ACTIVATION OF THE FIBRILLAR MUSCLES IN THE BUMBLEBEE DURING WARM-UP, STABILIZATION OF THORACIC TEMPERATURE AND FLIGHT

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INTRODUCTION

The fibrillar muscles of bumblebees may be used to drive the wings during flight and during fanning for nest-temperature regulation (Hasselrot, 1960), to produce buzzing sounds, to vibrate the substrate during pollen collecting (Michener, 1962), and to generate heat prior to flight or during the incubation of brood (Heinrich, 1972b). During fanning, buzzing and the production of other vibrations, the generation of heat is incidental to the performance of mechanical work, but during warm-up and brood incubation the primary task of the fibrillar muscles is heat production.

During warm-up the indirect muscles are usually excited by synchronous impulses (Mulloney, 1970; Kammer & Heinrich, 1972), but the motor patterns during the stabilization of thoracic temperature in stationary bumblebees, and during flight at different ambient temperatures, have not been examined previously. Little is known about the relationship between muscle activation and (1) the rate of heat production during warm-up at different thoracic and ambient temperatures, and (2) the temperature excess maintained during stabilization of thoracic temperature at various ambient temperatures.

Bumblebees fly at low as well as at high ambient temperatures (Heinrich, 1972a) and require a high thoracic temperature in order to be able to continue flying (Krogh & Zeuthen, 1941). However, during flight the primary function of the fibrillar muscles is to drive the wings, and the production of heat is obligatory and continuous. Since the fibrillar muscles can be used specifically for heat production during warm-up, it is of interest to know whether their activation is varied also during flight in order to control heat production.

We here report on the activation of the fibrillar muscles at different muscle temperatures during warm-up, during the stabilization of thoracic temperature at a variety of ambient temperatures, and during fixed flight at different ambient temperatures.

MATERIALS AND METHODS

Concurrent observations were made on the thoracic temperatures and the electrical activity of the fibrillar flight muscles of queen bumblebees, *Bombus vosnesenskii* Radowskowski. Muscle potentials were recorded extracellularly from bees glued to

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a support, as in previous experiments (Kammer & Heinrich, 1972). The data wer
recorded on magnetic tape and film for subsequent processing. The number of
muscle potentials (spikes) per second was determined by manually counting the
filmed recordings. When the firing occurred in bursts it was sometimes difficult to
make accurate counts of individual spikes because the extracellular potentials became
small and were summed. They could thus be confused with the 'noise' of small
potentials representing distant muscle units (cf. Fig. 7). Each small potential change
considered to come from the unit in question was counted as one spike.

Thoracic temperatures (T_{Th}) were measured by means of a small bead thermistor
placed about 2 mm below the thoracic notum. These data were also recorded on
magnetic tape with the muscle potentials. Ambient temperatures (T_A) were measured
with a mercury thermometer placed 1–3 cm from the bee. The ambient temperature
was changed by placing the bee and its support inside either a Styrofoam box con-
taining a layer of ice or a glass box within a heated water bath.

Thoracic temperatures were also measured on bumblebees free to move about
within a wooden box. In these bees thoracic and abdominal temperatures were
measured with 46-gauge copper-constantan thermocouples with cotton insulated leads.
Temperatures were recorded at 3 sec intervals with a Honeywell recording potentio-
meter.

Rates of heat production were calculated from the observed temperature excesses
($T_{Th} - T_A$) and the rates of change of T_{Th}. In making these calculations we assumed
that: (1) only the thorax was heated; (2) cooling was passive and, according to
Newton's Law of Cooling, a function of the temperature excess; and (3) the thermal
capacity of the thorax was 0·8 cal $°C^{-1}.g^{-1}$ (Krogh & Zeuthen, 1941). Data from the
rate of cooling in still air of a dead *B. vosnesenskii* queen which weighed 0·4 g were
used to calculate the rate of heat loss as different values of ($T_{Th} - T_A$). An average
value of 0·23 g was used as the thoracic weight of the experimental insects. Using a
mean thoracic weight is a valid approximation because the thoracic weights of queen
B. vosnesenskii were similar (0·22–0·25 g, $N = 5$). The assumption that only the
thorax is heated appears to be valid during preflight warm-up, but during the stabiliza-
tion of T_{Th} in stationary bees the abdomen may also be heated (Heinrich, 1972b) and
calculations of heat production based on T_{Th} therefore underestimate the actual rates
of heat production.

RESULTS

A. *Warm-up*

The rate of heat production by the bumblebees during warm-up depended on their
thoracic temperature. At low T_{Th} (< 10 °C) the bees were unable to warm them-
selves. However, when a bee commenced activity at higher T_{Th}, activation of the
fibrillar muscles (Fig. 1) resulted in a progressive rise in thoracic temperature (Fig. 2).
As the warm-up proceeded and T_{Th} increased, spike frequency increased (Figs. 2, 3).

