
REVIEW

ASYNCHRONOUS MUSCLE: A PRIMER

ROBERT K. JOSEPHSON^{1,*}, JEAN G. MALAMUD¹ AND DARRELL R. STOKES²

¹*School of Biological Sciences, University of California at Irvine, Irvine, CA 92697, USA* and ²*Department of Biology, Emory University, Atlanta, GA 30322, USA*

*e-mail: rkjoseph@uci.edu

Accepted 16 June; published on WWW 22 August 2000

Summary

The asynchronous muscles of insects are characterized by asynchrony between muscle electrical and mechanical activity, a fibrillar organization with poorly developed sarcoplasmic reticulum, a slow time course of isometric contraction, low isometric force, high passive stiffness and delayed stretch activation and shortening deactivation. These properties are illustrated by comparing an asynchronous muscle, the basalar flight muscle of the beetle *Cotinus mutabilis*, with synchronous wing muscles from the locust, *Schistocerca americana*. Because of delayed stretch

activation and shortening deactivation, a tetanically stimulated beetle muscle can do work when subjected to repetitive lengthening and shortening. The synchronous locust muscle, subjected to similar stimulation and length change, absorbs rather than produces work.

Key words: asynchronous muscle, muscle, insect, *Cotinus mutabilis*, *Schistocerca americana*, work, power, ultrastructure, stretch activation, shortening deactivation.

Introduction

The success of insects as a major and often the dominant animal group in most terrestrial environments may be attributed in part to the evolution of asynchronous flight muscle in several insect taxa. Asynchronous muscle (also known as fibrillar muscle or myogenic muscle) is that type of muscle which, when turned on by neural input and resulting muscle action potentials, can contract in an oscillatory manner if attached to an appropriate, resonant load such as is offered in life by an insect's wings and thorax. The frequency of the oscillatory contractions is different from, and typically much higher than, that of the activating neuronal impulses. Asynchronous muscle is so-named because during oscillation there is no direct correspondence between individual muscle contractions and muscle action potentials, which contrasts with synchronous muscle in which there is a 1:1 correspondence between muscle contractions and muscle action potentials or bursts of action potentials. Asynchronous muscle is only known to occur in insects. The muscles that power flight in dipteran flies, beetles, many of the true bugs and in some, but perhaps not all, bees and wasps are asynchronous muscles, as are the singing muscles of some cicadas (Cullen, 1974; Pringle, 1976; Josephson and Young, 1981; Dudley, 2000). The distribution of asynchronous muscle in different insect taxa suggests that asynchronous operation has evolved independently as many as 7–10 times (Cullen, 1974; Pringle, 1981; Dudley, 2000). Approximately three-quarters of the known insect species have asynchronous flight muscles

(Dudley, 1991). It follows, therefore, that asynchronous muscle powers flight in most animal species that fly.

Asynchronous muscles are high-frequency muscles. The wing-stroke frequency during flight of insects using asynchronous muscle ranges from approximately 20 Hz in large belostomatid bugs (Barber and Pringle, 1966) to 500–1000 Hz in small midges (Sotavalta, 1947, 1953). But high-frequency muscles need not be asynchronous. A number of synchronous muscles are known whose operating frequency is within the frequency range of asynchronous muscles. Among high-frequency, synchronous muscles are the shaker muscle of the rattlesnake rattle (up to 90 Hz; Schaeffer et al., 1996; Rome and Lindstedt, 1998), the toadfish sound-producing muscle (over 200 Hz; Fine, 1978; Fine and Mosca, 1989; Rome et al., 1996), the muscles used in stridulation by the katydid *Neoconocephalus robustus* (200 Hz; Josephson and Halverson, 1971) and the tymbal muscle used in sound production by the cicada *Okanagana vanduzeei* (550 Hz; Josephson and Young, 1985).

It has been postulated that, for high-frequency operation, asynchronous muscles have a higher mass-specific power output than do synchronous ones (Josephson and Young, 1985; Lindstedt et al., 1998). The limitation on power output in high-frequency synchronous muscles is thought to be largely one of packing. The thick and thin myofilaments of striated muscles are gathered into cylindrical or ribbon-shaped bundles, the myofibrils. In synchronous muscles, the myofibrils are

enveloped by a layer of sarcoplasmic reticulum; in asynchronous muscles, the sarcoplasmic reticulum is sparse and scattered. Muscle activation in both synchronous and asynchronous muscle results from the release of Ca^{2+} from the sarcoplasmic reticulum in response to fiber depolarization; relaxation follows re-uptake of the released Ca^{2+} by the sarcoplasmic reticulum. High-frequency operation requires brief contractions. The short twitch duration in synchronous muscle is achieved in part by hypertrophy of the sarcoplasmic reticulum and by a reduction in the diameter of the contractile myofibrils (Josephson and Young, 1987).

Elaboration of the sarcoplasmic reticulum and reduction of myofibrillar diameter decrease the distance between the sarcoplasmic reticulum and the myofilaments within the myofibrils and, hence, the diffusion time for Ca^{2+} into and out of the contractile myofibrils. In addition, hypertrophy of the sarcoplasmic reticulum increases its surface area and therefore increases the space available for the membrane-bound pumps that take up Ca^{2+} and terminate contraction. However, increasing the volume of the sarcoplasmic reticulum necessarily reduces the space available for myofibrils in a given volume of muscle. In extreme cases, the volume of sarcoplasmic reticulum within a fiber exceeds that of myofibrils (Rosenbluth, 1969; Josephson and Young, 1985). Further, the high rate of Ca^{2+} cycling required for high-frequency, synchronous operation is metabolically costly. The high cost of cycling Ca^{2+} reduces the efficiency of the muscle as a source of mechanical power and also requires greater investment in mitochondria within the fibers to support the costs of Ca^{2+} pumping, further reducing the space available for myofibrils.

All the known synchronous muscles that operate at very high frequencies, 100 Hz or more, are involved in sound production or reception. High-frequency contraction often seems to be important in sound production, but high mechanical power output is probably less so (Rome et al., 1996; Rome and Lindstedt, 1998). The high-frequency muscles used in sound production apparently can tolerate better the loss of power output associated with hypertrophy of the sarcoplasmic reticulum than can fast, locomotory muscles.

During normal operation, asynchronous muscles are turned on and maintained in an active state by relatively low-frequency neural input. Oscillatory contraction results from the intrinsic properties of the activated muscle and mechanical features of the load. High-frequency operation is achieved without high rates of Ca^{2+} cycling and without large investment in sarcoplasmic reticulum. Because the cost of Ca^{2+} cycling is low, asynchronous muscle is likely to be more efficient than is synchronous muscle. Because asynchronous muscle contains little sarcoplasmic reticulum, more of the fiber volume can be allocated to myofibrils, with concomitantly increased mechanical power output. Presumably, it is these advantages, greater efficiency and greater power output, that have favored the evolution of asynchronous muscles in several insect groups.

The asynchronous mode of operation is an interesting and

biologically significant solution to the problem of creating high-frequency movements, in particular those required for flight. The following is a review of some of the properties of asynchronous muscle. The emphasis is on the mechanical properties of asynchronous muscle that support oscillatory contractions and not on the molecular mechanisms that underlie the mechanical performance; introductions to the latter are provided in recent reviews by Dickinson and Tu (1997) and Maughan and Vigoreaux (1999). We try to bring the special features of asynchronous muscle into sharper focus by comparing an asynchronous muscle, a flight muscle from a beetle, with a synchronous one, a flight muscle from a locust. This review is intended to complement and extend an earlier one that compared the properties of asynchronous and synchronous muscles used in sound production by cicadas (Josephson and Young, 1981).

