

## COMPARATIVE STUDY OF CHILL-COMA TEMPERATURES AND MUSCLE POTENTIALS IN INSECT FLIGHT MUSCLES

By FRANZ GOLLER AND HARALD ESCH

*Department of Biological Sciences, University of Notre Dame, Notre Dame,  
IN 46556, USA*

*Accepted 12 December 1989*

### Summary

Muscle potentials in insect flight muscles decrease in amplitude and increase in duration with decreasing temperature. Amplitudes fall to zero at distinct temperatures, which are characteristic for different species. Chill-coma temperature is thus defined here as the critical temperature below which flight muscles cannot be activated. Chill-coma temperatures were 2°C in the species of butterflies measured and –2 to 0°C in the moths. In the species of Hymenoptera, Coleoptera and Diptera measured, chill-coma temperatures ranged from 3 to 14°C. The rate of decline of muscle potential amplitudes with decreasing temperature was different for different species. Rates were smaller over most of the temperature range for species that warm up from low environmental temperatures to reach thoracic temperatures necessary for flight. Amplitudes decreased faster at higher thoracic temperatures in species that start shivering only at higher ambient temperatures. Temperature has a similar effect on durations of muscle potentials in different species. Between 25 and 15°C, durations increased by 100 % in all species. The results suggest that cold-adaptation is not strongly related to chill-coma temperature but is strongly related to the rate of decline of muscle potential amplitudes.

### Introduction

Daily and annual activity periods in most insects depend to a great extent on environmental temperature. Physiological mechanisms to produce or retain heat have evolved to cope with low-temperature situations. Endothermic heating through shivering with flight muscles has been studied in a great number of species (for a review see Kammer, 1981). Remarkable differences have been reported for environmental temperatures at which certain insects can fly. Cuculiine winter moths and winter-active scarabs are able to heat their flight muscles from temperatures near 0°C to temperatures above 30°C as required for flight (Heinrich, 1987*a,b*; Morgan, 1987). Bumblebee queens can fly at ambient temperatures of –3.6°C (Bruggemann, 1958, cited in Heinrich, 1979), which

**Key words:** chill-coma temperature, muscle potentials, insect flight muscles.

requires warm-up from these low temperatures to near 36°C. Most larger insects, however, initiate warm-up at temperatures above 10°C.

Esch (1988) showed that electrical activity in flight muscles of a winter moth and honeybees ceased at distinct temperatures characteristic for the respective species. Temperatures determined with this method corresponded well with the lowest temperatures at which locomotor activity (walking) in these animals had been observed (chill-coma temperature). In winter moths, amplitudes of muscle potentials fell to zero near 0°C, and in honeybees they fell to zero at around 10°C. These differences support the idea that the environmental temperatures that are normally encountered are reflected in chill-coma temperatures (Mutchmor and Richards, 1961).

Observation of electrical activity in flight muscles seems to be a suitable physiological method for determining chill-coma temperatures. We therefore tried to address the following questions using this method. Are ecological conditions, in particular temperature, reflected in chill-coma temperatures, as might be suggested by results from winter moths and honeybees? Are physiological findings in accordance with what is known about thermoregulatory behaviour from field studies? We addressed these questions by studying members of closely related taxa.

### Materials and methods

Experimental animals were collected a few hours before the experiment from the surroundings of Notre Dame campus (for temperature conditions prior to capture see Table 1). *Trichoplusia ni* and *Heliothis zea* were taken from laboratory cultures which had been kept at room temperature (22°C). Honeybees were caught at the entrance of a hive in the apiary of the Department of Biological Sciences. More details about species and the number of animals are listed in Table 1.

Prior to preparation, animals were cooled to chill-coma. The motionless animals were waxed to a wooden rod on the notum. Wire electrodes (50 µm in diameter) were inserted into the dorsoventral and dorsal longitudinal flight muscles. For details of recording procedures see Esch (1988). The wooden rod was attached to a force-displacement transducer (Grass FT03C) to monitor wing beats. A thermocouple (copper/constantan 50 µm) was waxed onto the notum to determine thoracic temperature. A second thermocouple hanging 1 cm above the animal recorded ambient temperature. Animals were cooled at a rate of 1°C min<sup>-1</sup>. Muscle potentials, wingbeats and thoracic temperature were recorded on an instrumental tape recorder.