The estimated rate of heat production during warm-up was correlated with spike
frequency (Fig. 4). Within the limits of error in the estimated rate of heat production,
there was no effect of temperature on the heat produced per muscle activation (Fig. 5).
Therefore, heat production was primarily a function of spike frequency. This
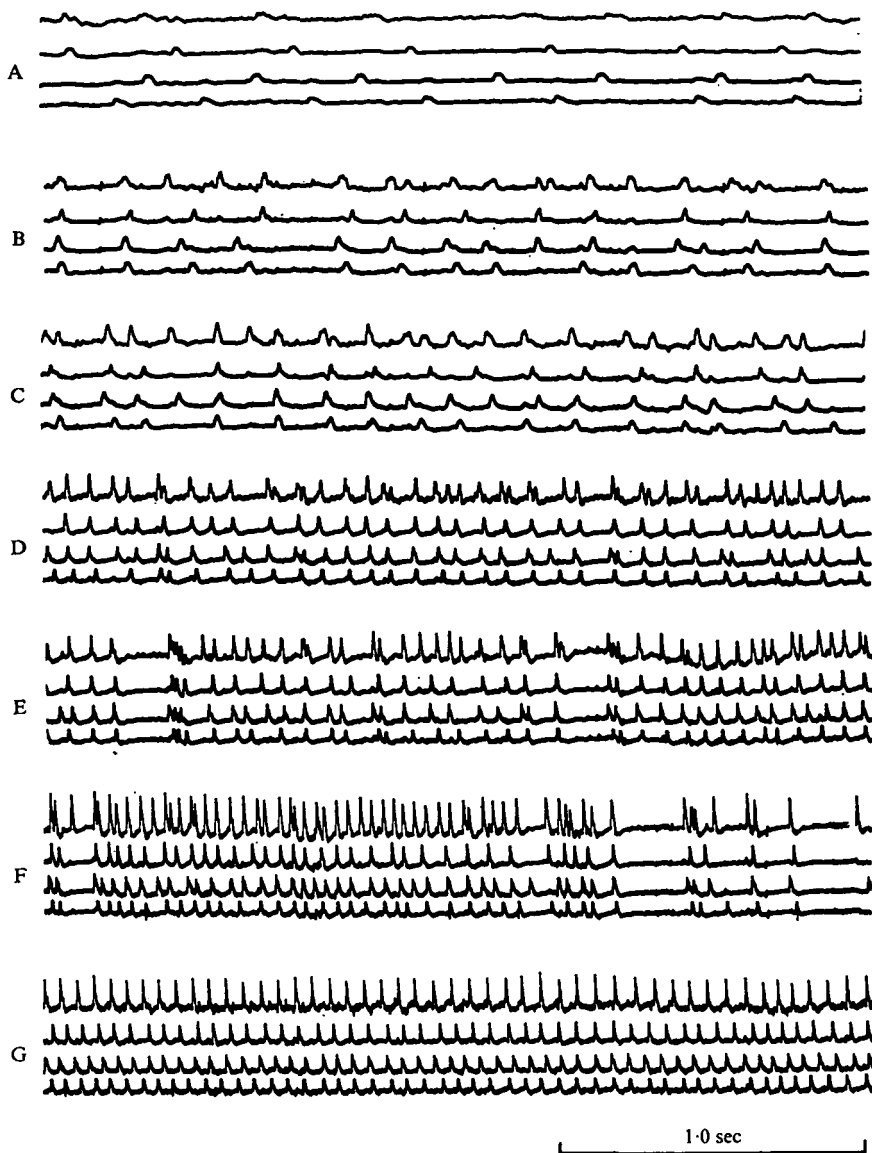frequency was always low at low T_{Th} and higher but more variable at higher T_{Th}.

Fig. 1. Muscle potentials recorded from four different motor units of the fibrillar muscles during warm-up (A–F) at different thoracic temperatures and during flight (G). Ambient temperature: 7·5 °C during warm-up and 11 °C during flight. Thoracic temperature: A = 13·0 °C, B = 17·0 °C, C = 19·9 °C, D = 25·6 °C, E = 30·1 °C, F = 37·7 °C, G = 38·1 °C. In each set of four tracings in the seven records the second is from the left DV muscle, the fourth is from the right DV muscle and the first and third are unidentified.

