

## THERMOREGULATION BY WINTER-FLYING ENDOTHERMIC MOTHS

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### SUMMARY

Noctuid moths of the subfamily Cuculiinae fly in any month of the winter in the north-eastern United States when air temperatures are at least 0°C. At lower temperatures they hide under leaf litter rather than perching on trees like many summer-flying noctuid moths. Like moths of similar mass and wing-loading that fly in the summer or that reside in tropical regions, the nocturnal cuculinids fly with a thoracic temperature of 30–35°C. The generation and maintenance of such high thoracic temperatures at low air temperatures depends on (1) the ability to begin shivering at very low muscle temperatures, (2) a thick insulating pile, and (3) counter-current heat exchangers that retard heat flow to the head and to the abdomen, respectively.

### INTRODUCTION

Endothermy is well-known for various taxa of moths (see for example Heinrich, 1971*a,b*, 1974; Heinrich & Bartholomew, 1971; Bartholomew, 1981; Bartholomew & Heinrich, 1973; Casey, 1981; Casey, Hegel & Buser, 1981; Casey & Joos, 1983). Endothermy results from shivering in pre-flight warm-up (Kammer, 1968) and from heat produced as a by-product of flight (Heinrich & Casey, 1973; Kammer, 1981). For many species so far studied a limitation for continuous flight is often an excess of heat production, and physiological mechanisms for dissipating heat have evolved (Heinrich, 1970; Kammer, 1981).

It has not been clear if the physiological patterns of thermoregulation in moths so far elucidated are the result of phylogeny or ecological adaptation. Part of the difficulty in differentiating between the two alternatives is that most of the data on thermoregulation in moths are from species that may fly over a similar range of ambient temperature; species that have been investigated from temperate regions fly at relatively high temperatures in the spring or summer. Many tropical species have been examined (Bartholomew & Heinrich, 1973; Bartholomew & Casey, 1978; Bartholomew & Epting, 1975), but some of these occur at high elevations.

The purpose of this study is to provide insights into ecological adaptation by examining the thermoregulatory behaviour and physiology of the Cuculiinae, a group of noctuid moths from northern temperate regions that have a seasonally

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reversed life cycle. In the *Cuculiinae* the larvae aestivate through the summer and the adults are active in the winter (Franklemont, 1954; Schweitzer, 1974).

#### MATERIALS AND METHODS

Measurements of head, thoracic and abdominal temperature during pre-flight warm-up were taken with 0.001 mm diameter duplex copper-constantan thermocouples as described previously (Heinrich & Mommsen, 1985). Temperatures were recorded every 10 s or less with a Honeywell potentiometric recorder.

Body temperatures of moths in the field, and of those after flight or pre-flight warm-up in a temperature-controlled room, were taken with a Bailey copper-constantan microprobe thermocouple in a hypodermic needle (Type MT-2911, time constant = 0.025 s, needle diameter = 0.33 mm). Temperatures were read to the nearest 0.1 °C on a Bailey Bat-12 digital thermocouple thermometer. The temperatures were referenced to a US Bureau of Standards thermometer.

Unlike many other insects, the moths often stopped beating their wings immediately upon being captured by net (particularly at low ambient temperature,  $T_a$ ). This behaviour could result in an error in the measurement of thoracic temperature ( $T_{th}$ ), since 3–4 s of cooling elapsed before a captured moth was stabbed through the netting to measure its body temperature.

The magnitude of the error could be estimated. For example, mean  $T_{th}$  ( $N = 14$ ) of moths captured by net at  $T_a = 7^\circ\text{C}$  was  $26.4^\circ\text{C}$ . However, moths grabbed during flight (by a wing or the abdomen) (when they were fluttering against a lighted screen) and measured within 1 s had  $T_{th}$  values (at  $T_a = 4.5^\circ\text{C}$ ) averaging  $4.7^\circ\text{C}$  ( $N = 14$ ) higher than net-caught moths.

When abdominal temperature ( $T_{ab}$ ) was measured first (which required approximately 1 s) followed by  $T_{th}$ ,  $T_{th}$  averaged  $2.0^\circ\text{C}$  lower ( $N = 14$ ) than when  $T_{th}$  was measured first. I conclude that without implanted thermocouple probes no more than one body temperature could be reliably taken for any one moth, and that  $T_{th}$  measurements from net-captured moths are not reliable because of the animals' small size and habit of stopping wingbeats immediately after being captured by net. Therefore, body temperatures of flight-captured moths all refer to single measurements of animals captured by hand (gloved with light leather) so that  $T_{th}$  or  $T_{ab}$  could be measured within 1 s after capture.

Oxygen consumption was measured with an S-3A Applied Electrochemistry oxygen analyser system capable of measuring to 0.001 %  $\text{O}_2$ . Measurements were made using a closed system of 6.0 ml. The excurrent air was dessicated and passed through a  $\text{CO}_2$  absorbant before being pumped to a Model N-37M sensor. Readings were taken every 5 min for 1.5 h or until the rate of decrease of oxygen concentration became linear with time. The moths were provided with filter paper so that they had secure footing and would come to rest. If the moths walked then the data were discarded. Temperatures around the moths were varied by immersion of the respirometer into a water bath, and a thermocouple was placed within the respirometer to monitor  $T_a$ .

Temperatures were visually recorded with an AGA Infrared System measuring in 10 distinct colours. The temperature range of any one colour depended on the total scale of temperature assessed. Moths were placed over ice and allowed to warm up while the temperatures were recorded on a television screen and photographed with a polaroid camera at various intervals throughout the warm-up cycle and/or after subsequent flight.

Cooling constants were measured with 0.001 mm diameter thermocouples near the centre of the thorax, with  $T_{th}$  (and  $T_a$  next to the moth) read out every 10 s or less with a Honeywell potentiometric recorder. The moths were heated with warm air to approximately 45°C and then placed facing a laminar air flow at room temperature (near 20°C). Various wind speeds in the wind tunnel were created by variously sized baffles, and air speed was determined with a hot-wire anemometer. After steady-state cooling had been achieved, when the  $\log(T_{th} - T_a)$  became linear with time, the linear regression of  $T_{th}$  on time was used to determine the cooling constants as previously described (Casey, 1981).

The coupling between head and thoracic temperatures in moths was measured by locally heating the thorax and then simultaneously monitoring head and thoracic temperature. In these experiments the thorax was heated by inserting a 1.5 mm diameter wire into the abdomen and heating the wire several centimetres from the moth with a soldering iron until  $T_{th}$  was near the  $T_{th}$  value observed in live moths.

Anatomy was determined from dissections under a light microscope.

Moths used in the laboratory were collected locally near Burlington, Vermont, after luring them to trees painted with either a solution of fermented apples, bananas and beer, or with honey diluted in water. The latter mixture attracted the most moths. Moths were maintained in closed jars with moist leaves in the laboratory at -3 to -7°C (for up to 3 weeks) until use.

## RESULTS

### *Flight activity*

Many of the moths were feeding on the flowers of witch hazel (*Hamamelis virginiana*) at the end of October after the foliage of the trees had fallen. There was usually no moth activity from December to February. However, in three winters they flew regularly before the last of the major snowstorms of the year. For example, on March 25, 1981, dozens of moths were flying at dusk, but then on April 11 a large snowstorm curtailed moth activity as temperatures declined to -10°C. Similarly, on March 18, 1983, one person captured 25 moths of numerous species in 1 h, but two major snowstorms occurred subsequently, on March 26 and April 20. In 1984 moths were captured on February 13, the first day of an early thaw that exposed bare patches of ground on south-facing slopes. The winter of 1985-1986 brought unusual amounts of snow, but despite a total cover of 0.5-1.5 m of compact snow at the beginning of March, occasional moths (*Eupsilia morrisoni*) were flying on the first nights that temperatures reached 5°C. Activity for most species of winter moths

ended near May 1, when the first migrant warblers (predators on moths) returned and the first leaf buds started to unfurl.

In general, moth activity was greatest in the evening. Temperatures usually dropped rapidly through the night, and moths that fly in the evening often face potentially lethal temperatures a few hours later. Given their lack of exceptional cold-hardiness (B. Heinrich & J. G. Duman, unpublished observations), the moths must rely on selection of appropriate microhabitats to keep from freezing to death.

