METABOLIC RATES RELATED TO MUSCLE ACTIVITY IN BUMBLEBEES

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SUMMARY

1. Oxygen consumption, thoracic temperature, and action potentials from the fibrillar muscles were concurrently recorded in queens and workers of *Bombus vosnesenskii*.

2. The oxygen consumption of 'inactive' bees (quiescent and/or producing fewer than 0·3 spikes/sec) was markedly temperature-sensitive, with a Q_{10} of 3·4 for thoracic temperatures of 25–35 °C.

3. The oxygen consumption of bees with active fibrillar muscles was directly correlated with spike frequency; a single spike was associated with the consumption of 2·3–2·6 μl O_2/g_{th}. No oxygen debt was observed.

4. The oxygen consumption per spike during flight was similar to that during warm-up.

5. Oxygen consumption per gram thorax was similar in queens and workers engaging in similar activity.

6. The results are compared with those from other insects and their adaptive significance is discussed.

INTRODUCTION

Bumblebees, like other large insects, produce heat during flight at such high rates that the temperature of flying bees is considerably greater than ambient (Sotavalta, 1954). Non-flying bees may maintain a high thoracic temperature continuously, as well as warming up prior to flight (Heinrich, 1972*a, b*). In both cases heat production is accomplished, without apparent movement of wings or thorax, by activation of the large flight muscles (Kammer & Heinrich, 1972). The same mechanism of endothermy is presumably employed during incubation of brood (Heinrich, 1972*c*, 1974) and during foraging, when thoracic temperatures are usually regulated at about 35 °C over a range of ambient temperatures.

We have previously shown that endothermy in bumblebees is correlated with the occurrence of spikes in the fibrillar muscles, and that the rate of temperature increase during warm-up is a function of the spike frequency (Kammer & Heinrich, 1972; Heinrich & Kammer, 1973). By using the difference between thoracic and ambient temperature during warm-up to calculate the rate of heat production, we estimated

that approximately 10 cal/g_{th} are produced per spike in the fibrillar muscl█ (Heinrich & Kammer, 1973). However, heat production during flight and during stabilization of thoracic temperature cannot be accurately calculated from the difference between thoracic and ambient temperatures because variable amounts of heat are transferred from the thorax to the abdomen, thus complicating the assessment of the rate at which heat is lost from the body. Hence from the data previously obtained we cannot determine whether or not the muscles are producing more work-plus-heat during flight than during warm-up. In order to answer this question we have measured the oxygen consumption of active bees. We have correlated this measure with spike frequency in order to determine if the oxygen consumption per spike is the same in warm-up, when the wings are not moving, as in fixed flight, when the muscles are doing work in moving wings and air, as well as producing heat. In addition we have measured the metabolism of bumblebees which were not activating their fibrillar muscles. Since an apparently quiescent bee may in fact be exciting its muscles, resting metabolism cannot be measured with confidence in the absence of simultaneous recording of the electrical activity of the muscles.

MATERIALS AND METHODS

The bumblebees used in these experiments were queens and workers of *Bombus vosnesenskii* Radowskowski, collected in the San Francisco Bay region. During an experiment a bee was supported by a small rod waxed to its notum. A quiescent or shivering animal held or 'walked' on a small styrofoam ball. Flight was initiated in bees with thoracic temperatures > 30 °C by removing the ball from the tarsi. Thoracic temperatures were recorded by means of a small-bead thermistor (Veco GC 32A130) inserted dorsally about 1 mm into the thoracic muscles. The thin thermistor leads were insulated with Duco cement. The thermistor and other recording leads were waxed in place, and no bleeding was observed. Although the thermistor was slightly larger than the thermocouple used previously (Heinrich & Kammer, 1973), similar results were obtained with the two methods.

