

The efficiency of an asynchronous flight muscle from a beetle

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Accepted 17 September 2001

Summary

Mechanical power output and metabolic power input were measured from an asynchronous flight muscle, the basalar muscle of the beetle *Cotinus mutabilis*. Mechanical power output was determined using the work loop technique and metabolic power input by monitoring CO₂ production or both CO₂ production and O₂ consumption. At 35 °C, and with conditions that maximized power output (60 Hz sinusoidal strain, optimal muscle length and strain amplitude, 60 Hz stimulation frequency), the peak mechanical power output during a 10 s burst was approximately 140 W kg⁻¹, the respiratory coefficient 0.83 and the muscle efficiency 14–16 %. The stimulus intensity used was the minimal required to achieve a maximal isometric tetanus. Increasing or decreasing the stimulus intensity from this level changed mechanical power output but not efficiency, indicating that the efficiency measurements were not contaminated by excitation of muscles adjacent to that from which the mechanical recordings were made. The CO₂ produced during an isometric tetanus was approximately half that during a bout of similar stimulation but with imposed sinusoidal strain and work output, suggesting that up to 50 % of the energy input may go to muscle activation costs. Reducing

the stimulus frequency to 30 Hz from its usual value of 60 Hz reduced mechanical power output but had no significant effect on efficiency. Increasing the frequency of the sinusoidal strain from 60 to 90 Hz reduced power output but not CO₂ consumption; hence, there was a decline in efficiency. The respiratory coefficient was the same for 10 s and 30 s bursts of activity, suggesting that there was no major change in the fuel used over this time range.

The mass-specific mechanical power output and the efficiency of the beetle muscle were each 2–3 times greater than values measured in previous studies, using similar techniques, from locust flight muscles, which are synchronous muscles. These results support the hypothesis that asynchronous flight muscles have evolved in several major insect taxa because they can provide greater power output and are more efficient than are synchronous muscles for operation at the high frequencies of insect flight.

Key words: muscle, asynchronous muscle, efficiency, work, work loop, metabolic rate, respiratory quotient, *Cotinus mutabilis*.

Introduction

The flight muscles of insects can be characterized as being synchronous or asynchronous, depending on the relationship between muscle electrical activity and contraction during flight (for reviews, see Dudley, 2000; Josephson et al., 2000b). Both synchronous and asynchronous muscles are turned on by impulses in motor neurons to the muscles and the resulting action potentials in the fibers of the muscles. In a synchronous muscle, each contraction is evoked by a muscle action potential or a burst of action potentials, and there is a one-to-one correspondence between the electrical activity in a muscle and contraction of that muscle. The term 'synchronous' refers to this congruence between electrical and mechanical events. An asynchronous muscle is turned on by neural input, but when it is activated it can contract in an oscillatory manner if it is attached to an appropriate mechanically resonant load, such as is offered in life by an insect's wings and thorax. The

contraction frequency of an oscillating, asynchronous flight muscle is determined by the resonant frequency of the load and is generally different from, typically much higher than, that of the muscle action potentials, which is why the muscles are termed asynchronous. The features of asynchronous muscle that allow maintained, oscillatory contractions are delayed contractile deactivation following shortening and delayed activation following stretch (for details, see Josephson et al., 2000b).

With the exceptions of flight muscles in many insects and sound-producing muscles of some but not all cicadas, all insect skeletal muscles appear to be synchronous muscles, as do all skeletal muscles in arthropods other than insects. Synchronous control of contraction is certainly the ancestral state and asynchronous control a derived condition (Pringle, 1957; Dudley, 2000). Asynchronous flight muscles are found widely

in extant insects, and they power flight in several of the more speciose orders, including Coleoptera, Diptera and Hymenoptera. The distribution of asynchronous muscles among insect taxa suggests that asynchronous flight muscle has evolved independently at least six times in different insect lines (Cullen, 1974; Pringle, 1981; Dudley, 2000). Several suggestions have been put forth as to the advantages offered by asynchronous control that might have led to its having been favored by evolution in several different insect groups.

(i) The potential for high operating frequency (Pringle, 1957, 1981; Josephson and Young, 1981; Dudley, 1991; Dudley, 2000). In synchronous muscles, each contraction begins with depolarization of the muscle fiber membrane. The depolarization triggers the release of Ca^{2+} into the cytoplasm from an internal store, the sarcoplasmic reticulum (SR). The released Ca^{2+} diffuses into the myofibrils where it activates the myofilaments. The contraction is terminated by the re-uptake of the released Ca^{2+} by the SR. Several of these control steps are potentially slow and rate-limiting, in particular the diffusion of Ca^{2+} into and out of myofibrils and the uptake of Ca^{2+} by the SR. The maximum operating frequency of a synchronous muscle is presumably set, in part, by the rapidity with which released Ca^{2+} reaches myofilament control sites and the rapidity with which Ca^{2+} can be taken up by the SR to terminate activity. Asynchronous muscles bypass cycle-by-cycle Ca^{2+} control and the limitations that it imposes. As indicated above, the operating frequency of an asynchronous muscle is the mechanically resonant frequency of the muscle and its load. The maximum frequency for an asynchronous muscle is probably limited only by the mechanical properties of the muscle and the parts to which it is attached and by the kinetics of the delayed shortening deactivation and delayed stretch activation that underlie oscillatory performance and allow positive power output by the muscles during cyclic contraction. The contractile frequencies reached by asynchronous muscles can be very high. The highest contractile frequency reported for any skeletal muscle is that from asynchronous wing muscles of a tiny midge (Diptera), for which the oscillation frequency was approximately 1000 Hz in intact animals and over 2000 Hz for animals in which the wings had been surgically reduced to small stumps (Sotavalta, 1953).

(ii) Increased power during high-frequency operation (Josephson and Young, 1981; Lindstedt et al., 1998). Some synchronous muscles do operate at quite high frequencies; 200 Hz for sound-producing muscles of the toadfish (Rome et al., 1996), over 200 Hz for the wing muscles used in sound production by a katydid (Josephson and Halverson, 1971) and over 500 Hz for the tymbal muscle of a cicada (Josephson and Young, 1985). High-frequency operation in synchronous muscles is achieved, in part, through hypertrophy of the SR, which reduces Ca^{2+} diffusion distances into and out of myofibrils and increases the capacity of the SR to remove Ca^{2+} rapidly from the cytoplasm. In extreme cases, the volume of the SR within the muscle can substantially exceed that of the myofibrils (Rosenbluth, 1969; Josephson and Young, 1985). Hypertrophy of the SR reduces the fraction of a muscle

available for myofibrils, so there is a trade-off between rapidity of contraction on the one hand and force and power on the other. Synchronous muscles that can contract at high frequency are expected to be, and have been found to be, weak and to have low power output (Josephson, 1984; Rome et al., 1999).

Asynchronous muscles achieve high contraction frequencies without hypertrophy of the SR; in fact, in asynchronous muscles, the SR is rather sparse. Because there is relatively little SR in asynchronous muscles compared with high-frequency, synchronous ones, there is potentially more space per unit muscle volume in asynchronous muscles for myofibrils. This is the basis for the proposal that high-frequency asynchronous muscles should have a greater mass-specific power output than synchronous ones. The best available information on the power output of asynchronous muscle comes from a recent study of a beetle flight muscle (Josephson et al., 2000a). The beetle muscle did indeed have a higher power output than reported values for high-frequency synchronous muscles. The increased power output in the beetle muscle was actually greater than could be accounted for simply on the basis of an increase in relative myofibrillar volume. The power output per unit mass of myofibril was higher in the asynchronous beetle flight muscle than in those synchronous muscles that have been examined. Specifically, the power output was approximately 243 W kg^{-1} myofibril for the beetle flight muscle [operating frequency, 60–90 Hz; power output, 141 W kg^{-1} muscle (Josephson et al., 2000a); myofibrillar volume density, 58.1% (Josephson et al., 2000b)]. The equivalent power output for synchronous flight muscle from a locust (contraction frequency during flight, 20 Hz) is approximately 105 W kg^{-1} myofibril [power output, 68 W kg^{-1} muscle (Malamud et al., 1988); volume density of myofibrils within the muscle, 65% (Josephson et al., 2000b)]. Asynchronous muscle does seem to be capable of producing more mechanical power during high-frequency operation than can synchronous muscle.

