TEMPERATURE DEPENDENCY OF WING-BEAT FREQUENCY IN INTACT AND DEAFFERENTED LOCUSTS

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Summary

Locusts do not regulate thoracic temperature during flight and as a result the thoracic temperature of a flying locust generally exceeds ambient temperature by 5–8°C. Elevated thoracic temperatures were shown to affect wing-beat frequency in intact and deafferented *Locusta migratoria*. Tethered locusts were flown in a wind tunnel. Temperature was elevated by increasing the ambient temperature of the apparatus and by exposing flying animals to heat pulses. Electromyographic (EMG) recordings were made in deafferented locusts perfused with salines at different temperatures. Wing-beat frequency was shown to vary with thoracic temperature in both the intact and the deafferented situation. The slope of the rise in wing-beat frequency with experimental increases in thoracic temperature was similar in intact and deafferented animals. These experiments demonstrate an effect of temperature on the central flight circuitry. Further intracellular investigations are needed to determine the neural basis of these effects.

Introduction

With a minimum permissive thoracic temperature for initiation of flight at 24°C (Weis-Fogh, 1956) and the maximum temperature for flight muscle contraction at 42°C (Neville and Weis-Fogh, 1963), the thoracic nervous system of a flying locust is able to operate in the 24–42°C range. Flight muscle activity generates heat in the thorax, resulting in thoracic temperatures in excess of ambient temperature: such increases in temperature are likely to maximise the power output of flight muscles (e.g. Stevenson and Josephson, 1990). In contrast to other insect species, which have evolved behavioural and physiological mechanisms for thermoregulation during flight (for reviews, see Casey, 1981; Kammer, 1981), locusts are not known to regulate their thoracic temperature during flight. Nevertheless, the excess thoracic temperature tends to be greater at lower ambient temperatures (Yurgenson, 1950, cited in Uvarov, 1977), indicating that the thoracic nervous system may have some measure of protection from large variations in ambient temperature.

During level flight, the excess thoracic temperature in locusts is usually between 6 and 7°C (Weis-Fogh, 1956). At other times, thoracic temperature can exceed ambient temperature by more than 10°C (Weis-Fogh, 1964). It is therefore likely that the thoracic nervous system of a flying locust could experience temperatures within the full extent of the potential operating range of 24–42°C. In early studies on the flight system of the locust *Schistocerca gregaria*, Weis-Fogh (1956) demonstrated that flight performance, including wing-beat frequency, was independent of air temperature in the 25–35°C range (thoracic temperatures from 32 to 42°C). A similar independence of wing-beat frequency in *Locusta migratoria manilensis* has been claimed for ambient temperature in the 26–31°C range (Chen and Chin, 1961, cited by Uvarov, 1977).

Several aspects of neural function are highly temperature-dependent (reviewed by Montgomery and Macdonald, 1990). For example, action potential threshold decreases and conduction velocity increases with increasing temperature (Sjodin and Mullins, 1958). In insects, Abrams and Pearson (1982) showed that temperature affects frequency of firing and synaptic potential amplitude in central neurones involved in locust jumping. More recently, Burrows (1989) has shown that a rise in temperature will increase amplitude and decrease latency of a postsynaptic potential at a central synapse between neurones that are also involved in locust jumping. Temperature also affects locust sensory systems. The responses of mechanoreceptors associated with flight in locusts show temperature-dependent effects (Miles, 1985; Pfau *et al.* 1989). Also, membrane properties, including rebound spike amplitude and input resistance, of locust ocellar neurones have been shown to be temperature-dependent (Simmons, 1990), and the conduction time, from the head to the thorax, of action potentials in ocellar interneurones is markedly affected by temperature (Reichert and Rowell, 1985). Thus, one implication of Weis-Fogh's (1956) results is that increases in the rate of neural function caused by increased temperature are compensated for by the locust flight system. This makes functional sense for, without any compensatory process, flight performance would be influenced by environmental variation including temperature fluctuations. Nevertheless, rates of neurally controlled phenomena in poikilotherms generally show linear relationships with temperature (Walker, 1975). Indeed, the wing-beat frequencies of most insects (synchronous and asynchronous) are affected by temperature (reviewed by May, 1981; see also Oertli, 1989) and Weis-Fogh's results are unusual in indicating temperature-independence.

These previous investigations of the effect of temperature on flight performance examined *Schistocerca gregaria* (Weis-Fogh, 1956) and a preliminary investigation of *Locusta migratoria manilensis* was confirmatory (Chen and Chin, 1961, cited by Uvarov, 1977). However, our initial observations in intact *Locusta migratoria migratoria* showed that increased temperature promotes more vigorous flight and increased wing-beat frequency (R. M. Robertson, unpublished observations) and it is known that, in *Locusta*, wing-beat frequency is positively correlated with flight speed (Kutsch and Gewecke, 1979). Variables of the wing beat during flight,

including wing-beat frequency, are controlled by central (Robertson and Pearson, 1985a,b) and peripheral (Pearson *et al.* 1983; Reichert and Rowell, 1986) circuits. The role of peripheral input can be investigated using intact tethered locusts. Intact animal studies in which the animal experiences ambient temperatures that result in a range of thoracic temperatures similar to the range of thoracic temperatures of normally behaving animals (e.g. Miles, 1985; Weis-Fogh, 1964) are likely to mimic natural conditions. In contrast, several investigations of the central circuits capable of generating flight motor patterns in dissected preparations have been carried out with the thoracic nervous system at room temperature (approx. 25°C) (e.g. Robertson and Pearson, 1985a). It is important to determine how a more natural, elevated thoracic temperature affects the operation of the locust flight circuitry.

