

How to Build Fast Muscles: Synchronous and Asynchronous Designs¹

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SYNOPSIS. In animals, muscles are the most common effectors that translate neuronal activity into behavior. Nowhere is behavior more restricted by the limits of muscle performance than at the upper range of high-frequency movements. Here, we see new and multiple designs to cope with the demands for speed. Extremely rapid oscillations in force are required to power cyclic activities such as flight in insects or to produce vibrations for sound. Such behaviors are seen in a variety of invertebrates and vertebrates, and are powered by both synchronous and asynchronous muscles. In synchronous muscles, each contraction/relaxation cycle is accompanied by membrane depolarization and subsequent repolarization, release of activator calcium, attachment of cross-bridges and muscle shortening, then removal of activator calcium and cross-bridge detachment. To enable all of these to occur at extremely high frequencies a suite of modifications are required, including precise neural control, hypertrophy of the calcium handling machinery, innovative mechanisms to bind calcium, and molecular modification of the cross-bridges and regulatory proteins. Side effects are low force and power output and low efficiency, but the benefit of direct, neural control is maintained. Asynchronous muscles, in which there is not a 1:1 correspondence between neural activation and contraction, are a radically different design. Rather than rapid calcium cycling, they rely on delayed activation and deactivation, and the resonant characteristics of the wings and exoskeleton to guide their extremely high-frequency contractions. They thus avoid many of the modifications and attendant trade-offs mentioned above, are more powerful and more efficient than high-frequency synchronous muscles, but are considerably more restricted in their application.

INTRODUCTION

The most complex ganglia are inert, the most elaborate brains mute, without effectors to translate their neuronal firing patterns into behavior. By far the most important effectors for animal behavior are muscles. The behavioral capabilities of an animal are determined, to a large extent, by the functional capacities of its muscles—the ability of the muscles to produce force and to do work, the speed of their responses, their efficiency in converting chemical to mechanical energy. Muscles from different sources vary widely in their performance. A sloth is necessarily a slow, deliberate creature, even when agitated, because its muscles are incapable of a rapid response (Enger and Bullock, 1965). A mosquito can fly because its wing muscles are capable of oscillating its wings at frequencies more than an order of magnitude higher than those achievable by the muscles that move human appendages. At first glance it might seem that every animal would benefit from having fast, strong, indefatigable muscles, and that evolutionary selection should lead to this end. But each of these properties—speed, strength, and fatigue resistance—comes with a cost in other aspects of performance. Nowhere are the cost-benefit trade-offs associated with muscle performance seen so clearly as in those muscles that operate at the upper end of the frequency range for animal behaviors. The following examines the design of high-frequency muscles and the factors that limit muscle performance at high

frequencies. The muscles used for high-frequency operation can be divided into two broad classes, synchronous muscles and asynchronous muscles, and these two modes of operation will be considered separately.

HIGH SPEED SYNCHRONOUS MUSCLE

Synchronous, skeletal muscles are those in which there is a 1:1 correspondence between muscle action potentials evoked by incoming neuronal activity and mechanical contractions. The depolarization of muscle cells resulting from neuronal activation triggers, through a series of cell and vesicular membrane-bound proteins, the release of activator calcium from the sarcoplasmic reticulum (SR). This calcium has several potential destinations. It can remain free in the cytosol. It can bind to sarco(endo)plasmic reticulum calcium ATPases (SERCA pumps) and be pumped back into the SR. It can bind to other proteins in the cytosol. Or it can bind to a subunit of the troponin complex that resides on the actin filament. If calcium binds to troponin, a conformational change in the troponin/tropomyosin complex occurs, such that myosin binding sites are exposed on the actin filament, and myosin cross-bridges can then attach causing contraction. For the muscle to relax, myosin cross-bridges must detach from actin, calcium must be released from troponin to restore thin-filament inhibition (*i.e.*, prevent further cross-bridges from forming), and the free, cytoplasmic calcium concentration must be lowered to prevent further calcium binding to troponin. All of these processes are repeated every time a twitch occurs in the muscle.

Skeletal muscles can be classified as relatively fast

¹ From the Symposium *Recent Advances in Neurobiology* presented at the Annual Meeting of the Society for Integrative and Comparative Biology, 2–6 January 2002, at Anaheim, California.