Action potentials from one or two motor units of the fibrillar muscles were recorded with respect to a ground in the abdomen by means of copper wires, 50 μm in diameter and insulated except at the tip. Only preparations from which single units were clearly recorded were used, because the action potentials were counted electronically (Time Systems Model 410 and Faratron Corp. Model 410). In a few instances the spikes were recorded on magnetic tape and recounted in order to check on the accuracy of the counting. In other instances the same spike-train was counted simultaneously by the two counters. These procedures produced cumulative counts which agreed within 10%. In most animals recordings were obtained from two different motor units which gave similar cumulative counts; in others the two motor units were active at different frequencies, one sometimes as much as 50–80% higher than the other. That such large differences in spike frequency were real was confirmed by visual observations of the spike-trains displayed on an oscilloscope. Since it was necessary to assume that the unit being sampled was representative of the population when in fact it may have been active at a frequency higher or lower than some unknown fraction of units, there is reason to expect scatter in the data. When two different values we█

⬤tained, both were plotted and included in the calculations. However, since a unit could fire at a higher frequency than a second unit and then within a few seconds fire at a lower frequency, these differences may have averaged out during the several minutes of recording. The effect was further minimized by using for calculations only those portions of the recordings during which the rate of oxygen consumption and the spike frequency were constant (cf. Fig. 2).

Oxygen consumption was measured by means of a Beckman E2 paramagnetic oxygen-analyser. After the animal was prepared for recording thoracic temperature and muscle spikes, it was enclosed in a sealed jar which was connected to the oxygen analyser. Air was circulated through the closed system by a peristaltic pump, and the percentage of oxygen in the air was determined. Readings to the nearest 0·001 % were made at frequent intervals during an experiment. The oxygen concentration was not allowed to fall below 20 %; it was maintained by flushing the animal-chamber with room air. The volume of the air contained in the system was 248 ml or 216–222 ml in different experiments. The lag-time between a marked change in the oxygen concentration of the air in the animal-chamber and the appearance of this air in the analyser was estimated to be a maximum of 30 sec. The data on oxygen consumption were corrected to STP (0 °C and 760 mmHg) and except where indicated are given in millilitres of oxygen per gram thorax per hour. Thoracic weight has been used instead of the more usual body weight because the fibrillar muscles which consume much of the oxygen occupy most of the thorax, and because the weight of the abdomen varies greatly depending on the honey content of the crop. For the bees used in this study, the average weight was three times average thoracic weight (mean of the ratio body weight/thoracic weight = 2·95, n = 9, s.d. = 0·46).

The oxygen content of the respirometer and the cumulative counts of spikes were noted every 15 sec (occasionally 30 sec) and plotted as in Fig. 2, which illustrates part of an experiment with one bee. From such raw data, time periods during which both oxygen content and spike-counts were changing linearly were selected, and the values observed during these periods were used to calculate rates of oxygen consumption and spike frequency.

In some experiments the air temperature around the bee was changed by placing the animal-chamber into warm water or onto ice. Ambient temperatures were measured by a second thermistor inside the glass jar.

