were made to account for and reduce variation. Since age is a important determinant of rate of egg output (Stern and Smith 1960), I used only data from females collected at the start of the flight period. Thereby, the possible range in age was reduced from a maximum of 25 days (Chapter One) to two to three days. Daily oviposition rate, and daily egg maturation rate were compared to both natural colour and colour which was artificially altered.

Natural Coloration

Oviposition Rate

Because of the very high variance of these data, natural melanism was treated as a continuous character (number of black scales), rather than a binary character (dark half, light half of each sample). Oviposition rate was correlated with the degree of melanism (Table VIII). All subsequent analyses involving natural coloration were treated similarly.

Predicted results are supported largely as trends. At low temperatures (12°C), increase in melanism increases the rate of egg oviposition (positive correlation). At higher temperatures
Table VIII. Correlation between the degree of melanism (number of black scales), and mean daily number of eggs oviposited by Colias females under various temperature regimes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Temp (°C)</th>
<th># Days</th>
<th># Females</th>
<th>Correlation Coefficient*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. nastes</td>
<td>12</td>
<td>14</td>
<td>12</td>
<td>.6416</td>
<td>p&lt;.05</td>
</tr>
<tr>
<td>C. nastes</td>
<td>22</td>
<td>14</td>
<td>39</td>
<td>-.2435</td>
<td>.10&lt;p&lt;.20</td>
</tr>
<tr>
<td>C. nastes</td>
<td>34</td>
<td>6</td>
<td>20</td>
<td>-.0553</td>
<td>p&gt;.90</td>
</tr>
<tr>
<td>C. meadii</td>
<td>22</td>
<td>11</td>
<td>12</td>
<td>.4668</td>
<td>.10&lt;p&lt;.20</td>
</tr>
</tbody>
</table>

* Spearman's Rank Correlation

(22°C), the relationship is reversed. At the highest experimental temperature (34°C), survivorship of females was very low, and no apparent difference in oviposition rate occurred (there was also no difference in survival between dark and light females). Colias meadii were kept at only one temperature (22°C) where dark females tended to produce more eggs than lighter females.

Maturation Rate

When the number of matured but unoviposited eggs is included in the calculation of fecundity, results do not differ markedly (Table IX). This would indicate that differences in egg output are due to actual maturation rate differences and not to difficulties in oviposition.

Under cold temperatures dark females mature slightly more eggs than lighter females. Higher temperatures result in greater rate of maturation by lighter coloured females. The tendency for higher reproduction in light females decreases somewhat at
Table IX. Correlation between degree of melanism (number of black scales) and mean number of eggs matured by *Colias* females.

<table>
<thead>
<tr>
<th>Species</th>
<th>Temp (°C)</th>
<th># Days</th>
<th># Females</th>
<th>Correlation Coefficient*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. nastes</em></td>
<td>12</td>
<td>14</td>
<td>12</td>
<td>.3846</td>
<td>.10&lt;p&lt;.20</td>
</tr>
<tr>
<td><em>C. nastes</em></td>
<td>22</td>
<td>14</td>
<td>39</td>
<td>-.4455</td>
<td>p&lt;.01</td>
</tr>
<tr>
<td><em>C. nastes</em></td>
<td>34</td>
<td>6</td>
<td>20</td>
<td>-.3583</td>
<td>.10&lt;p&lt;.20</td>
</tr>
<tr>
<td><em>C. meadii</em></td>
<td>22</td>
<td>11</td>
<td>12</td>
<td>.6416</td>
<td>p&lt;.05</td>
</tr>
</tbody>
</table>

*Spearman's Rank Correlation

34°C.

**Manipulated Colour**

**Oviposition Rate**

No significant difference in the mean daily number of eggs oviposited occurred between artificially darkened and undarkened individuals (Table X).

Oviposition rate was not different for natural and manipulated groups (Table X). From these results, I conclude that increasing the darkness of female coloration does not increase her rate of oviposition.

**Maturation Rate**

The inclusion of non-oviposited but mature eggs in the measure of fecundity does not result in significant difference between darkened and undarkened females (Table XI). Artificially darkening females does not greatly increase the
Table X. Mean number of eggs oviposited per day for manipulated and naturally dark and light groups of Colias females.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Temp (°C)</th>
<th>N</th>
<th>Mean # Eggs</th>
<th>Significance**</th>
</tr>
</thead>
<tbody>
<tr>
<td>manipulated</td>
<td>22</td>
<td>20</td>
<td>2.96</td>
<td>2.02</td>
</tr>
<tr>
<td>natural*</td>
<td>22</td>
<td>39</td>
<td>2.75</td>
<td>1.70</td>
</tr>
</tbody>
</table>

*dark and light groups based on median black scale count.  
**Student's t-test.