Recordings were digitized for analysis of muscle potentials, and amplitudes and durations were determined (for details see Esch, 1988). In Lepidoptera and Coleoptera, potentials of dorsal longitudinal muscles were analysed. In Hymenoptera, potentials of dorsoventral and dorsal longitudinal muscles were compared and found to respond equally to different temperatures. The data on bees presented here are from dorsoventral muscles. Only potentials from cooling-down

periods are included in this analysis to exclude possible hysteresis effects. Amplitudes of muscle potentials from various individuals were normalized, since extracellular recordings resulted in different absolute amplitudes (between 2 and 5 mV). Values for amplitudes are therefore given in relative numbers not in millivolts. Chill-coma temperature is defined here as the temperature at which muscle potential amplitude reaches 0.

## Results

### *Chill-coma temperatures*

Chill-coma temperatures could be determined very accurately. Intraspecific variability was very low (Table 1). In Diptera, Coleoptera and Hymenoptera, flight muscle activity stopped with a very distinct burst of muscle potentials. This burst also occurred in smaller species that showed no spontaneous muscle potentials during the cooling period (syrphid flies and small beetles). Lepidoptera did not have a distinct burst of potentials before reaching chill-coma.

Table 1 summarizes the results for all species and gives the temperature conditions that the animals were exposed to prior to the experiments. However, these environmental temperatures may not reflect typical conditions for all species. All Lepidoptera showed muscle potentials at thoracic temperatures below 3°C. Flight muscle activity in butterflies stopped at 2°C and in moths between -2 and 0°C. In Hymenoptera, chill-coma temperatures were quite variable for different species. *Vespula germanica* had the lowest chill-coma temperature, between 6 and 7°C. Bumblebees were not able to activate their flight muscles below 7°C. This was true for all investigated individuals (workers and queens of *Bombus bimaculatus*, *B. fraternus* and *B. pennsylvanicus*). Highest chill-coma temperatures were found in sphecid wasps and honeybee drones (12–14°C). Within the Coleoptera, the large *Lucanus elaphus* showed muscle potentials at thoracic temperatures as low as 3°C. Flight muscle activity in two smaller beetles stopped between 7 and 8°C (*Popillia japonica* and *Photinus marginellus*). In the two dipteran species studied, the final burst of muscle potentials occurred near 7°C (Table 1).

### *Changes in amplitude and duration*

The amplitude of muscle potentials declined with decreasing thoracic temperature and the duration increased. The rate of decline of amplitude was different in different species. Amplitudes decreased very little over most of the temperature range in *Eupsilia devia* (data from Esch, 1988). The steepest decline occurred between 5 and 0°C. Amplitudes decreased at a much higher rate between 15 and 5°C in other moths (Fig. 1A; Table 2). Within the Hymenoptera, lowering of temperature below 20°C affected amplitudes of muscle potentials most in honeybees and least in bumblebees. Muscle potential amplitude decreased to half the value at 25°C at 9.5°C in bumblebees and at 15.5°C in honeybees (Fig. 2A; Table 2). The duration of muscle potentials increased in all species at about the

Table 1. *Taxa, approximate body mass, chill-coma temperature (mean and standard deviation), and mean of average daily temperature for 5 days prior to capture*