RESULTS

1. *Metabolism in the absence of fibrillar muscle activity*

Data on the oxygen consumption of 'inactive' bumblebees were obtained from individuals which produced fewer than 0·3 spikes/sec over several minutes of observation. Such bees were sometimes apparently motionless, but at other times they were 'walking' on the styrofoam ball which they were holding. The thoracic temperature of these bees was altered by altering the ambient temperature. The rate at which oxygen was consumed by the 'poikilothermic' bees increased markedly as thoracic temperature increased (Fig. 1). The Q_{10} for the range 15–25 °C was 3·8, and for thoracic temperatures of 25–35 °C the Q_{10} was 3·4. (To calculate these Q_{10}'s, values ⬤oxygen consumption at the given temperatures were read from the curve in Fig. 1.)
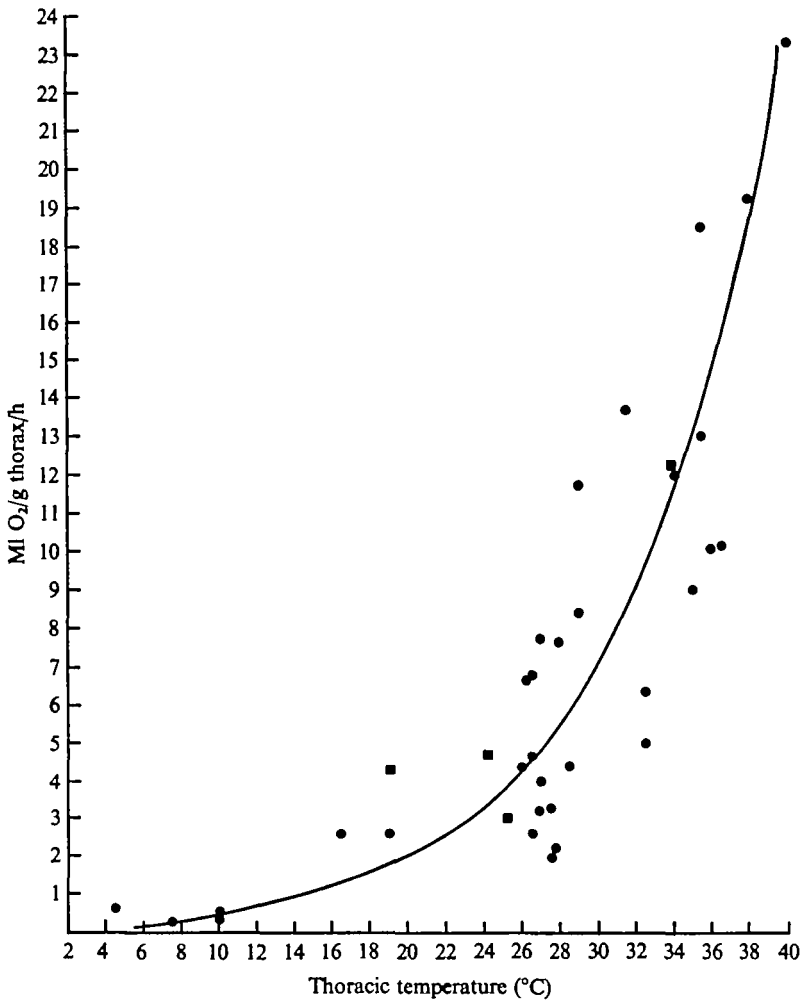
Fig. 1. Metabolic rates of inactive *Bombus vosnesenskii* at different thoracic temperatures. In this experiment 'inactive' means fewer than 0·3 spikes/sec recorded from the flight muscles. Thoracic temperatures were changed by changing the ambient temperature. The curve was fitted by eye (● = queens, ■ = workers).

On a per-gram-thorax basis, the two sizes of bumblebees had similar 'resting' rates of metabolism; the worker thorax weighs *ca.* 0·06 g (mean of 6 bees), the queen thorax *ca.* 0·2 g (mean of 11 bees).

2. *Metabolism and muscle activation during warm-up and temperature regulation*

The rate at which oxygen was consumed increased as the rate of excitation of the fibrillar muscles increased. Early in a bout of muscle activity thoracic temperature increased as spike frequency increased; later T_{th} typically was maintained higher than ambient (Fig. 2). A bumblebee regulating its thoracic temperature by 'shivering' at a rate of 10 spikes/sec has a metabolic rate almost 25 times higher than when it is quiescent with a thoracic temperature of 25 °C. The relationship between the spike frequency and the oxygen consumption was linear (Fig. 3) and the correlati
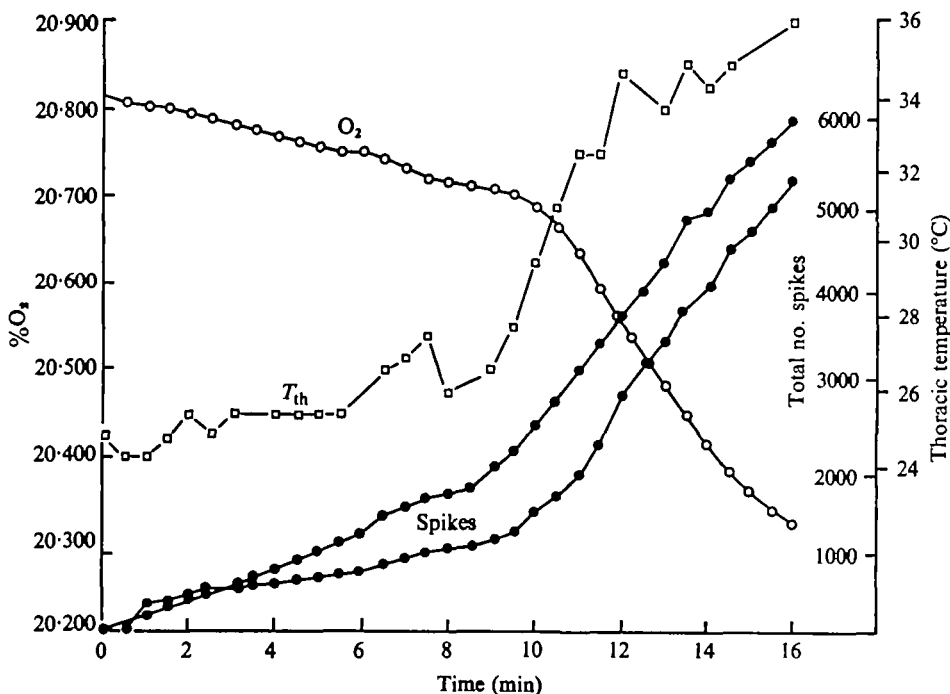
Fig. 2. Sample of data obtained during 16 minutes of observation of one *Bombus* worker, to illustrate concurrent changes in: oxygen concentration in the experimental vessel (O), thoracic temperature (□), and cumulative numbers of spikes in two different units of the fibrillar muscles (●).