#### *Microhabitat selection*

Moths should be protected from freezing while under the leaves of the forest floor. During the winter, after a snow cover of 10 cm, I measured temperatures of  $-2$  to  $0^{\circ}\text{C}$  under the leaves on the ground at  $T_a$  down to  $-25^{\circ}\text{C}$ . If the moths hide on or under the bark of tree stems they could be subjected to  $T_a$  close to  $-30^{\circ}\text{C}$ . To determine whether or not moths after flight seek colour-matching on trees or shelter from potential cold, 173 moths were released into an  $3\times3\times3$  m enclosure (on the evening of April 8, 1983) that contained two vertical tree stems (diameter = 15–30 cm) each of white birch, cherry, black locust, sugar maple, elm and pine. The floor of the enclosure was the forest floor itself, covered with natural leaf litter (primarily sugar maple) to a depth of at least 15 cm. On the next morning the tree stems and the leaf litter were examined for moths. The following list gives the numbers of individuals of each species put into the enclosure, and the number recovered (in parentheses, u indicating under leaves, and o indicating on tree stems) during 6 h of search: 74 *Eupsilia morrisoni* (9u, 2o), 29 *E. vinulenta* (1u), 16 *Lithophane patefacta* (1u), 13 *L. innominata* (2u), 10 *L. hemina* (3u, 2o), 9 *L. grotei* (1u, 1o), 6 *E. tristigmata* (4u), 6 *Pyroferra* sp. (1u), 5 *Orthosia hibisci* (3u), 5 *Xylena currimaculata* (1o). In summary, at least 25 moths were under the leaves and six were on tree stems. However, since it was easy to locate the moths on the trees (they were in bark cracks or on stems touching netting) and very difficult to locate them in leaf litter (where many had crawled into curled-up dead leaves), it can be presumed that most of the 142 unrecovered moths could still have been buried in the loose leaf litter.

#### *Pre-flight warm-up*

Like other moths, the cuculinids shivered in pre-flight warm-up. Wing positioning of shivering moths was similar to that of resting moths. However, in shivering moths the details of the markings on wings looked slightly fuzzy or out of focus to the human eye, indicating that the amplitude of the wing vibrations was very low, possibly no more than about 1 mm.

The moths were unusual in comparison with all other insects so far examined in being able to initiate shivering at ambient (and body) temperatures  $<10^{\circ}\text{C}$ . Indeed, I observed warm-up from  $T_a$  as low as  $-2^{\circ}\text{C}$ . However, warm-up from  $T_a$  as low as  $0^{\circ}\text{C}$  was rare. To ensure observing warm-up at  $T_a$  near  $0^{\circ}\text{C}$  several dozen moths that had been kept at lower  $T_a$  for several days were usually subjected simultaneously to the appropriate conditions (dim illumination) for warm-up. At  $T_a$  near  $10^{\circ}\text{C}$ ,

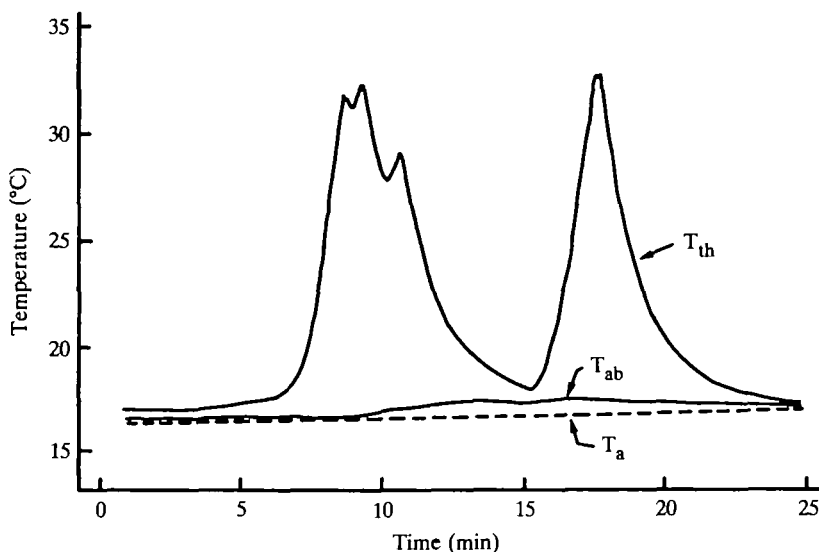


Fig. 1. Thoracic and abdominal temperatures during two consecutive warm-ups and cool-downs of a *Eupsilia morrisoni* within 30 min after it had been maintained at  $-7^{\circ}\text{C}$  for 2 weeks. Note that there is little or no increase in abdominal temperature ( $T_{ab}$ ) either during pre-flight warm-up or during subsequent cool-down.  $T_{th}$ , thoracic temperature;  $T_a$ , ambient temperature.

however, almost all the moths readily warmed up under relatively slight provocation (i.e. within several minutes of being taken out of the refrigerator).

Although the moths were often very reluctant to initiate warm-up at low  $T_a$ , they usually shivered without pause after starting (Figs 1, 2). Warm-up usually proceeded unbroken until a temperature suitable for flight was attained. Total duration of warm-up ranged from 20–25 min at near  $0^{\circ}\text{C}$  to approximately 1 min at  $20^{\circ}\text{C}$  (Fig. 2). Moths that were restrained at low  $T_a$  by thermocouple leads usually cooled again immediately after making a brief attempt to fly. Especially at the higher  $T_a$  values ( $>10^{\circ}\text{C}$ ), restrained moths often went through several warm-up cycles in rapid succession (Fig. 1).

The increase of thoracic temperature as a function of time was linear at high  $T_a$  values and relatively curvilinear at low  $T_a$  values (Fig. 2). The final  $T_{th}$  at the initiation of flight ranged from 26 to  $35^{\circ}\text{C}$  (Fig. 3), even within one genus (*Eupsilia*), but it was independent of  $T_a$  from  $-3^{\circ}\text{C}$  to  $28^{\circ}\text{C}$  in *Eupsilia* spp. as well as in *Lithophane* spp. and *Orthosia* spp.

Abdominal temperature in insects typically remains independent of thoracic temperature, although generally increasing to several degrees above  $T_a$ . The cuculid moths were unusual in showing either no or very little increase in  $T_{ab}$  during pre-flight warm-up (Fig. 1). Of 12 pre-flight warm-ups from  $14^{\circ}\text{C}$  where  $T_{th}$  and  $T_{ab}$  were simultaneously measured, the temperature in the ventrum of the second abdominal segment increased on average by only  $0.4^{\circ}\text{C}$ . In addition, there was at no time any increase in  $T_{ab}$  during post-flight (or post-warm-up) thoracic cooling. On

the contrary, of 22 cool-downs where  $T_{th}$  and  $T_{ab}$  were simultaneously measured, there was on average a slight decrease ( $0.1^{\circ}\text{C}$ ) of  $T_{ab}$  during the thoracic cooling.

Throughout any one pre-flight warm-up the moths necessarily lose heat convectively from the thorax. This passive heat loss is a direct function of temperature excess ( $T_{th} - T_a$ ), and it can be calculated (see Heinrich & Bartholomew, 1971) from the rate of change in  $T_{th}$  of a heated dead moth. Comparing the rates of heat production (Figs 4, 5) and heat loss as previously calculated (Heinrich & Bartholomew, 1971) of the moths at different  $T_a$  and  $T_{th}$  during warm-up shows the heat storage relative to heat loss (Fig. 5). For example, during warm-up from  $T_a = 0^{\circ}\text{C}$ , moths shivering with a  $T_{th}$  of  $5^{\circ}\text{C}$  ( $5^{\circ}\text{C}$  above  $T_a$ ) produce approximately  $2.2 \text{ cal g}^{-1} \text{ thorax min}^{-1}$  (Fig. 4). However, they are losing  $1.8 \text{ cal g}^{-1} \text{ thorax min}^{-1}$  due to