B. *Stabilization of thoracic temperature*

Bumblebee queens when relatively stationary during 'brooding' may maintain a high and relatively constant thoracic temperature independent of T_A for more than a day (Heinrich, 1972a). In bees walking about and in bees wired for the recording of muscle potentials there were short-term fluctuations of T_{Th} around a high value
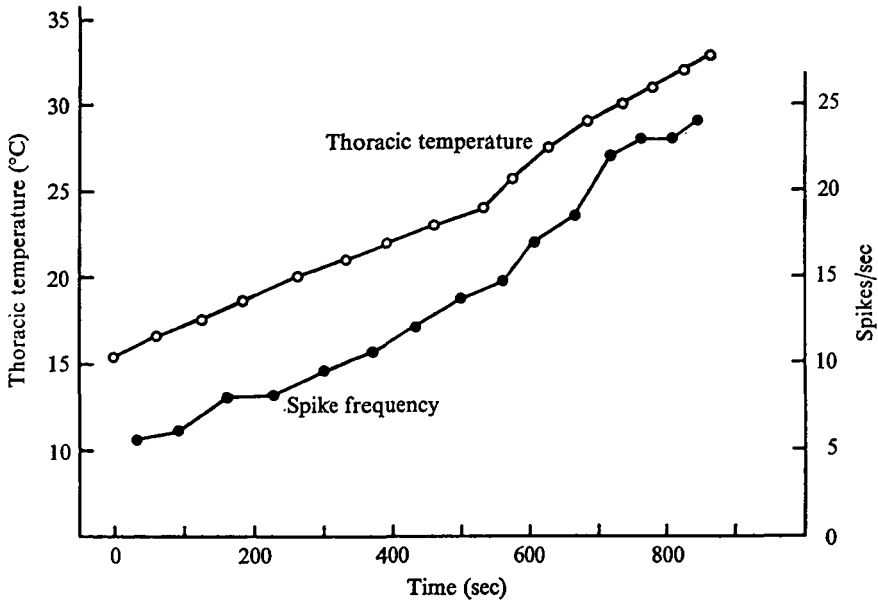
Fig. 2. Spike frequency and thoracic temperature during warm-up. The spike frequencies which are plotted represent the mean during the respective intervals between temperature measurements. Warm-up began with a T_{Th} of 13 °C after the bee was transferred to 11 °C from a higher T_A.
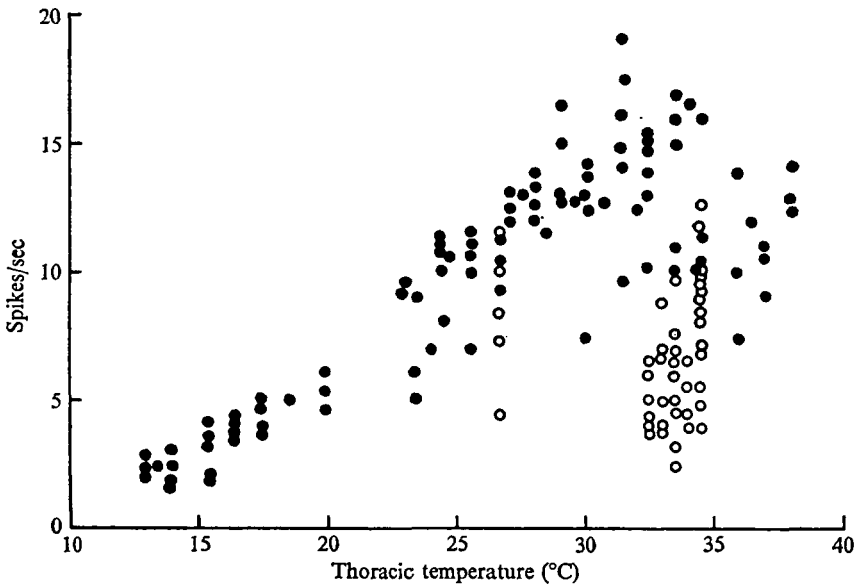