coefficient for the warm-up data was 0·85 ($n = 64$). Comparable values were obtained from both worker and queen bumblebees (Fig. 3).

Although oxygen was consumed at a high rate when the fibrillar muscles were activated, no noticeable oxygen-debt was accumulated. When an animal became quiescent after a bout of activity (either warm-up or flight), the rate of oxygen consumption returned to the resting value within 30 sec, the estimated lag-time of the oxygen analysis.

3. Metabolism and muscle-activation during flight

The metabolic rates and spike frequencies during fixed flight fell into a range similar to that observed during warm-up (Fig. 3). There is considerable scatter in the flight-data: the correlation coefficient is 0·45, which for $n = 17$ is not statistically significant at the 0·05 level (Table 7.6.1 in Snedecor, 1956). Part of the scatter is probably due to the fact that in our apparatus the bees did not perform long flights at a wide range of spike frequencies.* Because of the similarities in the distribution of the data points for warm-up and flight (Fig. 3), we conclude that the relationship between metabolic rate and spike frequency during fixed flight is the same as that during warm-up. In addition, there appears to be no difference between the metabolic

* It should be noted that some of the values for spikes/sec plotted in Fig. 3 do not represent actual spike frequencies in flight, since brief intervals during which flight ceased were included in the elapsed time over which the total number of spikes and oxygen consumption were measured.
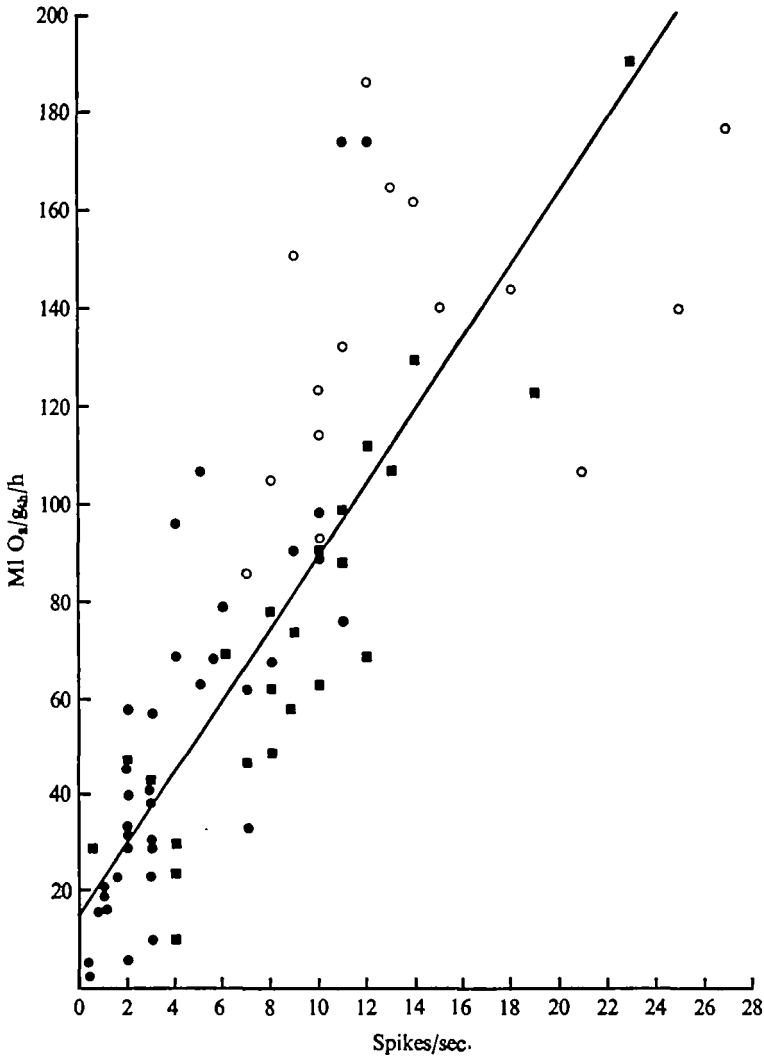
Fig. 3. Metabolic rates during warm-up (■ = workers, ● = queens) and flight (○ = queens) as a function of spike frequency. The regression line shown was calculated from the warm-up data (number of samples = 64).