(iii) Greater operating efficiency (Josephson and Young, 1981; Lindstedt et al., 1998; Dudley, 2000). The maximum efficiency (=work done/metabolic cost) of a synchronous muscle, operating as do flight muscles at moderately high frequency and with a moderately small length change (=strain) per cycle, is expected to be inversely related to operating frequency. As a first approximation, the maximum work per cycle available from a synchronous muscle during repetitive contraction is proportional to the cycle duration and, thus, inversely proportional to cycle frequency (Josephson, 1989). The mechanical power output, which is the product of work per cycle and cycle frequency, is therefore expected to be independent of cycle frequency or at least not strongly dependent on cycle frequency. The activation cost for turning muscle on and off is substantial; it is estimated that a quarter to half of the energy consumed by a muscle during an isometric twitch is associated with the release of Ca^{2+} to activate the muscle and the resequstration of Ca^{2+} to terminate activity (Homsher and Kean, 1978; Rall, 1982). The metabolic power input attributable to muscle activation can be expected to be

proportional to the frequency at which the muscle is turned on and off. The expected consequence of mechanical power output that is independent of operating frequency and activation costs that are proportional to frequency is declining efficiency with increasing operating frequency.

The activation costs are presumably much smaller for asynchronous muscles than for synchronous ones. In asynchronous muscles, the firing frequency of the activating motor neurons is low, generally much lower than the contractile frequency. The volume density of SR in asynchronous muscles is low and so too, therefore, must be the rate of Ca^{2+} release and uptake per unit muscle volume. It would be predicted that the higher mass-specific mechanical power output and the lower activation costs in asynchronous muscles compared with synchronous ones would result in asynchronous muscles being more efficient as motors for high-frequency operation. The following study was performed to determine whether this prediction is, indeed, true.

Materials and methods

General procedures and apparatus

The measurements were made from the metathoracic, basalar muscle of the beetle *Cotinus mutabilis* (Gory and Percheron). The beetles were obtained from a laboratory colony. All the animals used in this study were capable of ascending flight when they were provoked to fly by throwing them repeatedly into the air.

The methods used to raise animals and to prepare the muscle and record force and work from it have been detailed previously (Josephson et al., 2000a). The completed preparation consisted of a beetle with the legs, wings and head removed. The preparation was fixed, with its dorsal side up, to a platform on the moveable shaft of an ergometer (a device for imposing controlled length changes on the muscle). The ergometer shaft was vertical, and the animal was mounted such that one of the paired basalar muscles, that from which mechanical measurements would be made, was positioned directly above and in line with the shaft. Stimulating electrodes were placed in the ventral insertion of the muscle, and a force transducer was attached to the dorsal apodeme of the muscle. The stimuli used to activate the muscle were 1 ms shocks presented in bursts at 60 or 100 Hz. A thermocouple, inserted into the thorax on the side opposite to the experimental muscle, monitored thoracic temperature.

The preparation was enclosed in a rectangular chamber whose volume, without the animal, was 46 ml. The leads to the thermocouple and to connectors to which the stimulating electrodes were attached entered the chamber through leak-proof ports in the chamber wall. The ergometer shaft entered the chamber from the bottom through a flexible diaphragm that allowed the shaft to move along its axis. The rod holding the force transducer entered the chamber horizontally through a second flexible diaphragm that allowed some horizontal and vertical movement of the transducer and adjustment of the position of the muscle at the transducer attachment. Two sides

and the top of the chamber were removable to allow mounting of the animal, connection of the stimulating electrodes, insertion of the temperature thermocouple and moistening of the muscle with saline. When the animal had been mounted, the removable sides and top of the chamber were fixed in place with thumbscrews. The joints between the removable and the fixed walls of the chamber were made airtight by liberal application of stopcock grease.

The temperature of the insect within the chamber was controlled by adjusting the intensity of a heat lamp that shone on the chamber and that of a microscope lamp that illuminated the thorax of the animal within the chamber. The intensity of the heat lamp was adjusted manually to raise the thoracic temperature to slightly below the desired level when the microscope lamp was off. The final temperature control was achieved with the microscope lamp, whose intensity was controlled by a servo system, the input signal for which came from the thermocouple in the thorax of the preparation.

An air input port and an output port projected through opposite walls of the experimental chamber. The input air, before it reached the chamber, passed, in sequence, through a pump (Ametek R-1 Flow Control), a CO_2 scrubber (Ascarite), a water scrubber (Drierite), a mass flow controller (Sierra Side Track 840) and a hydrator consisting of a tube filled with folded, moistened filter paper (Fig. 1). Water and CO_2 were removed from the air before the mass flow controller to ensure that the volume of air measured as passing through the controller was dry and CO_2 -free. The flow rate through the controller was 100 ml min^{-1} in all experiments. The air was rehydrated after the mass flow controller to reduce drying of the preparation in the respiratory chamber. The air leaving the chamber passed through a second water scrubber and a CO_2 analyzer (Li-Cor LI-6251). In experiments in which both CO_2 production and O_2 consumption were monitored, the air leaving the CO_2 analyzer passed into one of the input ports of a differential oxygen analyzer (Sable Systems FC-2 'Oxzilla', Henderson, NV 89014). A second CO_2 scrubber was inserted between the CO_2 and O_2 analyzers in most but not all the experiments in which O_2 was measured. The reference air to the second input port of the differential oxygen analyzer came from a side arm on the supply side of the circuit after the CO_2 and water scrubbers. A flow meter with manual control was used to set the flow rate in the reference line to 100 ml min^{-1} .

When data collection in an experiment was complete, the respiratory chamber was opened and the muscle from which work had been obtained was fixed *in situ* by superfusion with 70% ethanol. After approximately 30 min of fixation, the muscle was detached from the force transducer, and the preparation was removed from the ergometer and stored in 70% ethanol. After one to several weeks in ethanol, the experimental muscle was dissected free from the preparation, rehydrated overnight in insect saline and weighed. An estimate of the original wet mass of the muscle was obtained by multiplying the mass of the rehydrated muscle by 1.177 to correct for the mass loss associated with fixation (Josephson et

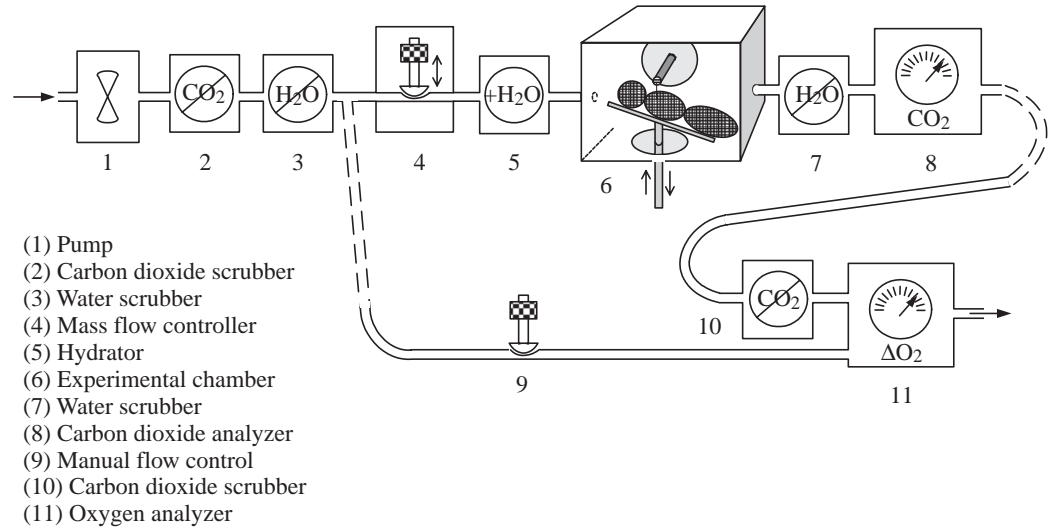


Fig. 1. Flow diagram for the apparatus. Components 9–11 were used only in series 2, in which both CO₂ production and O₂ consumption were measured.

al., 2000a). The mass of the muscles used in these experiments was 37.6 ± 5.8 mg (mean \pm s.d., $N=25$).

Setting parameters for stimulation and work output

Choosing an appropriate stimulus intensity and frequency

The basalar muscle is innervated by at least five excitatory axons and at least one inhibitor (Josephson et al., 2000a). The amplitude of a muscle contraction initiated by a stimulus or burst of stimuli is dependent on how many of the innervating axons are activated and, therefore, on stimulus intensity. When the animal had been mounted in the chamber, the thoracic temperature was set at 30 °C and isometric force was measured as the muscle was stimulated with 150 ms bursts of shocks of 1 ms duration at a stimulus frequency of 100 Hz. The stimulus intensity was initially below the threshold for contraction and was increased progressively from trial to trial (intertrial interval 1 min) until a maximal or nearly maximal contraction was produced. The stimulus intensity used in subsequent measurements was close to and sometimes slightly less than that required to evoke a maximal tetanic contraction. We will term this stimulus intensity 'adequate'. It should be emphasized that the 'adequate' stimulus intensity might be less than that required to obtain a maximal tetanic response. We reasoned that using a less-than-maximal stimulus might not activate all the motor units within the muscle, and the work output might be less than that obtainable with stronger stimuli, but that the increase in CO₂ production and in O₂ consumption above the baseline levels resulting from muscle stimulation would be more likely to be due to activity in the basalar muscle alone than might be true with stronger stimuli, which could excite muscles adjacent to the basalar. If muscles surrounding the basalar were to become activated, their activity would increase the amount of extra CO₂ produced and O₂ consumed above the resting level without increasing the measured work, thus reducing the apparent efficiency.