Fig. 3. Spike frequency over 5 sec intervals in relation to thoracic temperature during warm-up (●) and during the stabilization of thoracic temperature (O). These data are from one bee which was examined at several ambient temperatures.
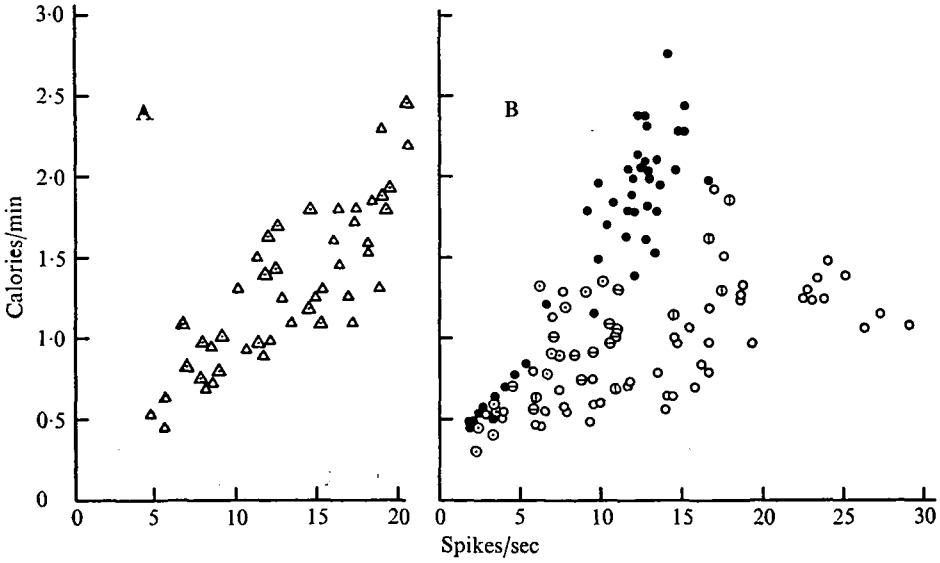
Fig. 4. Calculated rate of heat production during warm-up in relation to spike frequency. (A) Data obtained from one bee; $\triangle$, $T_A \geqslant$ 20 °C; $\triangle$, $T_A <$ 20 °C. (B) Data from five additional bees. Results from each bee are coded with a different symbol. Both (A) and (B) include results from different T_A and T_{Th}. Five out of the six bees show a direct correlation between calculated rate of heat production and spike frequency.
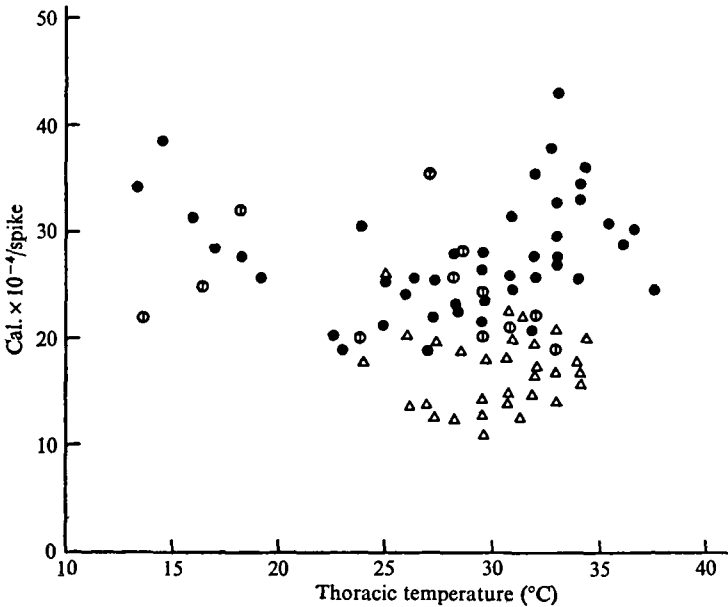
Fig. 5. The calculated heat production per spike during warm-up in relation to thoracic temperature. Data from three bees are shown. The symbols correspond to those in Fig. 4.
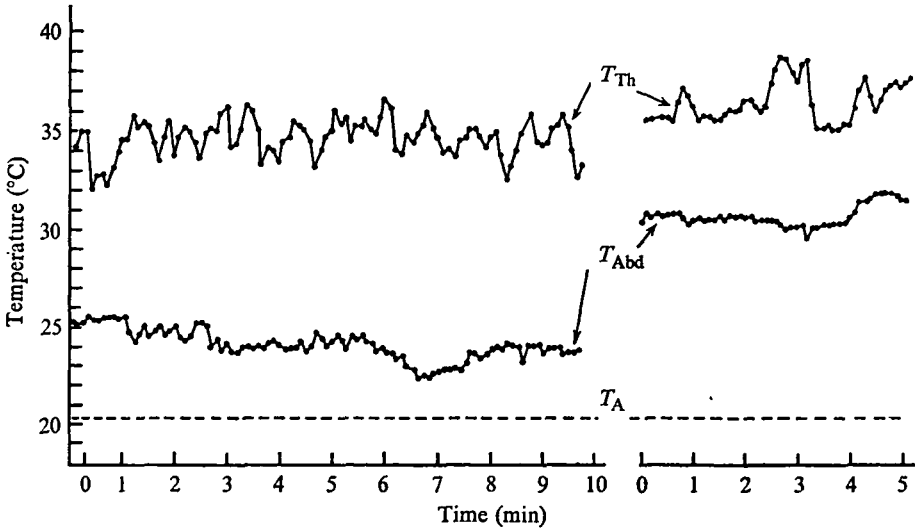
Fig. 6. Thoracic and abdominal temperatures ($T_{Abd.}$) of a *B. vosnesenskii* queen restrained only by thermocouple leads. In these two extracts from a continuous record, the thermocouples were in the same positions throughout. The temperature difference between T_{Th} and $T_{Abd.}$ here varies from 4·2 to 13·6 °C.

(Figs. 6–8). For purposes of data analysis in the present paper stabilization was defined as the maintenance of T_{Th} within 1 °C for at least 20 sec. Under the experimental conditions of the present study thoracic temperatures were usually stabilized at 33–36 °C over ambient temperatures ranging from 5–30 °C. However, the bees did not warm up at the lowest T_A unless T_{Th} was already partially elevated when they were placed in the lower T_A. Bumblebee queens may also stabilize the temperature of the abdomen, but at very different temperatures (Fig. 6; Heinrich, 1972a), indicating that different amounts of heat can be transferred from the thorax to the abdomen.

Muscle potentials recorded during the stabilization of T_{Th} showed bouts of intense activity, periods of relative inactivity, and wide ranges of intermediate frequencies (Fig. 7). The mean number of spikes/sec during the stabilization of T_{Th} was usually lower and more variable than that during warm-up at approximately the same thoracic temperature (Figs. 2, 8).