rate (O_2 consumption per gram thorax) of workers and that of queens at the same spike frequency.

The energy expenditure correlated with a single spike can be calculated from the regression line in Fig. 3. At the higher spike frequencies (10–20/sec, chosen because under this circumstance resting metabolism constitutes only a small fraction of the oxygen consumption), 2·3–2·6 μl of oxygen were consumed per gram thorax per spike.

The rate of oxygen consumption for an activity involving the fibrillar muscles, such as free flight, can be predicted from a measured spike frequency and the above result. In order to assess the metabolic rate in free flight, we allowed a bee to fly on a 50 cm long tether composed of only the light wire recording leads. When the bee flew approximately horizontally and was presumably supporting her full weight, inters█

Table 1. *Metabolic rates of various insects at rest (at the indicated temperature) and during flight*

Species	Oxygen consumption (ml/g body wt/h)			Source
	Inactive	Temperature	Flight	
Bombus vosnesenskii	1·3	(25 °C)	55–66[*]	Present paper
Schistocerca gregaria	0·63	(28 °C)	10–30[*]	Krogh & Weis-Fogh, 1951
Apis mellifera	3·16	(22 °C)	60·5[*]	Hocking, 1953
A. mellifera	—	—	70·3[*]	Bastian & Esch, 1970
Drosophila melanogaster	4·98	(25 °C)	32·9[*]	Hocking, 1953
D. repleta	1·7	(25 °C)	21·1[†]	Chadwick & Gilmour, 1940
Manduca sexta	—	—	21[*]	Heinrich, 1971
M. sexta and other sphingid moths	—	—	40–75[†]	Heinrich & Casey, 1973

[*] = fixed flight;   [†] = free flight.

intervals were measured with an oscilloscope. Under these conditions, the usual interspike interval was 50 msec, which corresponds to 20 spikes/sec. Shorter intervals of 35–45 msec were also observed occasionally, as were longer intervals of 60–70 msec. On the basis of 20 spikes/sec (the value observed most often) and 2·3–2·6 μl O_2/g_{th}/spike (the values determined above), we calculate that a bee in free flight consumes oxygen at the rate of 166–188 ml/g_{th}/hr. These calculated values correspond to minimum values of oxygen consumption measured in unimpaired free flight (Heinrich, in manuscript).

<center>DISCUSSION</center>

<center>1. *Metabolic rates: comparison with* Schistocerca</center>

In studies of insect flight-metabolism, comparison is often made with the locust *Schistocerca gregaria*, because that species has been relatively well studied. In comparison with results from the locust, our measurements of both resting metabolism and flight-metabolism are high (Table 1). However, they are not unreasonable, because: (1) The results are internally consistent. A datum obtained by extrapolating the results from active animals to 0 spikes/sec agrees with the result from inactive animals with the same thoracic temperature. That is, the Y-intercept of the regression line relating oxygen consumption to spike frequency (Fig. 3) is 15·1 ml/g_{th}/h, whereas the measured 'resting' metabolic rate at a T_{th} of 35 °C is 13–17·5 ml/g_{th}/h. (2) Metabolic rates of the same magnitude have been obtained from honeybees, *Apis mellifera*, by other workers using different methods (Table 1). (3) Two other estimates of the metabolic rate per spike in Hymenoptera are also consistent with the results of the present study. Bastian & Esch (1970) found that honeybees consumed 1·2 μl O_2/g body wt/spike, a value of the same order of magnitude as our result of 0·8 μl/g body wt/spike. In a previous study in which measurements of thoracic temperature were used to calculate the rate of heat production, we estimated that during warm-up the heat production per spike is approximately 10 cal/g_{th}/spike (Heinrich & Kammer, 1973). This result is equivalent to an oxygen consumption of 2·1 μl O_2/g_{th}/spike, which is close to the measured value of 2·3–2·6 μl/g_{th}/spike.