We reported previously that power output is maximal when the muscle is stimulated at 100 Hz (Josephson et al., 2000a). This result came from studies using bursts of stimuli lasting a

few hundred milliseconds. In the present study, in which the stimulus bursts during efficiency measurements were 10 or 30 s long, work output often declined irregularly and substantially throughout the period with muscle stimulation and sinusoidal oscillation when the stimulation frequency was 100 Hz. Reducing the stimulation frequency to 60 Hz appeared to give a more stable work output, and this stimulation frequency was used in most efficiency trials.

Determination of optimal muscle length and strain

Measurements of optimal muscle length and optimal strain amplitude were performed at a muscle temperature of 35 °C and a strain frequency of 60 Hz, conditions that were found previously to be approximately optimal for mechanical power output (Josephson et al., 2000a). When determining optimal muscle length, the muscle was set initially at a short length, one at which it was slack with little or no resting tension. The muscle was then stimulated tetanically at 100 Hz using the stimulus intensity identified as being adequate to excite most or all of the units in the muscle. During the plateau of the contraction, the muscle was subjected to 15–21 cycles of sinusoidal strain. The strain amplitude was typically 0.24 mm peak-to-peak, but was made slightly greater than this with particularly large beetles and slightly smaller for small ones. After every third cycle, the muscle length was increased, typically by 0.02 mm. Measurements of muscle length and force were collected with an analog-to-digital converter (sampling frequency 15 kHz per channel). A computer program, running on-line, created and displayed work loops for the second cycle of each set of three and calculated the work represented by the work loop. The optimal muscle length was taken as that length in the set that produced the greatest work per cycle.

In determinations of optimal strain, the muscle was held at that length determined to be optimal for work output and stimulated tetanically at 100 Hz. The muscle was subjected to 15–21 cycles of sinusoidal strain during the plateau of the resulting isometric contraction. The initial strain amplitude was

typically approximately 0.16 mm peak-to-peak (approximately 3% of muscle length) and was increased by approximately 0.02 mm (approximately 0.3% of muscle length) after every third cycle. The strain at which the work per cycle was greatest was accepted as the optimal strain. If the value determined for optimal strain differed significantly from that used earlier when measuring optimal muscle length, the optimal muscle length was again measured, as described above, using the newly determined value of optimal strain. All efficiency measurements were made with the muscle at that length determined to be optimal for work output and, except in some of the trials using a 90 Hz cycle frequency, with the optimal value of imposed strain.

Protocols

The results to be considered are drawn from two series of experiments. In the first series, CO₂ production, but not O₂ consumption, was measured. These experiments were designed to examine the effects on muscle efficiency of stimulus frequency, stimulus intensity and strain frequency. The second series of experiments, in which both CO₂ production and O₂ consumption were measured, was undertaken primarily to determine the respiratory coefficient (RQ) of working beetle muscle. The results discussed below will be identified as being from the first experimental series or the second. Experiments in both series consisted of a number of individual trials. Each trial included a 30 s pre-stimulation period, 10 or 30 s of continuous muscle stimulation with or without continuous sinusoidal strain, and a 500–600 s recovery period. The muscle temperature was 35 °C throughout. Trials in which the stimulus duration was

10 s, the stimulus frequency 60 Hz, the stimulus intensity 'adequate', and the sinusoidal strain frequency 60 Hz will be referred to as control trials. Control trials were interspersed among trials with other values for stimulation or strain parameters. The sequence of trials for experiments of the two series is shown in Table 1.

In both the first and second series, a few preparations, when first set up, were found to be either unresponsive or to produce unusually low isometric force and work; these were discarded without efficiency measurements being taken. The first series included a total of 10 and the second series 15 more-or-less successful experiments. In a few trials of the second series, the signal-to-noise ratio for the O₂ channel was too low to allow reasonable measurement of O₂ consumption. The sample size for data drawn from these experiments was 13–15 depending on the parameter being considered.

Data collection

During each trial, a block of interdigitated data samples was collected from the muscle force, muscle length, CO₂ and, if present, the O₂ channel at approximately 0.5 s intervals. The sampling frequency was 15 kHz per channel, and each block of data represented 200 ms of activity. If the data sample was from a period in which there was muscle stimulation and imposed strain, a work loop (a plot of muscle force against length) was collected and analyzed for the second complete cycle in the data set. The values saved from each block of data were: (i) t_n , the time at the onset of data collection for the block; (ii) W_n , the work done during the second complete strain cycle (if there was imposed strain) during the block; (iii) D_n , the duration of

Table 1. Parameters for the sequential trials in experiments of series 1 and series 2

Trial number	Stimulus frequency		Stimulus intensity			Burst duration		Sinusoidal frequency (strain)		
	30 Hz	60 Hz	Low	Adequate	2× adequate	10 s	30 s	0 Hz (isometric)	60 Hz	90 Hz
Series 1 (CO ₂ only)										
1		x		x		x				x
2		x		x		x		x		
3		x		x		x				x
4	x			x		x				x
5		x		x		x				x
6		x	x			x				x
7		x			x	x				x
8		x		x		x				x
9		x		x		x				x
10		x		x		x				x
Series 2 (CO ₂ +O ₂)										
1		x		x		x				x
2		x		x		x				x
3		x		x			x			x
4		x		x		x				x
5		x		x		x				x

Control trial numbers are in bold type.

the strain cycle; (iv) P_n , the power output for the strain cycle analyzed ($=W_n/D_n$); (v) $[CO_2]_n$, the value of the first sample read from the CO_2 analyzer and, for trials in the second series of experiments, (vi) $[O_2]_n$, the value of the first sample read from the oxygen analyzer. The individual values of P_n from each sample during which there was muscle stimulation and imposed strain were averaged to obtain a mean power output for the period of activity. The total work done during a trial, W_{total} , was calculated as the product of the mean power output and the duration of the burst of activation.

Analysis of CO_2 production and O_2 consumption

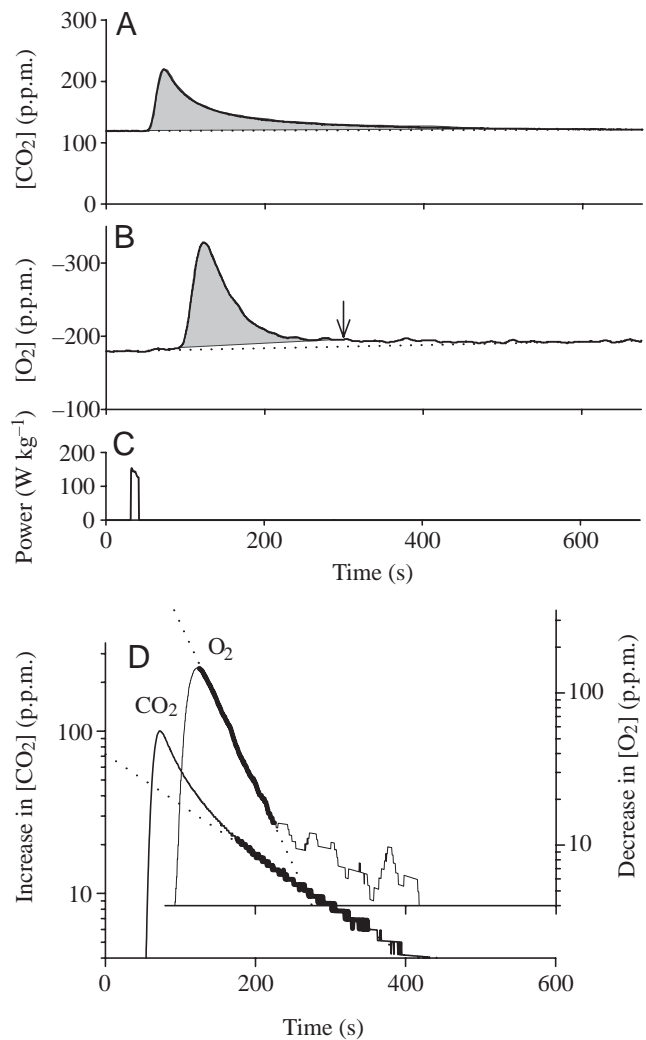
The basic response

A 10 s bout of muscle stimulation with imposed cyclic strain resulted in pronounced increases in the rates of CO_2