Experimental animal	Body mass (mg)	Chill-coma temperature (°C)	Environmental temperature (°C)
<b>Lepidoptera</b>			
Sphingidae: <i>Haemorrhagia diffinis</i> Boisduval (3)	140	-0.3±0.4	25.5–26.0
Lasiocampidae: <i>Malacosoma americanum</i> Fabr. (3)	90	-0.5±0.4	25.5–26.0
1 sp. not identified (2)	110	-0.9±0.3	21.7, 25.0
Noctuidae: <i>Trichoplusia ni</i> (Huebner) (3)	120	-0.6±0.4	22.0
<i>Heliothis zea</i> (Boddie) (2)	125	-0.1±0.1	22.0
<i>Autographa</i> sp. (1)	140	-1.0	26.0
<i>Eupsilia devia</i> (Huebner)* (3)	100	0.0±0.2	4.0
Geometridae: 2 spp. not identified (2)	75	-0.5±0.2	26.0
Pieridae: <i>Pieris rapae</i> (L.) (2)	90	2.1±0.2	18.7, 26.4
<i>Colias philodice</i> Godart (2)	110	1.8±0.7	26.4
Nymphalidae: <i>Polygonia interrogationis</i> (Fabr.) (1)	140	2.3	24.9
<b>Hymenoptera</b>			
Vespidae: <i>Vespula germanica</i> (Fabr.) (5)	130	6.7±1.8	21.0, 15.3
Apidae: <i>Bombus bimaculatus</i> Cresson (6)	160–450	7.0±0.6	10.1–25.2
<i>Bombus fraternus</i> (Smith) (3)	210	8.0±0.8	25.4, 21.0
<i>Bombus pennsylvanicus</i> (Degeer) (4)	170–340	7.1±0.7	21.0–21.6
<i>Apis mellifera</i> L. worker (9)	110	11.2±0.7	21.0
<i>Apis mellifera</i> L. drone (10)	195	13.3±1.2	19.4–20.2
Xylocopidae: <i>Xylocopa virginica</i> (L.) (13)	520	8.5±0.8	14.3–21.2
Sphecidae: <i>Sphecus speciosus</i> (Drury) (3)	650	13.5±0.2	28.0, 26.1
<i>Chlorion ichneumonium</i> (L.) (2)	250	13.5±0.5	23.4
<b>Coleoptera</b>			
Lucanidae: <i>Lucanus elaphus</i> Fabr. (1)	1300	3.0	20.2
Lampyridae: <i>Photinus marginellus</i> LeConte (8)	130	7.9±0.6	23.3
Scarabaeidae: <i>Popillia japonica</i> Newman (2)	250	7.2±0.2	25.3
<b>Diptera</b>			
Bombyliidae: 1 sp. not identified (3)	130	7.3±0.2	26.1, 24.9
Syrphidae: 1 sp. not identified (2)	30	7.2±0.8	25.3, 11.8

Numbers in parenthesis give number of individuals tested.

\* Data from Esch (1988), animals collected in Vermont and then kept in a refrigerator.

same rate between 36 and 15°C. At 15°C it was twice as long as at 25°C (Figs 1B, 2B; Table 2). Durations increased markedly near chill-coma temperatures, and at low temperatures some differences between species were found (Figs 1B, 2B).

The causes of these changes in muscle potentials are not known at present but are currently under investigation (see also discussion in Esch, 1988).