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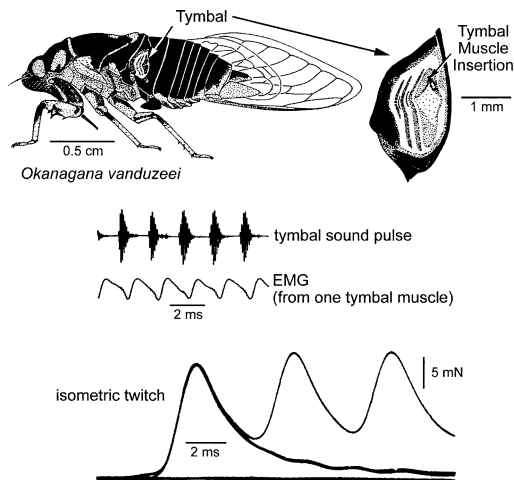


FIG. 1. Upper panel: Cicada, *Okanagana vanduzeei*, whose synchronous, tymbal muscles contract and relax at a frequency of over 500 Hz. The tymbals buckle and produce bursts of vibrations in response to tymbal muscle contraction. Middle panel: Sound pulses recorded from the tymbals, and EMGs record from one tymbal muscle. Lower panel: Isometric twitch from a tymbal muscle. Data from Josephson and Young (1985).

or slow based on their ability to shorten under load. In general, both “fast” and “slow” muscles can shorten at the speeds used to power most movements in animals; the startle response of fish is a well-described exception (Rome *et al.*, 1988) and there are likely others. Thus it is normally not the velocity of shortening *per se* that separates the usefulness of “fast” versus “slow” muscle for a particular activity, rather it is their ability to produce power. Power is the product of the velocity of shortening and the force produced. Fast muscles can not only shorten fast but they can produce substantial amounts of force while doing so, and thus can produce more power than slow muscles. They accomplish this by having cross-bridges that can cycle rapidly. Because of this, fast muscles also tend to use energy faster than slow muscles, and so, along with their higher power output, they are less efficient at doing work.

Muscles can also be “fast” in the sense that they are used to power activities that require rapid oscillations in force, such as flapping flight or the production of vibrations for sound. If a muscle powers an activity that requires cyclic, oscillatory movements, then it must be able to contract and relax in at least the same period as that of the movement (Young and Josephson, 1983; Josephson, 1984). The ability to simply shorten at high velocities and maintain tension is not adequate. Indeed, muscles can be very fast shorteners but not unusually fast twitchers (Josephson, 1984), and muscles that produce maximal power at dramatically different (50 \times) frequencies of cyclic contraction can have maximal velocities of shortening that are almost identical (Young and Rome, 2001). Several of the key modifications that allow synchronous muscles to operate at very high frequencies (*i.e.*, have very brief twitches) and how they affect the rate of energy use

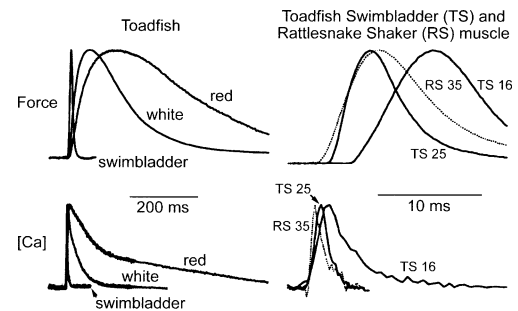


FIG. 2. Twitch force (upper panels) and cytosolic free calcium transient (lower panels) from toadfish swimbladder, slow red and fast white trunk muscles at 16°C (left), and from toadfish swimbladder muscle and rattlesnake tailshaker muscle at various temperatures (right). Data from Rome *et al.* (1996).

and in some cases limit the muscle’s ability to produce power will be discussed, using as examples sound producing muscles in cicadas, rattlesnakes and toadfish.

Cicadas produce a calling song by vibrating a pair of cuticular plates called tymbals (Fig. 1). The tymbals are buckled inward by contraction of a tymbal muscle, and recoil outward when released. Each buckling results in a burst of resonant vibrations from the tymbal, with the repetition rate of these bursts being determined by the contraction frequency of the tymbal muscle (Young and Josephson, 1983). The male cicada, *Okanagana vanduzeei*, produces a calling song with a pulse repetition frequency up to 550 Hz (Josephson and Young, 1985). In this species, recordings of muscle EMG’s during calling, recordings of sound pulses after destruction of one of the tymbals, and muscle ultrastructure, confirm that each tymbal muscle operates at this high frequency and that their tymbal muscles are indeed synchronous (Fig. 1).

