EFFECTS OF OPERATING FREQUENCY AND TEMPERATURE ON MECHANICAL POWER OUTPUT FROM MOTH FLIGHT MUSCLE

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Summary

1. Mechanical work output was determined for an indirect flight muscle, the first dorsoventral, of the tobacco hawkmoth *Manduca sexta*. Work output per cycle was calculated from the area of force–position loops obtained during phasic electrical stimulation (1 stimulus cycle^{-1}) and imposed sinusoidal length change. There was an optimal stimulus phase and an optimal length change (strain) that maximized work output (loop area) at constant cycle frequency and temperature.

2. When cycle frequency was increased at constant temperature, work output first increased and then decreased. It was always possible to find a frequency that maximized work output. There also always existed a higher frequency (termed the 'optimal' frequency in this paper) that maximized the mechanical power output, which is the product of the cycle frequency (s^{-1}) and the work per cycle (J).

3. As temperature increased from 20 to 40°C, the mean maximum power output increased from about 20 to about 90 W kg^{-1} of muscle (Q$_{10}$=2.09). There was a corresponding increase in optimal frequency from 12.7 to 28.3 Hz, in the work per cycle at optimal frequency from 1.6 to 3.2 J kg^{-1} muscle and in mean optimal strain from 5.9 to 7.9%.

4. Two electrical stimuli per cycle cannot increase power output at flight frequencies, but if frequency is reduced then power output can be increased with multiple stimulation.

5. Comparison of mechanical power output from muscle and published values of energy expenditure during free hovering flight of *Manduca* suggests that mechanical efficiency is about 10%.

6. In the tobacco hawkmoth there is a good correspondence between, on the one hand, the conditions of temperature (35–40°C) and cycle frequency (28–32 Hz) that produce maximal mechanical power output in the muscle preparation and, on the other hand, the thoracic temperature (35–42°C) and wing beat frequency (24–32 Hz) observed during hovering flight.

Introduction

Behavioural observations on insects have often identified a minimum ambient

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or body temperature for flight (Williams, 1940; Taylor, 1963; Heinrich, 1981). Muscle temperature has been proposed as the critical physiological factor that determines this behavioural threshold (Heinrich, 1974; Kammer and Heinrich, 1978; Josephson, 1981). This paper examines the mechanical power available from wing muscles of the hawkmoth *Manduca sexta*, and the relationship between muscle temperature and power output. Among the questions considered are (1) how does the mechanical power output obtainable from the flight muscle vary with muscle temperature and (2) at what temperature, if any, does the mechanical power output match that which has been estimated as being required for flight (Casey, 1981a; Ellington, 1984a)?

The tobacco hawkmoth *Manduca sexta* is an appropriate animal in which to examine the relationships between temperature and power output because its muscles operate at elevated temperatures. Before take-off at an ambient temperature of 20°C, *Manduca* increases its thoracic temperature from 21–22°C to about 36°C and nearly doubles its wing beat frequency (Heinrich and Bartholomew, 1971). Among sphingid moths generally, body temperature, wing beat frequency, muscle contraction rate and O_2 consumption rate all increase before flight occurs (Dorsett, 1962; Heinrich and Casey, 1973; Bartholomew and Casey, 1978). In accordance with a minimum power threshold for flight, Heinrich (1981) has suggested that all sphingids must achieve a thoracic temperature of about 36°C before they can fly.

Questions concerning the efficiency of muscle provided another motivation for this study. Muscles transform chemical energy into mechanical energy. The ratio of the mechanical power output to the chemical energy input is the mechanical efficiency (Hill, 1939). Among insect fliers this transformation is thought to be a totally aerobic process (Sactor, 1965), making the measurements of oxygen consumption during warm-up and free flight a good measure of the chemical energy input. Using oxygen consumption data, Casey (1981a) suggested that a flight muscle efficiency of 20 % for sphingids was consistent with the mechanical power output calculated from aerodynamic models. Ellington (1985) argued, however, that mechanical efficiency of insect flight muscle is closer to 10 % when improved aerodynamic models are used. The present study helps to resolve the differences in estimated muscle efficiency by making direct measurements of the mechanical power output.

Here we measure power output using the work-loop technique originally devised by Machin and Pringle (1959) and later adapted for synchronous muscle by Josephson (1985a). In the work-loop technique the muscle length is varied cyclically and the muscle length and force are recorded. The net work is the area of the work loop formed when force is plotted against length over a full length cycle. The net work output per cycle is calculated as the work produced during shortening minus the work necessary to re-lengthen the muscle. The mechanical power follows directly as the cycle frequency times the work per cycle. Josephson (1985a) and Johnston and Altringham (1988) discuss the rationale of this approach. Specifically the work-loop technique is suited to muscles that are used

cyclically, such as in locomotion or breathing, because (1) the work for stretching out the muscle is naturally incorporated into the measurement and (2) variables which must be adjusted during the measurements, such as cycle frequency, muscle strain and stimulation pattern, have a natural correspondence with the variables that are important in modulating force and power output of the muscle in the animal.

Materials and methods

Animals

Pupae of *Manduca sexta* were obtained from a laboratory culture. On the day after eclosion animals were weighed and thereafter maintained on long days (16 h: 8 h L: D) at approximately 20°C in a flight cage (0.8 m × 1.5 m × 1.5 m). The cage was supplied with tobacco plants for egg laying. Food was available from artificial flowers filled with 9:1 water: honey solution. Approximately 60 % of the animals began to feed. These animals lived as long as 3 weeks whereas non-feeders died within a week. Only feeders were used in experiments (mean age 9.5 days, mean decrease in body mass from eclosion 22 %).