During warm-up in most of the bees, heat production was a function of spike frequency (Fig. 4), and there is no reason to think that the same generalization does not apply to heat production during the stabilization of T_{Th}. As expected, there was a clear correlation between spike frequency and the temperature excess $(T_{Th} - T_A)$ maintained during the stabilization of T_{Th} in some bees (Fig. 9). However, in other bees the temperature excess, and the estimated heat production during the stabilization of T_{Th} (assuming only the thorax is warmed) was often less than half the predicted values at a given spike frequency. It follows that thoracic temperature is differently affected by a given spike frequency because of heat loss from the thorax. Since the abdomen may contain as much heat as the thorax at any one time (Heinrich, 1972b), we suggest that during the stabilization of T_{Th} heat may be transferred from the thorax to the abdomen, producing elevated abdominal temperatures (Fig. 6). How-
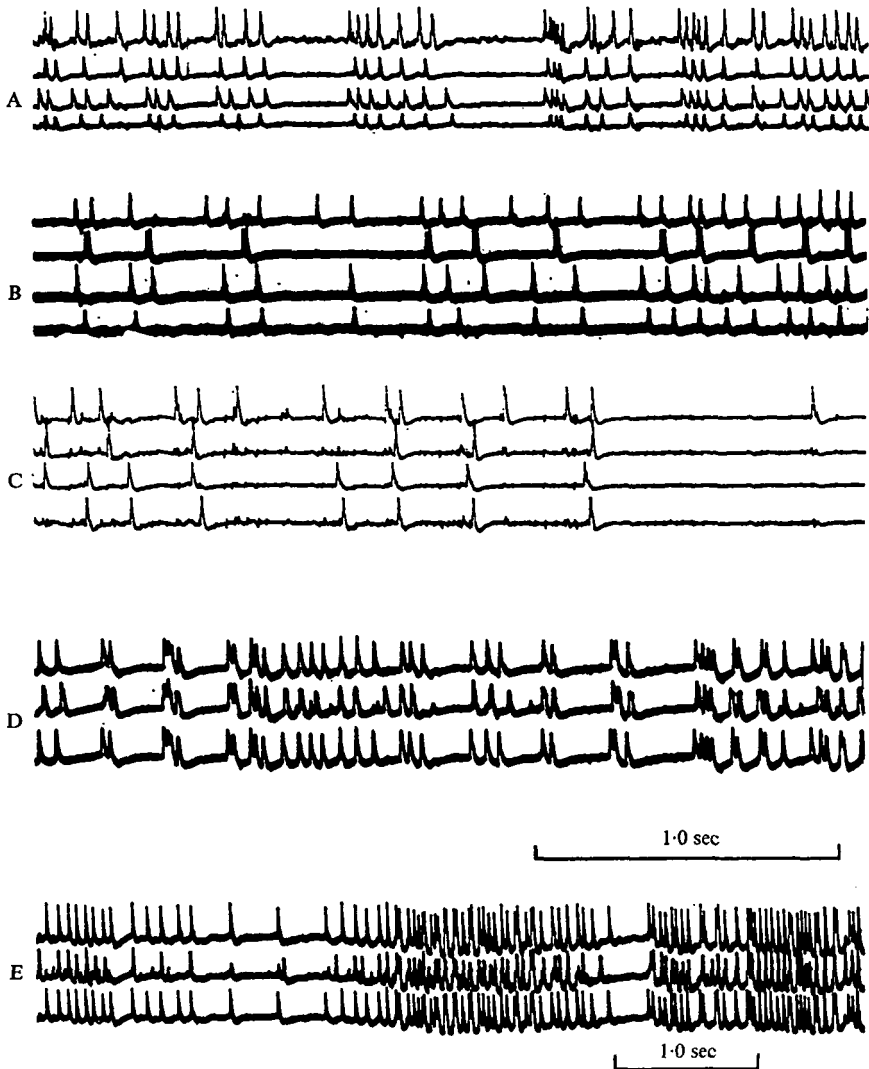
Fig. 7. Spikes recorded from the fibrillar muscles during the stabilization of thoracic temperature at different ambient temperatures. Thoracic temperature was similar (31–34 °C) in the different samples. Ambient temperature: A = 7 °C, B = 23 °C, C = 28 °C, D = 17 °C, E = 28 °C. A, B and C are from the same bee and from the same placement of electrodes as in Fig. 1. In D and E the three tracings from the top down are from right DV, left DV and right DL. This figure shows that spike frequency in any motor unit may vary over a wide continuum at a high T_{Th}. Spike frequencies were usually higher at the larger than at the smaller $(T_{Th} - T_A)$, although high spike frequencies, often in burst (E), were also observed at small $(T_{Th} - T_A)$.

ever, in stationary bees heat transfer is obviously not a prerequisite for the stabilization of T_{Th}.