Several vertebrates are also known to use high-speed synchronous muscles to produce sound. The male oyster toadfish, *Opsanus tau*, produces a 200 Hz “boatwhistle” mating call using rapid contractions of muscle that encircles its gas filled swimbladder (*e.g.*, Skoglund, 1961; Fine, 1978; Appelt *et al.*, 1991). The fish uses the swimbladder as a resonator, coupling the high frequency contractions of the muscle to high frequency sound production. Rattlesnakes shake their tail rattles as aposematic warnings. The shaker muscle is composed of six bands of red, aerobic muscle fibers at the base of the tail (Zimmerman and Pope, 1948), groups of which contract alternately and cause the rattle to shake from side to side (Schaeffer *et al.*, 1996). At 35°C, not atypical of a sunny, summer day in the southwestern U.S. deserts, the western diamondback rattlesnake, *Crotalus atrox*, shakes its rattle at frequencies up to 90 Hz (Chadwick and Rahn, 1954; Martin and Bagby, 1972; Schaeffer *et al.*, 1996). The twitches of toadfish sonic muscles and rattlesnake tail muscles are also extraordinarily brief (Fig. 2), a prerequisite for high frequency vibrations that produce sound.

To produce brief twitches and power very high-fre-

quency movements muscles must have the ability to contract and relax very rapidly and in a synchronised fashion. This requires precise neural control and a design allowing all of the above activation/relaxation processes to occur rapidly, and hence modifications of the regulatory and contractile machinery. These modifications will include rapid release and removal of activator calcium from the cytoplasm, rapid binding and release of calcium from troponin, and the ability for cross-bridges to attach and detach quickly (Josephson, 1975; Rome *et al.*, 1996; Tullis and Block, 1996; Rome and Lindstedt, 1998).

Co-ordination of high frequency contractions

The duration of a single twitch in high-speed, synchronous muscle is usually similar to the period of the vibrations required to produce the high-frequency sounds (Young and Josephson, 1984). Therefore it is critical that all muscle cells be activated simultaneously; if not, the muscle would become functionally tetanized and stiff, and could not power cyclic contractions. As such, muscles that operate at very high frequencies show designs that tend to synchronise contraction of all the cells in the muscle. The cells in synchronous tymbal muscles in cicadas all belong to a single motor unit (Young and Josephson, 1983) and are thus activated together. Similarly, EMG data suggest that all the cells in each of the six bands of muscle that shake the rattlesnake tail operate in unison (Schaeffer *et al.*, 1996). The muscle fibers in toadfish swimbladder are multiply innervated, and as they grow new synaptic terminals are added such that the spacing between terminals on each muscle cell is maintained; perhaps the distributed innervation compensates for a slow conduction velocity along the fibers and improves synchrony of contraction along the cell's entire length (Hirsch *et al.*, 1998). A similar design appears to be used in the high-frequency remoter muscle of lobsters (Mendelson, 1969). Manipulation of the path length of motor axons to different regions of the muscle cells may also ensure synchronous activation of some sonic muscles (Fine and Mosca, 1989).

Brief calcium transient

The very brief calcium transient observed in high-frequency synchronous muscles (Fig. 2) aids in initiating contraction and then promptly initiating relaxation, terminating the twitch (Appelt *et al.*, 1991; Block, 1994; Rome *et al.*, 1996; Rome and Lindstedt, 1998). The rate that calcium is released from the SR after depolarisation is quite fast in both slow and fast muscles (Fig. 2); this similarity, along with estimates of rates and distances of diffusion, suggest that calcium release is not a rate limiting factor for high-frequency operation (Appelt *et al.*, 1991; Josephson, 1975). However, despite the rates of rise of intracellular free calcium being very fast in both slow and fast muscles, the rate is indeed slightly faster in fast muscles, and the exclusive presence of the fast isoform of calcium release channels on the SR of swimbladder muscle

suggests the rate of calcium release is not unimportant to high-frequency operation (O'Brien *et al.*, 1993).

For a muscle to relax, calcium must dissociate from troponin, and this requires a relatively low cytosolic free calcium concentration. The rate that calcium is removed from the cytoplasm is much faster in fast muscles than slow (Fig. 2). The half width of the calcium transient is about 3–4 ms at 16°C in toadfish swimbladder muscle (Rome *et al.*, 1996), 2–3 times briefer than the calcium transient in fast twitch fibers of a frog (Konishi *et al.*, 1991), and is only 1–2 ms in rattlesnake shaker muscle at 35°C (Rome *et al.*, 1996). The high rate of calcium removal in fast muscles is due, in part, to the high relative volume of SR and the high density of calcium pumps (Josephson, 1975; Feher *et al.*, 1998; Rome and Lindstedt, 1998; Ferguson and Franzini-Armstrong, 1988; Mendelson, 1969; Rosenbluth, 1969; Appelt *et al.*, 1991; Ladich, 2001). However, possession of a high density of calcium pumps cannot explain entirely the rapid reduction in cytosolic calcium seen in these fast muscles. Based on pump densities and uptake rates (Feher *et al.*, 1998), the overall calcium uptake rate of the SR in swimbladder muscle is, surprisingly, perhaps 50% slower than for rat extensor digitorum longus, a muscle with a much longer calcium transient and twitch. Other factors must be considered to account for the rapid removal of calcium.