The experiments reported here were designed to examine the effect of thoracic temperature in the 24–40°C range on one measure of flight performance, wing-beat frequency, in intact *Locusta*. These results could then be directly compared with the effect of increases in thoracic temperature on the frequency of the flight rhythm produced by deafferented *Locusta*.

Materials and methods

Adult male and female *Locusta migratoria* at least 10 days post final moult were used. Animals were collected from a locust colony maintained at the Department of Biology, Queen's University.

Intact animals

Preparation

Locusts were cold-anaesthetized prior to beginning an experiment. The electrical activity of one of the first basalar muscles, a flight depressor muscle (M97, muscles numbered according to Albrecht, 1953), was monitored with implanted electromyographic (EMG) electrodes. A 40 gauge (approximately 0.1 mm in diameter) copper wire, insulated except at the tip, was inserted through the cuticle into the first basalar muscle and secured in place with a drop of wax. A similar indifferent electrode was inserted into the thorax through the arthrodial membrane underlying the pronotum. A 40 gauge copper/constantan thermocouple was inserted into the thorax, dorsally, to lie between the thoracic muscles and the gut. This position has been described as the place where the temperature of the working thorax is at a maximum (Weis-Fogh, 1964). The placement of the thermocouple was confirmed by dissection following each experiment.

Experimental apparatus

Fig. 1 shows the experimental apparatus for the intact animal experiments. The wind tunnel was constructed using a Plexiglas cylinder attached at one end *via* a circular piece of Plexiglas to a Rotron Muffin d.c. fan oriented to extract air from the tunnel. A honeycomb of 2 cm lengths of drinking straw was packed in the
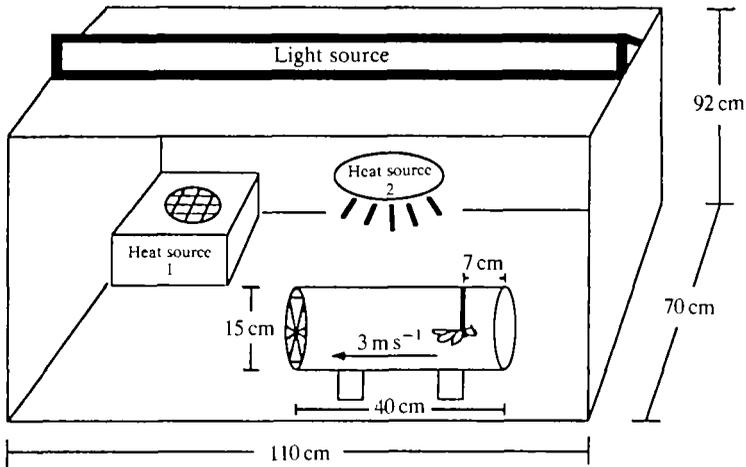
Fig. 1. The experimental apparatus for intact animal experiments. Experiments were carried out within a shielded cage. Animals were tethered and flown in a wind-tunnel with a wind speed of 3m s^{-1}. To increase ambient temperature, a small room heater with a fan was used (heat source 1) and to increase thoracic temperature at intervals during flight a 250W infrared lamp was used (heat source 2).

cylinder. The wind speed was adjusted to be about 3m s^{-1}. A temperature probe was placed in the wind stream to monitor ambient temperature. Locusts were tethered by waxing a rod to the pronotum and were suspended in the centre of the wind tunnel 7 cm from the opening. Experiments were carried out within a shielded box 110 cm wide by 92 cm high by 70 cm deep and illuminated by a light box (Shadow Ban fluorescent lamp, warm white deluxe). The back and sides of the box were solid, the top was enclosed with clear plastic, which could be extended to cover the front opening, to maintain elevated ambient temperatures within the box. A thermostatically controlled room heater with a fan (heat source 1) was placed on a stand in the back corner of the cage and was used to elevate and maintain the ambient temperature. The animal could be directly heated using a 250W infrared heat lamp (heat source 2). Using this apparatus two types of experiments were performed – constant ambient experiments and heat pulse experiments.

Constant ambient experiments

Animals were flown at room temperature (25°C) and at elevated ambient temperatures (28, 30°C). The apparatus was enclosed and heated to the desired temperature and the animals were allowed to acclimate for 45 min before the experiment was begun. During this time, the animal was suspended in the tunnel with no air flow and with a support for its legs to prevent flight. Flight was initiated by removing the support and turning on the fan. Experiments lasted from 1 to 3 h, during which time the thoracic temperature, ambient temperature and wing-beat frequency were monitored. The wing-beat frequency was determined using the

EMG recordings of the first basalar activity, which were amplified and displayed on a digital oscilloscope.

Pulse experiments

Animals were flown at room temperature (25°C) and exposed to pulses of heat using a 250W infrared heat lamp at regular intervals after flight had been initiated. In the first series of pulse experiments the animals were exposed to 3 min heat pulses at 15, 25, 35, 50, 65, 80 and 95 min after flight had been initiated. Experiments lasted 115 min. In the second series of pulse experiments the animals were exposed to 15 min heat pulses at 20, 45 and 70 min after flight had been initiated. Experiments lasted 95 min. Wing-beat frequency, thoracic temperature and ambient temperature were monitored throughout the pulse experiments.