Fig. 2. CO_2 production and O_2 consumption associated with mechanical work. (A) Increase in the CO_2 concentration of the air exiting the experimental chamber following 10 s of muscle stimulation and work output. The power output during the stimulation is shown in C. (B) The corresponding decrease in O_2 concentration of the air leaving the experimental chamber. The output signal from the O_2 analyzer was occasionally rebalanced between trials to compensate for drift. The background level for the O_2 trace is therefore arbitrary. The delay between the increase in CO_2 concentration and the decrease in O_2 concentration is a consequence of the O_2 analyzer being downstream from the CO_2 analyzer. The dotted lines in A and B connect the means of the first 50 samples collected in each trace with the means of the last 50 samples. These lines are taken as baselines for estimating the increase in CO_2 production or O_2 consumption during the period represented by the trace. The extra CO_2 produced as a result of stimulation was measured as the area between the CO_2 trace and the calculated baseline (shaded portion of A). (D) The increase in CO_2 emission above the calculated baseline and the corresponding decrease in O_2 concentration on semi-logarithmic plots. The graph of O_2 concentration has been displaced vertically from that for CO_2 to avoid overlap of the O_2 and CO_2 traces. The traces are thickened where they are approximately linear in the semi-logarithmic presentation and, therefore, where the relaxation of the evoked gas production or consumption is approximately exponential. The time constants for the exponential portions of the traces were determined from the slopes of least-squares regression lines (dotted lines) fitted to the thickened portions of the traces. The time constant for the CO_2 trace (τ_{CO_2}) was 140 ms, that for the O_2 (τ_{O_2}) 41 ms. To make the measurements of CO_2 produced and O_2 consumed as comparable as possible, the oxygen consumption was measured to the same end point relative to its signal decay rate as was the CO_2 production. Here, the end of the recording, which is also the end of the interval used in determining CO_2 production, came 603 s or $4.3\tau_{CO_2}$ after the peak in the CO_2 trace. The portion of the O_2 trace used to determine O_2 consumption was therefore terminated at $4.3\tau_{O_2}$ s (marked by an arrow in B) beyond the peak of the O_2 trace. In the example shown, the work output was 53.1 mJ, the extra CO_2 production was 13.4 μ l and the extra O_2 consumption, corrected for the change in volume flow through the O_2 analyzer caused by CO_2 absorption, was 15.8 μ l. Thus, the respiratory coefficient (RQ) was 0.85 and the efficiency, assuming an energy to oxygen ratio of 20.1 J ml $^{-1}$, was 16.7 %.

release and of O_2 uptake above those from a quiescent preparation (Fig. 2). The signal from the CO_2 analyzer began to rise 10–20 s after the onset of stimulation, the delay presumably resulting largely from transit time between the respiratory chamber and the analyzer. The delay to the onset of the response from the O_2 analyzer, which was further downstream, was 85–95 s. Both the CO_2 and O_2 signals rose rapidly to a peak and then decayed more slowly over the next several minutes. The decline in the efflux rate of extra CO_2 above the resting level, or of diminished O_2 below the resting level, when plotted as $\log(\text{efflux rate})$ versus time, was approximately linear for much of the decay portion of the response (Fig. 2D).

A relationship that is linear on a semilogarithmic plot can be represented as an exponential function. The time constants for the exponentially declining portions of the CO_2 and O_2 signals were determined from the slopes of least-squares regression lines fitted to the linear segments of semilogarithmic plots. The durations of the time segments considered ranged from 100 to 250 s. The average time constant for the CO_2 decay rate (τ_{CO_2}) in the first trials with



10 s of stimulation was 144 ± 62 s (mean \pm s.d., $N=25$). The measured time constants were substantially longer than that expected for the wash-out time from a well-stirred respiration chamber ($=\text{chamber volume}/\text{flow rate}=28$ s), suggesting that CO_2 production in the beetle and CO_2 diffusion out of the beetle into the chamber are major determinants of the time course of the CO_2 response. The equivalent time constant for the oxygen signals (τ_{O_2}) was 56 ± 18 s (mean \pm s.d., $N=14$). The longer time constant for the CO_2 signal as opposed to that for O_2 is probably a consequence of the greater aqueous solubility of CO_2 than of O_2 and therefore a longer time required for equilibration of CO_2 between outside air and insect hemolymph.

Throughout these experiments, the O_2 traces were noisier and with more long-term drift than were the CO_2 recordings. This is a consequence of the different conditions under which the two gases were measured. The CO_2 released was detected against a background level, that of the air flowing into the chamber, of zero, and small changes in the amounts of CO_2 produced gave fractionally large changes in the CO_2 signal. The oxygen signal, in contrast, was a relatively small change superimposed on a large background. In Fig. 2, for example, the maximum decrease after stimulation in the O_2 concentration of the effluent air from the respiratory chamber was approximately 150 parts per million (p.p.m.) in an airstream with approximately 210 000 p.p.m., or a fractional change of less than 0.1 %.

Calculating CO_2 production

The amount of extra CO_2 produced as a result of muscle stimulation is proportional to the area between the curve defining the effluent CO_2 concentration as a function of time following stimulation and the concentration of CO_2 emitted from the resting preparation (shaded portion of Fig. 2A). If, as in these experiments, the flow rate through the chamber is constant, the extra CO_2 produced is equal to the flow rate multiplied by the area between the stimulated

and the resting curves for CO_2 concentration as functions of time.

Evaluating the increased CO_2 production evoked by stimulation is complicated by drift in the resting level of CO_2 production. In most preparations, the resting level of CO_2 , as measured in the 30 s period immediately preceding stimulation in each trial, declined progressively through the set of trials of an experiment (Fig. 3). In a few cases, the resting CO_2 production rose initially over a few trials and then declined. We assume that the changing levels of resting CO_2 emission reflect recovery from tissue damage that occurred while preparing the muscle and possibly also cell and tissue death during the course of an experiment. The slow decline in the rate of CO_2 emission through the course of an experiment indicates that the rate of CO_2 release is approximately the same as the rate of CO_2 production during rest and activity and that there is no gradual accumulation of CO_2 dissolved in hemolymph and the applied saline. An increase in the CO_2 content of the preparation, and hence the CO_2 gradient between the inside and the outside of the animal, would lead to an increase in the rate of CO_2 release, rather than a decrease as was seen.

To extract the extra CO_2 production associated with activity from the slowly changing resting level, we assumed that the latter changed linearly over the duration of a single trial. An estimate of the CO_2 concentration reaching the analyzer early in the trial was obtained by averaging the first 50 of the saved values for CO_2 concentration and assigning the average value to the time after the trial onset at which the twenty-fifth sample was collected. Similarly, a value for the CO_2 concentration at the end of the trial was obtained by averaging the last 50 samples and assigning the average to the time at which sample 25 from the end was collected. Predicted values for the resting CO_2 concentrations that would have been expected at any time throughout the trial had there been no stimulation were obtained by linear interpolation between the early and late values for CO_2 concentration and the times at which they

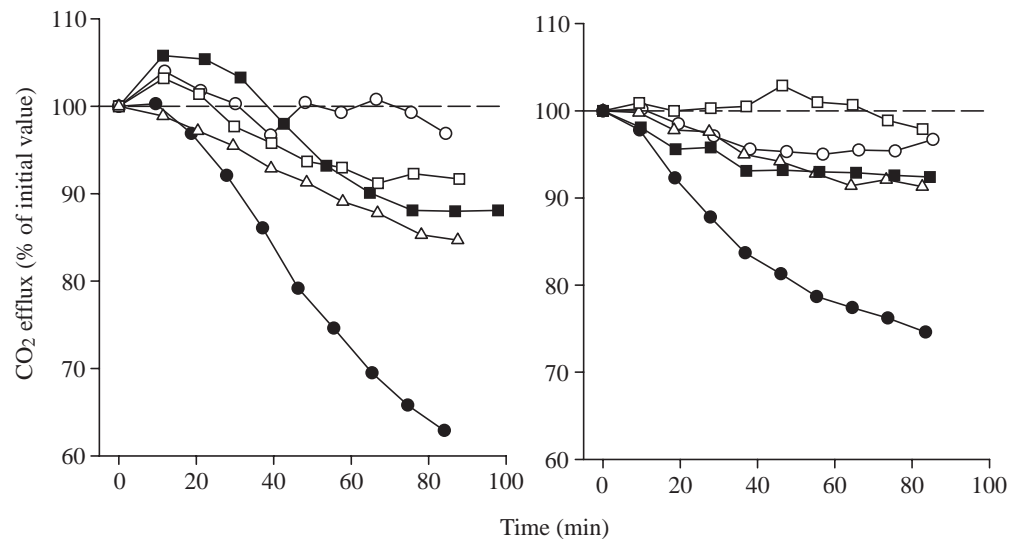


Fig. 3. Change in CO_2 efflux over the course of an experiment. Each of the set of points joined by a line is from a single preparation. The results from the 10 preparations of series 1 have been separated and displayed on two graphs to increase clarity. Each of the data points indicates the rate of CO_2 emission during the 30 s preceding stimulation in a trial relative to the value on the first trial.

occurred. The following equation was used to determine the total amount of CO₂ attributable to muscle stimulation and work:

$$V_{\text{CO}_2} = \sum_{i=1}^n [([\text{CO}_2]_{\text{meas},i} - [\text{CO}_2]_{\text{pred},i})(t_{i+1} - t_i)FR],$$

where V_{CO_2} is the total extra CO₂ volume, $[\text{CO}_2]_{\text{meas},i}$ is the measured CO₂ concentration in sample i , $[\text{CO}_2]_{\text{pred},i}$ is the predicted resting CO₂ concentration at the time of sample i , t_i is the time at onset of sample i , t_{i+1} is the time at onset of sample $i+1$, $(t_{i+1} - t_i)$ is the duration of the interval in which sample n was collected, n is the total number of samples in the trial and FR is the flow rate through the CO₂ analyzer. Reported volumes for CO₂ and O₂ are corrected to STP. We will use the term 'efficiency ratio' to indicate the ratio of mechanical work done to CO₂ produced in those experiments in which O₂ consumption was not measured.