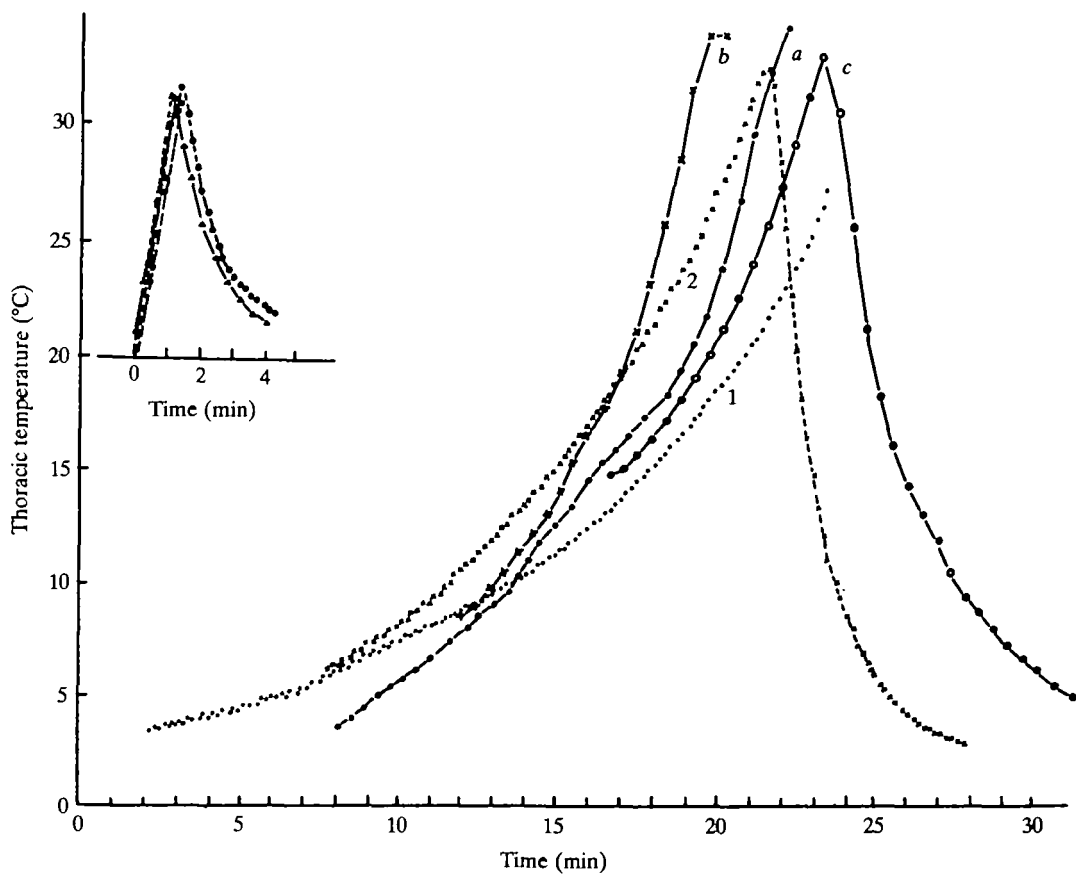


Fig. 2. Thoracic temperatures during five pre-flight warm-ups and two cooling curves at ambient temperature ( $T_a$ ) =  $0^{\circ}\text{C}$  (right) and four warm-up and two cooling curves at  $T_a = 20^{\circ}\text{C}$  (upper left). 1 and 2 indicate first and second warm-up of a *Eupsilia tristigmata* (193 mg), while a, b and c indicate first, second and third warm-ups of a *Eupsilia morrisoni* (192 mg). Warm-ups at  $20^{\circ}\text{C}$  are from the same two moths. Initial portions of some of the curves (right) are not included when temperature fluctuations occurred due to the process of implanting thermocouples or due to incomplete cool-down from the previous warm-up.

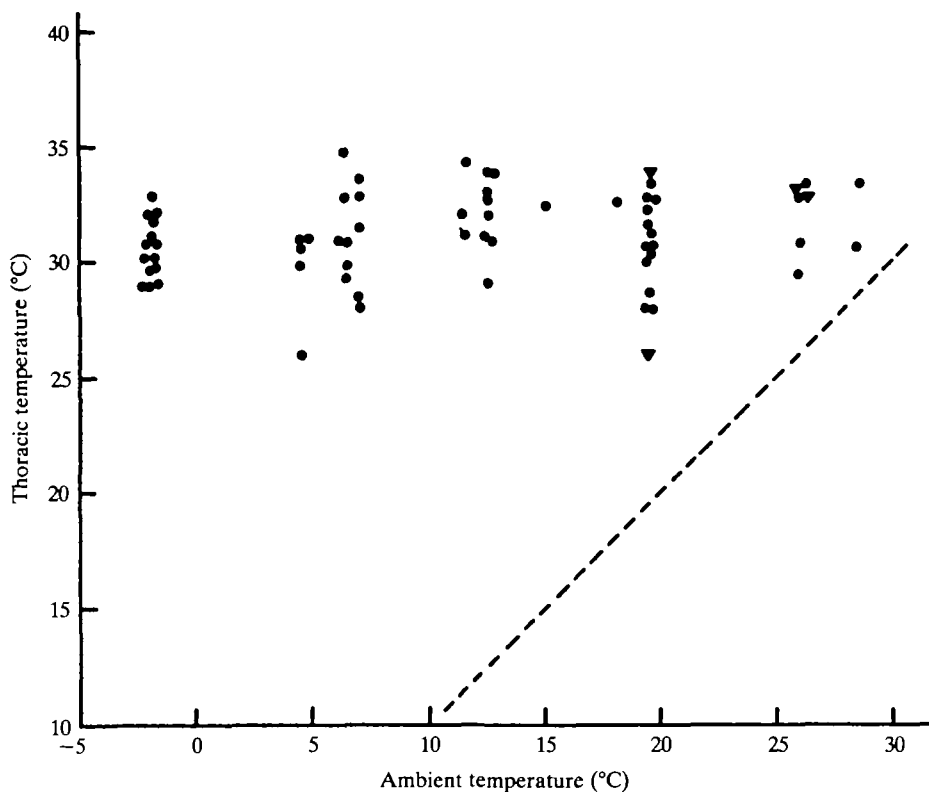


Fig. 3. Thoracic temperatures after pre-flight warm-up in *Orthosia* ( $\blacktriangle$ ), *Eupsilia* ( $\bullet$ ) and *Lithophane* ( $\circ$ ). Dashed line shows the isothermal, where thoracic equals ambient temperature.

convection. In contrast, during warm-up from 20°C, moths with a  $T_{th}$  5°C above  $T_a$  produce approximately  $11 \text{ cal g}^{-1} \text{ thorax min}^{-1}$ , even though they are still losing only  $1.8 \text{ cal g}^{-1} \text{ thorax min}^{-1}$  (Fig. 4). Thus, during warm-up from 5°C most (82 %) of the heat produced is lost through convection without increasing  $T_{th}$ , while at 20°C only 15–20 % of the heat generated throughout any one warm-up is lost to convection.

As in other moths, maximum observed rates of energy expenditure during pre-flight warm-up were independent of  $T_a$  and strictly dependent on  $T_{th}$  (Fig. 5), as would be predicted if the moths warm up as rapidly as they can without stopping, thereby minimizing the energy cost of any one warm-up. The rate of energy expenditure on  $T_{th}$  extrapolates to zero at near  $-1$  to  $-2^\circ\text{C}$ , confirming that the lowest  $T_a$  (and  $T_{th}$ ) at which warm-up was observed is indeed the lowest temperature at which the moths could theoretically warm up.

### Flight

Unlike other moths so far examined, the cuculinids were able to remain in continuous free flight at  $T_a$  near 5°C while maintaining thoracic temperatures near

30–35°C (Fig. 6). Using 2 min of uninterrupted flight as the criterion for 'continuous' flight ( $T_{th}$  stabilized after <1 min of flight), the moths flew continuously only between  $T_a$  of 4°C and at least 13°C. They also flew at -3°C. However, at  $T_a < 2^\circ\text{C}$  they interrupted flight to land and shiver, before resuming flight. Moths in intermittent flight at -3°C maintained  $T_{th}$  between 28.5 and 33°C, as at the initiation of flight following pre-flight warm-up.

At  $T_a$  from 4 to 13°C, where flight was continuous, the  $T_{th}$  was nearly indistinguishable from that at the end of pre-flight warm-up. At  $T_a$  of 19°C and above, however, all the  $T_{th}$  values of flying moths were greater than at take-off suggesting that overheating was occurring. At 19°C some of the moths stopped flying in under 2 min, presumably to prevent further heating as  $T_{th}$  approached 39.5°C.