Deafferented animals

We used a dissected preparation of the locust capable of expressing centrally generated flight motor patterns (Robertson and Pearson, 1982). The thoracic cavity of the animal was opened dorsally. The gut and overlying tissue were removed to expose the mesothoracic and metathoracic ganglia. Nerves 3, 4 and 5 of the mesothoracic and metathoracic ganglia (nerves numbered according to Campbell, 1961) were cut to reduce mechanical interference and to sever the motor supply to the majority of wing muscles. The nerves that innervate the mesothoracic and metathoracic dorsal longitudinal muscles (flight depressors) were left intact. The wing stumps were pinned to eliminate residual movement caused by dorsal longitudinal contractions. These preparations largely, possibly totally, deafferented the nervous system by preventing movement of the wings and thus preventing phasic proprioceptive feedback. Extracellular recording from nerve 1 confirmed there was no phasic sensory input to the central circuitry. A pair of EMG electrodes was inserted into one of the dorsal longitudinal muscles to monitor the flight motor pattern. The thoracic cavity of the animal was perfused with locust saline. The saline was heated by a heating coil located on the outlet pipe of the saline reservoir. A thermocouple was placed next to the mesothoracic ganglion to monitor thoracic temperature. Following a change in the temperature of the saline around the thoracic ganglia, the preparation was left for 3 min to stabilize to the new temperature before recordings were made. Flight sequences were initiated by a puff of air to the head of the animal. EMGs were stored on video tape and later displayed on a digital oscilloscope. EMG recordings were analysed and measurements were taken from the beginning of the flight sequence to determine wing-beat frequencies.

Data analysis

The data presented were collected from 16 animals flown at constant ambient temperatures, 8 animals flown at room temperature and exposed to heat pulses and 6 deafferented animal preparations. The wing-beat frequencies and thoracic temperatures recorded were analysed using a least-squares regression analysis.

Within the range of temperatures and wing-beat frequencies observed in this study a linear regression of the form $y=a+bx$ (where y is wing-beat frequency in Hz and x is thoracic temperature in °C), where a is the y-intercept and b is the slope, gave the best fit to the data. This is consistent with what has been found previously for the effect of temperature on rates of neural phenomena in poikilotherms (Walker, 1975) and on wing-beat frequencies in other insects (May, 1981). The average slopes of the resulting regressions were compared using analysis of covariance (ANCOVA). The number of observations/data points for each individual experiment varied; therefore, when the data were pooled to compare the three treatments, an identical number of points from each experiment was selected for the analysis.

Results

Intact animals

Constant ambient experiments

Tethered locusts flown at constant ambient temperatures assumed flight posture (Uvarov, 1977) within 5 min of the initiation of flight and flew continuously for periods of 1–3 h. Flight was characterized as having two stages: start-up and steady-state flight. During the start-up stage (first 25–40 min of flight) there was a rise and fall in thoracic temperature. Thereafter, thoracic temperature more closely followed ambient temperature but was elevated by between 2 and 4°C. Accompanying this rise and fall in thoracic temperature was a corresponding rise and fall in wing-beat frequency (Fig. 2). Fig. 2 shows the rise and fall in thoracic temperature and wing-beat frequency observed in three different locusts. Fig. 3 shows the average rise and fall in wing-beat frequency during the first 60 min of flight in 8 animals flown at 25°C. In different animals the range of wing-beat frequencies observed varied. To compensate for variation between individuals the wing-beat frequencies (WBF) from each experiment were converted to a percentage. 100 % WBF represented the maximum WBF reached in an individual experiment. In all experiments 100 % wing-beat frequency was reached within 10 min after flight had been initiated.

Regression analysis was performed on the wing-beat frequencies and thoracic temperatures recorded from intact animals flown at 25, 28, and 30°C (Fig. 4). Linear regression equations are given in Table 1. In all experiments an increase in wing-beat frequency was correlated with an increase in thoracic temperature. The slopes of the linear regressions ($b_{25}=1.38\pm0.15$, $b_{28}=1.41\pm0.02$, $b_{30}=1.02\pm0.16$) did not differ between individuals flown at different ambient temperatures (ANCOVA; $F_{(1)7,277}=1.31$, $P>0.05$), although the range of frequencies observed differed slightly.