As indicated above, much of the falling phase and especially the latter part of the falling phase of the extra rate of CO₂ production initiated by muscle stimulation is approximately exponential. Theoretically, it would take an infinitely long time for the rate of extra CO₂ release to reach zero; practically, the CO₂ signal becomes indistinguishable from noise after a few hundred seconds. The trials in our CO₂ measurements were generally 10–12 min long. The average length of the decay phase of the CO₂ signal, from peak to the end of the record, was $4.4 \pm 1.6\tau_{\text{CO}_2}$ (mean \pm S.D., $N=25$), where τ_{CO_2} is the time constant of the signal decay measured from the same preparation. The area beneath an exponentially declining curve, integrated from the onset of the decline to a time equal to 4.4 time constants, is over 98% of the value that would be obtained were the integration to be carried from peak time to infinity. The error in our CO₂ measurements attributable to terminating the trial after several hundred seconds rather than continuing it indefinitely is quite small.

Calculating O₂ consumption

To make the measurements of O₂ consumed and CO₂ produced as comparable as possible, O₂ consumption was measured to the same end point relative to its signal decay rate as was CO₂ production. Thus, if the end of the signal trace, and therefore the end of the time interval over which CO₂ production was measured, came n times the CO₂ decay time constant after the CO₂ peak, the interval over which O₂ consumption was measured was terminated at the time after the O₂ peak equal to n times the O₂ decay time constant (see Fig. 2 and legend for an example) (Fig. 2). The baseline from which the increased O₂ consumption was measured was a straight line drawn between $x_{\text{pre}}, y_{\text{pre}}$ and $x_{\text{post}}, y_{\text{post}}$, where x_{pre} is the mean of the 50 O₂ concentration values in the interval just preceding the rise in O₂ consumption, y_{pre} is the time at the mid-point of these samples, x_{post} is the mean of the 50 O₂ concentration values bracketing the selected endpoint of the analysis interval and y_{post} is the time of the selected endpoint. An initial estimate of the extra O₂ consumed above this

baseline was calculated in the same way as was the extra CO₂ production described above. In the CO₂ recordings, the measurement error introduced by the change in total flow rate due to the CO₂ produced and the O₂ consumed is tiny and can be ignored. In the O₂ measurements, however, the air flowing through the system has a large background O₂ concentration, and the small changes in the flow rate due to CO₂ addition and O₂ removal result in changes in the concentration of O₂ passing through the detector that are significant relative to those due to O₂ removed by metabolism. The initial estimate of O₂ consumption obtained from the area beneath the curve defining extra O₂ consumption was corrected for changes in volume flow using the volume equivalents of the flow rate equations given by Withers (equation 3b for the case in which CO₂ is not absorbed before the O₂ analyzer, equation 4a for the case in which it is) (Withers, 1997).

In several of the sections below, results from an experimental trial are compared with those in control trials preceding and following the experimental trial. When the dependent variable being considered was a ratio (RQ, efficiency, efficiency ratio), the value of the ratio for the control trials was calculated as the mean value in the pre- and post-trials for the parameter of the numerator divided by the mean value in the pre- and post-trials for the parameter of the denominator. This approach was adopted, rather than using the average value of the pre- and post-trial ratios themselves, to avoid biases that can arise when ratios are used in statistical calculations (Sokal and Rolf, 1981).

Results

Work output, CO₂ production and O₂ consumption (series 1 + series 2)

We believe that it is the first experimental trial that is likely to be most indicative of the work output and efficiency in a flying beetle, for it is on the first trial that the muscle is freshest and the power output generally the greatest. Table 2 summarizes work output and CO₂ production on the first trial for the preparations of both series 1 and series 2. The maximum power reached during the 10 s stimulation period (144 W kg^{-1}) is similar to the power output reported earlier for beetle muscle (127 W kg^{-1}) (Josephson et al., 2000a). Assuming that the respiratory coefficient (RQ) for these preparations is 0.83 and

Table 2. Maximum power, total work and extra CO₂ output in the first trial from each preparation

	Maximum power (W kg ⁻¹)	Total work (J kg ⁻¹)	CO ₂ produced (ml kg ⁻¹)	Efficiency ratio (J ml ⁻¹ CO ₂)
Mean	144	1087	295	3.80
S.D.	32	288	74	1.07

Strain frequency, 60 Hz; work duration, 10 s ($N=25$).

Mass-specific values are referenced to the mass of the basalar muscle.

Table 3. Work, metabolic cost and efficiency in the first trial for each preparation from which both CO₂ and O₂ were measured

	Total work (J kg ⁻¹)	CO ₂ produced (ml kg ⁻¹)	O ₂ consumed (ml kg ⁻¹)	RQ	Efficiency (%)
Mean	1045	307	378	0.83	14.0
S.D.	273	67	95	0.16	2.6

Mass-specific values are referenced to the mass of the basalar muscle.

Efficiency is calculated assuming that the work equivalent of the O₂ consumed is 20.1 J ml⁻¹ (Schmidt-Nielsen, 1997).

RQ, respiratory quotient.

N=14.

that the work equivalent of O₂ consumed is 20.1 J ml⁻¹ (see below), the ratio of work done to CO₂ produced is equivalent to an efficiency of 15.7%. The relationships between work, CO₂ production and O₂ consumption for the subset of the preparations of Table 2 from which both CO₂ and O₂ were measured, i.e. the preparations of series 2, are summarized in Table 3. The principal conclusion to be drawn from the results in Table 3, and by extension to those in Table 2 as well, is that this asynchronous muscle is approximately 14–16% efficient in converting chemical energy to mechanical work.

Cost of muscle activation (series 1)

Part of the cost of operating a working muscle is the energy input associated with turning the muscle on and off with each contractile cycle if it is a synchronous muscle or turning it on and maintaining it active if it is an asynchronous muscle. The major parts of these activation costs are related to Ca²⁺ release from the sarcoplasmic reticulum (SR) to initiate activity and Ca²⁺ uptake by the SR to terminate it.

We evaluated the cost of muscle activation, relative to that of doing work, by comparing the CO₂ produced by a muscle when it was stimulated and held at constant length so that no external work was done (trial 2, Table 1) with that done when the muscle was stimulated in an identical pattern but did external work (trials 1 and 3). In the 10 preparations of this series, the extra CO₂ produced above the resting level in the isometric trial averaged 50.0±15.2% (mean ± s.d.) of the mean for the two adjacent trials with work. Some internal work may have been done in the isometric trials, so the CO₂ production during these trials may slightly overestimate the cost of activation alone. Apparently, approximately half of the increased energy expenditure when a muscle is stimulated and does maximal work is attributable to activation costs, and half to the cost of actually doing work.

Efficiency at reduced stimulation frequency (series 1)

The stimulation frequency used in the control trials, 60 Hz, is somewhat less than that required for maximum muscle power output (Josephson et al., 2000a). However, 60 Hz is

substantially greater than the muscle activation frequency actually recorded from animals during tethered flight, which was approximately 20 Hz. Corresponding to the low activation frequency in tethered animals, the lift generated by the wings was also low, only approximately 20% of that required to keep a freely flying beetle airborne. The flight system of beetles is apparently structured to use low activation frequencies when, as in tethered flight, high power output is not necessary. We wondered whether muscle efficiency was different at low power output from that at high power output. To examine this, we compared muscle power output and efficiency when the muscle was stimulated at 30 Hz (trial 4, Table 1) with that in the usual control trials with 60 Hz stimulation (trials 3 and 5). Control values for work output and CO₂ production were, for each preparation, the average of those in the two control trials bracketing the trial with 30 Hz stimulation. In one preparation of the 10 in the series, the CO₂ signal became somewhat irregular during the second of the control trials (trial 5) and did not approach an obvious asymptote during recovery. This preparation has been omitted from the analysis, and the results are based on the other nine.