Preparation

A moth was weighed and then chilled to inactivate it. Scales of the thorax were rubbed off, and the wings and legs were removed. The thoracic ganglia were exposed by ventral dissection and removed in order to block spontaneous contractions of the muscle of interest and to give a quiescent preparation. Next, the ventral attachment of the dorsal ventral muscle, dv_1 (Eaton, 1971), was freed by making a series of cuts into the sternal exoskeleton (Fig. 1) which isolated a triangular piece of cuticle to which a part of the muscle was attached. dv_1 is an indirect power muscle used to elevate the wings. The isolated portion of the muscle contained only one motor unit, as indicated by a single, sharp threshold for twitch contractions in response to single stimuli. Bits of surrounding cuticle were trimmed away from the sternum so that movement of the muscle was unhindered. The head end of an insect pin (size 000), which had been shortened to 5 mm and shaped into a hook, was glued to the freed cuticle using cyanoacrylate cement (gel Super glue). This hook could then be attached to an ergometer arm *via* another hook.

Care was taken when preparing the muscle to keep the tracheal system attached to it. Preparations gave good contractions for up to 9 h. Saline (composition given in Tublitz and Truman, 1985) was occasionally added to keep the preparation moist. Protein clots, which formed around the free end of the muscle, were removed when necessary.

The muscle was stimulated through two silver wire electrodes (50 μm diameter), which were implanted in the dorsal end of the muscle and waxed into place. The

stimuli were 0.5 ms voltage pulses, one per cycle unless otherwise noted. When the electrodes were in place, the animal was glued on its back with 5 min Epoxy cement to a glass slide, which was then positioned beneath an ergometer.

The muscle was attached to either a Cambridge ergometer (model 300H, Cambridge Instrument Co., Cambridge, MA, USA) or an ergometer based on a shaker pot (102A Shaker, Ling Electronics, Anaheim, CA, USA) as a position control and semi-conductor strain gauge (Pixie 8101, Endevco Co, San Juan, Puerto Rico) for force measurement. Both instruments allowed control of muscle position and simultaneous measurement of both position and force. Custom-built electronics produced sinusoidal signals for control of muscle length and phasic trigger signals for controlling stimulation. (In a wide variety of insect species wing motion is sinusoidal; Weis-Fogh, 1973; Ellington 1984*b*. Here it will be assumed that muscle motion is also sinusoidal.) All control and output signals were
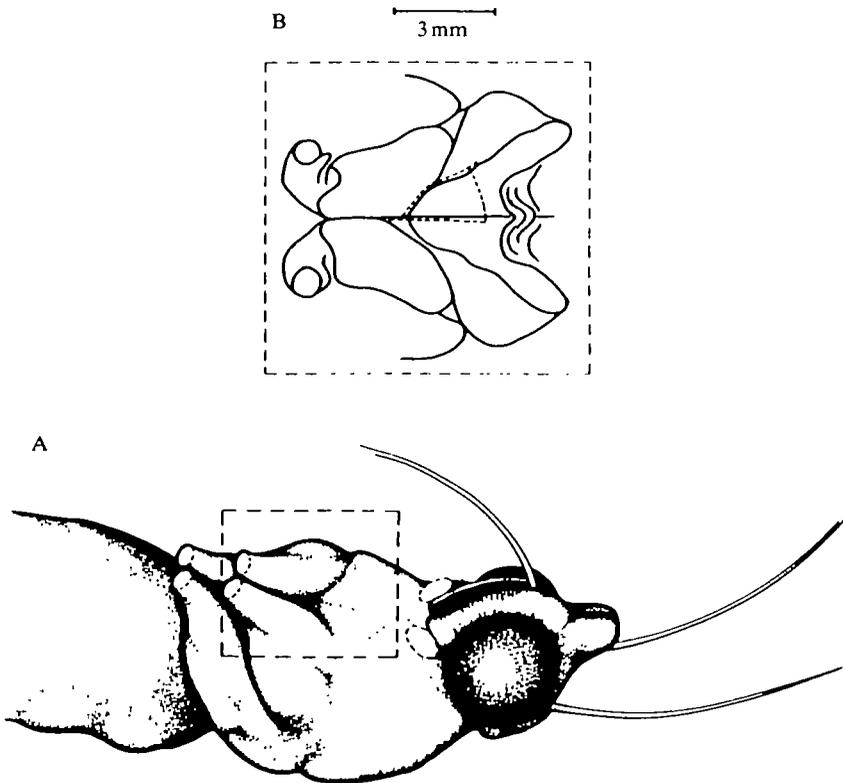

Fig. 1. (A) Perspective view of a *Manduca sexta* adult lying on its dorsal side. The prothoracic legs and portions of the other legs have been removed. (B) Ventral view of the mesothorax of *Manduca* at an enlarged scale when compared with A. The dotted lines outline a triangular piece of exoskeleton (basisternum) where three cuts were made to free the ventral attachment of a portion of the dorsal ventral flight muscle dv_1 used in these experiments. dv_1 is an indirect power muscle for elevating the wings. Madden (1944) provides additional drawings and a description of the anatomy.

recorded by an IBM PC computer through an A/D board (TECMAR Cleveland, Ohio, USA).

Experimental protocol

Before work measurements began, data to specify the 'phase' were gathered. Phase is a measure of the relative timing between the capacity of a muscle to generate force and the imposed sinusoidal length change. We follow the definition given by Josephson (1985*a*). Briefly, from an isometric twitch recording, the time between muscle stimulation and the peak tension was determined. (A separate determination was made for each new temperature.) Phase was then calculated as the time difference between the maximum length of the muscle and the expected time of maximum isometric force, based on the isometric twitch, divided by the cycle duration. This fraction was multiplied by 100 so that phase was expressed as a percentage from 0 to 100 %. As will be shown, the optimum phase for power output was about 25 %, which means that the expected time of peak isometric force would coincide with the mid-point of the shortening half-cycle.