Pulse experiments

The first series of heat pulse experiments was done with 3 min heat pulses. Fig. 5A represents an individual heat pulse experiment. Heat pulses (shown as
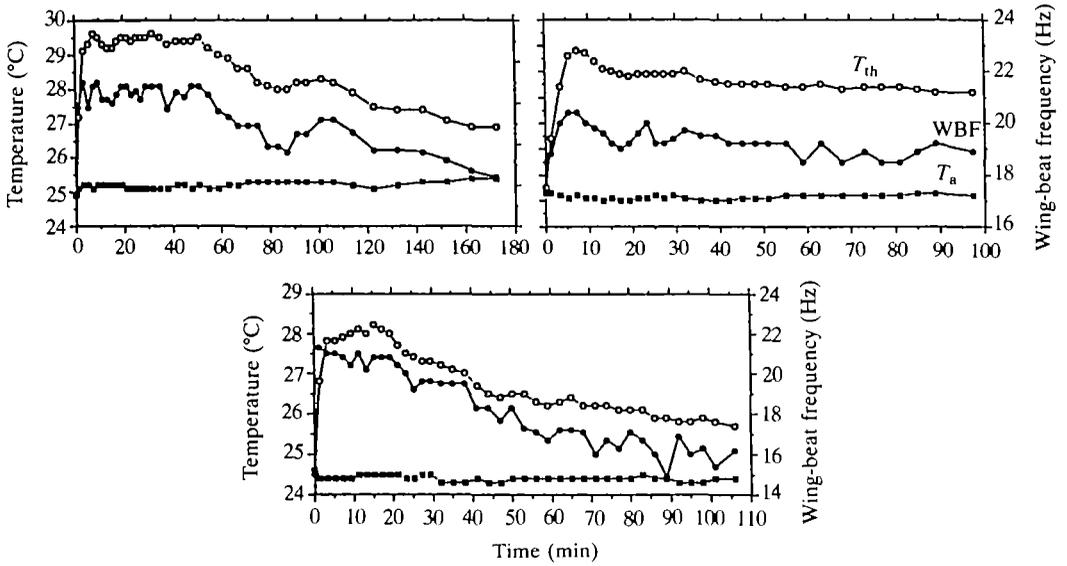
Fig. 2. Wing-beat frequency, thoracic and ambient temperature for three different locusts flown at room temperature (25°C). In each experiment there was a rise and fall in thoracic temperature and a corresponding rise and fall in wing-beat frequency. Thoracic temperature (T_{th}) is represented by open circles (O), ambient temperature (T_a) is represented by closed squares (■) and wing-beat frequency (WBF) is represented by closed circles (●).
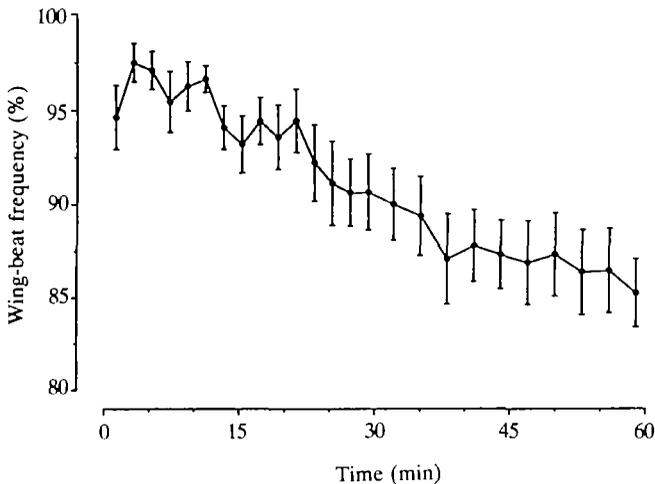

Fig. 3. The rise and fall in wing-beat frequency (WBF, mean±S.E.) observed during the first 60 min of flight for eight animals flown at 25°C. 100 % WBF represents the maximum WBF reached in an individual experiment. WBF was recorded every 30 s and was plotted at 2 min intervals (an average of four consecutive readings).

Table 1. *Linear regression equations of thoracic temperatures and wing-beat frequencies in intact* Locusta migratoria *flown at constant ambient temperature*

| Reference number	Slope	y-intercept	Correlation coefficient	Range of T_{th} measured (°C)
Ambient temperature 25°C				
6A	1.51	−24.04	0.971	26.9–29.6
6B	0.90	−4.87	0.800	26.2–27.8
6C	2.16	−39.75	0.962	25.7–28.2
6D	0.94	−10.15	0.938	29.2–32.9
6E	1.39	−21.68	0.698	27.9–30.2
6F	1.01	−8.68	0.775	25.8–27.3
6G	1.64	−26.76	0.840	26.8–28.2
6H	1.50	−21.68	0.926	25.1–27.3
Ambient temperature 28°C				
2N	0.96	−11.63	0.736	30.5–32.9
2M	1.42	−24.89	0.985	30.0–32.7
Ambient temperature 30°C				
2A	0.69	−1.73	0.421	31.9–34.4
2B	1.58	−35.89	0.861	34.8–36.9
2C	0.48	4.13	0.644	33.0–36.0
2D	1.07	−16.18	0.761	33.2–35.7
2E	0.96	−15.64	0.599	34.8–37.0
2F	1.32	−25.65	0.705	34.0–35.7
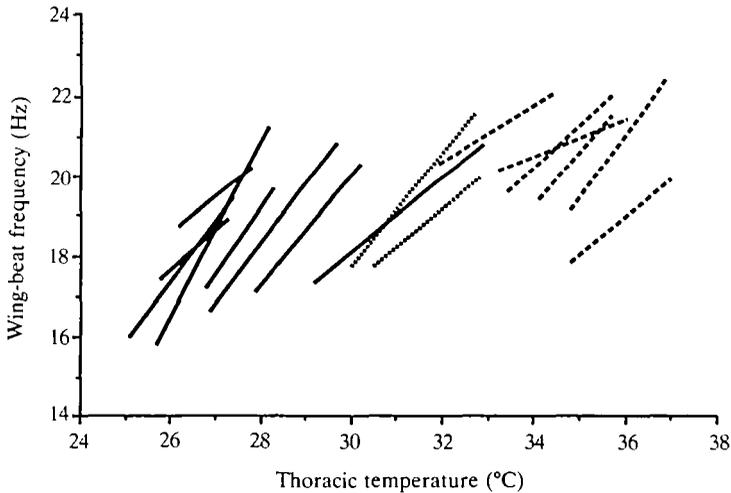
Fig. 4. Regressions of wing-beat frequency on thoracic temperature at different ambient temperatures: 25°C (solid lines), 28°C (dotted lines) and 30°C (dashed lines). Each line represents data from a single experiment.
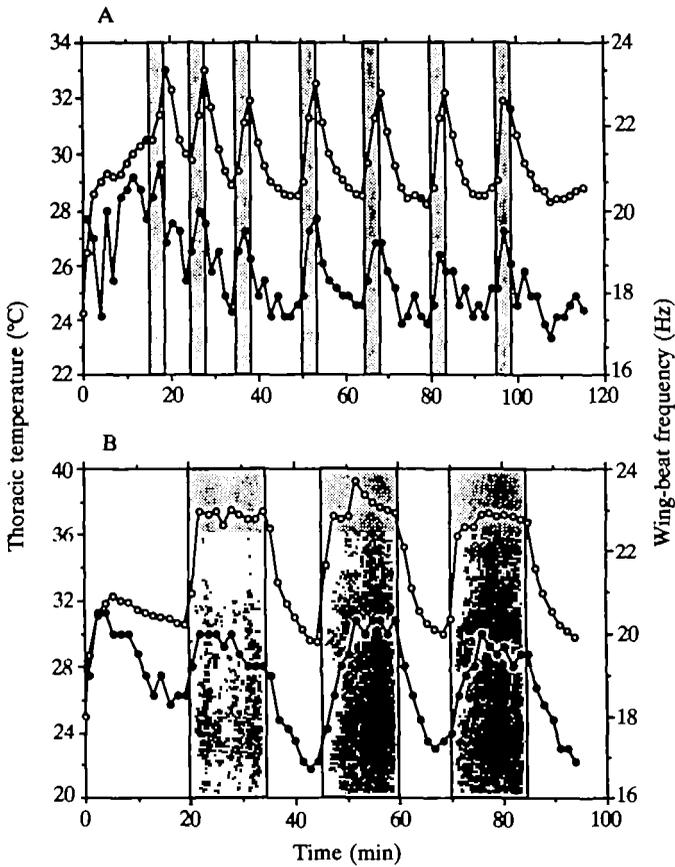
Fig. 5. Examples of a short-pulse experiment (A) and a long-pulse experiment (B). Each graph represents an individual experiment. Pulses of heat are shown as shaded columns. Thoracic temperature is represented by open circles (O) and wing-beat frequency is represented by closed circles (●). Wing-beat frequency and thoracic temperature were recorded every 30 s and were plotted at 1.5 min intervals (an average of three consecutive readings).