The muscles to be used as examples are the basalar muscle of the scarab beetle *Cotinus mutabilis* and either the metathoracic tergosternal (electromyogram recordings, ultrastructure) or the metathoracic posterior tergocoxal (force recordings) muscle of the locust *Schistocerca americana*. The beetles were raised in our laboratory; the locusts were obtained as adults from a laboratory culture at the California Institute of Technology. Adult beetles weighed approximately 1.3 g, adult locusts approximately 1.4 g. The techniques used to obtain the data are reported elsewhere as follows: raising beetles and preparing beetle muscles for mechanical recordings, Josephson et al. (2000); recording electromyograms, Full et al. (1998); monitoring wing thrust during flight, Josephson and Young (1981) and Josephson et al. (2000); stimulating insect flight muscles and recording isometric force or the force changes during muscle stretch and release, Josephson (1984), Malamud et al. (1988), Malamud and Josephson (1991), Josephson and Ellington (1997) and Josephson (1997); fixing muscles for electron microscopy and quantifying muscle ultrastructure, Stokes et al. (1994).

Features of the beetle muscle

The muscle is 'asynchronous'

The first indication that the physiology of some insect flight muscles was different from that of ordinary skeletal muscles came in a study by Pringle (1949), in which it was shown that muscle action potentials recorded during flight from the wing muscles of a fly are not correlated temporally with each wing movement. These observations were extended by Roeder (1951), who found that action potentials and muscle contractions in flight muscles did occur in a 1:1 relationship during flight in cockroaches and moths but not in several dipteran flies or in a wasp. These two types of muscle – those in which each contraction had associated muscle action potentials and was essentially a twitch or brief tetanus and those in which there was no strict correlation between electrical and mechanical events – were termed synchronous and asynchronous, respectively, by Boettiger (1957).

Electrical recordings from the beetle basalar muscle during

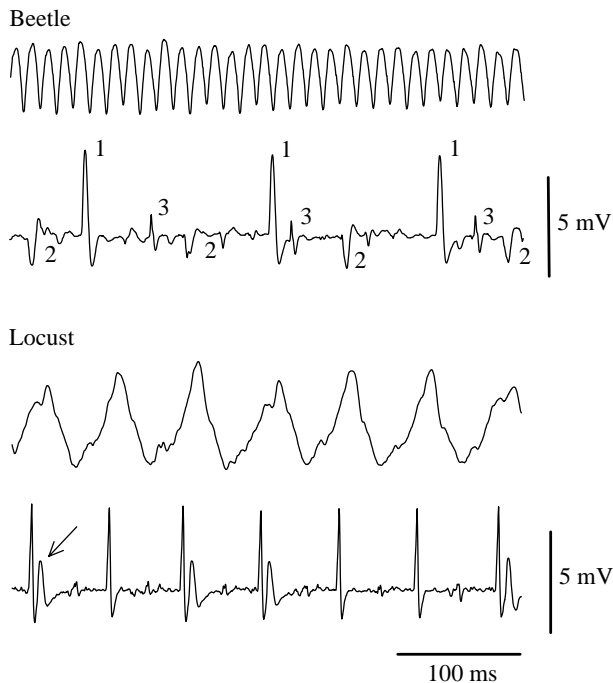


Fig. 1. Wing thrust (top trace in each pair) and muscle action potentials (electromyogram recordings, EMGs) during tethered flight. Animals were suspended from a force transducer attached to the dorsal prothorax, which recorded thrust associated with wing beats during flight. EMGs were recorded with 100 μ m silver wires implanted in the wing muscles. The EMG trace in A is from the basalar muscle of the beetle *Cotinus mutabilis*; that in B is from the tergosternal muscle of the locust *Schistocerca americana*. Action potentials from three motor units are evident in the beetle EMG trace. These units are labeled 1–3 in order of decreasing size. Each wing stroke of the locust muscle is associated with an action potential or a pair of action potentials (an example of a second action potential is marked with an arrow). The wing-stroke frequency of the beetle muscle was 76 Hz, that of the locust muscle 16 Hz.

tethered flight indicated clearly that it is an asynchronous muscle (Fig. 1A). The basalar contains a number of motor units that were distinguishable in electrical recordings by the differing amplitude and shape of their electrical signals, but neither singly nor collectively was there a 1:1 correspondence between motor unit action potentials and wing movements. In all preparations, there were far fewer muscle action potentials than wing strokes. In contrast, in the locust flight muscle, which is a synchronous muscle, there was a single muscle action potential or a pair of action potentials with each wing stroke (Fig. 1B).

The muscle is slow

The wing-stroke frequency during tethered flight was nearly five times greater in the beetle than in the locust (mean 78 Hz for beetles, 16 Hz for locusts). Nonetheless, isometric twitches recorded from the beetle flight muscles were much slower than those from locust muscles (Fig. 2). The mean twitch duration (time from onset to 50 % relaxation) found in an earlier study of the posterior tergocoxal muscle of *S. americana* was 40 ms

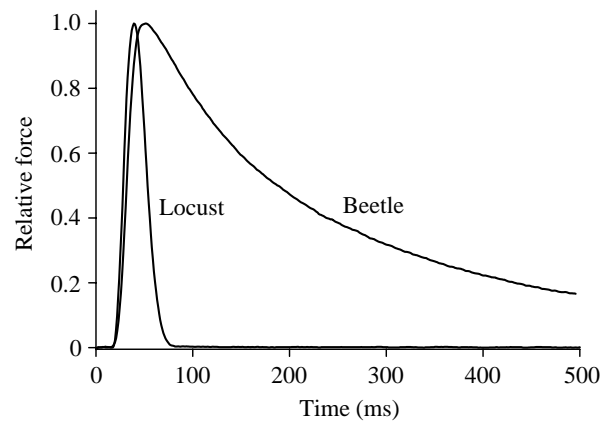


Fig. 2. Isometric twitches from beetle and locust flight muscle at 30 °C.

at 30 °C (Malamud et al., 1988). The mean isometric twitch duration for the basalar muscle of *C. mutabilis* at 30 °C was approximately 125 ms (Josephson et al., 2000). The rise time alone of the twitch from the beetle muscle (20 ms) is approximately twice the total wing-stroke duration during flight. Clearly, the time course of a stimulus-induced muscle contraction of the beetle muscle is far too long to support the rapid wing movements of normal flight. In fact, the muscle action potential frequency of individual motor units in the beetle muscle during tethered flight (mean 21 Hz) is sufficient to produce a nearly smoothly fused isometric tetanus in the beetle muscle.