Mating Frequency

Females which contained no spermatophores were included in the analysis of mating frequency. Dark females contained on average fewer spermatophores than did light females (Fig. 17), indicating that they are less likely to mate. Mean spermatophore count for dark and light groups were 1.14 and 1.37 respectively (p<.05, Student's t-test).
Figure 17. Frequency distribution of spermatophore counts for dark and light *Colias nastes* females.
# Spermatophores

<table>
<thead>
<tr>
<th># Spermatophores</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

- **Dark**
- **Light**
DISCUSSION

In theory, a dark female butterfly should be able to produce eggs at a rate more rapid than lighter females under cool conditions. I have shown that under relatively cool conditions (12°C), this does in fact happen. Clear cut results at warmer temperatures were not obtained, but the advantage for dark females does decrease. At warm temperatures, there is at least an indication that light females will have some advantage in egg production. Ambiguity might result from one of a number of reasons:

1. The very high variance in maturation rate among females would make the detection of any difference in fecundity difficult. Very large sample sizes would be required to detect this difference.

2. Thermocouple measurements of body temperature of dark and light individuals in sunlight (Chapter One), show a maximum temperature difference of four °C. Because of the relatively low radiation level in the growth chamber, and the likelihood of a warm boundary layer in the cages from heating of the soil and plants, temperature difference among colour types in these experiments are likely much less.

3. Egg production rate is predictable from body temperature (Stern and Smith 1960), but these were not measured for individuals in growth chambers. Body temperature can be predicted if its relationship to ambient temperature, solar radiation, and degree of melanism is known. Without this relationship, it is not possible to determine whether the
conditions used in the growth chambers were such that a difference in fecundity could occur. Three possible ambient temperature regimes exist which might result in no effective difference in fecundity:

a. ambient temperature too low, such that body temperatures lie to the left of the curve in Figure One

b. ambient temperature too high, such that body temperatures lie to the right of the curve in Figure One

c. ambient temperature is at the point where the two curves for dark and light individuals (Fig. 2) intersect, resulting in the same fecundity.

A simulation model was used to determine whether the ambient temperatures I used could result in any of the above three conditions. The relationship between body temperature and fecundity for *Colias* was derived from data of Stern and Smith (1960) and scaled to the maximum output of my females.

\[ E = 0.00002T_b^5 - 0.0044T_b^4 + 0.33T_b^3 - 12.3T_b^2 + 224.7T_b - 1602 \]

where: \( E \) = the number of eggs produced per day

\( T_b \) = body temperature (°C)

Body temperature, as a function of ambient temperature, light intensity, and degree of melanism, was obtained from multiple regression analysis in Chapter One.

\[ T_b = 0.625T_a + 0.025R + 0.047B + 6 \]

where: \( T_b \) = body temperature (°C)
\[ Ta = \text{ambient temperature (°C)} \]
\[ R = \text{solar radiation (gm.cal/cm}^2/\text{min)} \]
\[ B = \text{melanism expressed as number of black scales} \]

Therefore, fecundity can be calculated for any butterfly in the chamber provided melanism is known. Comparison was made between the predicted fecundity of the light half of the total sample (mean black scale count=113) and fecundity of the dark half of the total sample (mean black scale count=140). Light intensity in the chamber (.4 gm.cal/cm\(^2\)/min) was used in the model, and simulation was run for ambient temperatures from 0 through 50°C. Output consisted of fecundity of dark individuals minus that of light individuals. This value equals zero at chamber temperatures at which no difference in fecundity would occur. Simulation results are presented in Figure 18. Assuming that the model is correct, ambient temperatures which would not be predicted to result in no difference in fecundity are: 1) less than 12°C, and 2) approximately 24-25°C. From my high temperature experiment, it is obvious that at 34°C and higher, survivorhip is so low as to make fecundity estimates impossible. In retrospect, therefore, the temperatures used in this experiment (12°C, 22°C, and 34°C) are not ideal. Better results would be predicted if temperatures of 17-19°C had been used as a "cold" treatment, and 28-29°C used as a "hot" treatment.