shaded columns) were given at 15, 25, 35, 50, 65, 80 and 95 min after flight had been initiated. When a heat pulse was applied, there was a rise in thoracic temperature and a corresponding rise in wing-beat frequency. When the heat pulse was terminated, there was a fall in thoracic temperature and in wing-beat frequency. The average increase in thoracic temperature observed in response to a 3 min heat pulse was 3.9±0.8°C (*N*=28).

It is possible that the observed increase in wing-beat frequency in response to an increase in thoracic temperature might be a transient effect and if the heat pulse were longer the wing-beat frequency might adapt. This possibility was eliminated using 15 min heat pulses. In the second series of heat pulse experiments, 15 min heat pulses were administered at 20, 45 and 70 min after initiation of flight. A
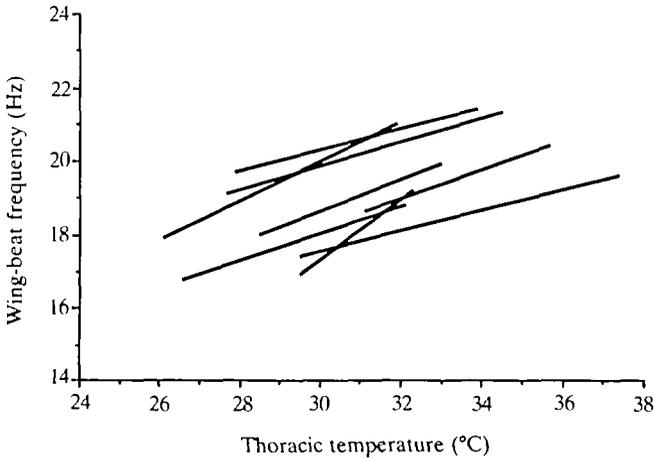
Fig. 6. Linear regressions of wing-beat frequencies on thoracic temperatures recorded during the experimental rise in thoracic temperature in eight heat pulse experiments. Each line represents an individual experiment.

representative long pulse experiment is shown in Fig. 5B. In this series of experiments when the thoracic temperature was increased and maintained at an elevated level for 15 min, the wing-beat frequency remained elevated for the duration of the pulse. The average rise in thoracic temperature observed in response to a 15 min heat pulse was $6.0\pm0.7°C$ ($N=12$).

Regression analysis was performed on the wing-beat frequencies and thoracic temperatures recorded during the experimental rises in thoracic temperature observed in response to heat pulses (Fig. 6). Short and long pulse experiments are combined in Fig. 6. The wing-beat frequencies and thoracic temperatures recorded between the time the heat lamp was turned on and the time when the thoracic temperature stopped increasing in each pulse of heat were used in this analysis. Wing-beat frequency was observed to increase with increasing thoracic temperature. Linear regression equations are given in Table 2.

Deafferented animals

In the deafferented animal experiments the temperature of the thoracic cavity was varied from 24 to 40°C. Fig. 7 shows sample traces of EMG signals recorded from the dorsal longitudinal muscle of a deafferented locust at 25, 35, and 40°C. These traces were taken from the beginning of flight sequences when the flight motor pattern is most robust. This figure clearly shows a temperature effect that includes both an increase in wing-beat frequency and an increase in the number of spikes per burst. This increase in spike number was consistent in all of the deafferented locusts.