Experimental trials were given regularly at 30 s intervals to keep the preparation at an approximately steady state. Each trial consisted of 5–6 imposed sinusoidal length changes with phasic electrical stimulation. The work output per cycle for the next to the last cycle was calculated by the computer. In the early cycles there was some facilitation but by the fourth or fifth cycle loop closure was very good, indicating a steady state had been reached. The optimum stimulus phase and muscle strain for work output were determined using a systematic search procedure. First, a value of the optimum strain was estimated and phase was varied until a work maximum was found. Then, having fixed the phase at the point which produced the local maximum, strain was varied until work output was again maximal. By repeating this procedure, the optimal phase and the optimal strain for maximizing work output were found for each cycle frequency. In total, the search required 6–15 min to complete. Next, the cycle frequency was altered and the process described above repeated. A series of 5–9 frequencies was investigated at five equally spaced temperatures from 20 to 40 °C.

Muscle fatigue

In early experiments it became obvious that work output usually decreased during the course of an experiment. In addition to a steady decrease, there was often an abrupt decline in performance. When a preparation exhibited an abrupt decline, the experiment was terminated. To monitor fatigue, maximal work output was determined at standard conditions, 30 °C and 24 Hz, between the trials at each new temperature tested. In addition, fatigue during the trials at a constant temperature was monitored by interspersing tests at a reference frequency between every two tests at new frequencies. Thus, a typical sequence of parameter changes might be: standard conditions (30 °C, 24 Hz), followed by a new temperature (e.g. 35 °C) and a scan of frequencies (28, 16, 20, 28, 24, 32, 28, 36, 40, 28 Hz), followed by standard conditions. The reference frequency chosen was the

frequency for which maximum power output was anticipated at the temperature being tested. For the data reported here, the time-dependent decrease in work output at standard conditions was approximately linear.

A fatigue index (FI) was defined as follows:

$$FI = 1 - \left[\frac{\text{Standard work, time} = t}{\text{Standard work, time} = 0} \right] .$$

The denominator within the brackets was the work determined at standard conditions in the first set of trials. The standard work at any other time, t, was estimated by linear extrapolation between the nearest determinations of work under the standard conditions before and after time t. Experimental sessions usually lasted 2–6 h. The average fatigue index at the end of experiments was 30 %. Unless otherwise indicated, all the values of work and power presented below have been corrected for expected fatigue by dividing individual values by $(1-FI)$. Thus, values given for work or power are estimates of those that would have been obtained from the preparation at the beginning of the experimental period.

Temperature control

Muscle temperature was controlled by placing the preparation in a Styrofoam box, where it was cooled by surrounding it with beakers of salted ice or heated by a microscope light. The temperature of the contralateral dv_1 muscle was measured continuously ($\pm 0.1\,°C$) with a thermistor probe. The output of the probe served as an input for a feedback circuit controlling the intensity of the microscope light. The muscle temperature was held within $0.5\,°C$ of the set temperature.

Determination of muscle length and mass

Initial muscle length was adjusted to *in vivo* length as judged by the surrounding cuticle of the thorax. After an experiment the body cavity was injected with 70 % ethanol while the muscle was still attached to the ergometer at its *in vivo* length. The muscle became fixed in place. After 30 min the muscle was detached from the ergometer and the animal stored in ethanol (70 %) for at least a week. Then the muscle was dissected out, and its dimensions were measured with an ocular micrometer. The muscle is composed of a parallel bundle of fibres that reach between the tergum and the sternum. The muscle is triangular in cross-section; its three-dimensional form is an elongate triangular prism. On the dorsal end there is a perpendicular termination of the fibres while on the ventral end there is an oblique termination. Thus, two of the edges of the prism are approximately equal in length, and the third somewhat longer (about 14 %) than the others. Based on this geometry, the average fibre length was calculated as $\frac{2}{3}L1 + \frac{1}{3}L2$, where $L1$ is the length of one of the shorter edges and $L2$ is the length of the longest edge. The contralateral dv_1 muscle was also removed and its length compared with that of the experimental muscle to determine if the length of the experimental muscle during the measurements was reasonably close to the *in vivo* length. In all instances the
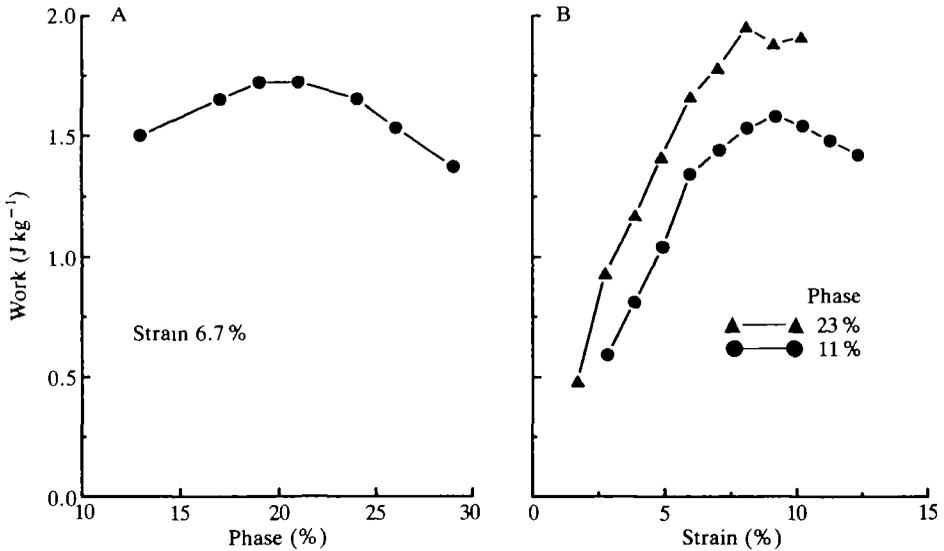
Fig. 2. Examples from two preparations of the data collected when searching for the maximum mechanical work output at fixed frequency and temperature. (A) The phase of electrical stimulation was varied while muscle strain was held constant at 6.7%. A local optimum exists for a phase of about 20%. (B) The strain was varied while the phase of stimulation was held constant at 11 or 23%. For each phase, there is an optimal strain of about 7% for maximum work output.

length of the muscle used in experiments was within 5% of the length of the contralateral dv_1. Muscle lengths averaged 7.0 ± 0.4 mm ($N=19$).