In summary, experiments would have been greatly improved if:

1. only females of known age were used.
2. higher light intensity was used.
Figure 18. Simulation results of fecundity difference between dark and light females as a function of ambient temperature. Arrows indicate temperatures used in experiments.
3. there was no soil in the cage bottom.
4. temperatures of approximately 17-19°C and 28-29°C were used.

From these data I conclude that any effect which melanism has on fecundity is minimal. This is particularly so considering the great microclimatological variance inherent in tundra habitats (Mani 1968). Oviposition experiments by Stern and Smith (1960), have shown that raising ambient temperature increases egg production by its effect on body temperature. It is interesting, however, that similar experiments by them, using increasing levels of light intensity, did not increase fecundity despite an increase in body temperature. This apparent discrepancy is not addressed by the authors. The range of body temperatures which resulted from the light intensities used was 26-32°C, which is the region of maximal egg output. Within this range body temperature has no effect on fecundity (Fig. 15a).

That males express the same amount of melanin variation as females would imply a more general explanation for the benefit of melanism in cold climates. A 10% increase in sperm by males would not convey the same selective advantage as would a 10% increase in egg production by females. Melanism would be of greater selective advantage to females, but they are no darker than males.

The apparent body temperature threshold for egg maturation of 20-22°C (Fig. 15a) would not be as great a limiting factor to these insects as the threshold body temperature for flight
of 30°C (Watt 1968). The range of ambient temperatures at which melanism is an advantage for fecundity are well below those for which it is an advantage for flight. The relative rates of egg maturation are irrelevant if these eggs cannot be oviposited, a behaviour for which flight is normally a requirement. In cold alpine and arctic habitats the probability of body temperatures reaching critical fecundity temperatures are much higher than for reaching critical flight temperatures.

I conclude therefore, that although melanism may have some effect on *Colias* fecundity at cold temperatures, it is not the major benefit for which it has been selected, and occurs only as an added bonus to the greater effect which melanism has on increasing activity under cold conditions.
MELANISM AND PREDATION

INTRODUCTION

Melanism in many insects has been demonstrated to occur in response to the need for crypsis to avoid detection by visual predators (Isley 1938, Kettlewell 1955, 1956). Within a species variation in melanism for crypsis is attributable to variation in colour of the backgrounds upon which these insects rest. The ability to select "optimum" coloured resting sites has been demonstrated for many lepidoptera (Boardman et al. 1974). Clines in melanism, if they are the result of differential predation, infer a clinal change in darkening of resting sites. The best documented case of clines of melanism occurring in response to differential predation is the peppermoth Biston betularia in Britain (Kettlewell 1955, 1956). If increased melanism of invertebrates, with increase in altitude and latitude, occurs in response to predation and the concomittant "need" to be cryptic, backgrounds on which these animals occur must also be darker at high altitudes and latitudes. This could result for one of two reasons: 1. The colour of all substrates become darker at higher elevations and further north, therefore, regardless of where the animal rests, dark individuals would be less visible. 2. Selection of an "optimal" resting site by these animals changes over altitudinal and latitudinal gradients. Dark coloured resting sites (which absorb more solar radiation) would be warmer and might be preferentially selected by butterflies at cold temperatures.
Since temperatures decrease with increase in altitude and latitude, darker resting sites should be selected at high altitude and latitude, and would result in reduced predation on darker individuals.

Sulphur butterflies of the genus *Colias* Fabricius (Pieridae), are frequently cited as examples of insects showing latitudinal and altitudinal clines of melanism (Leussler 1935; Freeman 1958; Watt 1968; Kettlewell 1973; Roland 1978). Colour variation within this group is not polymorphic, but is expressed as a continuum, and occurs both within and between species. Melanism in these butterflies is generally believed to occur in response to a need for thermoregulation (Chapter One). The majority of work on adaptive coloration in insects, however, has shown predation to be a very strong selective force (Sheppard 1975). Colour patterns which are cryptic (eg. Kettlewell 1955), aposematic (eg. Blest 1957), or mimetic (eg. Brower 1958), act to decrease rates of predation on individuals which bear them.