Regression analysis was performed on the wing-beat frequencies and thoracic temperatures recorded (Fig. 8). As in the intact animal, the wing-beat frequency

Table 2. *Linear regression equations of thoracic temperatures and wing-beat frequencies in intact* Locusta migratoria *exposed to heat pulses of 3 or 15 min duration*

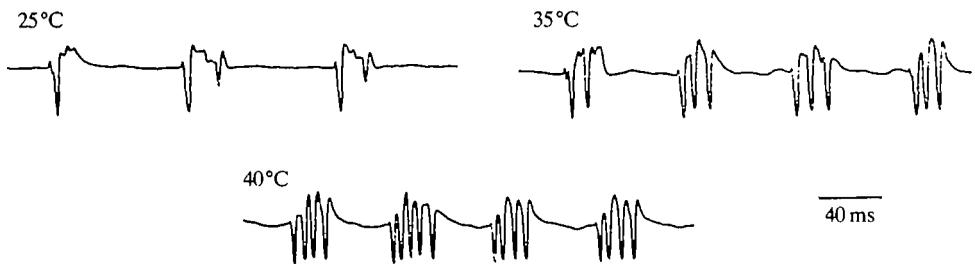| Reference number	Slope	y-intercept	Correlation coefficient	Range of T_{th} measured (°C)
Short-pulse experiments				
5A	0.38	6.82	0.417	31.1–35.7
5B	0.42	5.99	0.694	28.5–33.0
5C	0.32	10.23	0.782	27.7–34.5
5D	0.81	7.00	0.757	29.5–32.3
Long-pulse experiments				
1F	0.53	4.11	0.782	26.1–31.9
1H	0.28	9.14	0.801	29.5–37.4
1L	0.28	11.86	0.614	27.9–33.9
1R	0.37	6.91	0.848	26.6–32.1

Fig. 7. Samples of EMG traces recorded from a dorsal longitudinal muscle in a deafferented *Locusta migratoria* at three different thoracic temperatures. With increased temperature, an increase in the number of spikes per burst and an increase in the wing-beat frequency (12.5 Hz at 25°C, 14.3 Hz at 35°C, 16.1 Hz at 40°C) was observed.

was observed to increase with thoracic temperature. Table 3 gives the linear regression equations for the deafferented animal experiments.

Comparison of effects of temperature

The average slope of the regressions for the constant ambient temperature experiments, 1.25 ± 0.10 ($N=16$), differed significantly (ANCOVA; $F_{(1)23,397}=24.4$, $P<0.001$) from the average slope of the regressions for the pulse experiments, 0.42 ± 0.06 ($N=8$), and from the average slope of the regressions observed in the deafferented preparation, 0.35 ± 0.03 ($N=6$) (ANCOVA; $F_{(1)21,351}=28.2$, $P<0.001$). However, the average slope of the regressions observed in the deafferented preparation did not differ from the average slope of the regressions observed in the intact pulse experiments ($F_{(1)13,194}=1.13$, $P>0.05$). In the pulse
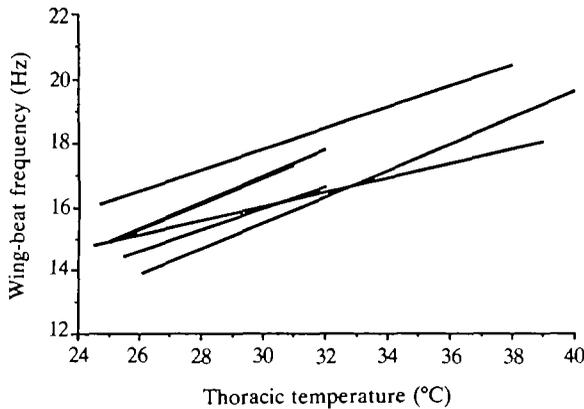
Fig. 8. Linear regressions of wing-beat frequencies on thoracic temperatures recorded from six deafferented animals perfused with saline at different temperatures. Each line represents data from a different animal.

Table 3. *Linear regression equations of thoracic temperatures and wing-beat frequencies in deafferented* Locusta migratoria

Reference number	Slope	y-intercept	Correlation coefficient	Range of T_{th} measured (°C)
3A	0.41	3.24	0.929	26.1–40.0
3B	0.22	9.41	0.709	24.5–39.0
3C	0.33	7.90	0.848	24.7–38.0
3D	0.33	5.99	0.883	25.0–31.0
3E	0.42	4.38	0.861	25.5–32.0
3F	0.40	4.86	0.748	26.0–35.0

experiments and in the deafferented animal experiments the thoracic cavity of the animal was heated directly and the effect of temperature on the rhythmical output was the same. It is clear that in the constant ambient temperature experiments wing-beat frequency is affected by more than just the experimental increase in thoracic temperature.