The muscle is 'fibrillar'

As discussed above, the rapidity of twitches in skeletal muscles is inversely correlated with the diameter of the myofibrils and directly correlated with the development of the sarcoplasmic reticulum. The slow time course of twitches from the beetle muscle leads to the prediction that the fibers of these muscles should have large myofibrils and little sarcoplasmic reticulum. This is indeed the case (Fig. 3; Table 1). The myofibrils in the beetle muscle have approximately twice the diameter and four times the cross-sectional area of those in the locust muscle (Table 1). The muscle fibers also have a larger cross-sectional area in the beetle muscle than in that from the locust (Table 1). We do not know if having large fibers is a consistent feature of asynchronous muscles. The sarcoplasmic reticulum of the beetle muscle is sparse and scattered, while that of the locust muscle, as expected for a fast, synchronous muscle, is a major structural component of the muscle fibers (Fig. 3). The distribution of the sarcoplasmic reticulum in the beetle flight muscle is quite like that found in the asynchronous tymbal muscle of a cicada (Josephson and Young, 1981). Single thin sarcoplasmic reticulum tubules partially encircle the myofibrils at the center of the sarcomere, and at the Z-line, and are connected to scattered longitudinal sarcoplasmic reticulum tubules. Occasional dyadic connections between sarcoplasmic reticulum and transverse tubules (T-tubules) occur near the middle of the sarcomere. In the myofibrils of all

Table 1. *Structural features of beetle (Cotinus mutabilis) and locust (Schistocerca americana) flight muscle*

	Beetle	Locust
Myofiber cross-sectional area (μm^2)	8069 \pm 520 (20)	3206 \pm 219 (20)
Myofibril cross-sectional area (μm^2)	3.7 \pm 0.1 (36)	0.82 \pm 0.03 (36)
% Muscle fiber as		
Myofibril	58.1 \pm 1.2 (6)	65.0 \pm 2.7 (4)
Mitochondria	36.7 \pm 1.6 (6)	23.5 \pm 2.1 (4)
SR and T system	1.6 \pm 0.1 (6)	9.6 \pm 0.5 (4)
Tracheoles	1.2 \pm 0.2 (6)	0.5 \pm 0.2 (4)
Other	2.4 \pm 0.5 (6)	0

Values are means \pm S.E.M. (*N*).

SR, sarcoplasmic reticulum.

The sample sizes, *N*, for myofiber area and myofibril area are the total number of fibers analyzed from muscles of 2–6 individual animals. The sample sizes for fractional volume are the number of muscles examined, each from a different animal. Five fibers were analyzed for each muscle.

See Stokes et al. (1994) for details of the stereological methods used.

beetle muscles examined, and in one of the four examples of locust tergosternal muscles, each thick myofilament was surrounded by six thin filaments, which is the usual thick:thin filament arrangement found in fast insect muscles (Fig. 3C,E). However, in three of the locust muscles, each thick filament was surrounded by 9–12 thin filaments (Fig. 3D), an arrangement commonly found in insect slow muscles. Why there was diversity in the filament organization of different locust tergosternal muscles is unknown.

The myofibrils of asynchronous muscle are large enough to be visualized with a light microscope. Presumably because there is little sarcoplasmic reticulum binding the myofibrils together, the fibers of asynchronous muscle, even when unfixed, can be teased apart readily to produce isolated myofibrils. Because of their large and conspicuous fibrils, the flight muscles of some insects were recognized by early microscopists as representing a distinct muscle type, which came to be called fibrillar (for a review, see Pringle, 1977). The term 'fibrillar muscle' is now widely used as a synonym for 'asynchronous muscle'. 'Fibrillar' is a somewhat misleading way to identify asynchronous muscle, since the contractile material of essentially all striated muscle is organized as myofibrils; it is just that, with the exception of some insect wing and tymbal muscles, the myofibrils are too small to be detected without electron microscopy. But 'fibrillar' is a commonly used descriptor and, structurally, the beetle basalar muscle is a 'fibrillar' muscle.

The muscle shows delayed stretch activation and shortening deactivation

The features of asynchronous muscle that allow it to contract in an oscillatory manner when attached to a resonant load are delayed stretch activation and its shortening counterpart, delayed shortening deactivation. If a tetanically stimulated

beetle flight muscle is given a brief, rapid stretch, muscle force rises throughout the stretch, but then continues to rise for a few milliseconds after the end of stretch (Fig. 4A,B). The increase in force following the end of lengthening is a manifestation of delayed stretch activation. There is a corresponding delayed drop in force when a stretched muscle is allowed to shorten rapidly (see Fig. 6). The trajectory of the force change during and following rapid stretch of the beetle muscle is quite different from that in a synchronous skeletal muscle, as exemplified by locust flight muscle (Fig. 4C,D). In a tetanically stimulated locust muscle, force rises during stretch but then declines immediately at the end of stretch, at first rapidly and then more slowly, with no sign of delayed activation. If stretch of the locust muscle is rapid, the force rises to a yield point (marked by an arrow in Fig. 4D), beyond which the force continues to rise but at a distinctly slower rate. The yield in a rapidly stretched locust muscle is quite like that seen in a rapidly stretched crab, frog or mammalian muscle (for a review, see Josephson and Stokes, 1999a). Similar yield was not seen in the beetle muscle.

The wing-stroke frequency of *S. americana* during flight is approximately 20 Hz, and the optimal strain for work output by the wing muscles is approximately 4% peak-to-peak (Mizisin and Josephson, 1987). The maximum shortening velocity of a muscle at 20 Hz and 4% strain is 2.5 lengths s^{-1} . It might be proposed that delayed stretch activation/shortening deactivation is a feature of the locust wing muscle during normal flight and that the failure to see delayed activation and deactivation in the locust muscle of Fig. 4 was because the duration of the stretch (4.7 ms) was much shorter and the stretch velocity (47 mm s^{-1} = 6.3 muscle lengths s^{-1}) much faster than that experienced by the muscle during flight. However, no delayed stretch activation or shortening deactivation was seen even when the muscle was subjected to stretch and release at a duration and velocity similar to that of flight (see Fig. 7). Apparently, delayed stretch activation/shortening deactivation are processes that are pronounced only in insect asynchronous muscles.

Active isometric stress is low

The maximum active stress, that increase in force above the resting level when a muscle is held at a fixed length and stimulated tetanically, was substantially smaller in the beetle muscle than in the locust flight muscle (compare the stress reached by the onset of stretch in Fig. 4A and 4C). The maximum active stress during tetanic stimulation in a set of beetle muscles averaged 32.6 \pm 13.2 kN m^{-2} (mean \pm S.D., *N*=19, at 35 °C). In contrast, the active stress in locust flight muscle was found to be approximately 360 kN m^{-2} (Malamud and Josephson, 1991), and values reported for maximum tetanic stress in most synchronous muscles range from 100 to 400 kN m^{-2} (Josephson, 1993). Low active stress seems to be a general feature of asynchronous muscle. Reported values for maximum tetanic stress range from 20 to 70 kN m^{-2} in the asynchronous flight muscles of a bumblebee, a beetle and a bug (Josephson and Ellington, 1997; Machin and Pringle, 1959;