*Colias nastes* is a holarctic species typical of, and abundant in tundra environments (see Chapter One for species description). The wings of this insect are covered by yellow scales and black scales which together cause an overall green colour. This coloration makes them extremely cryptic on the tundra vegetation. Duration of flight activity is very much restricted in these insects due to the cold temperatures of their habitat (Chapter One). Even under favourable conditions, a large amount of time is spent basking on the ground in an
effort to raise body temperature (Watt 1968, 1969; Kevan and Shorthouse 1970). Normal basking position is with the wings folded over the back, with the melanised ventral hind wing exposed. While on the ground, these insects would be highly vulnerable to predation. If ground predation were common, any adaptation acting to reduce its intensity should be selected for.

Predation by birds on Colias butterflies can also be significant while the insect is in flight and especially when at rest (Shapiro 1974). Increased melanism can increase detection by a predator since a moving dark object is highly visible to any predator, whether it has colour vision or not (Kettlewell 1973). For non-palatable species, melanism could serve an aposematic function.

The intent of this chapter is to determine whether predation on Colias nastes acts differentially on dark and light individuals in a manner which could explain observed geographic clines of melanism. Three hypotheses were tested: 1. Because of a strong thermoregulatory requirement, Colias nastes select resting sites which are an "optimum" temperature. When ambient temperatures decrease, the chosen resting sites must be darker in colour for it to be within the optimum temperature range. Under cold conditions therefore, dark individuals would be more cryptic. 2. Ground predation on Colias nastes is most intense on light individuals at high elevation, and most intense on dark individuals at low elevation. This would imply differential detection by a visual
predator during periods when butterflies are unable to fly.  
3. Differential bird predation on dark and light Colias along the elevation gradient act to create the observed altitudinal cline of melanism.
MATERIALS and METHODS

Much of this study was carried out in conjunction with the field study of the effect of melanism on Colias activity described in Chapter One. Study site, weather records, capture methods, and quantification of melanism are therefore the same as previously described.

Rest Site Selection

Before an attempt was made to capture a specific butterfly, it was allowed to land and take up a normal resting or basking position. The exact position was noted and the insect captured while still on the ground. To obtain a measure of darkness of colour of the resting site, a Soligor Spot Sensor spotmeter was used. The amount of light reflected, from a circular area approximately three cm in diameter around the resting site used by the butterfly, was measured. Changes in solar angle and cloud cover required standardization of these measurements. A Kodak White Standard (90% reflective) was placed immediately adjacent to the resting site at the same angle as the site surface. Light reflected from this standard was measured with the spotmeter. Percent reflectance was calculated by comparing resting site reflectance to standard "white card" reflectance. Reflectance of chosen resting sites was then correlated with air temperature measured 1.5 metres above ground level.
Ground Predation

During the day, a series of butterflies was collected and their degree of melanism quantified. This series was then divided into a "dark" half and a "light" half. All individuals were marked with an identifying number on the ventral surface of the forewing. The number was totally occluded by the hingwing when the insect was at rest, thereby not affecting visual detection. In the evening, (after 20:00 MST), long after all butterfly flight activity had ceased (Chapter One, Fig. 7), collected specimens were set out in the following manner: 1. One half of the dark sample and one half of the light sample were set out on the ground or vegetation in a prescribed one-metre square area at 2480 m elevation. 2. The other half of each group was similarly set out at 2750 m elevation. At about 06:00 the following morning, long before butterfly flight activity had begun, plots were thoroughly searched for individuals which had not been preyed upon.

Bird Predation

Predation by birds was measured by recording the presence of beak marks (Shapiro 1974) on the wing margins of all Colias captured. Because butterfly wings fray with age, care was taken to only count marks which were symmetric on both left and right wings. This type of mark would reflect predation attempts on individuals at rest with wings folded over the back. Beak mark frequencies were compared between dark and light morphs at different elevation intervals.
RESULTS

Rest Site Selection

Percent reflectance of 95 daytime resting sites were measured. There was no tendency for Colias butterflies to choose darker coloured resting sites under colder ambient conditions ($r^2=0.038$, $N=95$, $p>0.05$). Darkness of resting sites was not correlated with the degree of melanism of either C. nastes ($r^2=0.004$, $N=76$, $p>0.05$), or C. meadii ($r^2=0.237$, $N=16$, $p>0.05$). No tendency for selection of "correct" backgrounds was apparent. In order for clinal melanism to provide increased protection against daytime visual predators, there would have to be some correlation between the altitudinal or latitudinal gradient, and the backgrounds on which the insects rest. Higher elevations do not provide darker coloured resting sites ($r^2=0.002$, $N=95$, $p>0.05$). Therefore, if melanism were an adaptation to reduce predation, it is not in response to coloration of selected daytime resting sites.