An initial rise and subsequent fall in wing-beat frequency during flight sequences have been previously reported (Weis-Fogh, 1956; Goldsworthy and Coupland, 1974; Gewecke and Kutsch, 1979). Many factors other than thoracic temperature, including metabolic changes (Goldsworthy and Coupland, 1974) and neurohormone levels (Sombati and Hoyle, 1984), may influence the wing-beat frequency of a flying locust and, therefore, may contribute to the observed rise and fall. To eliminate variables other than temperature, we pooled all constant ambient temperature experiments to examine the effect of experimental increases in thoracic temperature. This analysis showed a continuous rise in wing-beat frequency over the entire range of thoracic temperatures measured (Figs 9, 10).
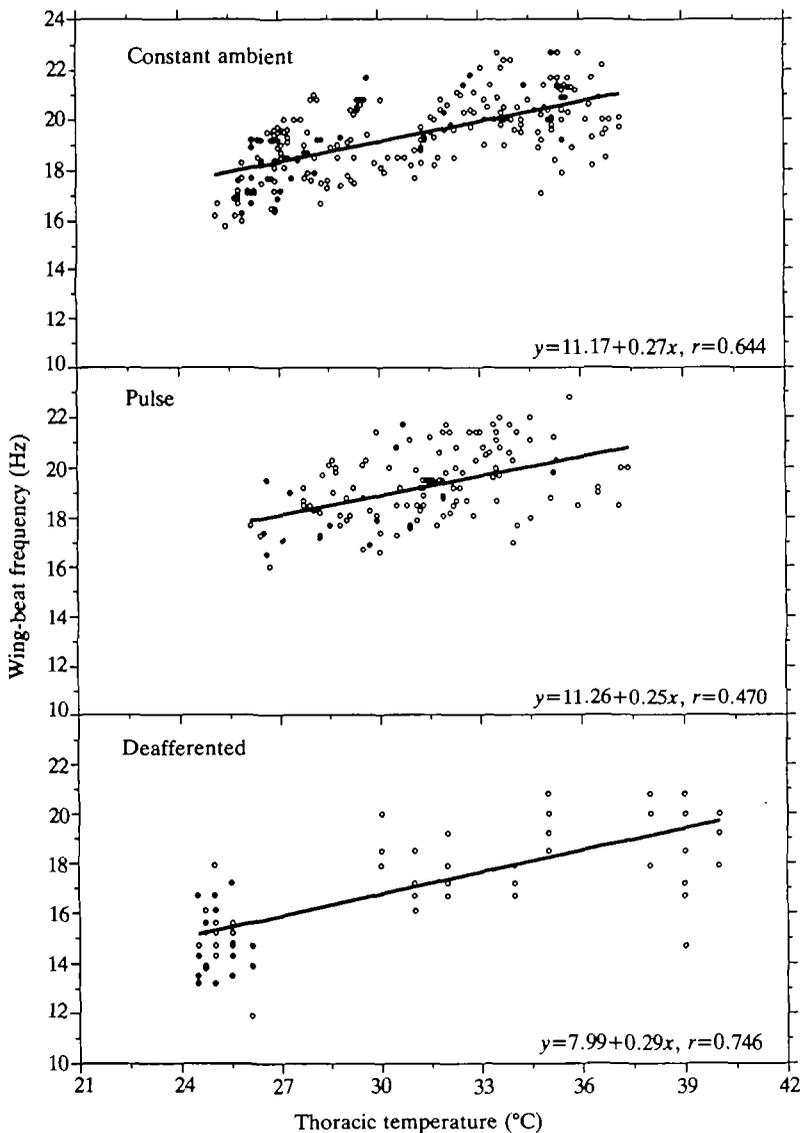
Fig. 9. Scatterplots and linear regressions of pooled data in constant ambient experiments, pulse experiments and deafferented animal experiments. Slopes of the resulting lines did not significantly differ between treatments (mean slope=0.27).

When a similar analysis was performed on the data pooled from all pulse experiments, the resulting linear regression was essentially the same as in the constant ambient experiment (Figs 9, 10). In addition, regression analysis performed on pooled data from all deafferented experiments, resulted in a linear regression (Fig. 9) in which the slope did not differ significantly from the slopes observed in the intact animal experiments. Fig. 9 shows the point scatter and
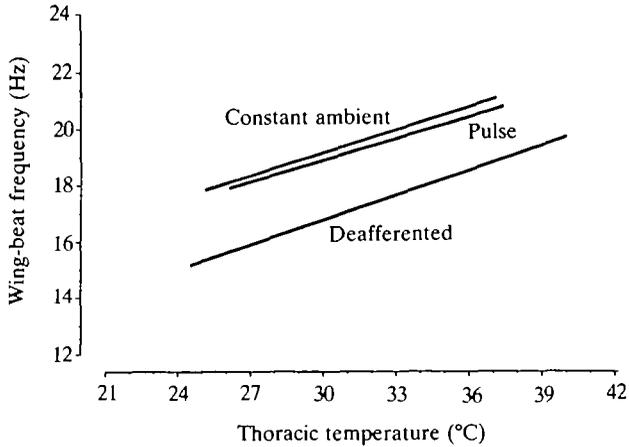
Fig. 10. An experimental increase in thoracic temperature resulted in a similar increase in wing-beat frequency in both the intact (constant ambient and pulse experiments) and the deafferented animal. Linear regressions for the average rise in wing-beat frequency in response to an increase in thoracic temperature are plotted.

linear regressions of wing-beat frequencies and thoracic temperatures resulting from experimental increases in thoracic temperature for intact and deafferented animal experiments. When the regressions alone are plotted together (Fig. 10), the effect of an experimental increase in thoracic temperature on the wing-beat frequency can be seen clearly. The three lines are essentially the same except for the shift in range of wing-beat frequencies observed in the deafferented situation (wing-beat frequency is observed to decrease following deafferentation, Wilson, 1961). Thus, these data show that an experimental increase in thoracic temperature affects wing-beat frequency in intact and deafferented locusts in a similar way, which suggests that this effect is probably due to a central effect of temperature.