At the time of these measurements, the preparations had been in the respiration chamber for 30 min or more, and the mean power in control trials, those trials with 60 Hz stimulation, had dropped to 101±32 W kg⁻¹ (mean ± s.d., N=9). The average power output in the trials with 30 Hz stimulation was 64±11 W kg⁻¹ (mean ± s.d.). The average ratio of power with 30 Hz stimulation to that in the control trials was 64±11% (mean ± s.d., N=9). Although reducing the stimulation frequency from 60 to 30 Hz reduced power output by approximately one-third, the efficiency ratio remained essentially unchanged. The values of efficiency ratio for trials with 30 Hz stimulation averaged 105±12% of those in the control trials with 60 Hz stimulation (mean ± s.d., N=9), which is not statistically different from the value in the control trials (P>0.2, two-tailed *t*-test with paired samples).

Efficiency and power at reduced and increased stimulus intensity (series 1)

As indicated above, we were concerned that stimulating the basalar muscle through implanted electrodes might also stimulate some motor units in adjacent muscles, units that when activated would contribute to the increase in CO₂ released and O₂ consumed above the resting levels without increasing measured work output, thus reducing the apparent efficiency. It was to lessen the probability of activating adjacent muscles that we used stimuli, termed 'adequate' stimuli, whose intensity was close to, and sometimes slightly less than, that required to initiate a maximal isometric tetanus from the basalar muscle. To evaluate the possibility that there was activation of motor units in addition to those of the basalar muscle, we measured power output and relative efficiency when the basalar muscle was activated with stimuli whose intensity was well below the 'adequate' intensity and when the stimulus intensity was twice the 'adequate' level (trials 6 and 7, respectively, Table 1). We reasoned that if, indeed, muscles

other than the basalar were contributing to the extra CO₂ and O₂ signals, decreasing the stimulus intensity should result in a proportionally greater reduction in the activation of the extraneous motor units than of basalar units since the extraneous units are more distant from the stimulating electrodes than are the basalar units. The efficiency, then, should increase with decreasing stimulus intensity to a maximum reached when the stimulus intensity was sufficiently small that only basalar units were excited. Similarly, increasing stimulus intensity should disproportionately increase the activation of extraneous motor units, since all or nearly all the motor units of the basalar muscle are already activated with 'adequate' stimuli, and efficiency should decrease.

In these experiments, the power and efficiency ratio at reduced and at increased stimulus intensity were compared with those in two control trials, one coming immediately before and the other immediately after the sequential trials with altered stimulus intensity (Fig. 4). In one of the preparations of this series, the first control trial was the one mentioned above in which the CO₂ signal was irregular and did not approach an obvious asymptote; this preparation is not included in the analysis.

Three conclusions emerge from the trials of this series. (i) Muscle efficiency in the control trials did not vary in a significant way with the power output of the muscle being examined (Fig. 4A) (correlation coefficient 0.44, $P > 0.2$). The efficiency ratio was approximately 4 J ml⁻¹ in muscles that were relatively weak, probably from degeneration and fatigue, with power outputs close to 50 W kg⁻¹, as well as in muscles with power outputs over 100 W kg⁻¹. (ii) Reducing stimulus intensity did result in a decrease in power output but, rather than there being an associated increase in efficiency as would be expected were motor units external to the basalar being excited by the 'adequate' stimuli, there was a decrease in efficiency with the decrease in stimulus intensity and resulting power output (Fig. 4B) (correlation coefficient 0.78, $P < 0.02$). Why efficiency should decline with decreasing stimulus intensity is not obvious. Perhaps, when only a few motor units of the basalar are activated, the surrounding inactive units act as a mechanical load, reducing the external work done by the active units. (iii) Doubling the stimulus intensity from the 'adequate' level had little effect on power output except in one preparation in which power output nearly doubled; apparently, in this preparation, we substantially underestimated the 'adequate' stimulus (Fig. 4C). The overall efficiency ratio at the higher stimulus intensity (mean 3.9 ± 1.4 J ml⁻¹, mean \pm S.D., $N=9$) was somewhat less than that in the control trials (mean 4.1 J ml⁻¹), but the difference between the efficiency ratio with the high stimulus intensity and that in control trials was not statistically significant ($P > 0.18$, two-tailed t -test for paired samples).

To summarize, decreasing the stimulus intensity from the 'adequate' level did not increase efficiency, nor did increasing the stimulus intensity decrease efficiency, indicating that there had not been significant activation of motor units other than those of the basalar itself during trials with 'adequate' stimuli.

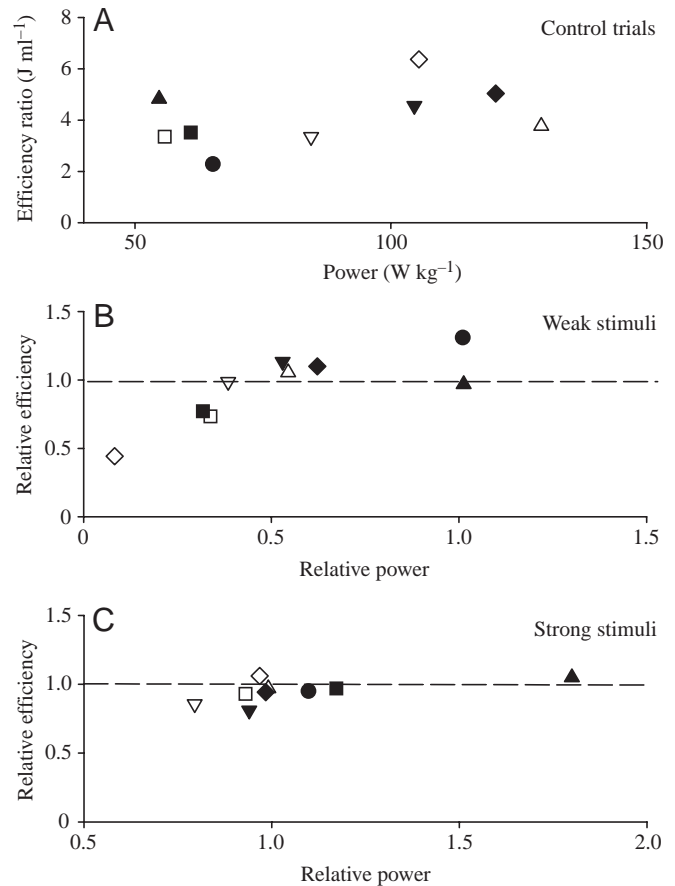


Fig. 4. (A) Efficiency ratio (=work done/extra CO₂ produced by the muscle) during the control trials for the experiments investigating the effects of varying stimulus intensity. Each of the symbols is the mean of the value in the control trial preceding and in that following the trials with altered stimulus intensity. (B) The efficiency ratio and the average power output, each relative to that in the preceding and following control trials, when the muscle was stimulated with shocks of reduced amplitude. (C) Relative efficiency and power when the muscle was stimulated with shocks twice as large as those in the control trials. Results from the same preparation are identified by a common symbol in A–C.

Efficiency with 90 Hz strain (series 1)

In an earlier study of beetle flight muscle (Josephson et al., 2000a), the muscle power output at 35 °C was greatest at a strain frequency of 60 Hz. However, the normal wing stroke frequency during flight is approximately 90 Hz. It seemed appropriate, therefore, to determine whether muscle efficiency at the normal flight frequency (90 Hz) was different from that at the usual test frequency (60 Hz). The approach to ascertaining this was as before; power output and efficiency under the test conditions were compared with those in the preceding and following control trials. The optimal strain for power output by the beetle basalar muscle declines with increasing cycle frequency and is expected to be approximately 10% less at 90 Hz than at 60 Hz (see fig. 10 in Josephson et al., 2000a). In three of the preparations of this experiment, the muscle strain used with both 60 and 90 Hz cycle frequencies

was that found to be optimal for work output at 60 Hz. In the remaining seven preparations, the strain used at 90 Hz was 10% less than that found to be optimal and used at 60 Hz. The results from the group with and from that without reduced strain at 90 Hz were similar and have been combined for simplicity of presentation.

Power output (P) was significantly less with 90 Hz than with 60 Hz cycle frequency ($P_{90\text{Hz}}/P_{60\text{Hz}}=0.79\pm 0.13$, mean \pm S.D., $N=10$, $P<0.002$, two-tailed t -test for paired samples). Although the power output decreased with an increase in cycle frequency from 60 to 90 Hz, the CO_2 production was essentially the same at the two frequencies (ratio for CO_2 consumption, $90\text{ Hz}/60\text{ Hz}=1.04\pm 0.18$, mean \pm S.D., $P>0.5$); consequently, the efficiency ratio was significantly smaller for 90 Hz than for 60 Hz oscillation [(efficiency ratio) $_{90\text{Hz}}/(\text{efficiency ratio})_{60\text{Hz}}=0.76\pm 0.06$, mean \pm S.D., $P<0.001$]. The wing stroke frequency used by the beetle in flight, 90 Hz, seems not to be the optimal frequency for power output nor for efficiency.