The moths also flew at 26–29°C. However, most of these moths flew for less than 1 min before stopping, and all of them had  $T_{th}$  values of 38–40°C. These data indicate that the moths attempt to achieve a  $T_{th}$  during flight between a minimum of 29°C and a maximum of 39°C and that thermoregulation involves alternating flight and shivering at  $T_a < 4^\circ\text{C}$  and alternating flight and cool-down at  $>19^\circ\text{C}$ .

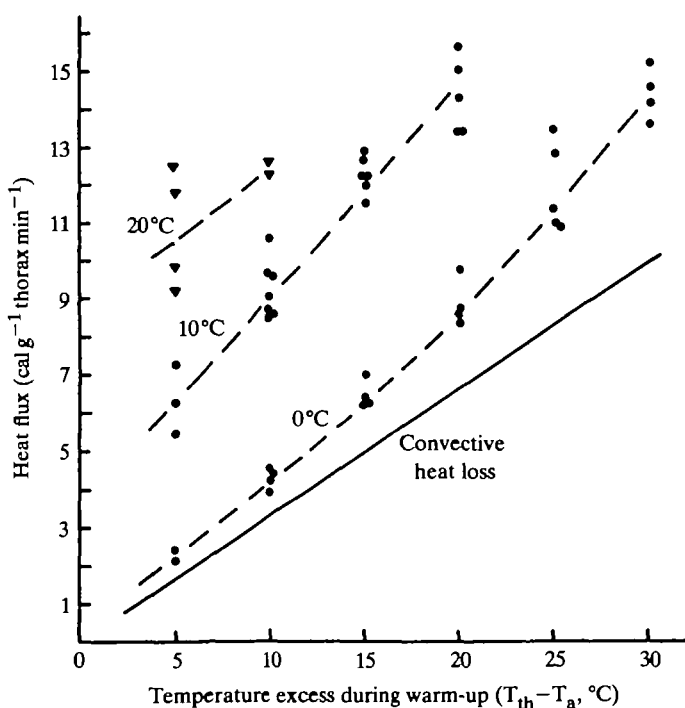


Fig. 4. Rate of energy expenditure during pre-flight warm-up from  $T_a = 0^\circ\text{C}$  (●),  $10^\circ\text{C}$  (○) and  $20^\circ\text{C}$  (▽), calculated from the temperature increase during uninterrupted warm-up and from the passive rate of thoracic cooling (in still air) at the respective temperature excess ( $T_{th} - T_a$ ), as determined from recently killed animals. The collected data are from one *Eupsilia tristigmata* and three *E. morrisoni* (thoracic masses, 38–42 mg).



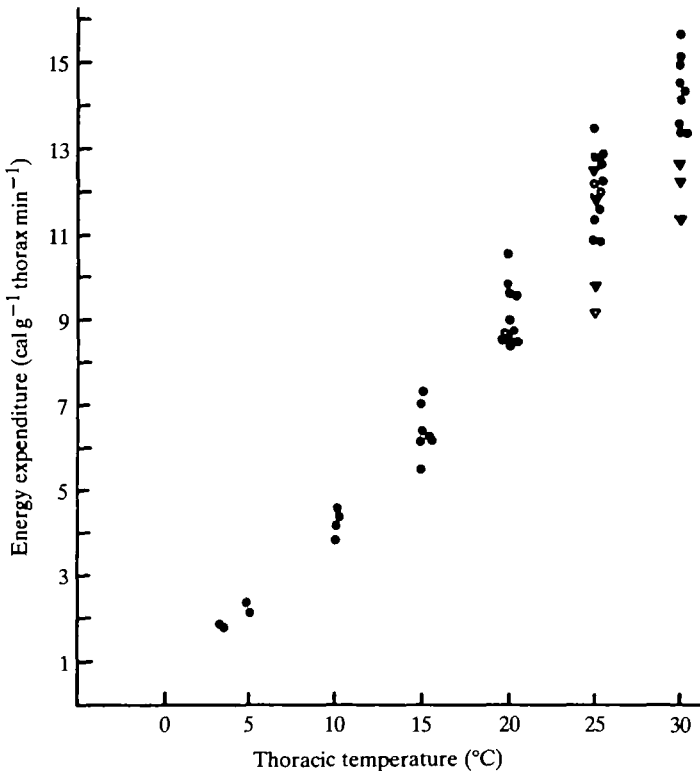


Fig. 5. Calculated energy expenditure (from warm-up curves) of *Eupsilia* during pre-flight warm-up at ambient temperatures of 0°C (●), 10°C (○) and 20°C (▽) as a function of temperature excess ( $T_{th} - T_a$ ).

Abdominal temperatures during flight were low, and the mean abdominal temperature excess ( $T_{ab} - T_a$ ) was nearly identical at 5, 15 and 23°C (Fig. 6). 5°C was near the lower limit for flight, and at  $T_a = 23^\circ\text{C}$  the moths stopped flying because of heat prostration. If the abdomen were used to dissipate heat, then the  $T_{ab}$  excess should increase at high  $T_a$ . The present results show that the abdomen is well-insulated from the thorax, and it is not used as a sink or radiator to dissipate heat.

#### *Foraging and thermoregulation*

In the field the moths began to fly at dusk, and in the winter they were attracted to wounds on trees from which sap was seeping and to bait applied to tree stems (see Materials and Methods). Feeding durations were highly variable inasmuch as they presumably depended on numerous factors such as crop capacity, fluid viscosity and the amount of spreading of the bait over the bark. No records were kept of feeding duration in the field, but my impression was that feeding durations were almost always at least several minutes.

*Eupsilia* and *Lithophane* moths captured in the field ranged in mass from approximately 100 to 210 mg. However, thoracic mass was usually within 3–4 mg of 40 mg, and the large range in body mass could be a reflection of crop contents. I offered

three *Eupsilia morrisoni* weighing 160.5, 153.1 and 141.0 mg a solution of 35 % sugar (honey and water) and at the end of feeding they had taken up 107, 102 and 82 mg. Three *E. devia* weighing 99, 94 and 109 mg took up 54.3, 89.0 and 87.5 mg of the sugar solution. Crop content alone can therefore account for as much as 94.5 % of total body mass.

A moth that stops flight has the choice of shivering to keep in flight readiness (and using its energy reserves), of immediately cooling back to  $T_a$ , or of shivering and maintaining an intermediate thoracic temperature. Tethered moths that had attained a  $T_{th}$  suitable for flight sometimes maintained an elevated  $T_{th}$  by intermittent shivering for up to about 30 min (Fig. 7), although they usually cooled immediately after a short flight.