#### *Shivering behaviour*

Shivering behaviour was different in various species. Most Hymenoptera

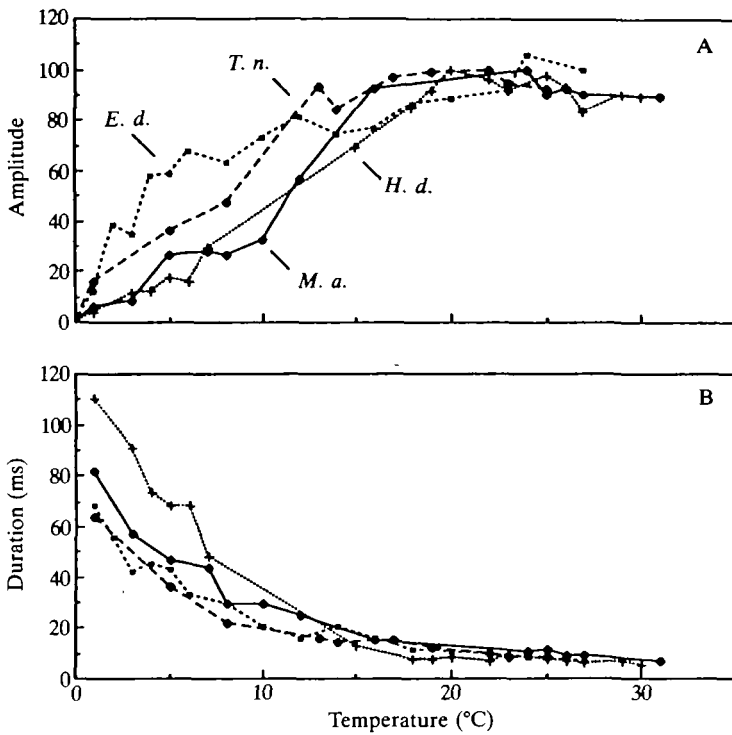


Fig. 1. Mean muscle potential amplitudes (A) (in relative units) and durations (B) and thoracic temperature for four moths (*E. d.* = *Eupsilia devia*; *T. n.* = *Trichoplusia ni*; *M. a.* = *Malacosoma americanum*; *H. d.* = *Haemorrhagia diffinis*). Variability was very low for all means and generally higher at low temperatures. Error bars are not included in the graphs because for most means they would be covered by the symbol. Standard errors were maximally 9 % of the mean amplitude and 10 % of the mean duration. Number of potentials measured and number of individuals (in parenthesis): *Eupsilia*, 957 (3); *Trichoplusia*, 664 (3); *Malacosoma*, 603 (3); *Haemorrhagia*, 830 (3).

Table 2. Temperatures (°C) at which amplitudes of muscle potentials were 50 % of values at 25°C (AMP50) and at which durations of muscle potentials were twice as long as durations at 25°C (DUR200) for four Lepidoptera and three Hymenoptera

Species	AMP50	DUR200
<i>Eupsilia devia</i> *	3	15
<i>Trichoplusia ni</i>	8	15
<i>Malacosoma americanum</i>	11	14
<i>Haemorrhagia diffinis</i>	12	16
<i>Bombus bimaculatus</i>	9.5	16
<i>Xylocopa virginica</i>	11.5	16
<i>Apis mellifera</i> worker	15.5	16

\* Data from Esch (1988).

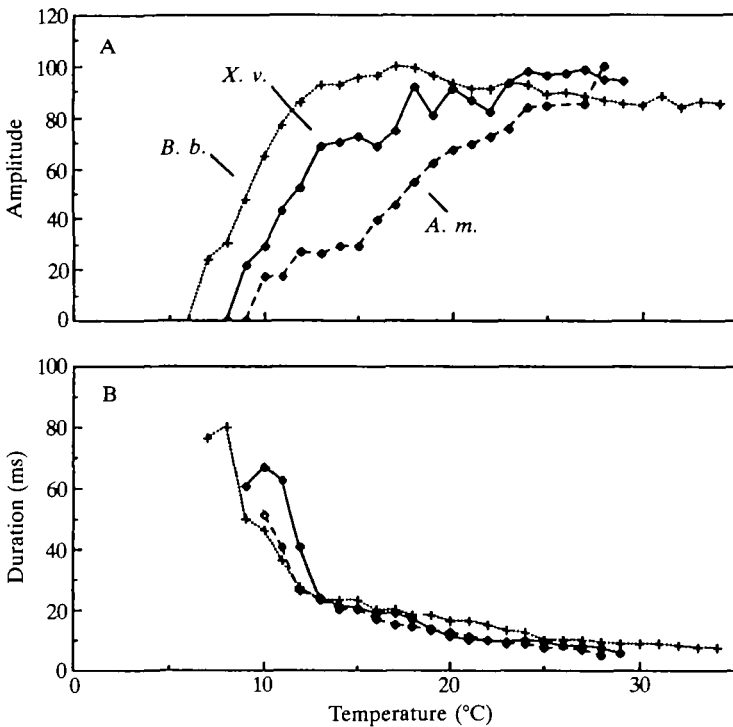


Fig. 2. Mean muscle potential amplitudes (A) (in relative units) and durations (B) and thoracic temperature for three hymenopterans (*B. b.* = *Bombus bimaculatus*; *X. v.* = *Xylocopa virginica*; *A. m.* = *Apis mellifera* worker). Maximal s.e. for amplitudes was 7% of mean, maximal s.e. for durations was 8% of mean at low temperatures. Number of potentials measured and number of individuals (in parenthesis): *Bombus* 10 048 (6); *Xylocopa* 8451 (13); *Apis* 2887 (4).