High concentrations of other calcium binding proteins, such as parvalbumin, in very fast muscles likely play an important role in rapidly lowering free cytosolic calcium and reducing the twitch duration (Heizmann *et al.*, 1982; Ferguson and Franzini-Armstrong, 1988; Appelt *et al.*, 1991; Hou *et al.*, 1991; Rome and Lindstedt, 1998; Feher *et al.*, 1998); but see Appelt *et al.* (1991) for a discussion of why parvalbumin may not help in this regard. Such proteins act as "calcium shuttles" between the cytoplasm and SR (Appelt *et al.*, 1991), effectively lowering the free calcium content of the cytosol and then transferring the calcium to the SR *via* pumps at a moderate rate between contractions (Feher *et al.*, 1998; Rome and Klimov, 2000). There are also potential energetic benefits to avoiding the use of ATP driven calcium pumps as the exclusive means of lowering the cytosolic calcium content, which will be discussed later.

Related to the need to release and sequester calcium rapidly is the need for calcium to reach the myofibrils quickly. To this end, the myofibrils of muscles that operate at very high frequencies tend to be thin as compared with those of slower muscles, and are sometimes ribbon-shaped as opposed to circular in cross-section (Josephson, 1975; Young and Josephson, 1984; Josephson and Young, 1985; Appelt *et al.*, 1991; Fine *et al.*, 1993; Ladich, 2001). Narrow fibrillar dimensions result in short diffusion distances for calcium, thus speeding rates of activation and inactivation. Small diameter cells may also be required to satisfy the need for rapid energy transfer. While toadfish and their swimbladder muscles continue to grow through-

out life, the cells appear to continue to divide and remain relatively small (Fine *et al.*, 1993).

Rapid release of calcium from troponin

Simply removing free cytosolic calcium is not enough to terminate the twitch; calcium must dissociate from troponin to prevent further cross-bridge formation. This requires a high kinetic off rate (k_{off}) of calcium from troponin (*i.e.*, troponin with a low affinity for calcium). While the calcium affinity of troponin in fast muscles has not been measured directly, other evidence suggests it is low. Kinetic modelling indicates that toadfish swimbladder muscle could not operate at the frequency it does if its troponin possessed the same k_{off} as fast muscles in frogs (Rome *et al.*, 1996). With a k_{off} like that of frogs, significant calcium would remain attached to troponin between activations and the muscle could not relax. Also, a rightward shift in the force-pCa relationship of fast muscle implies a relatively lower affinity of troponin for calcium, as might occur with a high k_{off} (Rome *et al.*, 1996).

Work on the flight muscles of dragonflies (Marden *et al.*, 1999, 2001) provides further evidence supporting an important role of troponin in modulating the performance of high-frequency synchronous muscles. Various transcripts of the troponin T subunit are expressed naturally in the muscles of dragonfly populations at different locations. Specific transcripts are correlated with a suite of changes in muscle contraction and flight kinetics, including changes in calcium sensitivity and co-operativity of the thin filaments, peak force, work and power output, and wing-beat frequency and amplitude. The changes are presumed to reflect the types of troponin T expressed, which in turn could affect muscle activation.

We have summarised some of the modifications required for high frequency operation, and will now focus on some consequences of these changes. In particular, high-speed synchronous muscles consume large amounts of energy, but not to the extent one might expect, and they are, somewhat surprisingly, relatively weak.

Low myofibrillar volume

The force production and power output of a given volume of muscle depends, in part, on how much of the muscle is composed of contractile machinery. A muscle cell is made up of myofibrils (the contractile machinery), SR, mitochondria, T-tubules and surface membrane, and to a lesser extent other intracellular organelles and undifferentiated cytoplasm. It is the first three elements that largely compete for intracellular space. Hypertrophy of one must come at the expense of the others (Josephson, 1975; Schaeffer *et al.*, 1996; Block, 1994), and muscles that operate at high frequencies have sacrificed myofibrils in exchange for machinery that supports fast contractions.