After dissection each muscle was rehydrated in saline solution for 24 h and then weighed on a Cahn electrobalance (model 21). Preliminary measurements showed that, after storage in 70% ethanol, muscles weighed an average of $73.0 \pm 3.7\%$ ($N=20$) of their initial mass. Therefore measured masses were multiplied by 1.37 to correct for the expected mass loss. Muscle masses averaged 13.8 ± 2.3 mg ($N=19$). Unless otherwise indicated, values for work and power output are given per unit mass of muscle.

Results

Stimulus phase, muscle strain and work output

The search for the stimulus phase and imposed strain which gave maximal mechanical work output was accomplished by varying one parameter at a time. The work output changed in a smooth fashion with changes in stimulus phase and strain (Fig. 2). The relative independence of the phase and strain optima made it possible to locate a maximum with 3–5 transections of the two-dimensional variable space. Several characteristics of work loops (loop shape, optimal phase of stimulation, optimal strain, independence of optimal phase and strain) for

Manduca flight muscle are very similar to those reported for wing muscles of katydids (Josephson 1985*a,b*) and locusts (Misizin and Josephson, 1987).

Cycle frequency, work and power

Mechanical work output first increased and then decreased when examined over a range of increasing frequencies (Fig. 3). Mechanical power output, too, exhibited an inverted U-shaped dependence on frequency (Fig. 3). The power curve is shifted to higher frequencies compared to the work curve. This shift is to be expected because power is the product of frequency and work. In the example of Fig. 3 the frequency that maximized work output was about 20 Hz while that for power output was about 26 Hz.

Temperature effects on power output

As temperature increased, the maximum power output and the optimal frequency for maximum power output both increased (Fig. 4). The mean maximum power output rose from about 20 W kg^{-1} at 20°C to about 90 W kg^{-1} at 40°C (Fig. 5A). For two individuals maximum values were close to 130 W kg^{-1}. The mean optimal frequency increased from 12.7 to 28.3 Hz over the experimental temperature range (Fig. 5B), and mean work per cycle at optimal frequency doubled, from 1.6 to 3.2 J kg^{-1} (Fig. 5C). There was considerable variation in the work output from animal to animal, which is reflected in the large error bars of Fig. 5C. In each individual the relationship between work output and temperature was a monotonically increasing function, roughly paralleling the mean slope shown in Fig. 5C.

The data in Fig. 5 suggest that frequency and work per cycle contribute approximately equally to the increase in power output with temperature, since
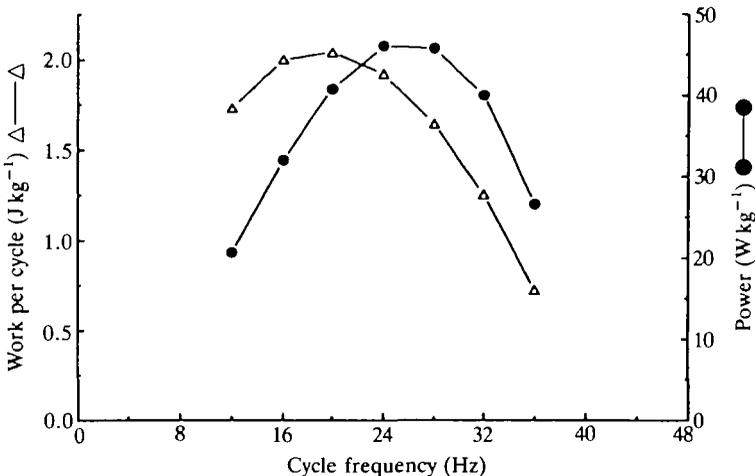

Fig. 3. Work output per cycle at the phase and strain that maximize work output and the corresponding power output as a function of cycle frequency from one preparation. Muscle temperature was 30°C.
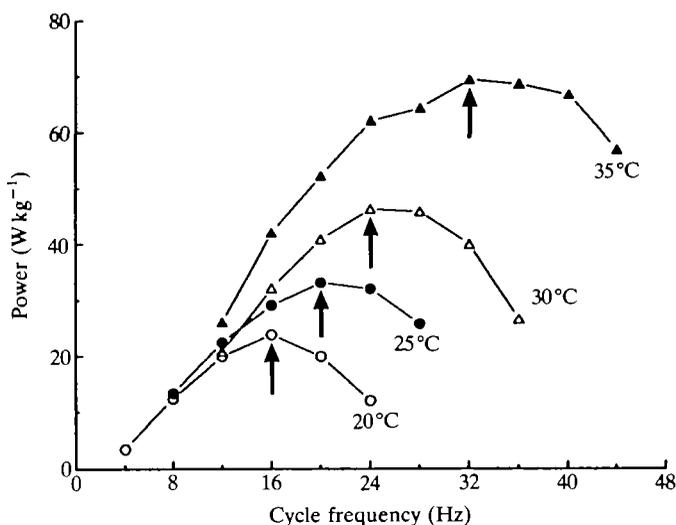
Fig. 4. The dependence of mechanical power output on cycle frequency in one individual at four different temperatures. Note that the power and the frequency at which power is maximum (the 'optimal' frequency, marked by arrows) both increase with muscle temperature.

both increased about twofold over the temperature range tested. However, these results are somewhat confounded because of variability among individuals and because the same individuals were not measured at all temperatures. In four preparations, data were obtained at each of four temperatures: 20, 25, 30 and 35°C. For this temperature range, cycle frequency increased an average of 2.4-fold (95% confidence interval=0.36) while work per cycle increased 1.5-fold (confidence interval=0.4). These comparisons suggest that changes in optimal frequency may contribute more to the increase in power output than do changes in the work per cycle at optimal frequency.