C. *Flight*

The bumblebees usually initiated flight spontaneously after a vigorous warm-up. At the beginning of flight spike frequency was high, and then decreased (Figs. 1, 10; fig. 1 in Kammer & Heinrich, 1972). Spike frequency during fixed flight was
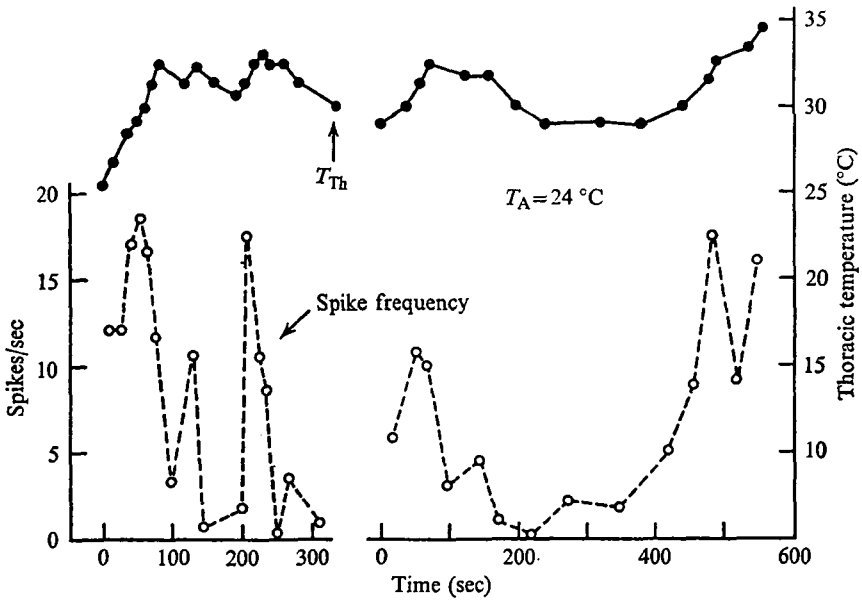
Fig. 8. Spike frequency and thoracic temperature during the later stages of warm-up and during the stabilization of thoracic temperature in stationary bees. Data are plotted as in Fig. 2. Thoracic temperature is stabilized by on-off warm-up behaviour as well as by variations of spike frequency.
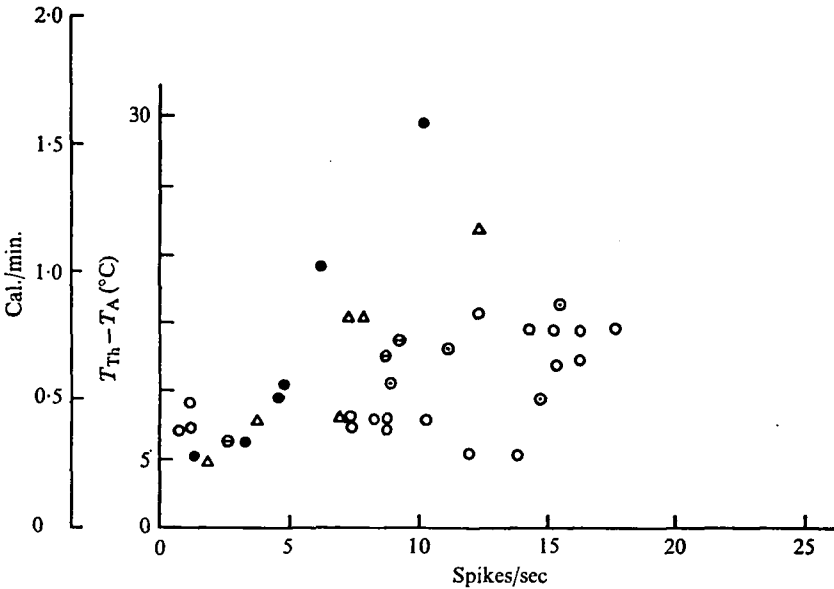
Fig. 9. Temperature excess $(T_{Th} - T_A)$ and calculated rate of heat production in relation to spike frequency during the stabilization of thoracic temperature in stationary bees. Data from different bees are coded with different symbols (the same symbols were used to indicate the same bees in Figs. 4 and 5). In bees (●) and (△) the calculated rate of heat production and spike frequency are directly correlated as in warm-up. In bees (☉) and (○) the two variables are not correlated and the apparent rate of heat production is much less than during warm-up.
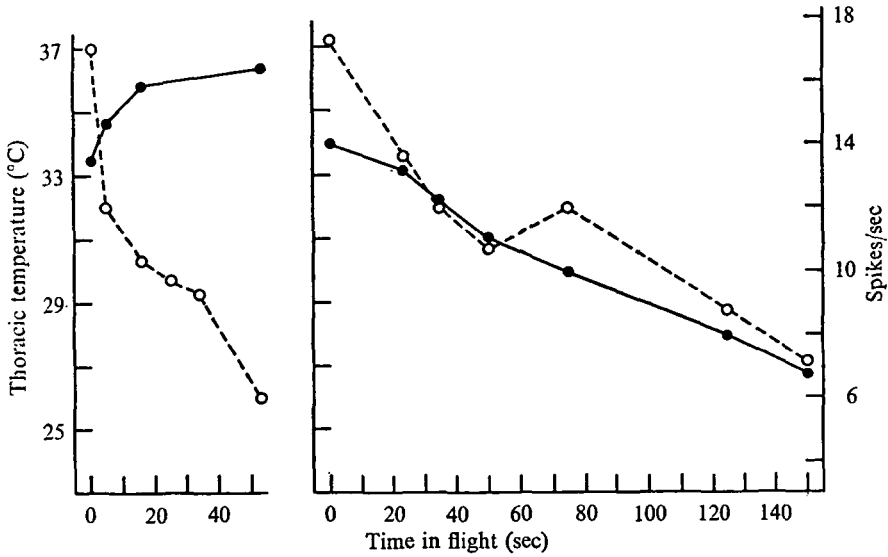
Fig. 10. Thoracic temperature (●) and spike frequency (O) during early portions of fixed flight at 29 °C (left) and 13 °C (right). At both ambient temperatures spike frequency decreases. At high T_A the heat production from the flight effort is sufficient to cause an increase in T_{Th}, but T_{Th} decreases along with spike frequency at low T_A.
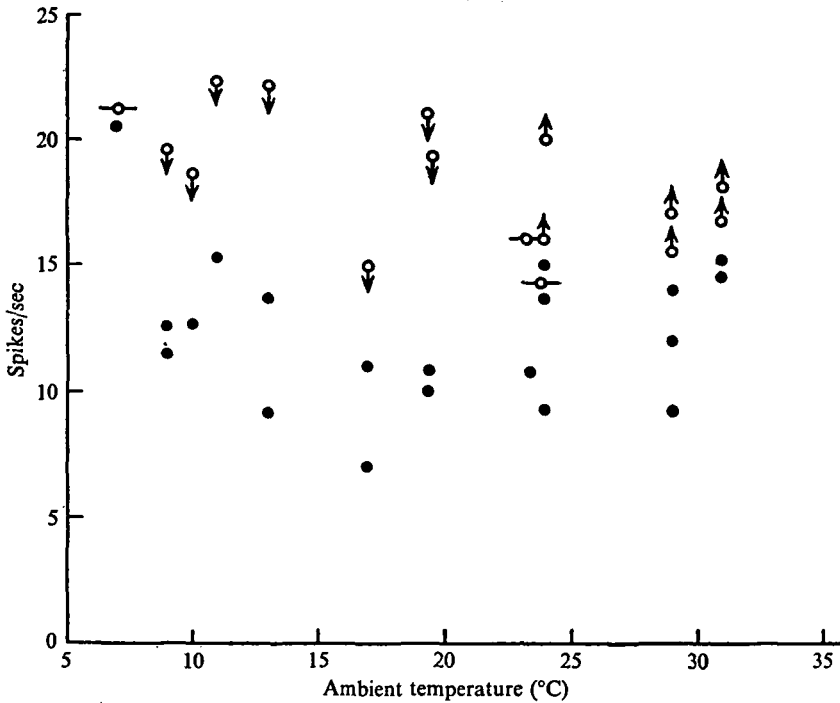