A brief twitch requires a brief calcium transient, which in turn is facilitated by having many calcium pumps and thus a large volume of SR (Rosenbluth,

1969; Mendelson, 1969; Josephson, 1975; Ferguson and Franzini-Armstrong, 1988; Appelt *et al.*, 1991; Rome and Lindstedt, 1998). In synchronous tymbal muscles of cicadas there is a good correlation between the calling frequency used (*i.e.*, the need for a brief calcium transient) and the volume of the cell occupied by SR (Josephson, 1975). SR occupies a remarkable 34% of the cell volume in the tymbal muscles of the extremely high-frequency caller *O. vanduzeei* (Josephson and Young, 1985). Similarly, about 30% of the volume of toadfish swimbladder cells is SR (Appelt *et al.*, 1991) and 26% is SR in rattlesnake shaker muscle (Schaeffer *et al.*, 1996). The SR occupies only about 10% and usually less of the cell volume in muscles not used to produce sound, including the frog sartorius and bat cricothyroid which are considered quite fast by most standards (*e.g.*, Josephson, 1975; Rosenbluth, 1969; and references therein). Not surprisingly, the volume of the muscle occupied by myofibrils is inversely related to calling frequency. Myofibrils occupy about 41% of the cell volume in the tymbal muscles of the low frequency caller *Arunta perulata* (Young and Josephson, 1983, 1984) but only 22% in the tymbal muscles of the high-frequency caller *O. vanduzeei* (Josephson and Young, 1985). The high frequency muscles, with less myofibrillar volume, are thus expected to be weaker (Josephson, 1984).

The volume of the muscle occupied by mitochondria appears more related to the requirement for sustained power production than to the frequency of contraction (Josephson, 1975; Rome and Lindstedt, 1998). Toadfish, which call only intermittently, dedicate only 4% of their swimbladder muscle cell volume to mitochondria, which is even less than in typical fast muscles of vertebrates (Appelt *et al.*, 1991). In accord, their metabolic enzyme profiles indicate a relatively low aerobic and high anaerobic capacity (Walsh *et al.*, 1987). In contrast, the shaker muscle of rattlesnakes, which can operate for hours without pause, contain an impressive 26% mitochondria by volume (Schaeffer *et al.*, 1996). The mitochondrial volume of cicada tymbal muscles is also extremely high, ranging between 34–42% in 11 cicada species studied, and is again seemingly not related to the calling frequency used by a particular species (Josephson and Young, 1985). Like the shaker muscle, tymbal muscles in cicadas are often used for prolonged periods of calling, and the uniformly high mitochondrial volumes likely reflect this requirement for sustained activity. High-frequency, stridulating muscles of katydids also have a mitochondrial volume of over 40% (Elder, 1971).

Fast cross-bridges

Rapid relaxation of muscle requires rapid cross-bridge detachment. In the cross-bridge model of Huxley (1957) cross-bridges attach and detach with defined rates. The rate that they detach limits the maximal velocity at which a muscle can shorten, and this velocity can thus be used as a relative predictor of the cross-bridge detachment rate. Toadfish swimbladder

muscle at 16°C has a maximal shortening velocity that is nearly double that of fast muscles from frogs and lizards, and rattlesnake shaker muscle at 35° has a maximal shortening velocity nearly double that of swimbladder muscle (Rome *et al.*, 1996). These high speed muscles have adopted very fast cross-bridge detachment rates, perhaps to allow faster relaxation.

However, the consequences of having fast cross-bridges go further than simply conferring a high shortening velocity and fast relaxation. Fast cross-bridge detachment appears also to limit force. We expect fast muscles to be relatively weak because more of their cell volume is dedicated to the overhead associated with high frequency contractions (*i.e.*, more SR and perhaps mitochondria). But even after accounting for the amount of myofibril present, muscles that operate at very high frequencies or that have very brief twitches (*i.e.*, muscles that have fast cross-bridges) generate considerably less force than other muscles (Josephson, 1984 and references therein; Rome *et al.*, 1999).

Toadfish swimbladder muscles generate about 56 kNm⁻² myofibrillar cross-section, while the slower red and white trunk muscles, used for locomotion, generate over 200 kNm⁻² (Rome *et al.*, 1999). These forces are recorded from "skinned" fibers that are maximally activated, so the force deficit is not due to an inability to maximally activate the high-speed muscle. Further, white, red and swimbladder muscles have about the same number of cross-bridges on their myosin filaments, and the force generated by each cross-bridge is about the same in all three muscle types (Rome *et al.*, 1999). Thus, the low force must be due to swimbladder muscles having fewer cross-bridges attached when the muscle is activated. Comparison of muscle stiffness during maximal activation and during rigor (when all of the cross-bridges are attached) reveals that between 60 and 70% of the total cross-bridge population is attached when red and white muscles are active, while in swimbladder muscle only about 10% are attached (Rome *et al.*, 1999).