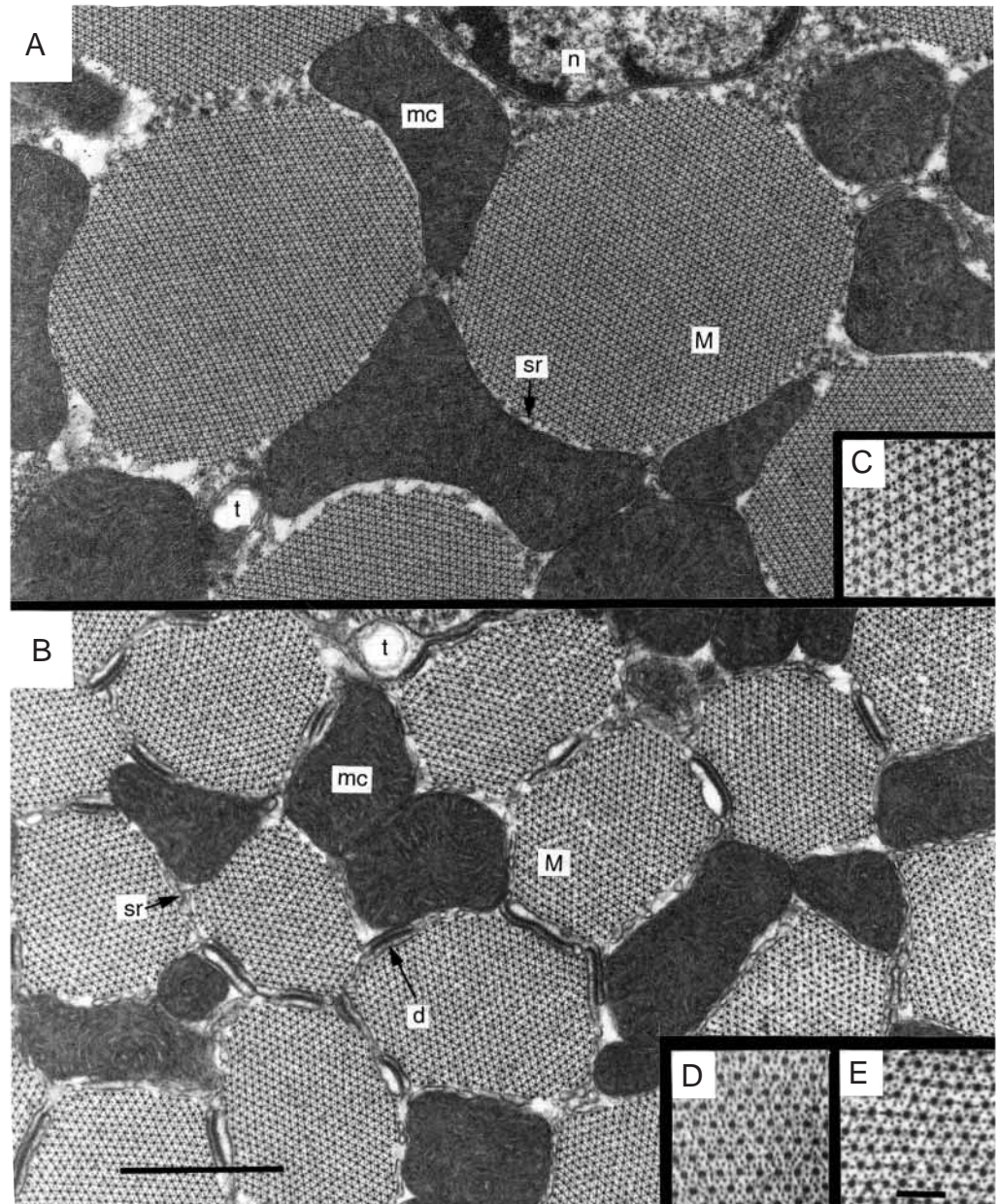


Fig. 3. Ultrastructure of asynchronous (A) and synchronous (B) flight muscle from *Cotinus mutabilis* and *Schistocerca americana* respectively. The beetle muscle is the basalar; the locust muscle the tergosternal. (A,B) Transverse sections at identical magnification. Scale bar, 1 μm . Insets C–E are at greater magnification to show myofilament arrays. The scale bar in E, which also applies to C and D, is 0.1 μm . d, dyad; M, myofibril; mc, mitochondrion; n, nucleus; sr, sarcoplasmic reticulum; t, tracheole.

Pringle, 1967; Granzier and Wang, 1993; see also Peckham et al., 1990).

The resting muscle is stiff

The locust flight muscle, like other synchronous muscles, is relatively compliant and easily extended when unstimulated and becomes stiff and resistant to stretch when stimulated (Fig. 5B). The beetle flight muscle, in contrast, was nearly equally stiff and resistant to stretch whether stimulated or unstimulated (Fig. 5A).

The stiffnesses of beetle and locust flight muscles were estimated from the rate of force increase during stretch when the muscles were unstimulated (passive) or stimulated (active). To avoid confounding the force increase during stretch resulting from intrinsic stiffness with the force increase that, in the beetle muscle, is due to delayed activation, we used

stretches that were quick (4–5 ms) and during which there was probably little delayed activation (Fig. 5). The stretch amplitude was similar for all muscles (0.18–0.20 mm) but, because of differing muscle length and slightly different stretch velocities, the strain rates ranged from 6.5 to 7.5 lengths s^{-1} in a non-systematic manner among the different preparations. Stiffness was measured as the ratio of the slope of the stress increase during stretch to that of the strain increase. In passive muscles, stress increased nearly linearly during constant-velocity stretch, and for estimating stiffness it made little difference when during the stretch the rate of force increase was measured. In active muscles, however, the rate of force increase was not constant during stretch (Fig. 5). The stiffness of active muscles is based on the steepest portion of the force rise, which occurred late in the stretch in beetle muscles and shortly before the onset of yield in locust muscles.