attempted to heat themselves throughout the cooling period. Vigorous shivering near chill-coma temperatures resulted in a temporary 1–2°C rise of thoracic temperature. Coleoptera and Diptera had to be stimulated to produce muscle potentials by pulling the paper the animals were holding onto (tarsal reflex). Once shivering had been initiated, individuals stayed active for several minutes. The final burst of potentials before chill-coma never had to be initiated by stimulation. Interspecific differences in flight muscle activity were especially pronounced in Lepidoptera. Butterflies moved their wings spontaneously below 10°C but at very low frequencies (<1 Hz). Above 10°C they had to be stimulated. No distinction was made between actual shivering and flapping of wings in ineffective flight. Moths shivered spontaneously below 20°C. However, the temperature dependence of muscle potential frequencies was quite different in different species (Fig. 3). All species have wingbeat frequencies near 60 Hz during flight (Heinrich, 1987a; Casey *et al.* 1981; F. Goller and H. Esch, in preparation). The highest observed muscle potential frequency at around 25°C was largest in *Haemorrhagi diffinis* (50–60 Hz) and lowest in *Eupsilia devia* (23 Hz). Frequencies in *Malaco*

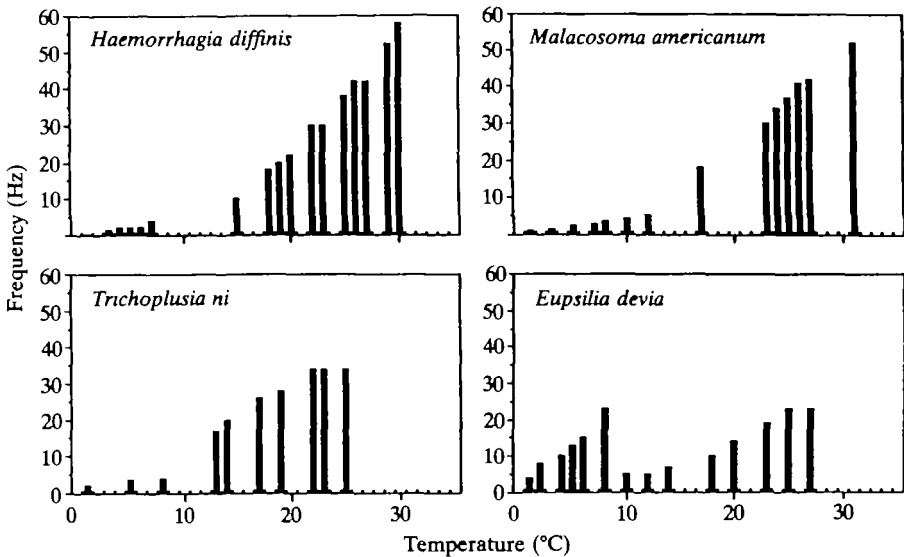


Fig. 3. Maximal muscle potential frequency and thoracic temperature for four moths. Data were taken from the most vigorously heating individual of each species, but all are typical for the respective species.

*soma americanum* and *Trichoplusia ni* were between these values. Below 10°C, this sequence was reversed, and *Eupsilia* produced muscle potentials at much higher frequencies than did the other species (Fig. 3). Only *Eupsilia devia* raised its thoracic temperature significantly (1–3°C) above ambient near chill-coma temperature.

### Discussion

Determination of chill-coma temperature by recording electrical activity in flight muscles is an objective and useful tool in insects which use their flight muscles for endothermic heat production. Earlier methods relied on behavioural criteria, defining chill-coma temperature as the temperature at which the animals became motionless, unresponsive to stimulation, or fell over because they had lost control over the righting reflex. Many Hymenoptera and Diptera do not show detectable movements when heating, whereas Lepidoptera shiver with wing movements of low amplitude (Esch, 1964; Kammer and Heinrich, 1972; Heinrich and Pantle, 1975; Kammer, 1981). Thus, many insects sit motionless at one spot during active heating at low temperatures. But, in contrast to an individual in chill-coma, they could raise their thoracic temperature by themselves and become active again. Chill-coma temperatures based on ceasing of electrical activity in the flight muscles were either equal to or lower than those determined by behavioural criteria (e.g. Mellanby, 1940; Colhoun, 1960; Mutchmor and Richards, 1961). During our experiments, movement of the appendices was never observed (even upon stimulation) below temperatures at which flight muscles failed.