Ground Predation

In some cases (15), the individual specimen was completely missing at the morning observation. The remainder of the specimens recorded as missing (13) had their wings neatly cut off by the predator prior to the body being carried away (Fig. 19). These wings were still located within the experimental plot at the morning census. Since wings had been
Figure 19. Photograph of wings found remaining after predation experiment.
marked, positive identification of these individuals were made. Those specimens still present and alive had moved very little or not at all during the ten-hour period. A large difference in predation intensity occurred over the altitudinal range which these experiments were carried out (Chisquare=7.667, df=1, N=78). Essentially no loss of butterflies occurred at the lower experimental plot (Table XII).

Table XII. Number of butterflies of differing darkness present or absent at each of two elevations following one night of exposure to ground predation.

<table>
<thead>
<tr>
<th>Number of Butterflies</th>
<th>2480 m</th>
<th>2750 m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>Present</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Absent</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

\[ p>.75^* \quad p<.07 \]

*Fisher's Exact Probability Test.

At the upper site, however, almost 50% of the butterflies set out at 20:00 in the evening were gone by 06:00 the following morning, with the greatest loss occurring among lighter individuals (Table XII). Unfortunately, the identity of the predator is as yet speculative. Characteristics of the predator are: 1. visual predator 2. capable of neatly cutting wings off butterflies
3. capable of preying upon three to four butterflies per night.

Since these experiments were carried out between 20:00 and 06:00, the predator would have to be active during this period, but not necessarily restricted to it.

Bird Predation

Birds were never censused for abundance nor distribution. It was observed, however, that total number of individuals were greatest at treeline, decreasing with increase in altitude. Primary species, in approximate order of relative abundance, were: robin (Turdus migratorius), white-crowned sparrow (Zonotrichia leucophrys), hermit thrush (Hylocichla gutata), varied thrush (Ixoreus naevius), horned lark (Eremophila alpestris), water pipit (Anthus spinoletta), white-tailed ptarmigan (Lagopus leucurus), and mountain bluebird (Siulia currucoides). Of these, all but the white-crowned sparrow would be potential predators on butterflies.

For the entire sample, there is no difference in beakmark frequency between dark and light groups (Chisquare = .386, df = 1, N = 791). When the altitudinal gradient is divided into four subplots, equal incidence of beak marks was recorded from each (Table XIII). If bird predation is greatest near treeline, and beak marks are an accurate measure of relative predation intensity, movement of butterflies along the altitudinal gradient is sufficient to mask any real predation gradient.
Table XIII. Frequency of beak marks on *Colias nastes* wings over the elevation gradient of the study area.

<table>
<thead>
<tr>
<th>Elevation (m)</th>
<th>Number of Butterflies</th>
<th>Elevation (m)</th>
<th>Number of Butterflies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with beak marks</td>
<td>without beak marks</td>
<td>proportion</td>
</tr>
<tr>
<td>2400-2500</td>
<td>8</td>
<td>44</td>
<td>.154</td>
</tr>
<tr>
<td>2500-2600</td>
<td>13</td>
<td>86</td>
<td>.131</td>
</tr>
<tr>
<td>2600-2700</td>
<td>86</td>
<td>487</td>
<td>.150</td>
</tr>
<tr>
<td>2700-2800</td>
<td>9</td>
<td>42</td>
<td>.176</td>
</tr>
</tbody>
</table>

Chi-square = 0.558, df=3, p>.90

Beak marks are in fact a measure of successful escape, a fact which confounds their usefulness as a measure of predation intensity (Shapiro 1974). At most elevations, differential predation on dark or light morphs is not apparent (Table XIV).

Table XIV. E elevational change in beak mark frequency for dark and light *Colias nastes*.

<table>
<thead>
<tr>
<th>Elevation (m)</th>
<th>Proportion with beak marks</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
<td>Dark</td>
</tr>
<tr>
<td>2400-2500</td>
<td>.140</td>
<td>.222</td>
</tr>
<tr>
<td>2500-2600</td>
<td>.110</td>
<td>.167</td>
</tr>
<tr>
<td>2600-2700</td>
<td>.169</td>
<td>.134</td>
</tr>
<tr>
<td>2700-2800</td>
<td>.042</td>
<td>.296</td>
</tr>
</tbody>
</table>

*Fisher's Exact Probability Test.