Discussion

From our constant ambient temperature experiments, two phenomena can be described. First, in individual locusts there is a correlation between the rise and fall of thoracic temperature and the rise and fall in wing-beat frequency. We suggest that many factors, including metabolic and hormonal factors, influence wing-beat frequency and that the observed correlation is a result of many complicating factors and is not exclusively a temperature-dependent effect. Second, in *Locusta migratoria*, there is a temperature-dependent effect on wing-beat frequency in response to experimental increases in thoracic temperature. Studies of flight performance of *Schistocerca gregaria* have reported that wing-beat frequency immediately after the start of flight was approximately the same for animals flown at different ambient temperatures (25, 30, 35°C) and decreased during the first 10 min of flight to a lower frequency (Weis-Fogh, 1956). Weis-Fogh (1956) concluded that wing-beat frequency in ambient temperatures between 25 and 35°C

was independent of temperature. The results of the regression analysis of our constant ambient experiments (Fig. 9) contradict Weis-Fogh's (1956) results. This contradiction may be due to a species difference between *Locusta* and *Schistocerca*. Alternatively, the method of analysis used may have made a difference. In Weis-Fogh's (1956) experiments, data from 5- and 10-min flight intervals for eight different animals were averaged. We have shown that the effect of temperature on wing-beat frequency is mild (only about $0.3\,Hz\,degree^{-1}$) and could easily been overlooked.

The motor patterns controlling wing-beat frequency during flight are generated in the thoracic ganglia (Wilson, 1961) and some of the circuitry has been described (Robertson and Pearson, 1985a). The wing-beat frequency in an intact locust is influenced by a number of factors. There is a gradual increase in the wing-beat frequencies observed in the first 2 weeks following the final moult (Kutsch, 1973). In addition, phasic sensory input from the wing hinge stretch receptor is known to influence the wing-beat frequency in the intact animal (Pearson *et al.* 1983). Nonetheless, in the deafferented animal preparation, in which all effective phasic sensory input was removed, varying the temperature of the thoracic cavity from 24 to 40°C resulted in a continuous rise in wing-beat frequency. In addition, the slope of the linear regression for the deafferented preparation paralleled that observed in both intact animal treatments. The single difference between the effect of temperature on the wing-beat frequency in intact and deafferented animals was the shift in wing-beat frequency observed in the deafferented preparation, which is known to be a direct result of deafferentation (Wilson, 1961). Also, the range of frequencies observed at the higher temperatures following deafferentation is greater than expected and the upper portion of this range approaches frequencies observed in the intact animal. Stevenson and Kutsch (1987) reported a similar increase in wing-beat frequency in a deafferented locust preparation following octopamine ionophoresis. With the removal of sensory input, the above results can be attributed to a direct effect on the flight circuitry.

In this study the effect of temperature in the intact animal can be attributed solely to an effect on the centrally generated rhythm and yet it is known that in the intact animal sensory input plays an important role in producing the final motor output (Pearson *et al.* 1983). In an intact flying locust, the wing hinge stretch receptor signals phasic information directly to flight motorneurones (Burrows, 1975) and to an oscillatory interneurone that is considered to be part of the pattern generator for locust flight (Pearson *et al.* 1983). Recently, Pfau *et al.* (1989) have demonstrated a temperature dependence of stretch receptor activity. The instantaneous frequency of action potentials from the stretch receptor was observed to depend both on the amplitude of the stretch cycle and on temperature. However, in our study there is no evidence for an additional temperature effect *via* wing hinge stretch receptor input on the frequency of the flight motor output in the intact animal. This may be because the flight circuitry is not sensitive to the temperature-induced increases in stretch receptor activity. Alternatively, stretch receptor activity in intact flying animals may be saturated as a result of modulation

of responsiveness of the stretch receptor *via* circulating octopamine (Ramirez and Orchard, 1990). It remains to be determined whether stretch receptor activity in intact flying animals is affected by changes in thoracic temperature and, if so, what compensatory mechanisms may exist.

To understand the control of natural flight in the intact animal it has been well established that a knowledge of peripheral and central components of the neural control system will be necessary. The operation of this system is affected by temperature. In other systems, temperature is seen to have profound effects on the operation of the circuitry (Johnson *et al.* 1991) and it is possible that phenomena not evident at room temperature may be induced. In addition, the nervous systems of poikilotherms experience ranges of different temperatures and at any one time different parts of the same nervous system could be at different temperatures as a result of differential heating and cooling. That this may be significant for understanding the control of behaviour is amply demonstrated in the analysis of the control of communication in grasshoppers. The frequency of syllables during stridulation is linearly related to ambient temperature (Walker, 1975). It has been shown that this is dependent on the temperature of the thoracic ganglia, implying a central effect, as shown here for locust flight, whereas recognition is dependent on the temperature of the head (Bauer and von Helversen, 1987). Under natural conditions this may not pose a problem for this particular behaviour. However, in other behaviours, such as flight, the integration of signals from parts of the nervous system at different temperatures could be important. Reichert and Rowell (1985) have shown that the transmission of ocellar information from the head to the thoracic flight circuitry is clearly affected by temperature (latencies at 23 °C are more than halved by an increase to 35 °C, from 29 ms to 12 ms). If internal temperatures vary with time and location, then compensatory mechanisms are to be expected. Understanding these mechanisms and the operation of the system at natural temperatures is of prime importance. Further investigation of the locust flight circuitry is necessary to discover the neural basis of the temperature effects described here. Intracellular experiments examining the components and connections of the locust flight central pattern generator will provide information on the cellular basis of the temperature effects on the operation of the flight circuitry.

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