The low proportion of attached cross-bridges in the swimbladder muscle is a consequence of the detachment rate of cross-bridges being 10 times higher than in white muscle and 39 times higher than in red muscle. The attachment rate is similar in all three muscle types. An increase in the detachment rate without a concomitant increase in the attachment rate results in fewer cross-bridges being attached at any given moment (Rome *et al.*, 1999), in turn resulting in a low force per cross-section of muscle or myofibril.

Thus the ability to contract and relax at very high frequencies comes with a mechanical price. The requirement of very fast cross-bridge detachment rates for rapid relaxation results in relatively few cross-bridges being attached at any instant. The requirement for a brief calcium transient results in a high volume of SR and thus less room for contractile proteins. Both lead to muscle that is fast, but weak (Rosenbluth, 1969; Mendelson, 1969; Josephson, 1975, 1984; Frueh

et al., 1994; Rome *et al.*, 1999, Rome and Lindstedt, 1998).

An energetic price?

It is now known that increasing the detachment rate of cross-bridges is important for making muscles fast, and that this contributes to the muscles being weak. It is not so clear why the attachment rate of cross-bridges has not kept pace with the detachment rate, as it appears to have done in most other muscles, and which would allow the muscle to maintain high levels of force. But it may have a basis in the costs of contraction.

ATP hydrolysis is a pre-requisite for cross-bridge detachment, thus high rates of cross-bridge detachment will translate into high rates of ATP consumption. The ATPase rate of cross-bridges in toadfish swimbladder muscle is about 6 times higher than that in slow, red muscle (Rome *et al.*, 1999). In addition, the swimbladder generates only about 1/4 the force of red muscle per myofibrillar cross-section. So swimbladder myofibrils are over 20 times less economical at generating force than they are in red muscle (about 7 times less economical than in white muscle). If the cross-bridges in swimbladder muscle had an attachment rate sufficiently high that force was the same as in red muscle, swimbladder would then be almost 40 times less economical than red muscle.

Perhaps the reason for the low attachment rate of cross-bridges in swimbladder, and presumably in other very fast muscles, is to avoid this extreme cost. It does not require a lot of power or force to produce sound. Thus the close coupling between rates of cross-bridge attachment and detachment, prevalent among most muscles, has been averted in swimbladder, allowing them to operate at very high frequencies but with low force and improved economy. While the economy of swimbladder muscle is still less than that of the slower red or white muscles, it is considerably better than had it retained the unnecessary capacity to generate high forces. The energetic savings associated with down-regulation of force production when it is not required seem to be nontrivial, as suggested by the important ecological and evolutionary benefits in matching capacity with demand in synchronous flight muscles (Marden *et al.*, 1998, 1999). The ability to modulate force and power production without compromising twitch speed, via molecular alterations affecting cross-bridges and troponin, may in fact be a clever achievement rather than a trade-off.

Just how inefficient might synchronous muscles that are designed to work at high frequencies be? The comparisons above reflect only the cost of generating force by the cross-bridges and exclude the cost associated with pumping calcium, which normally account for 25–40% of the total cost of contraction (Rome and Klimov, 2000). Based on the large size and the short duration of the calcium transient in swimbladder muscle, it has been estimated that the average uptake rate of calcium into the SR of this muscle might be 50

times greater than it is in red muscle at their respective normal operating frequencies (Rome *et al.*, 1996). The cost of calcium pumping is related to the rate of release and removal and, when combined with the already high cost of operating the cross-bridges, one might expect the real cost of operating swimbladder muscle to be exorbitant. To the contrary, for an equivalent volume of muscle the steady-state ATP utilisation rates of swimbladder muscle are slightly less than those of fast-twitch frog muscle (Rome and Klimov, 2000). Further, the calcium pumps in “skinned” swimbladder muscle use no more energy, as a fraction of the total, than they do in other muscles (Rome and Klimov, 2000).