Chill-coma temperatures, as defined above, differ over a range of 15°C in our experiments. They lie within 4°C for our 12 species of Lepidoptera. They have less variability than in our eight hymenopteran species, ranging from 5 to 14°C. Most interestingly, there is no difference in chill-coma temperature between the winter-flying *Eupsilia devia* and summer-flying moths of four different families. Butterflies have a chill-coma temperature which is about 2°C higher than that of moths. This is not simply because most moths are active at night and operate at lower ambient temperatures, because the sphingid *Haemorrhagia diffinis*, which is active during the day, has the same chill-coma temperature as all the other moths.

Heat production depends strongly on the amplitude of muscle contractions. The changes in muscle potential amplitude that we observed probably reflect changes of contraction force, since decreasing depolarization causes decreasing contraction amplitudes (Aidley, 1975). Flight muscles that have a slower decrease in muscle potential amplitude with decreasing thoracic temperature are therefore more effective for heating at lower temperatures. Major differences in the rate of decrease of amplitudes among the investigated species point in that direction. Amplitudes in species known to initiate warm-up at low ambient temperatures decrease much more slowly than in species that start to shiver only at higher ambient temperatures. The amplitude of potentials in flight muscles of *Eupsilia devia* decreased to 50 % of that at 25°C near 3°C. In other moths this decrease to 50 % was reached at considerably higher temperatures (e.g. 11°C in *Malacosoma americanum*). Warm-up rates become zero at approximately 0°C in *Eupsilia* (Heinrich, 1987a) but at 9°C in *Malacosoma* (Casey *et al.* 1981). Stroke work in *Malacosoma* decreases significantly below 25°C (Casey and Hegel-Little, 1987). The shape of the curve (Fig. 7 in Casey and Hegel-Little, 1987) correlates well with the decrease in muscle potential amplitudes.  $Q_{10}$  values are approximately 1.4 for stroke work (Casey and Hegel-Little, 1987) and 1.2 for muscle potential amplitude (this study) in the range 25–15°C. The small decline in muscle potential amplitudes in bumblebees suggests that they can use their flight muscles more effectively for heating at lower temperatures than can *Xylocopa virginica* or honeybees. In addition, their flight muscles' chill-coma temperature is lower than in the two other genera.

The durations of muscle potentials did not decrease at different rates between 25 and 15°C. At 15°C, durations were twice as long as at 25°C in all species. Heating in flight muscles is controlled by muscle potential frequency. A similar increase in duration suggests, therefore, that the highest possible muscle potential frequency (because of refractory periods) is not affected differently by temperature in our experimental animals. The effect of temperature on muscle potential duration at temperatures near chill-coma might be more significant.

How do physiological data compare with behavioural observations? Our physiologically determined chill-coma temperatures show that all the moths can activate their flight muscles to some extent near 0°C. Most moths for which behavioural and/or physiological data are available, however, do not start warm-up below 8°C (e.g. Mutchmor and Richards, 1961; Heinrich and Bartholomew,

1971; Casey *et al.* 1981; Casey and Joos, 1983). The only exceptions known are cuculiine moths (e.g. *Eupsilia* spp.) (Heinrich, 1987a). However, as Heinrich (1987a,b) pointed out, the difference between winter-flying moths and other moths is gradual and not absolute. This is supported by our data. Rates of decrease in amplitudes are lowest in *Eupsilia*, intermediate in *Trichoplusia* and *Malacosoma*, and highest in *Haemorrhagia*. *Eupsilia* flies in winter, *Trichoplusia* and *Malacosoma* in summer at night, and *Haemorrhagia* in summer during the day.

*Colias* butterflies, which have a chill-coma temperature of 1.8°C, have not been observed to shiver in the field. They raise their thoracic temperature by basking. They do not fly at air temperatures below 12°C (Watt, 1968; Kingsolver, 1983). Other butterflies fly at ambient temperatures as low as 8°C (Heinrich, 1981a).