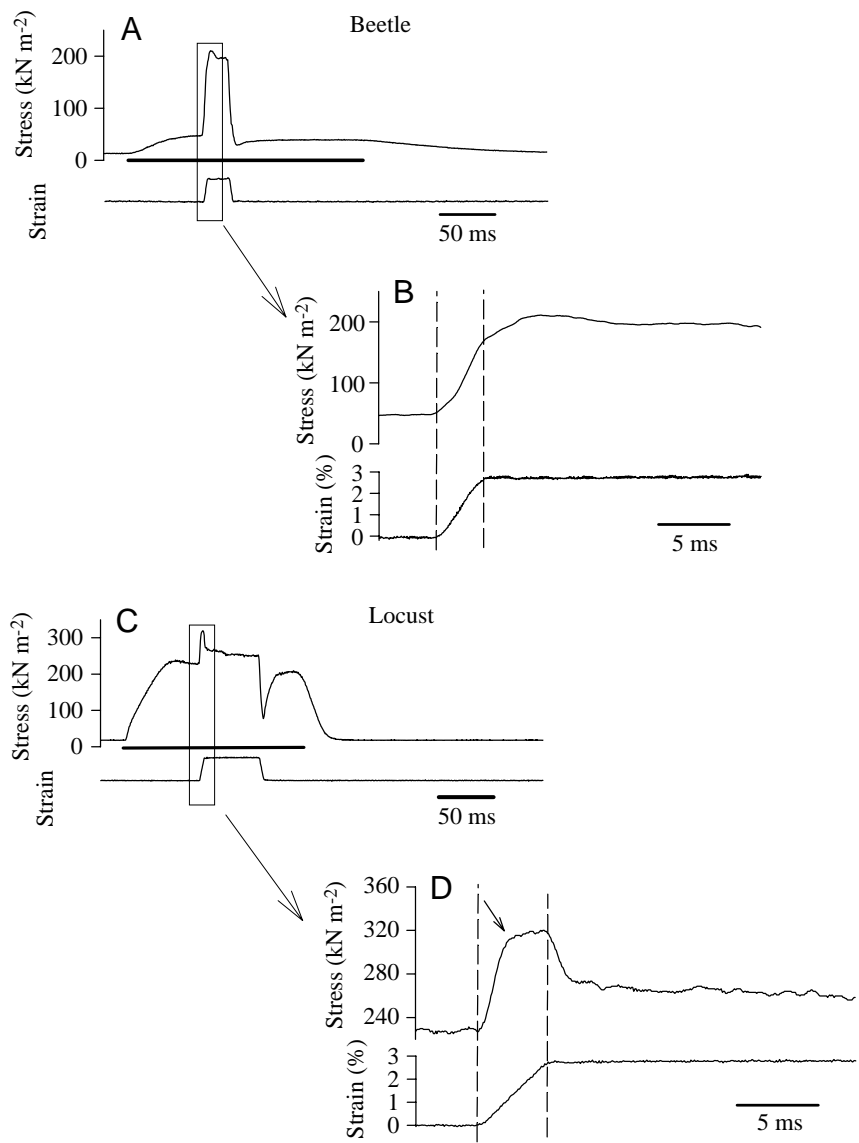


Fig. 4. Force increase during and following stretch in tetanically stimulated flight muscles from the beetle (A,B) and locust (C,D) at 30 °C. (A,C) Force (upper trace of each pair) and length change (lower trace) during stretch and release. The muscles were stimulated tetanically for the periods marked by the thick, horizontal lines. The stretch was timed to occur during the plateau of the tetanic contraction. The portions of the recordings outlined by the vertical boxes are shown on an expanded time base in B and D. The vertical broken lines in B and D mark the beginning and end of stretch and are included to aid comparison of the length and force changes. The arrow in D marks the onset of force yield.

The results of the stiffness evaluations are summarized in Table 2. The stiffness of the stimulated beetle muscle was significantly less than that of the locust muscle ($P<0.001$). The most striking difference between beetle and locust muscle, however, was in the passive stiffness, which was relatively much greater in the beetle than in the locust muscle. Stimulation increased the stiffness of the flight muscle in the locust by approximately a factor of 10, while in the beetle the stiffness increase upon stimulation was only approximately 40 %. High passive stiffness, like low tetanic force, seems to be a general feature of asynchronous muscles (for a review, see Josephson and Ellington, 1997).

Delayed stretch activation/shortening deactivation can produce oscillatory contraction

The wings and thorax of insects that use asynchronous flight muscles form mechanically resonant systems, and this may also be true of the flight apparatus in all insects. A mechanically

Table 2. *Muscle stiffness (the ratio of stress rate to strain rate) during linear stretch of unstimulated (passive) and stimulated (active) beetle and locust flight muscles*

	Passive stiffness (MN m ⁻²)	Active stiffness (MN m ⁻²)	Passive/active
Beetle muscle	4.1±0.5	5.8±0.9	0.71±0.06
Locust muscle	1.1±0.6	11.0±0.8	0.10±0.05

Values are means ± S.D. (N=4).

resonant system may be able to oscillate, but energy input is required if the oscillation is to be maintained. Energy must be supplied to overcome viscous energy loss in the moving parts and, for flight systems, energy must be supplied for the work done by the beating wings in accelerating the air passing over them. The delayed stretch activation/shortening deactivation of asynchronous muscle provides a mechanism for supplying the energy needed to maintain oscillatory contraction.

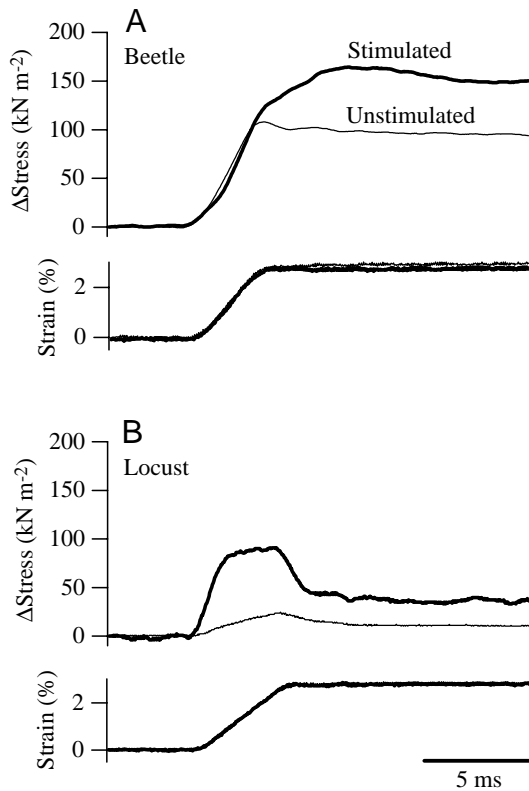


Fig. 5. Force increase during stretch of stimulated and unstimulated beetle (A) and locust (B) flight muscles. The stress and strain trajectories indicated by heavy lines are basically those of Fig. 4B,D. The stress at the onset of stretch has been subtracted from each stress trace to display better the change in stress associated with stretch. The thin lines indicate the stress and strain in similar trials in which the muscles were not stimulated. Note that in the beetle muscle the stress trajectory during stretch is nearly the same in the stimulated and unstimulated muscle and that the unstimulated muscle shows no delayed activation following stretch.

The process by which delayed stretch activation and shortening deactivation can lead to positive work output and maintained oscillatory contraction by asynchronous muscles is illustrated in Fig. 6. A beetle muscle was tetanically stimulated and, during the plateau of the tetanus, subjected to two cycles of stretch–hold–release–hold (Fig. 6A). The cycle duration (16.6 ms) was similar to that at which power output is maximal in intact muscles (approximately 17 ms at 35 °C; Josephson et al., 2000), and the stretch velocity was approximately 41 mm s⁻¹ (6.5 lengths s⁻¹). The letters a–d in Fig. 6B indicate the force trajectory over one complete cycle. The force falls during shortening (a) and continues to fall, because of delayed deactivation, during the following interval at constant length (b). The force rises during the subsequent stretch (c) and, because of delayed stretch activation, rises further to a peak during the next interval during which the length is constant (d). The net result is that a plot of stress against strain forms a loop, traversed counterclockwise, with the stress greater at any length during shortening than during lengthening (Fig. 6C). Loops formed by plotting stress against strain, or force against length,