This remarkable ability, to operate at very high frequencies with very fast cross-bridge cycling and very brief calcium transients yet use no more energy than typical fast muscles, is attributed to three qualities (Rome and Klimov, 2000). 1) A relatively small volume of swimbladder muscle is actually occupied by myofibrils (about 50%, Appelt *et al.*, 1991); thus although the cross-bridges have high ATPase rates there are not many of them. 2) Of this small population of cross-bridges, very few (about 10%) are actually attached when the muscle is active. 3) The muscle has developed mechanisms to reduce the duration of the calcium transient without requiring high rates of energy use. The high calcium pumping rate cited above assumes that the SR calcium pumps are the only mechanism to lower free, cytoplasmic calcium levels. As mentioned, high speed muscles have high levels of parvalbumin and appear to use this as an important mechanism to sequester calcium from the cytosol. These muscles can thus have very brief calcium transients without the need for extremely high rates of calcium pumping (Rome and Klimov, 2000).

It is not known if calcium pumping rates in skinned muscle cells (Rome and Klimov, 2000) faithfully reflect the rates in living cells operating at high frequencies. Thus, it remains to be validated if the energetic cost of operating high-frequency muscles (swimbladder specifically) is truly as modest as it appears. The metabolic rate of rattlesnake shaker muscles is in fact quite high; higher than for other ectotherms and higher than in the muscles of some endotherms, although the actual cost per contraction is among the lowest observed for vertebrates or invertebrates (Schaeffer *et al.*, 1996).

High speed synchronous muscles are thus no doubt expensive to operate, but because of their unique modifications they are certainly less expensive than expected based on extrapolation from the performance of slower muscles.

ASYNCHRONOUS MUSCLES

A half-century ago studies by Pringle (1949) and by Roeder (1951) established that two, fundamentally different mechanisms are used in different insects to control repetitive contractions of the main wing muscles. In many insects the control of flight muscle is like that

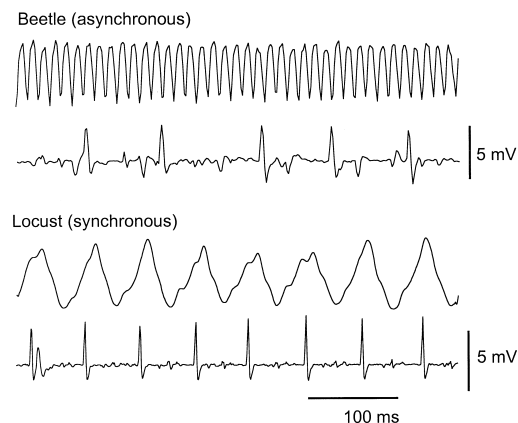


FIG. 3. Wing thrust (upper trace of each pair) during tethered flight and associated muscle action potentials (lower trace) from an asynchronous and a synchronous flight muscle. The asynchronous muscle was the basalar muscle of the beetle *Cotinus mutabilis*; the synchronous muscle the metathoracic tergo-sternal muscle of the locust *Schistocerca americana*. The animals were suspended from a force transducer that recorded the thrust developed by the beating wings. Action potentials were recorded with silver wires implanted in the relevant flight muscle.

in vertebrate skeletal muscle in that each contraction is initiated by neural input to the muscle and resulting action potentials or bursts of action potentials (Fig. 3). In other insects neural input and resulting muscle action potentials are needed to turn on a wing muscle, but when activated the muscle can contract in an oscillatory fashion if it is attached to a mechanically resonant load such as is offered, in life, by the wings and thorax. The oscillation frequency is the resonant frequency of the load, and is typically different from, generally much higher than, the frequency of the activating muscle action potentials (Fig. 3). These muscles are referred to as asynchronous muscles, reflecting the lack of congruence between electrical and mechanical events.

The features of asynchronous muscles that allow oscillatory contraction are delayed activation following stretch and delayed deactivation following shortening (reviewed in Josephson *et al.*, 2000b). Because of stretch activation and shortening deactivation, a cyclically-contracting muscle develops more force during shortening than it did at equivalent lengths during the preceding lengthening phase. Thus more work is produced by the muscle during shortening than is required to relengthen it following shortening, and there is net positive work output over a complete shortening-lengthening cycle; work that, in an intact insect, is available to drive the wings or, in some cicadas, to operate the tymbals. The work output does not come for free. Oscillation in asynchronous muscles, like contraction in synchronous ones, is powered by ATP hydrolysis (Pybus and Tregear, 1975). The metabolic rate of an active, oscillating asynchronous wing muscle is about 2 orders of magnitude greater than for the muscle at rest (Josephson *et al.*, 2001).