Fig. 11. Spike frequency during fixed flight at different ambient temperatures. O = early in flight, ● = later in the same flight. Changes in thoracic temperature (accompanying the recordings of spike frequency) during early flight: ↑ = increase, ↓ = decrease, — = no change.

independent of the ambient temperature. During fixed flight at high T_A thoracic temperature increased but at low T_A thoracic temperature usually decreased (Fig. 11). However, in one case we observed a $T_{Th} = 35.8$ °C at a T_A of 7 °C during fixed flight lasting approximately 1 min. Spike frequency also stayed high during this flight, namely between 20.3 and 21.7/sec. In the same animal while not flying, and presumably while losing less heat by convection, stabilization of T_{Th} at 34.7 °C at 5 °C was accomplished with only 10.2 spikes/sec. Thus a high T_{Th} can be maintained at a low T_A during fixed flight. In most of these experiments, however, T_{Th} was not stabilized during fixed flight, and varied with T_A and with spike frequency.

DISCUSSION

The thoracic temperature of bumblebees, as of any insect, depends on the balance between the rate of heat input and the rate of heat loss. The main determinant of heat input (in shade) is the activity of the flight muscles, as indicated by the frequency of muscle potentials. The rate of heat loss depends primarily on the difference between thoracic and ambient temperatures, but heat loss can be varied by the transfer of heat from thorax to abdomen. The relationships among these variables during warm-up, stabilization of T_{Th}, and fixed flight are considered in the following discussion.

A. *Warm-up*

Like many other insects, bumblebees warm up prior to flight and do not initiate flight until the thoracic temperature is near 30 °C (Krogh & Zeuthen, 1941). Heat production of sufficient magnitude to elevate body temperature above ambient temperatures occurs only when the flight muscles are activated. In honeybees the metabolic rate during warm-up as well as during flight is directly related to spike frequency (Bastian & Esch, 1970). Similarly, in bumblebees, spike frequency and rate of heat production are correlated during warm-up. Both increase with thoracic temperature (Figs. 2–4) so that T_{Th} rises slowly at low T_A (Fig. 2) and rapidly at high T_A (Fig. 8). At $T_{Th} < 10$ °C the rate of heat production is lower or equal to the rate of heat loss. Consequently the bees are unable to attain the high T_{Th} required for the rapid production of heat which is necessary to maintain a large temperature excess. Since bumblebees may forage with a T_{Th} near 36 °C, even at dawn at a T_A of 2 °C (Heinrich, 1972a), it is probable that they keep warm throughout the night in the nest and that, at such low T_A, they maintain a high T_{Th} during the day in order to remain active. Temperature regulation in the nest at low T_A (Hasselrot, 1960) may thus be functional not only in accelerating the rate of brood development (Himmer, 1932), but also in making it possible for the bees to initiate foraging early in the morning before T_A has risen to levels where warm-up from low T_{Th} (and low T_A) is possible.

In most bees the rate of heat loss is probably not varied during pre-flight warm-up. The linear relation between spike frequency (Fig. 4) and heat production (calculated from T_{Th} and passive rate of cooling) and the maintenance of a relatively low abdominal temperature (Heinrich, 1972b) suggest that heat is sequestered in the thorax rather than being dissipated to the abdomen. Such a retention of heat in the thorax occurs

In the sphinx moth, *Manduca sexta*, during flight at low T_A (Heinrich, 1970) and during warm-up (Heinrich & Bartholomew, 1972).

B. *Stabilization of thoracic temperature*

It is intuitively obvious that the minimum amount of heat which is required to stabilize a given T_{Th} is inversely related to T_A. However, in several bees we did not observe a correlation between spike frequency (and presumably heat production) and the difference between T_{Th} and T_A (Fig. 9). Abdominal temperature was often variable while T_{Th} was relatively stable (Fig. 6). These results suggest that, at the higher spike frequencies, these bees were producing more heat than the minimum which was necessary to stabilize T_{Th} and the additional heat was being transferred into the abdomen. Heating of the abdomen is functionally significant during brood incubation; heat is transferred from the thorax into the brood clump via the abdomen (Heinrich, 1972b). Transport of heat from the thorax to the abdomen has also been observed in flying moths. In *Manduca sexta* during free flight at ambient temperatures higher than 20 °C heat is carried by the blood into the abdomen, thereby preventing overheating of the flight muscles and stabilizing T_{Th} (Heinrich, 1970).