The area of a work loop depends on the muscle strain and forces throughout the strain cycle. The mean work output per cycle doubled when temperature increased from 20 to 40°C (Fig. 5C). What are the relative contributions of strain and force to this doubling, based on the expectation that work=strain×mean force difference for the shortening and lengthening half-cycles? Over a 20°C temperature range, mean optimal strain increased 34% (Fig. 6A), suggesting that mean forces are also changing. Unfortunately, we have no data for the average forces over the lengthening and shortening portions of the cycle. Furthermore, the precautions noted in the previous paragraph for interpreting mean data apply here also. When the analysis is restricted to the four preparations (see above) for a 15°C range in temperature, strain increases a mean of 1.3-fold (confidence interval=0.26). Together these data suggest that increases in both strain and in the force differences between the half-cycles contribute to the increase in work output, but their relative contributions are uncertain.

The optimal phase for maximum power output was approximately 25 % and showed no clear dependence on temperature (Fig. 6B). The step size used when searching for optimal phase was 2–3 % and the change in work output was very gradual near the optimum phase, both of which make it difficult to define accurately the optimum phase or to detect a trend with temperature.

Power output was the most temperature-sensitive variable. The thermal sensitivities of the variables examined decreased in the following order: optimal frequency>work>optimal strain (Table 1). The temperature dependence of all these parameters decreased as temperature increased. At the highest experimental temperatures of 35 and 40°C, all variables were relatively independent of temperature. This is the same range of temperatures which *Manduca* thoracic muscle experiences during flight.
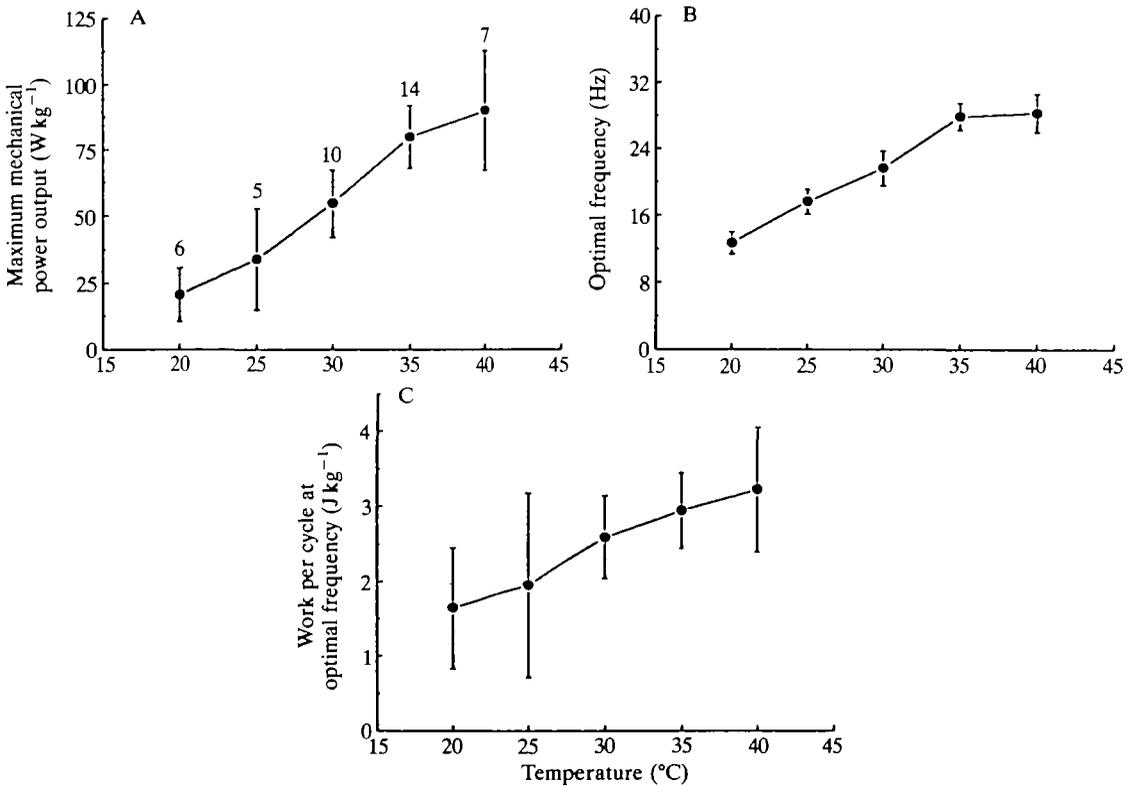