At the highest altitude interval, however, beak mark frequency is significantly higher on darker coloured *C. nastes*.
DISCUSSION

Darkness in colour of daytime rest sites shows no relationship with the degree of melanism of *Colias nastes* nor with the elevational gradient.

Intensity of ground predation, however, has a very strong elevational component. The higher rate of loss of individuals at high altitude is most intense on lighter coloured individuals. Escape flight by butterflies is impossible at low temperatures and/or low solar radiation. Ground predation would therefore not only be important at night, but anytime weather is cool or cloudy. Shapiro (1974), found that the greatest incidence of observed predation by birds on pierids (including *Colias eurytheme*), was while insects were at rest on vegetation. Any mechanism which might reduce this predation intensity, should be strongly selected for. Dark individuals, in my experiments suffered the lowest predation at high altitude, but had the highest beak-mark frequency at high elevation. These facts could be related if the predator is a bird, active in the morning and evening, and if beak marks are considered a measure of successful escape not predation. Since total beak mark frequency is even along the altitudinal gradient, an even distribution of avian predators is implicated. Absence of crepuscular or night predation at low elevation in the predation experiments implies that, if the predator is a bird, it is not the only species taking butterflies during the day. Different predators might well be involved in the two measures of predation.
Crypsis

Resting sites are not darker at high altitude. However, since predation occurs during periods when the darkness or length of shadows are greatest, refuges for darker Colias might exist. Certainly shadows would be just as dark or long at low elevation, but the apparent altitudinal distribution of the predator involved, renders this immaterial.

Aposemasis

There is some indication that pierids (including Colias eurytheme) might be non-palatable (Shapiro 1974). Larval food plants include locoweed (Oxytropis spp.) which may provide a chemical defense for the adults. The usefulness of melanism as a means of increasing visibility of the insect while at rest is unlikely, especially in view of the insect's extreme crypticity.

I suggest that the predator might be a small mammal, a carabid beetle, or a crepuscular bird. Exact determination of the predator would require a trapping program or direct observation of predation. Of the bird species noted in the area, white-tailed ptarmigan is the only one consistently observed at the highest elevation. In both years at least one hen with brood was seen in the vicinity of the upper sample-site. I have observed ptarmigan taking insects while feeding. Least Chipmunk (Eutamias minimus) is the only small mammal noted at the upper site, but ranges down to treeline. It is not likely, therefore, to be involved in the differential predation observed along the altitudinal gradient. Carabid beetles are
active at night, and would take insects on the ground. If the predator involved is a carabid beetle, its distribution would be restricted to higher elevations.

For the observed latitudinal cline of melanism to be the result of differential predation would require that the distribution of the same or similar predators be arranged along the latitudinal gradient in the same way as the altitudinal gradient. In this study, the degree of melanism does appear to have a marked, although statistically non-significant effect on the rate of ground predation in the evening, and at night. The gradient of predation is very steep, but the observed cline of melanism within the site is very weak. Movement of butterflies is great enough to swamp the effect of predation. From my experiments and observations, there is no apparent balancing predation on dark individuals at low elevation. Predation experiments along a latitudinal gradient would be very useful to determine its importance as a general selective force for melanism in these insects. Until further experiments are carried out to confirm the differential predation indicated, and to identify the predator involved, it is not possible to conclude that predation accounts for the broad geographical clines of melanism with increased altitude and elevation, observed both within and between species. Light Colias do not possess an advantage in reducing predation at low elevation. For light individuals to be present at all, would require the demonstration of some other advantage for light coloration, or a "cost" of being melanistic.
Predation has been demonstrated to be a very important factor in determining adaptive coloration of insects. My results indicate that predation may be important in causing the observed altitudinal cline of melanism, and should be pursued further.
GENERAL CONCLUSION

Using colour variation among individuals from a single population of Colias nastes butterflies, I have tested three alternative hypotheses regarding the importance of melanism in these tundra insects. I have attempted to provide empirical evidence, from laboratory and field observation and experiment, to demonstrate the mechanism by which melanism is adaptive.