For all hymenopteran species studied here, chill-coma temperatures match behavioural data. Honeybee workers have lower chill-coma temperatures than drones. Drones fall out of a bee cluster first when temperatures become very low (Heinrich, 1981b). Bumblebees fly at lower ambient temperatures than honeybees and *Xylocopa virginica* (Free and Spencer-Booth, 1960; Heinrich, 1979; Esch, 1988; F. Goller and H. Esch, in preparation). However, bumblebees fly at lower ambient temperatures than the chill-coma temperatures determined by us. According to our data, bumblebees could not shiver if their flight muscles were colder than 7°C. Individuals have to keep their thoracic temperature above this critical value if they are to remain active. Bumblebee nests provide very good insulation (Richards, 1973) and queens can shiver throughout the night while 'incubating' brood (Heinrich, 1979). The availability of insulated shelter and the ability to warm up for extended periods might explain why bumblebees can fly at ambient temperatures below chill-coma.

A possible mechanism of heat production below chill-coma temperatures is endothermic heat generation by enzymic action. High levels of activity of phosphofructokinase and fructose diphosphatase were found simultaneously in flight muscles of bumblebees, suggesting that heat might be generated by splitting ATP (Newsholme *et al.* 1972; Clark *et al.* 1973). However, in long-term experiments in which flight muscle activity and thoracic temperature were monitored simultaneously in bumblebees, an increase in thoracic temperature was never recorded unless flight muscles were activated (Kammer and Heinrich, 1974; F. Goller and H. Esch, in preparation).

The use of endothermic activity in the natural environment is dictated by economic factors. The costs of active heating need to be balanced by biological benefits. This might explain why many Lepidoptera do not use flight muscles for warm-up at temperatures where shivering is possible. Too small an amount of heat production as a consequence of reduced wing-stroke work and insufficient heat retention might be limiting factors (e.g. Casey *et al.* 1981). Animals were cooled rather rapidly in our experiments, and only *Eupsilia devia* could heat its thorax significantly near 0°C.

The small variation in chill-coma temperatures in Lepidoptera indicates that ecological adaptation is not strongly reflected in this physiological characteristic

(Mutchmor and Richards, 1961). The rate of decrease of muscle potential amplitudes, however, seems to be correlated with ecological conditions. This is also supported by acclimatization effects on honeybee flight muscles, where in a single species the rate of decrease of muscle potential amplitudes changed with the temperature to which the animals were exposed. At the same time, however, chill-coma temperatures did not change. These effects on muscle potential amplitudes were not seen on the durations of muscle potentials (F. Goller and H. Esch, in preparation). Acclimation affects the shivering behaviour of *Danaus plexippus*. Cold-acclimated butterflies shivered more readily and for a longer period at 15°C than did the warm-acclimated control groups (Kammer, 1971). In the hymenopteran species investigated here, chill-coma temperatures are more variable among species and generally higher than in Lepidoptera. One reason for this might be the specialization of their flight muscles (asynchronous type). Lepidoptera have synchronous flight muscles. Studies on other synchronous fliers should help clarify whether low chill-coma temperatures are a common characteristic of all synchronous muscles or just of those in Lepidoptera. This will give further insight into the role of phylogenetic determination of chill-coma temperatures in flight muscles.

We are grateful to Drs Timothy Casey and Bernd Heinrich and two anonymous reviewers for comments on the manuscript.