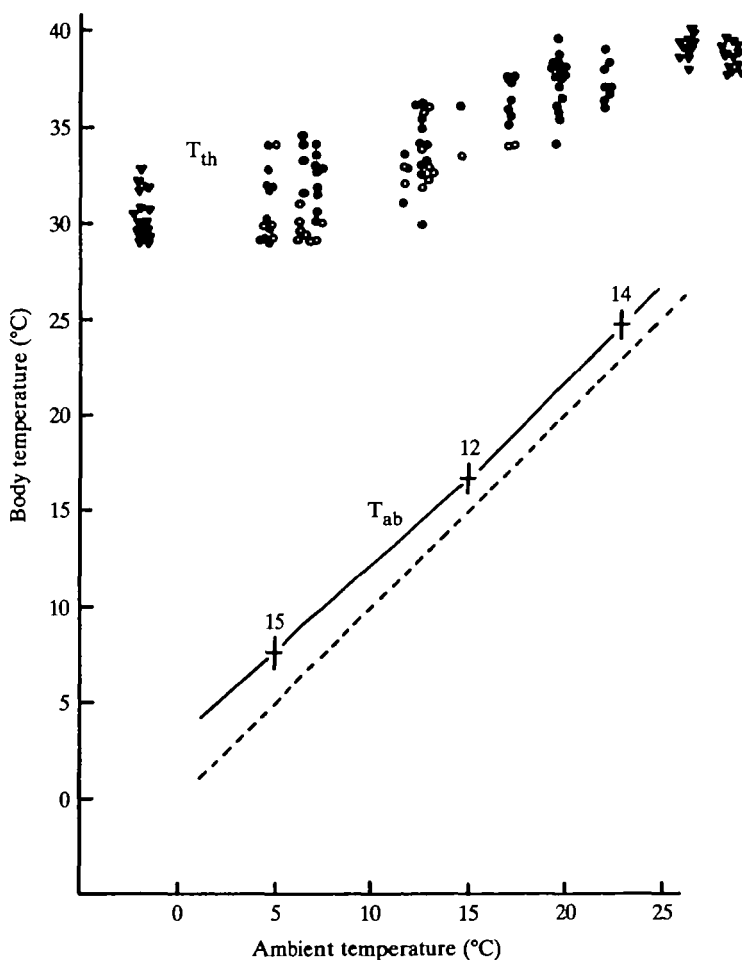


Fig. 6. Thoracic ( $T_{th}$ ) and abdominal ( $T_{ab}$ ) temperatures of cuculinid moths in flight as a function of ambient temperature in a temperature-controlled room. ●, *Eupsilia*, ○, *Lithophane*. Data for  $T_{ab}$  include mean  $\pm$  1 s.d. and *N*. Triangles show  $T_{th}$  of moths that stopped and restarted flight (i.e. flight durations were <2 min). Dashed line indicates the isothermal, where body equals ambient temperature.

In the field, feeding moths also allowed their  $T_{th}$  to fluctuate, and they generally did not attempt to regulate  $T_{th}$  while perched. At  $T_a$  near 6°C approximately half of the moths at any one time, that were lapping sugar solution, were not shivering but at least 80 % were shivering at  $T_a = 13^\circ\text{C}$  and  $17^\circ\text{C}$  (Table 1). The  $T_{th}$  values of shivering moths (at  $T_a = 6^\circ\text{C}$ ,  $13^\circ\text{C}$  and  $17^\circ\text{C}$ ) averaged 23–24°C, ranging from near  $T_a$  to 29°C (Table 1). The  $T_{th}$  of most of the non-shiverers was within 1–2°C of  $T_a$  although some, that were probably just starting to cool, had  $T_{th}$  values up to 10°C

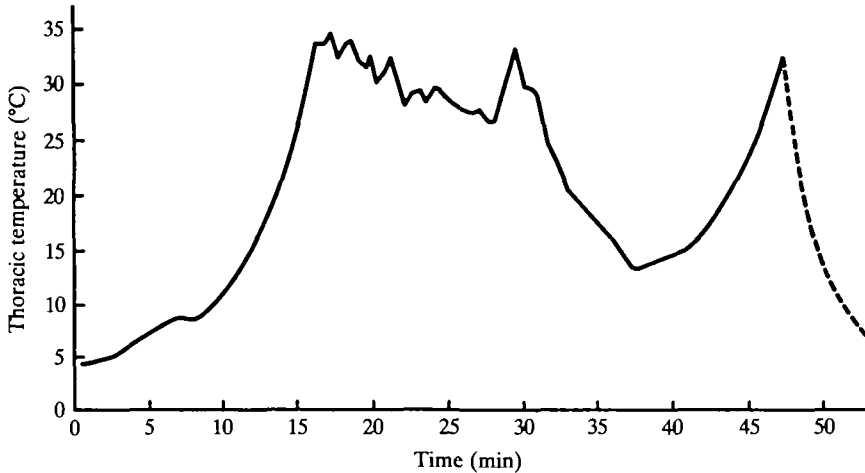


Fig. 7. Thoracic temperature of a tethered *Eupsilia morrisoni* (192 mg) at an ambient temperature ( $T_a$ ) of 0°C. Dashed line indicates passive cool-down.

Table 1. Thoracic temperatures ( $^{\circ}\text{C}$ ) and behaviour of winter moths disturbed at sugar bait (dilute honey, 50 % sugar) at three ambient temperatures

		Ambient temperature					
		6.0°C		13°C		17°C	
		$T_{th}$		$T_{th}$		$T_{th}$	
		No.	( $\bar{X}$ , range, S.D.)	No.	( $\bar{X}$ , range, S.D.)	No.	( $\bar{X}$ , range, S.D.)
Shivering	47 %			95 %		81 %	
Fly	16		22.8, 15.5–28.0, 3.1	40	23.9, 15.0–24.4, 3.15	26	24.4, 20.0–29.0, 2.5
Drop	13		8.8, 6.7–12.4, 2.0	0		0	
Not shivering	53 %			5 %		19 %	
Fly	1		21.5	2	22.4 and 23.5	6	21.1, 20.0–23.6, 1.41
Drop	32		8.9, 6.0–16.4, 2.9	0		0	
Total							
Fly	45 %			100 %		100 %	
Drop	66 %			0		0	

Means, range and standard deviation are shown for thoracic temperatures.

above  $T_a$ . At  $T_a$  near  $6^\circ\text{C}$  16 of the 29 shivering moths attempted to fly when disturbed, while 13 dropped to the ground, but almost all of the non-shiverers dropped. At  $T_a$  values of  $13^\circ\text{C}$  and  $17^\circ\text{C}$  all the moths attempted to fly and none dropped to the ground.

By not shivering the moths make considerable energy savings. At low  $T_a$  the cost of maintaining an elevated  $T_{th}$  by shivering is high. In addition, the resting metabolic rate when poikilothermic is low (Fig. 8). Both of these relationships combine to make the energy savings for not shivering progressively greater as  $T_a$  is lowered.

### Heat retention

The capacity of the moths to be active at low  $T_a$  depends in part on their ability to retain the heat generated by warm-ups and by flight. The thoracic pile serves as one effective barrier to heat loss (Fig. 12). The cooling rates of moths with and without pile in still air were, at  $0.55$  and  $0.65^\circ\text{C min}^{-1}^\circ\text{C}^{-1}$  difference between  $T_{th}$  and  $T_a$ , very similar. However, at wind speeds of  $5\text{--}6\text{ ms}^{-1}$ , near the moths' flight speed, depilated moths cooled about twice as fast (or maintained half the temperature excess) as those with their thoracic pile intact (Fig. 9). In furred moths an increase in wind speed from  $1.7$  to  $6.4\text{ ms}^{-1}$  increased mean cooling rate by only 14 %, but in depilated moths the same increase in wind speed resulted in a 34 % increase in cooling rate.

Apart from losing heat from the thorax directly through the thoracic pile, considerable amounts of heat could potentially be lost through the head and the abdomen. The head necessarily receives heat from the thorax by conduction. It was of interest, however, whether or not additional heat was shunted to the head either to

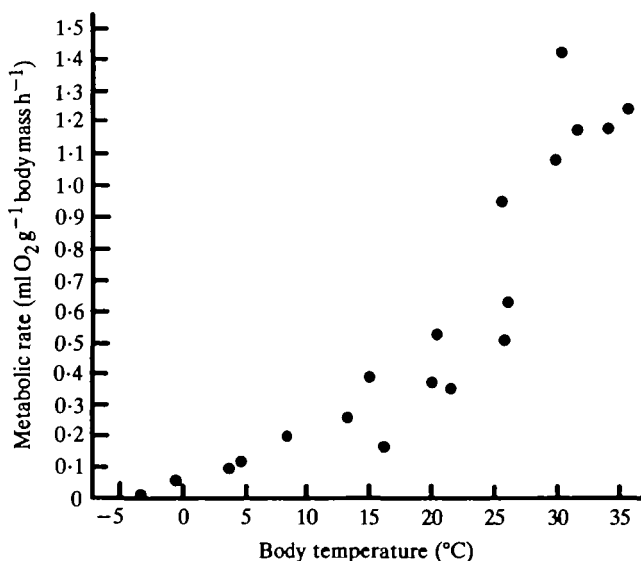


Fig. 8. Metabolic rates of *Eupsilia* spp. at rest as a function of ambient (and body) temperature.