Fig. 5. (A) Maximum mechanical power at optimal phase, strain and cycle frequency as a function of muscle temperature. Numbers are the sample sizes at each temperature obtained from 19 individuals (one at five temperatures, three at four temperatures, four at two temperatures, and eight at one temperature). The error bars are two times the s.e. For a 20°C temperature range, the Q_{10} of power output is 2.09. (B) Optimal frequency and (C) work per cycle at the optimal frequency. Samples sizes and confidence intervals are as in A. The variation in work output is much larger than that found for optimal frequency. The variation largely reflects variation among individuals (see text).
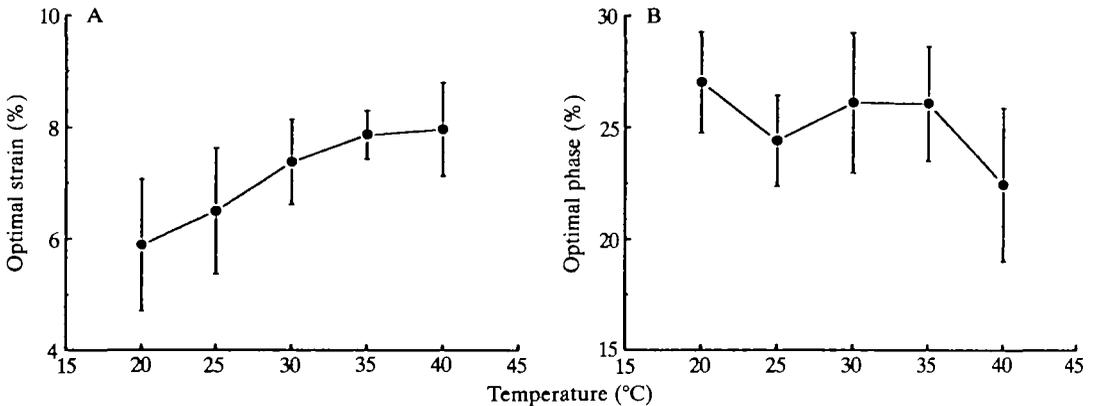
Fig. 6. Optimal strain and stimulation phase corresponding to the maximum power output data from Fig. 5. Sample sizes are the same as in Fig. 5. Confidence intervals are computed as described in the legend of Fig. 5A. Mean optimal strain is about 7%. Over the 20°C temperature range there is a 34% increase in strain from 5.9 to 7.9%. (B) Mean optimal phase varies between 22 and 27%, with no significant temperature trend.

Work output with paired stimuli per cycle

The experiments described above were all done with one stimulus per strain cycle. The effects of paired stimuli per cycle on work output were examined at several combinations of muscle temperature and cycle frequency. At 35°C, 28 Hz, and at 30°C, 24 Hz, paired stimuli at interstimulus intervals of 2–12 ms did not increase work output appreciably above that available with one stimulus per cycle (Fig. 7). In contrast to these results, at a lower operating frequency, 12 Hz at 30°C, the maximum work output increased significantly, 1.5–2 times, with paired stimuli.

Table 1. Q_{10} and R_{10} values for variables associated with mechanical power output of Manduca flight muscle

	Temperature ranges		
Variable	20–30°C	30–40°C	Flight 35–40°C
Power (W kg^{-1})	2.65	1.65	1.27
Optimal frequency (Hz)	1.70	1.31	1.03
Work per cycle (J kg^{-1})	1.58	1.24	1.20
Optimal strain (%)	1.26	1.08	1.03

R_{10} is a concept equivalent to Q_{10} but used for parameters which are not rates, here work and optimal strain (see Bennett, 1984).

Q_{10} values are compiled from a mean value of the variable at each temperature.
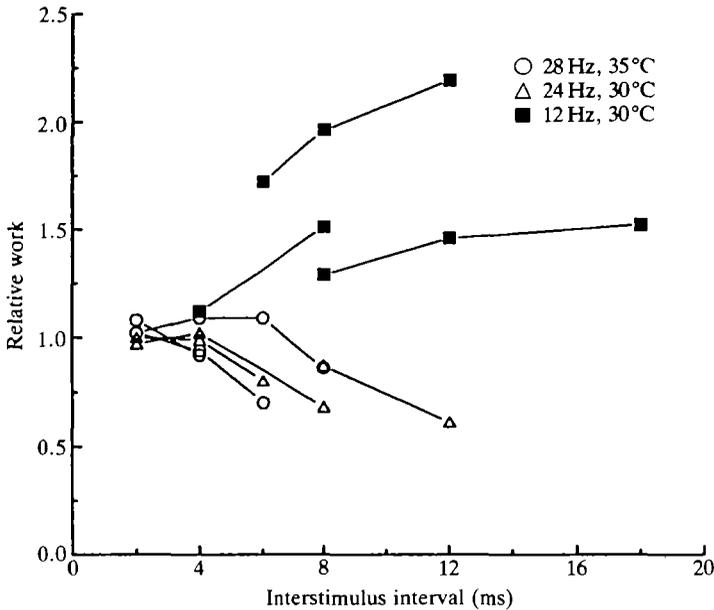
Fig. 7. The effects of two stimuli per cycle on work output for three preparations at 35°C, 28 Hz; 30°C, 24 Hz; and 30°C, 12 Hz. For each interstimulus interval, excursion amplitude and phase are adjusted to produce maximum work output. Relative work is the ratio of maximum work output at two stimuli per cycle and the work output for one stimulus per cycle at the identical temperature and frequency.

Discussion

Temperature, power and flight

It has often been observed that there is minimum body temperature below which an insect cannot fly, and that many insects warm up by basking or by muscle shivering before flight (Heinrich, 1981). The inability of cool muscles to provide the minimum power required for flight is thought to prevent take-off at low body temperature. Casey (1981a) has estimated that sphingid moths require a minimum mechanical power output of $350 \, W \, kg^{-1}$ of muscle in order to fly. This calculation is based on the assumptions that flight muscle is about 20 % of the total animal mass, and that none of the kinetic energy in the moving wing is stored elastically during deceleration of the wing. If perfect elastic storage is assumed, the required power is $80-110 \, W \, kg^{-1}$ of muscle. Ellington (1984a) has used improved aerodynamic theory and has estimated that at least $50 \, W \, kg^{-1}$ of muscle are required for insect flight. The power obtained from the dv_1 muscle of *Manduca* ranged from 20 to $90 \, W \, kg^{-1}$. Thus, the power available is within the range estimated to be required for flight, at least if it is assumed there is substantial elastic storage. We can take this comparison one step further by estimating a minimum thoracic temperature for flight. Our data directly show that the power available for flight increases with temperature (Fig. 5A). If $50-80 \, W \, kg^{-1}$ of muscle is taken as the

minimum power required for flight, muscle temperatures must be at least 30–35°C to deliver this power. Thus, these data support the view that muscle temperature could curtail flight in *Manduca* and other sphinx moths at body temperatures less than 30–35°C (Heinrich, 1981).