Efficiency during 30 s bursts of activity (series 2)

In some insects, there is a change in the fuel used during the course of a burst of flight. For example, in the African fruit beetle *Pachnoda sinuata*, the first few minutes of flight are powered by the oxidation of proline and later flight largely by the oxidation of glycogen (Zebe and Gäde, 1993). In *Locusta migratoria*, there is a shift from the metabolism of carbohydrate as the principal energy source to that of lipid during the course of long flights (Worm and Beenackers, 1980). To gain some insight into whether there might be a change in the fuel used during the rather short activity periods used in our measurements of muscle efficiency, we compared the respiratory coefficient and efficiency in trials with 30 s of stimulation and strain with those in preceding and following control trials with 10 s of stimulation and work output. Neither the RQ nor the efficiency was significantly different between trials with 10 s of stimulation and work output and those with 30 s of stimulation and work (Table 4). Our results provide no evidence for a change in fuel when the duration of a bout of activity is increased from 10 to 30 s.

Discussion

Efficiency of beetle flight muscle

Muscle efficiency is defined as the ratio of work done to energy expended in doing that work. The energy input associated with work output has been measured, in different studies, by heat production, fuel depletion or, for aerobic muscles such as the beetle flight muscle, by CO_2 produced or O_2 consumed. Evaluating the fraction of the total energy expended that may appropriately be assigned to the measured work output can be problematic. Living biological tissues have a resting metabolic rate reflecting the costs of maintaining cellular integrity. It is common in studies of muscle to subtract the resting metabolic rate from the elevated rate during activity when calculating the cost of muscle contraction. Stainsby et al. (1980), in a thoughtful review of problems associated with

Table 4. Respiratory coefficient and efficiency in trials with 10 s and with 30 s of stimulation and work

Duration of stimulation and strain (s)	RQ	Efficiency (%)
10	0.84 \pm 0.03	13.4 \pm 1.1
30	0.86 \pm 0.02	14.0 \pm 0.7

Values shown are mean \pm S.E.M. ($N=14$).

Neither the respiratory quotient (RQ) nor the efficiency is significantly different between the 10 s and 30 s trials ($P>0.6$ for RQ, $P>0.4$ for efficiency, two-tailed t -test for paired samples).

measuring muscle efficiency, distinguished and evaluated several different ways in which efficiency has been calculated (see also Syme, 1994).

Gross efficiency (=external work done/total energy used). Gross efficiency includes the resting metabolic rate as part of the cost of muscle activity.

Net efficiency (=external work done/energy expended during activity minus resting energy expenditure for an equivalent time interval). In this measure, the cost of contraction is based on the increase in metabolic rate above the resting level.

Work or apparent efficiency (=external work done/energy expended minus energy expended under similar conditions but with no work output). Here, the efficiency is based on the increase in metabolic output above that in muscles that are stimulated but do no work, e.g. muscles allowed to contract isometrically or to shorten against zero load.

Delta efficiency (=increment in work done/increment in energy expended). This measure is essentially the slope of the line relating work output and energy input.

The approach that we used with the beetle basalar muscle did not allow the resting metabolic rate of the muscle alone to be determined. The resting CO_2 production and O_2 consumption measured were those of the whole preparation, of which the basalar muscle made up a small fraction. In evaluating muscle efficiency, we took, as a measure of the energy expended, the increase in CO_2 production or O_2 consumption during muscle activity above the resting level for the whole preparation. Our value for efficiency of 14–16% is the net efficiency of the muscle. The metabolic rate of the basalar muscle during activity is enormous relative to the resting rate (see below) and, for calculations of efficiency, the resting metabolic rate of the muscle is negligible. In the beetle muscle, the net efficiency and the gross efficiency are essentially identical.

The power output from the basalar muscle tended to decline over the course of a 10 or a 30 s burst of activity (Fig. 2). It is possible that some or all of the drop in power was a result of inadequate ventilation of the tracheal system and an insufficient oxygen supply to the basalar muscle. This raises the possibility that the efficiency measurements themselves may have been influenced by a limitation in the oxygen supply. If there were developing oxygen starvation during bursts of activity, and if this did affect efficiency, one would expect the

efficiency to be different for long bursts of activity, during which oxygen starvation might become increasingly pronounced, than for short bursts of activity which should depend more on existing oxygen stores at the onset of stimulation. The decline in power output during 30 s bursts of activity was greater than that in the bracketing 10 s control trials, with the result that the mean power over the full 30 s was less than the mean power averaged over the preceding and following control trials (mean power for 30 s bursts is $88.5 \pm 6.6\%$ of the average for the control trials, mean \pm S.E.M., $N=14$, $P<0.001$, two-tailed t -test). Although the power dropped more in 30 s trials than in 10 s trials, possibly indicating greater oxygen insufficiency in the longer trials, the efficiency in the 10 and 30 s trials was similar (Table 4). If there were developing oxygen insufficiency in the longer trials, it apparently did not affect the muscle efficiency.

Metabolic scope of flight muscle

A single basalar muscle makes up approximately 3.2% of the mass of an intact beetle (Josephson et al., 2000a). The preparation placed into the chamber was a beetle without head, elytra, wings, legs and a few other small parts. We weighed three animals before and after they had been prepared for recordings and found the average mass of the preparation to be 83.1% of that of the original animal (range 81.7–84.2%). A single basalar muscle, then, represents approximately $3.2/0.831=3.9\%$ of the mass of the preparation. Some of the preparation is exoskeleton, which is likely to have a low metabolic rate, and some is nerve tissue and Malpighian tubules, which are likely to have relatively high metabolic rates. In the absence of good information on how best to partition the resting metabolism among the various tissues, we will assume that the metabolic rate is the same for all tissues and that a single basalar muscle contributes 3.9% of the total. The resting rate of CO_2 emission in the first experimental trial for the 25 animals used in this study averaged $14.6 \mu\text{l min}^{-1}$, of which, assuming equal rates for all tissues, $0.57 \mu\text{l min}^{-1}$ is the basalar's share. The extra CO_2 above the resting level produced over the 8–10 min following stimulation in these trials averaged $11.1 \mu\text{l}$. The question is, to what time interval do we ascribe this CO_2 emission in order to calculate a CO_2 emission rate? Imagine that the muscle was stimulated continuously and that it produced work continuously at the same efficiency and using fuel with the same RQ as during the 10 s trial. The rate of CO_2 emission would be expected to rise until it reached a steady state during which CO_2 emission just matched CO_2 production. In any 10 s period at steady state, an amount of CO_2 would be released that was equal to that produced in association with doing 10 s worth of work and, from the assumptions made, this would be the same as that released over several minutes during the trial with only 10 s of stimulation. Thus, we assert, the appropriate time interval to which to assign the CO_2 produced is that of the stimulation period, here 10 s, and that the CO_2 production rate by the stimulated muscle is approximately $67 \mu\text{l min}^{-1}$, or approximately 120 times the resting rate. This value for metabolic scope is somewhat less

than that for oxygen consumption between rest and flight in sphingid and saturnid moths (approximately 150-fold) (Bartholomew and Casey, 1978) but substantially greater than the relative increase in CO_2 production between rest and flight found in *Drosophila* (seven- to ninefold) (Dickinson and Lighton, 1995; Lehmann and Dickinson, 1997).

What is the fuel for flight?

The available evidence indicates that the flight muscles of insects are strictly aerobic; the mechanical power output of flight is supported solely by oxidative metabolism (Beenackers et al., 1984). There is variation among different insects in the fuels consumed during flight. In some, the principal fuel used by the flight muscles is carbohydrate, in some lipid and in some amino acids (for reviews, see Beenackers et al., 1984; Dudley, 2000). The RQ determined for the basalar muscle of *C. mutabilis* (0.83) does not identify the fuel used by this muscle, but it does eliminate some possibilities. The muscle cannot be relying solely on oxidation of carbohydrate (RQ=1) or solely on the burning of fat (RQ=0.71). It is likely that energy for work output by the flight muscle comes from the oxidative conversion of proline to alanine (RQ=0.8) or from the oxidation of both proline and carbohydrate, as has been shown to be the case in several other beetle species (Weeda et al., 1979; Zebe and Gäde, 1995; Auerswald and Gäde, 1995).

Muscle metabolism in vitro and in free flight

Chappell (1984) measured the oxygen consumption of a scarab beetle flying in a container. The beetle used by Chappell was described as being *Cotinus texana* but, judging by the description given of it and where it was collected, it is almost certainly the same species as the one we identified as *C. mutabilis*. The average oxygen consumption for the flying beetle was $61 \text{ ml h}^{-1} \text{ g}^{-1}$ animal. Assuming that all O_2 consumed was by the asynchronous flight muscles, and that the asynchronous flight muscles make up 19.3% of the total body mass (Josephson et al., 2000a), the metabolic rate during flight is equivalent to $5.3 \text{ ml O}_2 \text{ min}^{-1} \text{ g}^{-1}$ muscle. The extra O_2 consumption due to 10 s of activity in the semi-isolated beetle muscle (Table 3) was $2.27 \text{ ml O}_2 \text{ min}^{-1} \text{ g}^{-1}$ muscle, or approximately half that predicted from the metabolic rate of a flying beetle. Several factors probably contribute to the seemingly lower rate of O_2 consumption in the semi-isolated muscle compared with that in a flying beetle.