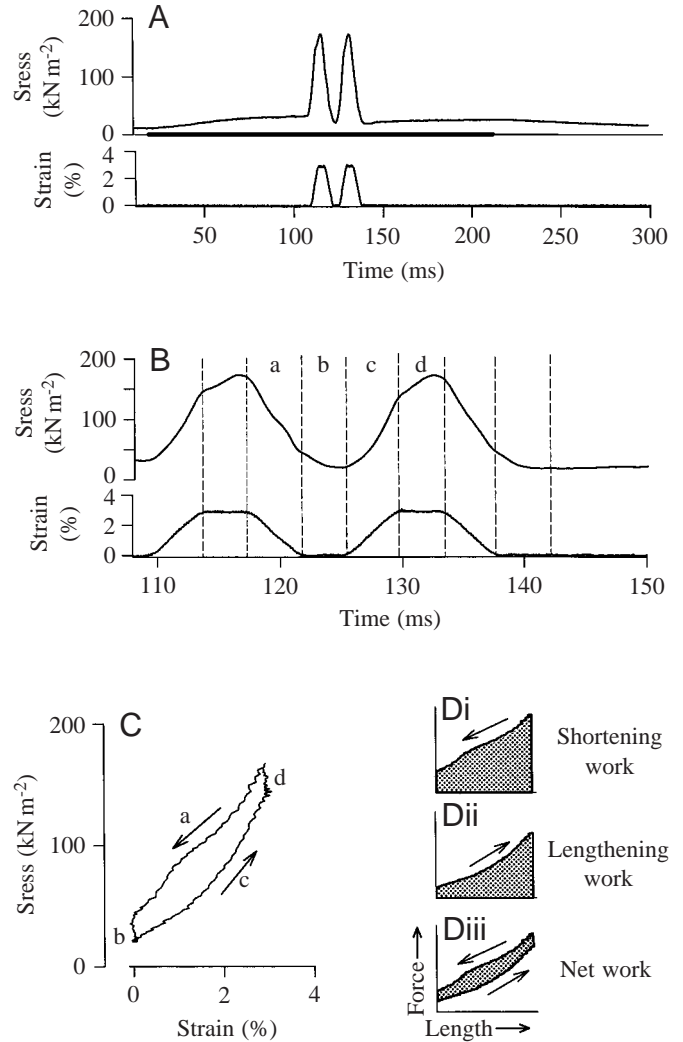


Fig. 6. A synthesized work loop from the beetle muscle. (A) The muscle was stimulated tetanically and subjected to two stretch–hold–release–hold cycles. The thickened portion of the line beneath the upper trace indicates the duration of tetanic stimulation. (B) The length and force changes during the stretch and release cycles at higher gain. The letters a–d identify the segments of the strain and stress trajectories that are plotted as a work loop in C. The arrows in C indicate the direction in which the loop was traversed. D illustrates that the area of a work loop is the net work done over a shortening–lengthening cycle (shortening work minus lengthening work = net work). The net work output for the loop in C is 0.97 W kg⁻¹ (see text for further details).

are termed work loops because their area is the work done on or by the muscle over a shortening–lengthening cycle. That such plots do give work output is perhaps most easily seen when the axes are force and length (Fig. 6D). The area between the shortening limb of the curve and the zero-force axis (Fig. 6Di) has dimensions of force × distance (=work) and is the work done by the muscle during shortening. The area between the curve and the force axis during lengthening (Fig. 6Dii) similarly has dimensions of work and is the work done on the muscle to restretch it. The difference between the work of

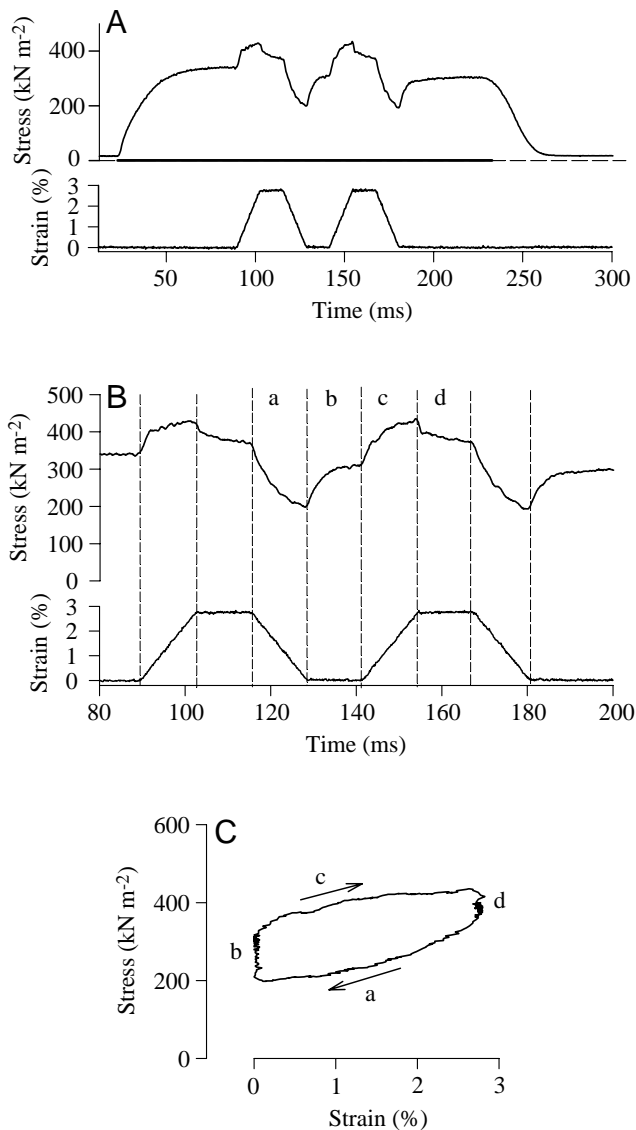


Fig. 7. A synthesized work loop from a locust flight muscle. Note that the loop is traversed in a clockwise direction (=net work absorption) rather than anticlockwise (=net work production) as in the beetle muscle. The net work absorbed over the cycle in C was 4.18 J kg^{-1} . See text and the legend to Fig. 6 for details.

shortening and the work of lengthening (Fig. 6Diii) is the net work done over the cycle. When the plot is of stress (=force/unit area) against strain (=change in length/initial length), the area of the work loop is work/unit volume \approx work/unit mass. It should be emphasized that the work loop for the active beetle muscle, when forced through a shortening-lengthening cycle, is traversed in a counterclockwise direction. The force is higher during shortening than during lengthening, and there is net work output by the muscle over the cycle.

Fig. 7 shows the force trajectory in a tetanically stimulated flight muscle of a locust when subjected to length changes similar to those it might experience in flight (Fig. 7A). The cycle duration here was 52 ms, and the stretch/release velocity was 15.6 mm s^{-1} ($2.1 \text{ lengths s}^{-1}$). The locust muscle behaved

like a partially damped spring. Force dropped during shortening (segment a) but partially recovered in the following interval at constant length (b). Force rose during stretch (c) and then relaxed somewhat in the following period at constant length (d). The net result was a work loop that was traversed in a clockwise direction, with the force during lengthening being greater than that during shortening. The locust muscle absorbed work, rather than produced work, when forced through a shortening-lengthening cycle. Obviously, locust wing muscles do produce net work during flight, but their power output depends on periodic neural input and neurally evoked activation followed by relaxation on each cycle rather than delayed stretch activation and shortening deactivation.

How consistent are the characteristics of asynchronous muscle?

A number of muscle features have been identified as being associated with the self-oscillatory mode of operation in the beetle flight muscle: asynchrony between muscle electrical and mechanical activity, a slow time course of isometric contraction, a fibrillar organization with a poorly developed sarcoplasmic reticulum, delayed stretch activation/shortening deactivation, low isometric force and high passive stiffness. As indicated above, asynchronous muscle has evolved independently a number of times in insects. It is appropriate, therefore, to ask whether asynchronous muscle shares the same suite of characteristics in each of its independent origins.