Temperature and limits on mechanical power output

The mechanical power output from a flight muscle of *Manduca* averaged 90 W kg^{-1} at 40°C. This value is higher than measurements of power output from other muscles using the same technique (9 W kg^{-1} for a crab respiratory muscle to 78 W kg^{-1} for tettigoniid wing muscle, Fig. 8). However, when allowances are made for the lower temperatures during earlier experiments with other muscles, the *Manduca* muscle is similar to those of other species (Fig. 8). It is noteworthy that all the direct measurements of mechanical power output are much less than the maxima of 230–250 W kg^{-1} calculated by Weis-Fogh and Alexander (1977) and by Pennycuick and Rezende (1984), based on the force–velocity properties of muscle and estimates of muscle strain. Even the maximum power output of 130 W kg^{-1}, observed in two *Manduca* preparations, is only about half of the theoretical estimates.

The thermal sensitivity of *Manduca* muscle (Table 1) is similar to the thermal sensitivity of muscle from other taxa, as summarized by Bennett (1984). Specifically, (1) where direct comparisons can be made, temperature sensitivities
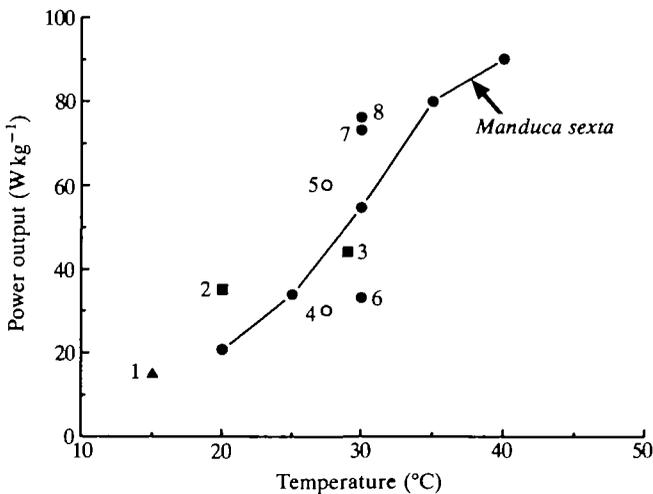

Fig. 8. Power output of muscles measured by the work-loop technique from a variety of animals plotted against the temperature at which the experiments were conducted. The solid line is taken from data in Fig. 5A. Literature data are as follows: (1) crab respiratory muscle (Stokes and Josephson, 1988); (2) frog sartorius (Stevens, 1988); (3) rat diaphragm (D. A. Syme and E. D. Stevens, in preparation); (4) beetle and (5) bumblebee flight muscle (Machin and Pringle, 1959); (6) tettigoniid flight muscle (Josephson, 1985*a*); (7) tettigoniid flight and singing muscle (Josephson, 1985*b*); (8) locust flight muscle (Mizisin and Josephson, 1987).
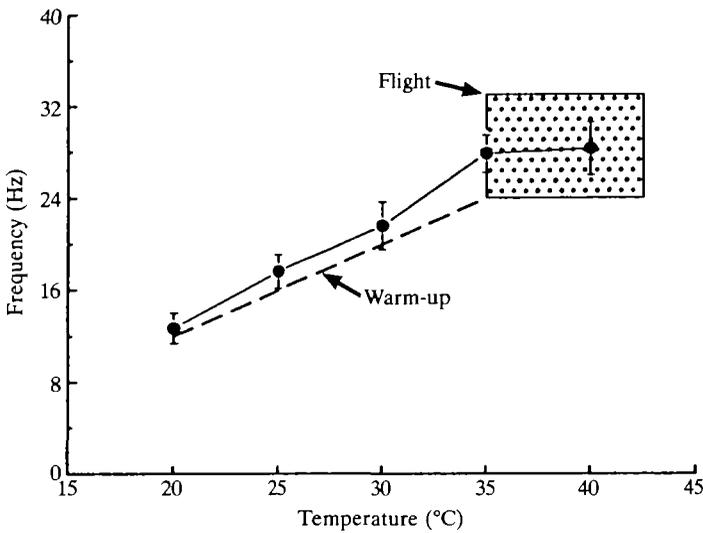
Fig. 9. Comparison of frequency–temperature relationships described in the muscle experiments of Fig. 5B (solid line) with data obtained from the whole animal during flight (dotted box, Heinrich, 1971; R. D. Stevenson, unpublished results) and warm-up (dashed line, Heinrich and Bartholomew, 1971).

of variables measured for *Manduca* muscle are similar to mean values presented by Bennett (1984), (2) rate processes (power and frequency) are more temperature-sensitive than non-rate processes (work and strain) and, (3) temperature sensitivity decreases at higher temperatures.