### References

- AIDLEY, D. J. (1975). Excitation-contraction coupling and mechanical properties. In *Insect Muscle* (ed. P. N. Usherwood), pp. 337-356. London: Academic Press.
- BRUGGEMANN, P. F. (1958). Insect environment of the high Arctic. *Proc. Int. Congr. Entomol.* X, vol. 1, pp. 695-702.
- CASEY, T. M., HEGEL, J. R. AND BUSER, C. S. (1981). Physiology and energetics of pre-flight warm-up in the eastern tent caterpillar moth *Malacosoma americanum*. *J. exp. Biol.* **94**, 119-135.
- CASEY, T. M. AND HEGEL-LITTLE, J. R. (1987). Instantaneous oxygen consumption and muscle stroke work in *Malacosoma americanum* during pre-flight warm-up. *J. exp. Biol.* **127**, 389-400.
- CASEY, T. M. AND JOOS, B. A. (1983). Morphometrics, conductance, thoracic temperature, and flight energetics of noctuid and geometrid moths. *Physiol. Zool.* **56**, 160-173.
- CLARK, M. G., BLOXHAM, D. P., HOLLAND, P. C. AND LARDY, H. A. (1973). Estimation of the fructodiphosphatase-phosphofructokinase substrate cycle in the flight muscle of *Bombus affinis*. *Biochem. J.* **134**, 589-597.
- COLHOUN, E. H. (1960). Acclimatization to cold in insects. *Ent. exp. appl.* **3**, 27-37.
- ESCH, H. (1964). Ueber den Zusammenhang zwischen Temperatur, Aktionspotentialen und Thoraxbewegungen bei der Honigbiene (*Apis mellifica* L.). *Z. vergl. Physiol.* **48**, 547-551.
- ESCH, H. (1988). The effects of temperature on flight muscle potentials in honeybees and cuculiinid winter moths. *J. exp. Biol.* **135**, 109-117.
- FREE, J. B. AND SPENCER-BOOTH, Y. (1960). Chill-coma and cold death temperatures of *Apis mellifera*. *Ent. exp. appl.* **3**, 222-230.
- HEINRICH, B. (1979). *Bumblebee Economics*. 245pp. Cambridge: Harvard University Press.
- HEINRICH, B. (1981a). Ecological and evolutionary perspectives. In *Insect Thermoregulation* (ed. B. Heinrich), pp. 235-302. New York: John Wiley.
- HEINRICH, B. (1981b). The mechanisms and energetics of honeybee swarm temperature regulation. *J. exp. Biol.* **91**, 25-55.

- HEINRICH, B. (1987a). Thermoregulation by winter-flying endothermic moths. *J. exp. Biol.* **127**, 313–332.
- HEINRICH, B. (1987b). Thermoregulation in winter moths. *Scient. Am.* **256**, 104–111.
- HEINRICH, B. AND BARTHOLOMEW, G. A. (1971). An analysis of pre-flight warm-up in the sphinx moth, *Manduca sexta*. *J. exp. Biol.* **55**, 223–239.
- HEINRICH, B. AND PANTLE, C. (1975). Thermoregulation in small flies (*Syrphus* sp.): basking and shivering. *J. exp. Biol.* **62**, 599–610.
- KAMMER, A. E. (1971). Influence of acclimation temperature on the shivering behavior of the butterfly *Danaus plexippus* (L.). *Z. vergl. Physiol.* **72**, 364–369.
- KAMMER, A. E. (1981). Physiological mechanisms of thermoregulation. In *Insect Thermoregulation* (ed. B. Heinrich), pp. 115–158. New York: John Wiley.
- KAMMER, A. E. AND HEINRICH, B. (1972). Neural control of bumblebee fibrillar muscle during shivering. *J. comp. Physiol.* **78**, 337–345.
- KAMMER, A. E. AND HEINRICH, B. (1974). Metabolic rates related to muscle activity in bumblebees. *J. exp. Biol.* **61**, 219–227.
- KINGSOLVER, J. G. (1983). Thermoregulation and flight in *Colias* butterflies: elevational patterns and mechanistic limitations. *Ecology* **64**, 543–545.
- MELLANBY, K. (1940). The activity of certain arctic insects at low temperatures. *J. anim. Ecol.* **9**, 296–301.
- MORGAN, K. R. (1987). Temperature regulation, energy metabolism and mate-searching in rain beetles (*Plecoma* spp.), winter-active, endothermic scarabs (Coleoptera). *J. exp. Biol.* **128**, 107–122.
- MUTCHMOR, J. A. AND RICHARDS, A. G. (1961). Low temperature tolerance of insects in relation to the influence of temperature on muscle apyrase activity. *J. Insect Physiol.* **7**, 141–158.
- NEWSHOLME, E. A., CRABTREE, B., HIGGINS, S. J., THORNTON, S. D. AND START, C. (1972). The activities of fructose diphosphatase in flight muscles from the bumble-bee and the role of this enzyme in heat generation. *Biochem. J.* **128**, 89–97.
- RICHARDS, K. W. (1973). Biology of *Bombus polaris* Curtis and *B. hyperboreus* Schoenherr at Lake Hazen, Northwest territories (Hymenoptera: Bombini). *Quaest. Entomol.* **9**, 115–157.
- WATT, W. B. (1968). Adaptive significance of pigment polymorphisms in *Colias* butterflies. I. Variation of melanin pigment in relation to thermoregulation. *Evolution* **22**, 437–458.