First, it should be emphasized that the assertion that asynchronous muscle has evolved independently in a number of insect lines is largely an inference. Asynchrony between electrical activity and mechanical contraction has been demonstrated directly in flight muscles of only a few of the insect groups in which it is thought to occur. Many insects are too small or too reluctant to fly when physically restrained to readily allow recordings of muscle action potentials during flight. Information on the distribution of asynchronous muscles is based largely on histological and ultrastructural information on the distribution of fibrillar muscle and the assumption that muscle that is fibrillar is also asynchronous. The latter assumption does seem reasonable for all muscles with a moderate to high operating frequency.

It follows from what is known about the mechanisms by which muscle activation is controlled through the release of Ca^{2+} from and re-uptake by the sarcoplasmic reticulum that the brevity of neurally evoked contractions should be related directly to the development of the sarcoplasmic reticulum and inversely to the diameter of the myofibrils. Indeed, it was in part the discovery that fast muscles have an unusually well-developed sarcoplasmic reticulum that drew attention to the role of the sarcoplasmic reticulum in the control of contraction (e.g. Porter, 1961), and much subsequent work has confirmed the close correlation between the abundance of sarcoplasmic reticulum in a muscle and the rapidity of neurally evoked contractions from that muscle (see, for example, Josephson and Young, 1987). The presence of fibrillar organization, with its

large fibrils and sparse sarcoplasmic reticulum, may be taken as *a priori* evidence that the muscle turns on and off slowly following neural activation. If the operating frequency of a muscle with fibrillar organization is at all high, it may be supposed that the muscle is not turned on briefly by neural input on each contraction. Some other mechanism must underlie the periodic contractions, and the only known candidate is delayed stretch activation/shortening deactivation. Muscles in which repetitive contractions are a consequence of delayed activation/deactivation and a resonant load need not be asynchronous. It is conceivable that there could be neural input, perhaps triggered by proprioceptive feedback, and muscle action potentials on each contraction, but this would be neither necessary nor economical. Because fibrillar muscles turn on and off slowly when stimulated, only a low stimulation frequency is needed to evoke a maximal tetanic contraction (Ikeda and Boettiger, 1965; Josephson and Young, 1981; Josephson and Ellington, 1997). Increasing the stimulation frequency above that required for full activation does not increase the force of the contraction, but it does increase the costs of the neural and synaptic activity and probably also of Ca^{2+} cycling within the muscle fibers. For maximal economy, the muscle should be stimulated at a frequency no greater than that required for full activation. The minimal stimulation frequency for full activation of fibrillar flight muscle is likely to be much lower than that of the wing strokes; hence, one expects asynchrony between electrical potentials and mechanical contraction.

It is not obvious why active force during isometric contraction should be low in asynchronous muscles, nor why high resting stiffness is closely associated and perhaps causally linked with the asynchronous mode of operation. The high passive stiffness is striking and has been noted in many studies on the mechanics of asynchronous muscle: beetle (*Oryctes rhinoceros*) flight muscle (Machin and Pringle, 1959); *Lethocerus* (hemipteran, several species) flight muscle (Pringle, 1974; Granzier and Wang, 1993; Peckham et al., 1990); *Drosophila melanogaster* flight muscle (Peckham et al., 1990); bumblebee (*Bombus terrestris*) flight muscle (Josephson and Ellington, 1997). The stiffness is due to molecules of the titin family that link the thick filaments to the Z-bands (Granzier and Wang, 1993; Moore et al., 1999). The high passive stiffness is probably an important clue to the underlying mechanisms of asynchronous operation, but how to interpret this clue is not yet clear.

In summary, muscles with fibrillar organization are expected to turn on and off slowly following neural activation and to rely on delayed stretch activation/shortening deactivation to achieve high-frequency contraction. Because the muscles are intrinsically slow, they can be, and economic operation dictates that they should be, activated with a relatively low neural input frequency, which results in asynchrony between electrical and mechanical events during normal operation. Many of the elements in the set of features listed above – in particular, asynchrony between electrical and mechanical activity, a fibrillar ultrastructure, the slow time course of isometric contraction and delayed stretch activation/shortening

deactivation – are likely to be general features of asynchronous muscles of insects wherever they occur. Low active force and high passive stiffness may be inescapable consequences of the muscle mechanisms allowing asynchronous operation, but there is no compelling evidence yet for this.

Delayed stretch activation/shortening deactivation is not unique to insect asynchronous muscles. Many muscles show one or more forms of deactivation following shortening (for a review, see Josephson and Stokes, 1999b). Shortening deactivation can reduce the work required to re-lengthen a muscle after it has shortened and, in this way, may increase the net work available from the muscle over a complete shortening–lengthening cycle (Josephson and Stokes, 1989). Shortening deactivation would be particularly effective in increasing net work output if it were delayed somewhat with respect to the length change; then, the muscle could be fully active or nearly so, producing maximal work, during shortening and deactivated during the following lengthening. Delayed stretch activation and shortening deactivation occur to some extent in a number of ordinary, synchronous skeletal muscles and in cardiac muscle, and in these muscles it can result in some positive work output during low-amplitude, oscillatory length change (Steiger, 1971; Kawai and Brandt, 1980). It is presumably evolutionary hypertrophy of intrinsic stretch activation/shortening deactivation, initially selected because it increased the work per cycle during neurally controlled, repetitive contraction of synchronous muscle, that has led to the multiple occurrences of asynchronous muscles in insects.

We thank Dr Gilles Laurent of the California Institute of Technology for providing the locusts used in this study. The work was supported by NSF grant IBN9603187.

References

- Barber, S. B. and Pringle, J. W. S. (1966). Functional aspects of flight in belostomatid bugs (Hemiptera). *Proc. R. Soc. Lond. B* **164**, 21–39.
- Boettiger, E. G. (1957). The machinery of insect flight. In *Recent Advances in Invertebrate Physiology* (ed. B. T. Scheer), pp. 117–142. Eugene, OR: University of Oregon Press.
- Cullen, M. J. (1974). The distribution of asynchronous muscle in insects with particular reference to the Hemiptera: an electron microscope study. *J. Ent. A* **49**, 17–41.
- Dickinson, M. H. and Tu, M. S. (1997). The function of dipteran flight muscle. *Comp. Biochem. Physiol.* **116A**, 223–238.
- Dudley, R. (1991). Comparative biomechanics and the evolutionary diversification of flying insect morphology. In *The Unity of Evolutionary Biology* (ed. E. C. Dudley), pp. 503–514. Portland, OR: Dioscorides Press.
- Dudley, R. (2000). *The Biomechanics of Insect Flight: Form, Function, Evolution*. Princeton: Princeton University Press.
- Fine, M. L. (1978). Seasonal and geographical variation of the mating call of the oyster toadfish *Opsanus tau* L. *Oecologia* **36**, 45–57.
- Fine, M. L. and Mosca, P. J. (1989). Anatomical study of the innervation pattern of the sonic muscle of the oyster toadfish. *Brain Behav. Evol.* **34**, 265–272.
- Full, R. J., Stokes, D. R., Ahn, A. A. and Josephson, R. K. (1998).