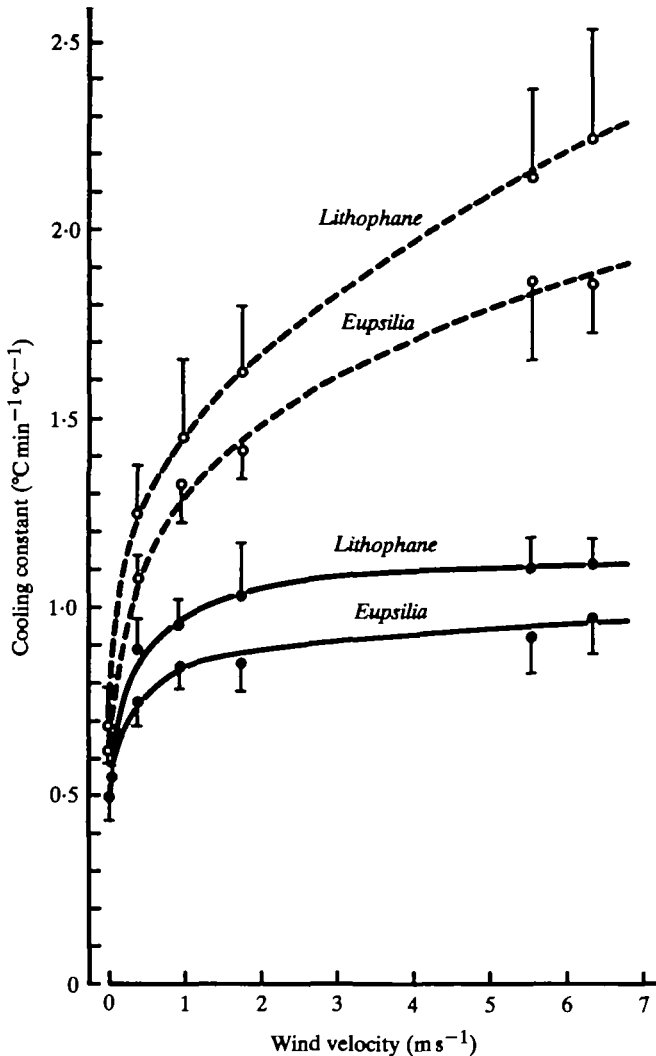


Fig. 9. Cooling constants of *Eupsilia* and *Lithophane* thoraxes, with insulating scales intact (solid lines) and insulating scales removed from the thorax (dashed lines), as a function of wind velocity. Each point represents a mean of six measurements. One standard deviation is indicated at one side of the mean. Mean body masses of the *Lithophane* and *Eupsilia* were 160 mg and 164 mg, respectively.

maintain an elevated head temperature at low  $T_a$  or to dissipate excess heat at high  $T_a$ . Neither seems to apply. Head temperature in the moths was tightly coupled with  $T_{th}$  (Fig. 10). In stationary moths that were shivering, head temperature rose in parallel with  $T_{th}$ , averaging 42 % ( $N = 14$ ) of thoracic temperature excess. In killed moths with heated thoraxes (see Materials and Methods) the head temperature excess was nearly identical (41 %). Passive heat conduction from the thorax therefore accounts for most or all of the observed head temperature excess. During flight, head temperature immediately plummeted to approximately 15 % of thoracic temperature

excess (Fig. 10), presumably because the small head (approx. 5 mg) had a much higher rate of convective cooling than the thorax weighing approximately 40 mg.

AGA infra-red emission photographs (Fig. 11), which show temperatures colourimetrically, confirm that head temperature remains coupled with  $T_{th}$ . In addition, the photographs also show, as already indicated, that abdominal temperature remains close to  $T_a$  during pre-flight warm-up as well as during flight.

An examination of the moths' anatomy (Fig. 12) helps to explain their unusual capacity to sequester heat in the thorax. In the four species of moths examined (*Eupsilia morrisoni*, *E. tristigmata*, *Lithophane grotei* and *Pyroferria citromba*) the complex of abdominal and thoracic air sacs, that should retard the passive conduction of heat from the hot thorax to the cool abdomen, is well developed. The only functional connection for exchange of materials between the thorax and the abdomen

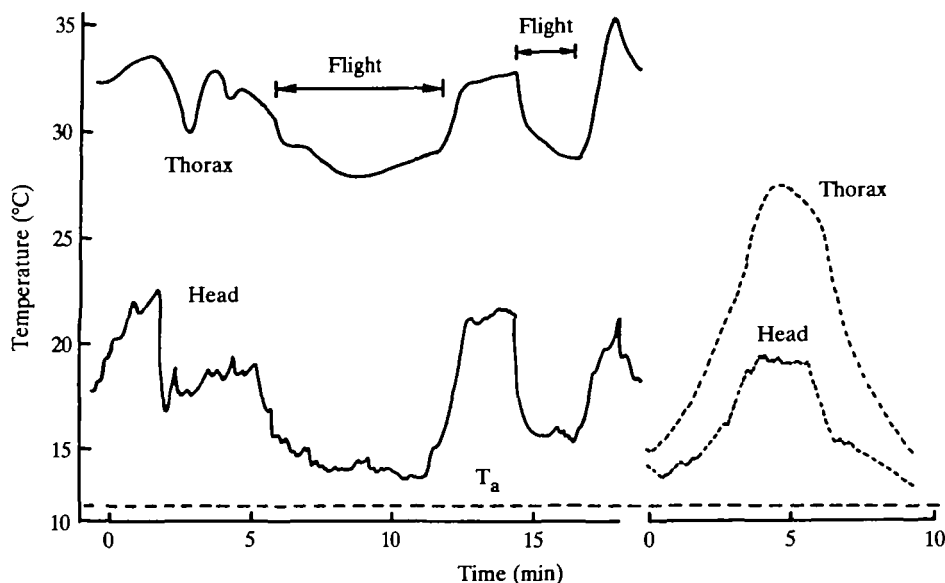


Fig. 10. Head and thoracic temperatures of a *Eupsilia morrisoni* during a prolonged bout of intermittent shivering, escape behaviour and forced flight (when the moth was held with forceps by its legs). Broken lines indicate the increase in head and thoracic temperature in the same moth (with thermocouples still in the same place) when dead and heated with a hot copper wire inserted into the abdomen (the air sacs between thorax and abdomen were destroyed). Ambient temperature ( $T_a$ ), 11.5°C.

Fig. 11. Infra-red AGA thermovision photographs of a *Eupsilia morrisoni* (length = 1.7 cm) from the beginning (A) to the end (E) of a pre-flight warm-up, and after 1 min of flight (F). A and B show dorsal views of the moth perched on top of a diagonal stick and facing the top righthand corner of picture. C and D show lateral views, facing in the same direction. E shows a dorsal view in which the stick is horizontal and the moth is facing the upper lefthand corner of the picture. In F the moth is being held with forceps by its left wing while being photographed. The photographs indicate the locations of the temperature increases and show heat retention in the thorax with lack of heat transfer to head, abdomen and wings. The background (black) is ice. Purple = 11.0–13.9°C, light blue = 14.0–17.1°C, green = 17.2–19.5°C, dark blue = 19.6–22.3°C, pink = 22.4–24.7°C, red = 24.8–26.5°C, yellow = 26.6–30.9°C.