Temperature and frequency relationships

During free flight, thoracic temperatures in *Manduca* range between 36 and 42°C and wing beat frequencies range between 26 and 32 Hz (Heinrich, 1971; R. D. Stevenson, unpublished results). This range of values obtained from flying animals encompasses the temperature and optimal frequency values that maximize power output of the muscle preparation (Fig. 9). At a lower range of temperatures (20–35°C), a close ,correspondence was also found between the thoracic temperature *vs* wing beat frequency regression obtained from animals during warm-up (Heinrich and Bartholomew, 1971) and the temperature *vs* optimal frequency relationship that maximizes power output (Fig. 9). The agreement between wing beat frequency during warm-up and the optimal frequency for power output was unanticipated. During warm-up the wing movements are of a low amplitude and the functional significance of the behaviour is thought to be heat production not mechanical power output (Heinrich and Casey, 1973). The correspondence may reflect some underlying property of the muscle or of the nervous circuitry involved in the generation of the output patterns, or it may be coincidental.

Muscle activation and work output

Among insects with synchronous wing muscles, the motor neurones to the muscle may fire repetitively on each cycle, resulting in bursts of up to seven muscle action potentials per wing beat (reviewed in Kammer, 1985). Species with higher wing beat frequencies (20–50 Hz), including sphinx moths, have shorter burst lengths and fewer spikes per cycle than species with low wing beat frequencies (Kammer, 1985). Multiple activation per cycle is generally thought to increase power output (Kammer, 1985). At cycle frequency and temperature conditions normally experienced during flight, multiple activation per cycle does increase mechanical power output from locust and katydid wing muscles above that available from a single stimulus per cycle (Josephson 1985a,b; Mizisin and Josephson, 1987). However, for the dv_1 muscle of *Manduca* at cycle frequencies similar to those during flight (24 Hz, 28 Hz), pairs of stimuli did not increase power output above that obtained with single shocks (Fig. 7). In contrast, at low operating frequencies (12 Hz), multiple stimulation did increase power output.

The effect of multiple activation on power output can be expected to depend on the length of the shortening phase of the operating cycle compared with the duration of the force evoked by a stimulus. In a sinusoidal length cycle at 24 Hz, the shortening half-cycle lasts 21 ms. The twitch duration of the dv_1 muscle at 30°C is about 37 ms (onset to 90 % relaxation, R. D. Stevenson, unpublished data). Thus, the shortening half-cycle is significantly shorter than the duration of a twitch initiated by a single shock. Multiple stimulation increases the duration of muscle activation; one effect of this would be to increase muscle force during the lengthening half-cycle at 24 Hz. Because of the increased force, more work would be required to re-lengthen the muscle than would be true for a muscle activated but once per cycle. It is presumably because of increased work input required for muscle lengthening, which reduces the net work output, that multiple stimulation does not increase power output at normal operating frequencies. At the lower frequency examined (12 Hz), the shortening half-cycle lasts about 42 ms, which is longer than the length of a twitch initiated by a single stimulus. At the low frequency, multiple stimulation results in the muscle being active through more of the shortening half-cycle. In this instance, there is an increase in work during the shortening portion of the cycle which is greater than any change in the work necessary to re-lengthen the muscle. Therefore, both work and power output increase with multiple stimulation at low frequency.

The dv_1 muscle is an indirect flight muscle, a muscle that does not attach directly to the wings and whose contraction drives the wings indirectly by bending the thoracic cuticle. Kammer (1971) has recorded muscle action potentials from the dorsal longitudinal muscle (dlm) of *Manduca*, which is another indirect flight muscle, and from several direct flight muscles which insert on wing sclerites. During both straight flight and turns, the dlm typically produces one action potential per cycle, while the direct flight muscles (basalar, subalar, third axillary muscle) often fired several times per cycle. It may be that the direct flight muscle produces twitches which are shorter than those of the dv_1 and that multiple

activation of the direct flight muscles does increase their power output. Alternatively, the multiple activation may change the phase relationships between activity in the various wing muscles but not their power output, and it is the changing phase relationships which are important for manoeuvring during flight.

Estimates of muscle efficiency

The mechanical efficiency of muscle is defined as the ratio of mechanical power output of the muscle to the chemical power input (Woledge et al. 1985, p.209; Hill, 1939). For flight conditions in Manduca of 40°C and 28 Hz, average mechanical power output was 90 W kg^{-1} of muscle while maximum values reached 130 W kg^{-1} of muscle. An estimate of power input can be calculated from published values for oxygen consumption. During free hovering flight, oxygen consumption of Manduca is approximately 44 ml O$_2$ g^{-1} body mass h^{-1} (=12.2 ml O$_2$ kg^{-1} body mass s^{-1}) (Heinrich, 1971; Casey, 1976). The wing muscles of Manduca make up about 21 % of the body mass (R. D. Stevenson, unpublished). Assuming that essentially all the oxygen consumption during flight is by the wing muscles (see Casey, 1981b), the estimated power input is 1170 W kg^{-1} of muscle. [Because fat is the main source of fuel when Manduca flies (Ziegler and Schultz, 1986), we assumed that 1 ml of O$_2$ is approximately equivalent to 20 J (Schmidt-Nielsen 1983).] Therefore overall muscle efficiency is calculated as 8–11 %. This is lower than most published values of 20–25 % for the mechanical efficiency of muscle (data cited in Ellington, 1984a). Muscle efficiency may be more variable and sometimes lower than previously thought. Ellington (1985) calculated values of 10 % for asynchronous insect flight muscle. Other estimates of overall efficiency calculated by Heglund et al. (1982) for locomotion in small mammals and by Stokes and Josephson (1988) for mechanical power output of a crab respiratory muscle are also close to 10 %.

In summary, the work-loop technique has provided direct measurements of mechanical power output as a function of temperature. These data are consistent with the hypothesis that body temperatures below 30°C prevent Manduca from flying because its muscles have insufficient power. The technique has also been used to derive estimates of muscle efficiency and it has provided information about the patterns of nervous stimulation and muscle strains that might be expected during flight.

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