C. *Flight*

During fixed flight in bumblebees spike frequency was not related to T_A. The heat production, which is a function of spike frequency, was sufficient to cause an increase of T_{Th} during fixed flight at high T_A. At low T_A, however, the heat production was insufficient to maintain the temperature ($\geqslant$ 30 °C) which is necessary for level free flight. These results indicate that T_{Th} is not regulated at specific lower set-points during fixed flight. However, the bees must have a $T_{Th} \geqslant$ 30 °C during free flight in the field, and bumblebees in the field are capable of continuous free flight even at $T_A <$ 10 °C. If a bee in free flight at these T_A allowed T_{Th} to decrease to the same extent as in fixed flight, it would soon become incapable of generating sufficient lift for further flight. Nevertheless, the bees could remain in free flight without regulating their T_{Th}, *per se*, if they control their altitude and flight velocity on the basis of information received from exteroceptors such as the compound eyes and/or mechanoreceptors. Increasing the flight effort would automatically increase T_{Th} especially in an insect that is sufficiently insulated with pile (Church, 1960) to retard the rate of convective cooling.

SUMMARY

1. Extracellular action potentials and thoracic temperatures (T_{Th}) were simultaneously recorded from the fibrillar flight muscles of *Bombus vosnesenskii* queens during preflight warm-up, during stabilization of T_{Th} in stationary bees, and during fixed flight.

2. In most stationary bees during warm-up and during the stabilization of T_{Th} the rate of heat production, as calculated from thoracic temperature and passive rates of cooling, is directly related to the frequency of action potentials in the muscles.

3. The rate of heat production increases throughout warm-up primarily because of a greater spike frequency at higher T_{Th}.

4. In stationary bees during the stabilization of T_{Th} at different ambient temperatures (T_A) the fibrillar muscles are activated by any in a continuous range of spike frequencies, rather than only by on-off responses.

5. Regulation of T_{Th} in stationary bees may involve not only changes in the rate of heat production but also variations of heat transfer from the thorax to the abdomen.

6. During fixed flight the fibrillar muscles are usually activated at greater rates at the initiation of flight than later in flight, but the spike frequency and thus heat production are not varied in response to differences in T_A and heating and cooling rates.

7. During fixed flight T_{Th} is not regulated at specific set-points; T_{Th} appears to vary passively in accordance with the physical laws of heating and cooling.

8. Differences in the T_{Th} of bees in free and in fixed flight are discussed with regard to mechanisms of thermoregulation.

REFERENCES

BASTIAN, J. & ESCH, H. (1970). The nervous control of the indirect flight muscles of the honeybee. Z. vergl. Physiol. 67, 307–24.

CHURCH, N. S. (1960). Heat loss and body temperature of flying insects. II. Heat conduction within the body and its loss by radiation and convection. J. exp. Biol. 37, 186–212.

HASSELROT, T. B. (1960). Studies on Swedish bumblebees (genus Bombus Latr.). Their domestication and biology. Opusc. ent. Suppl. 17.

HEINRICH, B. (1970). Thoracic temperature stabilization by blood circulation in a free-flying moth. Science, N.Y. 168, 580–2.

HEINRICH, B. (1972a). Patterns of endothermy in bumblebee queens, drones and workers. J. comp. Physiol. 77, 65–79.

HEINRICH, B. (1972b). Physiology of brood incubation in the bumblebee queen, Bombus vosnesenskii. Nature, Lond. 239, 223–5.

HEINRICH, B. & BARTHOLOMEW, G. A. (1972). An analysis of pre-flight warm-up in the sphinx moth, Manduca sexta. J. exp. Biol. 55, 223–39.

HIMMER, A. (1932). Die Temperaturverhältnisse bei den socialen Hymenopteren. Biol. Rev. 7, 224–53.

KAMMER, A. E. & HEINRICH, B. (1972). Neural control of bumblebee fibrillar muscle during shivering. J. comp. Physiol. 78, 337–45.

KROGH, A. & ZEUTHEN, E. (1941). The mechanism of flight preparation in some insects. J. exp. Biol. 18, 1–10.

MICHENER, C. D. (1962). An interesting method of pollen collecting by bees from flowers with tubular anthers. Rev. Biol. Trop. 10, 167–75.

MULLONEY, B. (1970). Impulse patterns in the flight motor neurones of Bombus californicus and Oncopeltus fasciatus. J. exp. Biol. 52, 59–77.

Note added in proof

It has recently been proposed (on the basis of enzyme activities in muscle homogenates) that bumblebees elevate their T_{Th} by non-shivering thermogenesis (Newsholme et. al., 1972, Biochem. J. 128, 89–97). However, the elevation of T_{Th} in bumblebees with inactive muscles has so far not been demonstrated by direct measurement. On the basis of our correlation of action potentials and heat production, and on the basis of Ikeda & Boettiger's (1965) correlation of action potentials and mechanical activity in bumblebee fibrillar muscle (J. Ins. Physiol. 11, 779–789), we conclude that the fibrillar muscles produce heat of sufficient quantity to elevate T_{Th} only when they are excited by impulses from the central nervous system, when they are mechanically active.