11 A

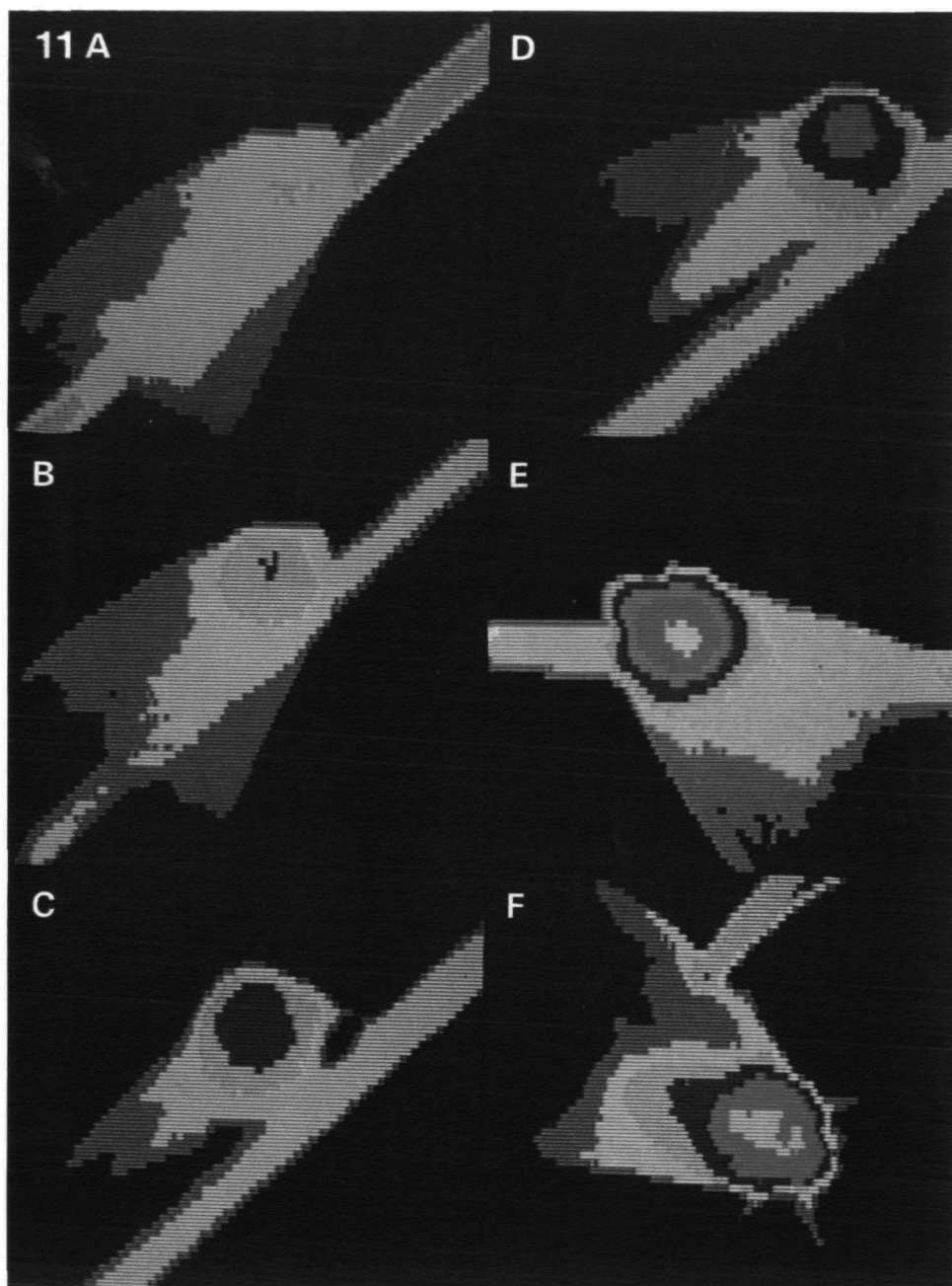
D

B

E

C

F







is a relatively long (3 mm), narrow passage in the anterior ventrum of the abdomen below the air sacs that accommodates the oesophagus, the ventral nerve cord and the aorta. All the blood from the cool abdomen flows confined in the aorta through this passage (see dimensions, Fig. 12), and all the blood heated in the thorax returning to the abdomen flows confined in the narrow space over and around this vessel. This anatomical arrangement conforms to a counter-current heat exchanger which may account for the low abdominal temperatures observed.

Within the thorax the aorta ascends between the thoracic muscle to the scutellum, makes a hairpin loop and descends closely pressed against the ascending portion of the aorta before proceeding anteriorly into the head. When the thoracic muscles are contracting and producing heat, cool blood from the abdomen ascending through the aorta (Jones, 1964) would withdraw heat from muscles. But heat from this blood should be recovered by the lower portion of the ascending loop lying next to it. This second counter-current heat exchanger should retain some of the heat in the thorax that would otherwise be shunted into the head. Nevertheless, the aorta runs through muscle after leaving the presumptive heat exchanger, so that some heat transfer to the head should still occur.

Direct measurements (Fig. 13) are consistent with the temperature distributions required to satisfy the thoracic counter-current model. Surface temperatures at the top of the thorax directly above the thoracic heat exchanger (at the scutellum) during pre-flight warm-up in *E. morisoni* were  $0.5^{\circ}\text{C}$  below thoracic surface temperature. During fixed flight (with the thermocouples still in the same place), however, scutellar surface temperatures decreased (from  $1.0$  to  $2.0^{\circ}\text{C}$  below surface  $T_{\text{th}}$ ) as predicted if cool blood flowed anteriorly in the aorta into the scutellum. With internal

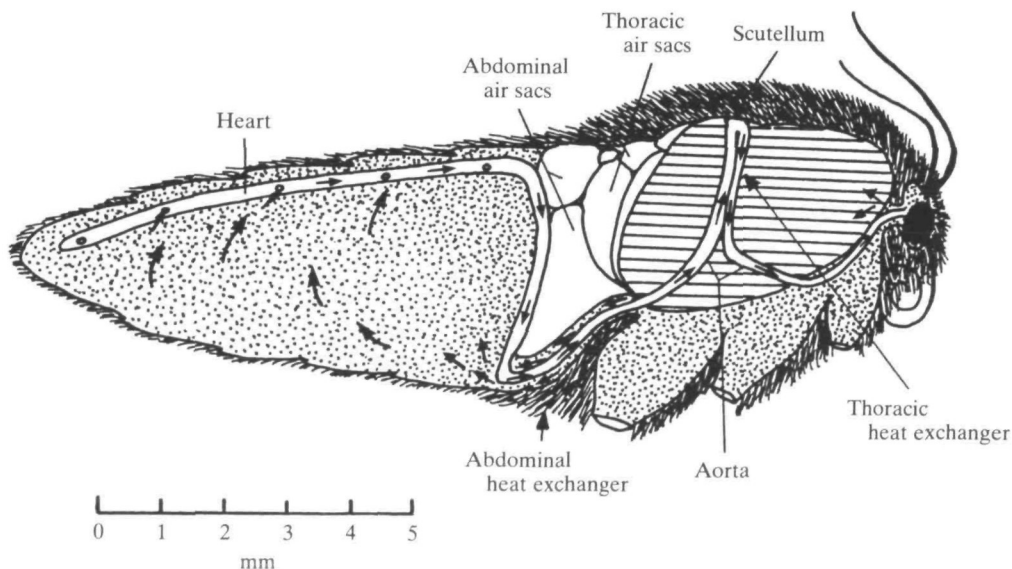


Fig. 12. Diagrammatic representation of a sagittal section of a winter moth. Direction of blood flow is indicated by arrows. Only the anteriorly flowing blood is confined by vessels. Horizontal lines in thorax indicate flight muscle.

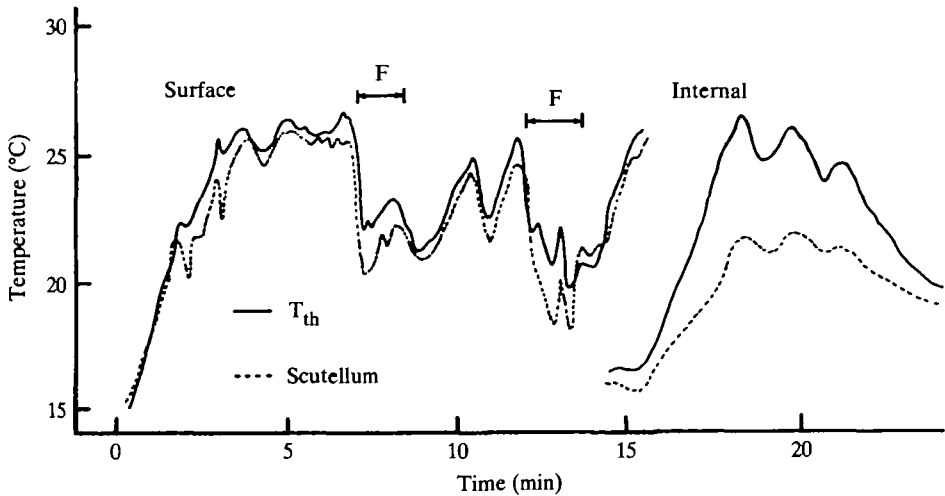


Fig. 13. Record of thoracic ( $T_{th}$ ) and scutellar temperatures of a *Eupsilia morrisoni* during pre-flight warm-up and flight on the thermocouple tether (F). Left: probes were applied to the surface. Right: probes were inserted to a depth of approximately 1 mm. Ambient temperature, 15°C.

probes, however, temperatures inside the thorax near the aorta were 3.0–4.5°C below those of the surrounding muscle, corroborating the temperature distribution required by the heat exchanger and indicating that heat is recovered as the blood ascends the heat exchanger. These data are also consistent with the notion that blood circulation increases during flight following warm-up. Comparative anatomy and physiology of other moths may provide added insights (see below), but the micro-details of blood flow and heat exchange in these tiny moths remain to be explored.