The oxygen consumption in flight of *Bombus* is two- to sixfold greater than that of *Schistocerca* if expressed as a rate per gram of body weight (Table 1). The

difference is apparently not due to the presence of different types of muscle: *Drosophila* has fibrillar muscles like *Bombus* but its oxygen consumption is similar to that of *Schistocerca*, and sphingid moths have non-fibrillar muscles like *Schistocerca* but consumption is similar to that of *Bombus* (Table 1). The difference appears to be largely due to the fact that a greater proportion of the body of *Bombus* is flight-muscle, since the results for *Bombus* can be expressed as 87–186 ml O_2/g muscle/h, and these values are similar to those of 53–158 ml/g muscle/h obtained for locusts on a flight-mill (Weis-Fogh, 1952).

Bumblebees in free flight have, however, greater metabolic rates than those reported here (Heinrich, in ms.). The upper values for *Schistocerca*, on the other hand, are a fair representation of values for free flight, since they correspond to a lift equal to or greater than the body weight (Weis-Fogh, 1964). Therefore the oxygen consumption expressed per gram of muscle is greater for *Bombus* in free flight than for *Schistocerca*.

The spike frequencies and oxygen consumption recorded during warm-up also represent less than maximal performance. Bumblebees subjected to less manipulation can produce higher spike frequencies and more rapid increases of thoracic temperature than those observed in the present experiments (Heinrich & Kammer, 1973, and unpublished observations).

2. *The mechanism of heat production*

Within the resolution of the data obtained, the oxygen consumption per spike in *Bombus* is the same during warm-up and flight. Thus there appears to be no additional activation of the fibrillar muscles by the stretching and shortening which occur during flight. A similar conclusion was reached by Bastian & Esch (1970) in their study of honeybees.

In *Bombus* there also is no evidence for increased oxygen consumption other than that related to increased thoracic temperature (Fig. 1) and increased muscle activity (Fig. 3). We have previously proposed that the main mechanism for control of heat production in bumblebees is alteration in the spike frequency (Heinrich & Kammer, 1973). However, production of heat for endothermy by biochemical mechanisms other than those which depend on muscle activation has been postulated recently (Newsholme *et al.* 1972; Clark *et al.* 1973). We challenge this hypothesis; there is as yet no evidence for increased thoracic temperatures in the absence of muscle activity. Furthermore, the rate of substrate cycling reported by the latter authors yields only 0·104 cal/min/g muscle (using the maximum rate observed and assuming 1 mole of ATP hydrolysed produces 10^4 cal). For a bee with a thoracic weight of 0·23 g the yield from the proposed biochemical mechanisms would be 0·024 cal/min. In order to maintain a thoracic temperature only 10 °C greater than ambient, a *Bombus* bee with a thoracic weight of 0·23 g must expend approximately 0·5 cal/min (Heinrich & Kammer, 1973). This amount is about 20 times more than that which can be produced by the observed rates of ATP cycling. Furthermore, the bees are able to generate heat at rates two or more times higher than our example (Heinrich & Kammer, 1973).

3. *Why do some insects warm-up and regulate thoracic temperature?*

Bumblebees and some other large insects have to raise their body temperature before they can fly, if the ambient temperature is less than about 25 °C (Krogh

Zeuthen, 1941; Sotavalta, 1954). Why have these insects not become adapted to flight over a range of low thoracic temperatures, thus sparing themselves the metabolic expense of warming up ? It is proposed that muscles adapted to function over a limited temperature range are more efficient than muscles which are adapted to function over a wide range of temperature. It is further proposed that large insects regulate at a high temperature because limited mechanisms of cooling make such temperatures unavoidable during flight at normal ambient temperatures (cf. Neville, 1965). The first hypothesis is supported by the observation that during flight at high lift the muscles of *Bombus* have a higher metabolic rate than the muscles of *Schistocerca*, but the latter are less temperature sensitive (Q_{10} for glucose oxidation = 1·4; Candy, 1970). Running and flying vertebrates are also subject to obligatory heating so the above hypothesis may also apply to them.

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