Melanism does convey some advantage by reducing the incidence of nocturnal ground predation at high elevations. A balancing advantage does not, however, exist for light coloured butterflies at low elevation. That differential predation is acting to cause the altitudinal and latitudinal clines of melanism both within and between several species would require that the distribution of the predator (or predators) be consistently organised parallel to the clines of melanism. The very sharp gradient of predation pressure and almost non-existent gradient of melanism at this site would indicate melanism to be serving some other function. Until identification of the predator is determined and its distribution ascertained, it is not possible to conclude that crypsis is a general explanation for arctic and alpine melanism.

Body temperature is significantly affected by the darkness of Colias butterflies. Physiological processes and behaviour can be ordered with respect to increasing body temperature (Fig. 20). Fecundity is increased by melanism, but only at low temperatures. There is some indication that at high ambient
Figure 20. Physiology and behaviour of *Colias* butterflies as a function of body temperature. (Developmental threshold from Ae 1958, egg maturation from Stern and Smith 1960, flight thresholds from Watt 1968).
Egg production upper limit
Flight upper threshold
Flight lower threshold ♀
Flight lower threshold ♂
Egg production peak
Egg production lower limit
Developmental threshold
temperatures light females have a higher fecundity. Although fecundity differences are small, there appears to be a balanced advantage for both dark and light individuals with respect to fecundity over a temperature range.

Great differences occur in duration of activity among Colias butterflies with differing degrees of melanism. The darkest individuals of this population are active up to 55% longer than the lightest individuals. This results in a much greater amount of time available for feeding, mate locating, and ovipositing; all of which are determinants of fecundity. There is some indication that light butterflies are able to remain active (flying) under the hottest sunny conditions, during which dark individuals must remain inactive. In terms of duration of activity, therefore, there appears to be a balanced advantage for both dark and light butterflies.

Alpine and arctic tundra are typically cold (monthly mean daily temperature never exceeds 10°C) and have a short growing season. Time is therefore a major constraint on any ectotherm in these habitats. Body temperature threshold for egg production (Fig. 20) of 20°C, would be easily attained compared to that for flight (30-35°C). Any differences in fecundity caused by melanism would be overshadowed by the ultimate requirement of reaching the temperatures necessary to successfully fly (feed, mate, oviposit). This is especially true considering Watt's (1968) finding that females require a body temperature of 35°C to fly, compared to the male requirement of 30°C. Although slightly increased fecundity may result from
darkening in colour, the ultimate advantage of melanism is the increased absorption of visible radiation to raise body temperature to the critical flight threshold. Although this has been suspected in the past, this is the first empirical demonstration that it is so.

If, throughout the geographical range of Colias nastes, the mean daily temperature never exceeds 10°C, why are all populations not equally dark along the latitudinal gradient? The reason, I think, is that although mean temperatures are similar, diurnal temperature fluctuation in alpine areas is far greater (Remmert and Wunderling 1969, LeDrew and Weller 1978). This results in frequent, but short, periods of very high temperature in alpine meadows (Mani 1968). Under these conditions, a very definite advantage for light individuals would be envisaged. Cold temperatures tend to merely slow activity and metabolism down, and can be endured by insects for long periods. High temperatures can be endured for only relatively short periods, and the upper lethal threshold is usually very sharp (Heinrich 1981). It is possibly for these reasons that the mechanisms for the advantages of being light (heat tolerance) are so difficult to observe as compared to those for being dark (cold tolerance).

On a global scale, Colias butterflies are primarily inhabitants of arctic and alpine habitats (Hovanitz 1950). Their association with these habitats has probably been very long, and they have certainly evolved with it. There is no a priori reason to consider these animals offshoots of temperate
groups, living at the extreme limits of their tolerances by the
evolution of special adaptations such as melanism. A more
plausible description of their biogeography, might be that
melanism is the norm for these and many other arctic
invertebrates; the clinal change is simply an increase in
lighter pigments with decrease in altitude and latitude. This
implies that the environmental pressure to be melanistic is
relaxed in southern latitudes or at lower elevations, or that a
specific advantage for light colour exists in these regions. I
therefore feel that future work might best be spent on
determining the advantage of light colour in southern areas and
at low elevation.


Walsingham, Lord 1885. On some probable causes of a tendency to melanic variation in Lepidoptera of high altitudes. Trans. Yorks. Nat. Union, 113-140 (original reference not consulted).