(i) The estimated value of O_2 consumption by wing muscles in a flying beetle is based on the assumption that all the measured O_2 consumption is attributable to asynchronous flight muscles. This calculation will be an overestimate to the extent that tissues other than asynchronous wing muscle contribute to the total O_2 consumed.

(ii) For technical reasons, Chappell's (1984) measurements of O_2 consumption during flight were from those animals, somewhat less than half of the total examined, that maintained flight for 45 s or more in the respiration chamber. The selection criterion used for the animals in our study was only that they could produce ascending flight. Chappell's beetles may well

have been more vigorous than ours, with greater metabolic rates.

(iii) The ambient air temperature was 25–31 °C in Chappell's measurements of O₂ consumption (Chappell, 1984). On the basis of the relationship between ambient temperature and thoracic temperature that Chappell measured for flying animals, the flight muscle temperature in his measurements probably ranged from 35 to 38 °C. Thus, Chappell's measurements were from muscles at a higher average temperature, and associated higher metabolic rate, than were ours, which were all examined at 35 °C.

Given that there are several sources of error in the calculation of flight muscle metabolism based on O₂ consumption of flying beetles, some of which lead to overestimation, we think that there is reasonable agreement between the metabolic rates of active muscle determined from whole animal performance and from individual muscles.

Efficiency of synchronous and asynchronous muscles

Information about the efficiency of insect flight muscles is available from several studies in which oxygen consumption was measured during hovering flight and mechanical power output was estimated from flight performance. The mechanical power output during flight is conveniently divided into aerodynamic power, the power required to accelerate air to provide lift and thrust and to overcome drag on the body and beating wings, and inertial power, the power required to accelerate the wings during the first half of the upstroke and downstroke (Casey, 1981). Dickinson and Lighton (1995) pointed out that, if the inertial power is less than the aerodynamic power, the inertial power can be recovered, converted to aerodynamic power, during the deceleratory phases of each wing cycle. In addition, inertial energy can be stored elastically during wing deceleration and used to accelerate the wing during the next half-cycle. Because of uncertainties about the extent to which inertial energy is recycled, two values are given in many studies for the efficiency of flight muscles, one calculated with the assumption that there is complete storage of excess inertial energy and the other with the assumption that there is no elastic storage. Casey (1981) measured flight performance and metabolic rates for a series of sphingid moths (synchronous flight muscles) of varying size. The mean muscle efficiency, calculated from data in table 3 of Casey (1981), is 5% (range 2–8%) if it is assumed that there is perfect elastic storage and 17% (range 8–24%) if it is assumed that there is no elastic storage of inertial energy. In a number of bees and flies (asynchronous flight muscles), the calculated mechanical efficiency ranged from 4 to 16% if there was perfect elastic storage and from 8 to 34% with no elastic storage (Casey and Ellington, 1989; Casey et al., 1992; Ellington, 1984). Given the likelihood that there is at least some elastic storage in insect flight systems (Ellington, 1985), the values calculated on the basis of perfect elastic storage may be the more realistic. In insects with synchronous and in those with asynchronous flight muscles, there is a direct relationship between animal size and

calculated muscle efficiency (Casey, 1976, 1981; Casey et al., 1992).

Some recent studies are of note because they were performed with insects in which the inertial power during flight was less than the aerodynamic power, so the total power output is equal to the aerodynamic power whether or not there is elastic storage. Wakeling and Ellington (1997) estimated the efficiency of flight muscle in a dragonfly and in a damselfly (synchronous flight muscles) on the basis of flight performance and metabolic energy input determined from the thoracic temperature excess above ambient maintained during flight. The calculated efficiency for the flight muscle of the dragonfly *Sympetrum sanguineum* was 12.6% and that for the damselfly *Calopteryx splendens* 8.7%. Dickinson and Lighton (1995) calculated muscle efficiency in the fruitfly *Drosophila hydei* and Lehman and Dickinson (1997) in *D. melanogaster* on the basis of flight performance and measured CO₂ production. The efficiency was approximately 11% in both species. In general, values of flight muscle efficiency in insects, based on metabolic power input and calculated mechanical power output during flight, are substantially lower than the 20–50% usually measured for vertebrate muscles (Woledge et al., 1985). The range of values for the efficiency of insect flight muscles based on flight performance is rather large, in part because of the varying size and associated differences in the wingbeat frequency of the animals examined, and published results allow no clear conclusions about the relative efficiencies of synchronous and asynchronous muscles.

An informative comparison of the performance of synchronous and asynchronous muscles is given by the results described above from an asynchronous muscle of a beetle and those published earlier (Josephson and Stevenson, 1991) from a synchronous flight muscle of the locust *Schistocerca americana* (Table 5). In both studies, mechanical power output was measured directly from a single muscle using the work loop approach, and metabolic power input was determined from the increased oxygen consumption above the resting level associated with the power output. The beetle and the locust were of similar mass. The locust did have a substantially lower wingbeat frequency, but this, if anything, should bias the efficiency in favor of the locust since there appears to be an inverse relationship between wingbeat frequency and efficiency in related insects (Casey, 1976; Casey et al., 1992). The power output of the locust muscle in Table 5 is generally similar to that found with the same technique and at the same temperature in several other synchronous flight muscles [68–75 W kg⁻¹ for metathoracic first tergocoxal muscles of the locusts *Schistocerca gregaria* and *S. nitens* (Mizisin and Josephson, 1987; Malamud et al., 1988); 76 W kg⁻¹ for a flight muscle from a tettigoniid (Josephson, 1985); 55 W kg⁻¹ for a flight muscle from the moth *Manduca sexta* (Stevenson and Josephson, 1990)]. The power output and the efficiency of the beetle muscle are each 2–3 times greater than those of the locust muscle. The sample size is small – one asynchronous muscle and several synchronous ones for power output, one asynchronous and one synchronous muscle for efficiency – but

Table 5. Power output and efficiency of asynchronous (beetle) and synchronous (locust) flight muscle

	Beetle	Locust
	<i>Cotinus mutabilis</i>	<i>Schistocerca americana</i>
Animal mass (g)	1.2	1.5 (male), 3.0 (female)
Muscle	Basalar	Metathoracic tergosternal
Test frequency (Hz)	60	20
Temperature (°C)	35	30
Maximum power output (W kg ⁻¹)	144	42*
Efficiency (%)	14–16	6.4

*Calculated from data in table 1 of Josephson and Stevenson (1991) and the expected power increase of 13% when there are two stimuli per cycle rather than one, as was the case for the data of their table 1.

The locust values are from Josephson and Stevenson (1991).

the results support the assertion that asynchronous muscles are, as has been predicted, more powerful and more efficient than their synchronous counterparts. It is presumably the greater power output and greater efficiency offered by asynchronous muscle that have led to the independent evolution of this mode of muscle control in the flight systems of several major insect groups.

One factor in the greater efficiency of beetle than of locust muscle is the much lower activation cost, relative to the work output, in the beetle muscle. An indication of activation cost is given by the energy consumed during an isometric contraction. The CO₂ produced by a stimulated beetle muscle during an isometric contraction is half as great as when the muscle is oscillated at optimal strain and does work. Not all the energy expenditure in an isometric contraction can be attributed solely to activation costs. There is some internal work done even during an isometric contraction. In addition, it is likely that the increased intracellular Ca²⁺ concentration resulting from stimulation leads to an increase in the rate of crossbridge cycling with associated energetic costs. The energy consumption during an isometric contraction is an upper limit for an estimate of activation costs. In the beetle flight muscle, the cost of activation is no more than half the total energy consumed by a muscle doing maximal work. In the locust flight muscle, the oxygen consumption during isometric contraction was 87% as great as during maximal work output. Apparently, in the locust muscle, most of the energy input supports the costs of cyclically turning the muscle on and off. As mentioned above, one measure of efficiency that has been used is the ratio of work done to the extra energy consumed above that during similar conditions of stimulation but during which work is not done ('work efficiency' or 'apparent efficiency'). By this measure, which excludes activation costs, the beetle muscle is 28% efficient, the locust muscle 49% efficient. But the real cost of locomotion includes both activation costs and the additional costs of doing work and, as a motor for flight, the beetle muscle is substantially more efficient than that of the locust.

This work was supported by NSF IBN9603187. We thank T. Bradley for generously allowing us to use his oxygen analyzer and J. Lighton for helpful suggestions on measuring gases.

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