- Energy absorption during running by leg muscles in a cockroach. *J. Exp. Biol.* **201**, 997–1012.
- Granzier, H. L. M. and Wang, K.** (1993). Interplay between passive tension and strong and weak binding cross-bridges in indirect flight muscle. *J. Gen. Physiol.* **101**, 235–270.
- Ikeda, K. and Boettiger, E. G.** (1965). Studies on the flight mechanism of insects. II. The innervation and electrical activity of the fibrillar muscles of the bumble bee, *Bombus*. *J. Insect Physiol.* **11**, 779–789.
- Josephson, R. K.** (1984). Contraction dynamics of flight and stridulatory muscles of tettigoniid insects. *J. Exp. Biol.* **108**, 77–96.
- Josephson, R. K.** (1993). Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* **55**, 527–546.
- Josephson, R. K.** (1995). The mechanical power output of a tettigoniid wing muscle during singing and flight. *J. Exp. Biol.* **117**, 357–368.
- Josephson, R. K.** (1997). Power output from a flight muscle of the bumblebee *Bombus terrestris*. II. Characterization of the parameters affecting power output. *J. Exp. Biol.* **200**, 1227–1239.
- Josephson, R. K. and Ellington, C. P.** (1997). Power output from a flight muscle of the bumblebee *Bombus terrestris*. I. Some features of the dorso-ventral flight muscle. *J. Exp. Biol.* **200**, 1215–1226.
- Josephson, R. K. and Halverson, R. C.** (1971). High frequency muscles used in sound production by a katydid. I. Organization of the motor system. *Biol. Bull.* **141**, 411–433.
- Josephson, R. K., Malamud, J. G. and Stokes, D. R.** (2000). Power output by an asynchronous flight muscle from a beetle. *J. Exp. Biol.* **203**, 2667–2689.
- Josephson, R. K. and Stokes, D. R.** (1989). Strain, muscle length and work output in a crab muscle. *J. Exp. Biol.* **145**, 45–61.
- Josephson, R. K. and Stokes, D. R.** (1999a). The force–velocity properties of a crustacean muscle during lengthening. *J. Exp. Biol.* **202**, 593–607.
- Josephson, R. K. and Stokes, D. R.** (1999b). Work-dependent deactivation of a crustacean muscle. *J. Exp. Biol.* **202**, 2551–2565.
- Josephson, R. K. and Young, D.** (1981). Synchronous and asynchronous muscles in cicadas. *J. Exp. Biol.* **91**, 219–237.
- Josephson, R. K. and Young, D.** (1985). A synchronous insect muscle with an operating frequency greater than 500 Hz. *J. Exp. Biol.* **118**, 185–208.
- Josephson, R. K. and Young, D.** (1987). Fiber ultrastructure and contraction kinetics in insect fast muscles. *Am. Zool.* **27**, 991–1000.
- Kawai, M. and Brandt, P. W.** (1980). Sinusoidal analysis: a high-resolution method for correlating biochemical reactions with physiological processes in activated skeletal muscles of rabbit, frog and crayfish. *J. Muscle Res. Cell Motil.* **1**, 279–303.
- Lindstedt, S. L., McGlothlin, T., Percy, E. and Pifer, J.** (1998). Task-specific design of skeletal muscle: balancing muscle structural composition. *Comp. Biochem. Physiol.* **120B**, 35–40.
- Machin, K. E. and Pringle, J. W. S.** (1959). The physiology of insect fibrillar muscle. II. Mechanical properties of a beetle flight muscle. *Proc. R. Soc. Lond. B* **151**, 204–225.
- Malamud, J. G. and Josephson, R. K.** (1991). Force–velocity relationships of a locust flight muscle at different times during a twitch contraction. *J. Exp. Biol.* **159**, 65–87.
- Malamud, J. G., Mizisin, A. P. and Josephson, R. K.** (1988). The effects of octopamine on contraction kinetics and power output of a locust flight muscle. *J. Comp. Physiol. A* **162**, 827–835.
- Maughan, D. W. and Vigoreaux, J. O.** (1999). An integrated view of insect flight muscle: genes, motor molecules and motion. *News Physiol. Sci.* **14**, 87–92.
- Mizisin, A. P. and Josephson, R. K.** (1987). The effects of octopamine on contraction kinetics and power output of a locust flight muscle. *J. Comp. Physiol. A* **162**, 827–835.
- Moore, J. R., Vigoreaux, J. O. and Maughan, D. W.** (1999). The *Drosophila* projectin mutant, bentD, has reduced stretch activation and altered indirect flight muscle mechanics. *J. Muscle Res. Cell Motil.* **20**, 797–806.
- Peckham, M., Molloy, J. E., Sparrow, J. C. and White, D. C. S.** (1990). Physiological properties of the dorsal longitudinal flight muscle and the tergal depressor of the trochanter muscle of *Drosophila melanogaster*. *J. Muscle Res. Cell Motil.* **11**, 203–215.
- Porter, K. R.** (1961). The sarcoplasmic reticulum: its recent history and present status. *J. Biophys. Biochem. Cytol.* **10** (Suppl.), 219–226.
- Pringle, J. W. S.** (1949). The excitation and contraction of the flight muscles of insects. *J. Physiol., Lond.* **108**, 226–232.
- Pringle, J. W. S.** (1967). The contractile mechanism of insect fibrillar muscle. *Prog. Biophys. Mol. Biol.* **17**, 3–60.
- Pringle, J. W. S.** (1974). The resting elasticity of insect flight muscle. *Symp. Biol. Hung.* **17**, 67–78.
- Pringle, J. W. S.** (1976). The muscles and sense organs involved in insect flight. *Symp. R. Ent. Soc. Lond.* **7**, 3–15.
- Pringle, J. W. S.** (1977). The mechanical characteristics of insect fibrillar muscle. In *Insect Flight Muscle* (ed. R. T. Tregear), pp. 177–196. Amsterdam: North Holland Publishing Co.
- Pringle, J. W. S.** (1981). The evolution of fibrillar muscle in insects. *J. Exp. Biol.* **94**, 1–14.
- Roeder, K. D.** (1951). Movements of the thorax and potential changes in the thoracic muscles of insects during flight. *Biol. Bull.* **100**, 95–106.
- Rome, L. C. and Lindstedt, S. L.** (1998). The quest for speed: muscles built for high-frequency contractions. *News Physiol. Sci.* **13**, 261–268.
- Rome, L. C., Syme, D. A., Hollingworth, S., Lindstedt, S. L. and Baylor, S. M.** (1996). The whistle and the rattle: The design of sound producing muscles. *Proc. Natl. Acad. Sci. USA* **93**, 8095–8100.
- Rosenbluth, J.** (1969). Sarcoplasmic reticulum of an unusually fast-acting crustacean muscle. *J. Cell Biol.* **42**, 534–547.
- Schaeffer, P. J., Conley, K. E. and Lindstedt, S. L.** (1996). Structural correlates of speed and endurance in skeletal muscle: the rattlesnake tail shaker muscle. *J. Exp. Biol.* **199**, 351–358.
- Sotavalta, O.** (1947). The flight-tone (wing-stroke frequency) of insects. *Acta Ent. Fenn.* **4**, 1–117.
- Sotavalta, O.** (1953). Recordings of high wing-stroke and thoracic vibration frequency in some midges. *Biol. Bull.* **104**, 439–449.
- Steiger, G. J.** (1971). Stretch activation and myogenic oscillation of isolated cardiac, skeletal and insect flight muscle. In *Insect Flight Muscle* (ed. R. T. Tregear), pp. 221–268. Amsterdam: North Holland.
- Stokes, D. R., Malamud, J. G. and Schreihofer, D. A.** (1994). Gender specific developmental transformation of a cockroach bifunctional muscle. *J. Exp. Zool.* **268**, 364–376.