#### DISCUSSION

The subfamily Cuculiinae is dominantly north temperate in its distribution. It contains nearly 50 species in the north-eastern United States (Franklemont, 1954). Many of the species that develop eggs in the winter apparently do not diapause, and they have been observed to fly in the field on some nights with air temperatures as low as 2.2°C (Schweitzer, 1974).

Although they are physiologically capable of flight at low  $T_a$ , their major adaptations for being winter-active involve behaviour. Many, if not most, other noctuid moths selectively rest in those trees where their colouration matches the substrate (Sargent, 1966, 1981). The diverse colouration of the winter noctuid species (grey, brown, yellow, white, black, sienna) appears to be unrelated to colour matching with the substrate. The group is polyphyletic (Franklemont, 1954), suggesting that the habit of hiding under leaves by these moths has evolved independently, possibly from previously summer- or autumn-active ancestors that invaded colder seasons.

The moths do not need to undergo prolonged physiological adjustments before becoming active. Instead, they can become active almost immediately, flying on the

first warm night of late winter, or after being taken out of a refrigerator where they have been kept at  $T_a < -5^\circ\text{C}$ .

One of the problems of overwintering concerns energy supplies. So far it is not known what energy reserves the moths carry with them in the winter. However, given the opportunity, they bloat themselves on sugar syrup. Moths increased their body weight up to 94.5 % on 35 % sugar (honey) solution. Assuming  $3.7 \text{ cal mg}^{-1}$  sugar, a moth with 90 mg of 35 % sugar solution in its crop should have energy reserves of at least 115 cal, and given a resting metabolic rate of  $0.24 \text{ cal g}^{-1} \text{ body weight h}^{-1}$  at  $0^\circ\text{C}$  (Fig. 8), this should be sufficient reserves for 31 days. This may be a rather slim margin, because it is unlikely that moths will normally be so well-loaded with concentrated sugar in the field. Subnivian soil temperatures where many of the moths hide are probably near  $-2^\circ\text{C}$  to  $0^\circ\text{C}$  (Schweitzer, 1974 and personal observations). However, the exact temperatures the moths experience in the field in winter are unknown. I observed moths long before the first patches of ground free of snow appeared, suggesting that some of them overwinter above ground, possibly under loose bark where they would experience temperatures considerably lower than  $-2^\circ\text{C}$ . It is also possible that their metabolic rates in the field are lower than those observed under laboratory conditions. Nevertheless, my measurement of mass-specific resting metabolic rate is approximately 30 % lower than the mass-specific rates previously reported for tropical moths (Bartholomew & Casey, 1978).

The increase in metabolism from rest to flight at low  $T_a$  is enormous – approximately 8000 times from  $-3^\circ\text{C}$ , and 1600 times from  $0^\circ\text{C}$ , and to warm up and fly on a regular basis would appear to require the availability of food. However, sap begins to run in trees even in late autumn, as soon as a frost–thaw cycle starts, and although sap is dilute sugar (2–6 % in sugar maple), concentration of the sap should occur at open wounds where there is evaporation. Wounds may occur at broken limbs or at chew-marks left by squirrels who lick at ‘taps’ chewed into limbs (personal observations).

The moths require a  $T_{th}$  near  $30^\circ\text{C}$  during free flight, similar to that of other moths of their size and wing-loading from a tropical site in Central America (Bartholomew & Heinrich, 1973). However, no other moths are known to be able to warm up from near  $0^\circ\text{C}$ . Lack of observed warm-up from low  $T_a$  could in part be because the animals are reluctant to perform, so that large sample sizes of moths must be surveyed. However, the rate of warm-up as a function of  $T_a$ , when extrapolated to a zero rate of warm-up, should indicate the minimum temperature at which warm-up is physiologically possible. Such data indicate, for example, that the winter cuculinids should be able to warm up at  $T_a$  as low as  $0^\circ\text{C}$  (Fig. 5). The tent caterpillar moth *Malacosoma americanum*, in contrast, cannot warm up from  $T_a < 8^\circ\text{C}$  (Casey *et al.* 1981), while the sphinx moth, *Manduca sexta*, requires a minimum temperature near  $10^\circ\text{C}$  for pre-flight warm-up (Heinrich & Bartholomew, 1971). It is puzzling why the flight muscles of the winter moths are able to function at some  $10^\circ\text{C}$  lower than those of many if not all other moths so far examined, especially since some of the key catabolic enzymes have temperature characteristics similar to those of moths that fly with much higher  $T_{th}$  (Heinrich & Mommsen, 1985). However, the

cuculinids suffer from heat prostration when  $T_{th}$  approaches  $40^{\circ}\text{C}$ , a  $T_{th}$  that is close to that physiologically regulated by many moths during free flight (Heinrich, 1970, 1974; Casey, 1981; Bartholomew & Heinrich, 1973).

The moths appear to be able to fly at very low  $T_a$  not only by having muscles that can begin to shiver at low temperatures, but also by being able to sequester heat within the thorax to a greater extent than other moths. However, the ability to retard physiologically mediated heat flow to the head and abdomen appears to be gained at the expense of heat-dissipating ability at high  $T_a$ . Unlike bumble-bees, which fly at both low and high  $T_a$  and which have a reversible counter-current heat exchanger (Heinrich, 1976), the winter moths' heat exchanger is not reversible. Moths forced to remain in free flight heated to  $38^{\circ}\text{C}$  even at relatively modest  $T_a$  ( $22^{\circ}\text{C}$ ), and they were incapable of continuous flight at  $22^{\circ}\text{C}$ , apparently because of heat prostration; when allowed to cool they immediately resumed flight. However, these moths are limited in their activity in the winter almost exclusively by low temperatures, and  $T_a$  values as high as  $22^{\circ}\text{C}$  (or even  $15^{\circ}\text{C}$ ), which are rarely encountered at night in the winter, may not be ecologically relevant.

The maintenance of a high  $T_{th}$  is also potentially a general problem in small endothermic insects that do not fly in the winter (Bartholomew, 1981), and anatomy that may be functionally similar in kind (though not necessarily in degree) to that described here is found also in many other small nocturnal Lepidoptera (Hessel, 1969). For example, in *Malacosoma americanum* (Lasiocampidae), a well-studied endothermic moth (Casey, 1981; Casey *et al.* 1981) of similar mass to most winter noctuids, the ascending and descending portions of the aorta also lie close to each other in the thorax (Hessel, 1969), although they are not actually pressed together as in winter moths (personal observation). In *M. americanum* there is greater apparent heat leakage to the extremities. Head temperature excess averaged 64% of thoracic temperature excess (Casey *et al.* 1981), some 1.5 times greater than in winter moths. Similarly,  $T_{ab}$  in flight (personal observations) averaged  $7.0^{\circ}\text{C}$  above  $T_a$  at  $14^{\circ}\text{C}$  ( $N = 10$ ), some 3.9 times greater than that in the winter noctuids reported here. Although the small summer-flying moths only lose heat physiologically at the high end of their activity range (Casey *et al.* 1981), summer-flying sphinx moths, in part because of their considerably greater mass, must physiologically dissipate heat over most of their activity range of  $T_a$  (Heinrich, 1970, 1971a,b, 1974).

The circulatory anatomy in Lepidoptera (Hessel, 1969) and in some other insects (Wille, 1958) has been considered to show phylogenetic relationships. My results here indicate that the anatomy also functions in thermal balance. However, the data so far are insufficient to draw general conclusions about possible adaptive physiological patterns of thermoregulation in moths. The winter-flying moths appear to be anatomically and physiologically similar to summer-flying moths their size, but they are physiologically better able to warm up and fly at low  $T_a$  than other moths, both because they can retain more heat in the thorax and because they can begin to shiver at lower temperatures. Nevertheless, their major adaptations for being active in the winter appear to be behavioural.

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