Asynchronous flight muscle occurs in several of the

TABLE 1. *Properties of flight muscles from a locust (Schistocerca americana) and a beetle (Cotinus mutabilis).*

	Locust (synchronous)	Beetle (asynchronous)
Wing beat frequency during free flight, Hz	20	90
Twitch duration, onset to 50% relaxation, 30°C, ms	40	125
Power, W kg ⁻¹	42 (30°C)	144 (35°C)
Efficiency	6%	15%

Data from Josephson *et al.*, 2000a, 2001. The locust muscle was the metathoracic second tergocoxal, the beetle muscle the metathoracic basalar.

more speciose insect orders, including beetles, true bugs, wasps and bees, and dipteran flies. Approximately three-quarters of known insect species have asynchronous flight muscles; hence most animals that fly do so using asynchronous muscles. Asynchronous muscle also powers sound production in some but not all cicadas (Josephson and Young, 1981). The distribution of asynchronous muscle among extant insect taxa suggests that this mode of control has evolved independently some 7–10 times (Dudley, 2000).

For aerodynamic reasons there is an inverse relationship between the size of a flying animal and the wing-stroke frequency needed to keep it in the air. Insects are small and their normal wing stroke frequencies are high, ranging from a few Hz in large butterflies to 1,000 Hz or more in small midges (Sotavalta, 1947). Insects that power flight with synchronous muscles presumably achieve their high operating frequencies using the same kinds of muscle modifications, with the same drawbacks and inefficiencies, as do the high-frequency sound producing muscles considered above. Asynchronous muscles, when oscillating, do not need to be turned on by neural input on each contraction. Only low-frequency neural input is required to keep an asynchronous muscle active and oscillating. The non-oscillatory contraction kinetics of asynchronous muscles are rather slow. Their isometric twitches are quite long (Table 1) and, as would be expected from this, there is very little sarcoplasmic reticulum within the fibers (volume density = 2–3%, see Fig. 4). Although it has not been measured directly, it may be inferred from the long twitch duration and the low frequency of muscle action potentials that the rate of calcium cycling during oscillatory contraction is low. It is clear that high frequencies in asynchronous muscles are achieved without hypertrophy of the sarcoplasmic reticulum, and without rapid cycling of calcium between sarcoplasmic reticulum and myofibrils and its attendant cost.

Because there is not major investment in sarcoplasmic reticulum in asynchronous muscles, a greater fraction of their volume is available for myofibrils, which are the contractile elements, than is true for high-frequency, synchronous muscles. Therefore it would be predicted that power output per unit muscle volume would be greater in a high-frequency, asynchronous muscle than in a high frequency, synchronous one. Further, the asynchronous mode of operation achieves high-frequency contraction without massive calcium

cycling and its high cost. Therefore it would also be predicted that asynchronous muscles should be more efficient in doing work than are high-frequency synchronous ones. Both these predictions are met, at least in the two examples of insect flight muscles, one synchronous and the other asynchronous, for which we have appropriate data (Table 1).

It is not known whether stretch activation and shortening deactivation allow asynchronous muscles to operate at high frequencies without requiring extremely high detachment rates, and the associated low forces, but it is quite possible that this is so. The asynchronous flight muscles of *Drosophila* have an unusual extension on the regulatory light chains of myosin, which appears to be associated with the pronounced stretch activation characteristic of asynchronous muscle. Site directed mutagenesis of a single amino acid in this protein (phosphorylatable serine to nonphosphorylatable alanine) results in a large drop in the fly's ability to accelerate upwards, a drop in maximum force and oscillatory power output, and notably, a 50% reduction in the degree of stretch activation with no effect on the kinetics of cross-bridges (reviewed in Maughan and Vigoreaux, 1999). The authors suggest that simply by de/phosphorylating the serine residue on the regulatory light chain the fly may be able to alter the characteristics and thus function of its muscles, from perhaps low power for courtship song to high power for flight. They retain the efficient design of asynchronous muscle to power high-frequency oscillatory behaviors, but have found a way to modulate force independently of cross-bridges kinetics and, like synchronous muscle, without slowing the contractions down.

It should be pointed out that there is a downside to the use of asynchronous rather than synchronous control of contraction, namely a reduction in the capacity for fine neural control. As indicated, in an oscillating asynchronous muscle the contraction frequency is typically several times greater than that of activating neural impulses, and there is not the possibility of cycle-to-cycle control of muscle force and power by variation in neural input as there is in a synchronous muscle. Further, the operating frequency itself in asynchronous muscle is determined principally by the resonant frequency of the load and is largely independent of the pattern of activating neural impulses. Asynchronous control works well for high power output during high-frequency contraction, but it would not be

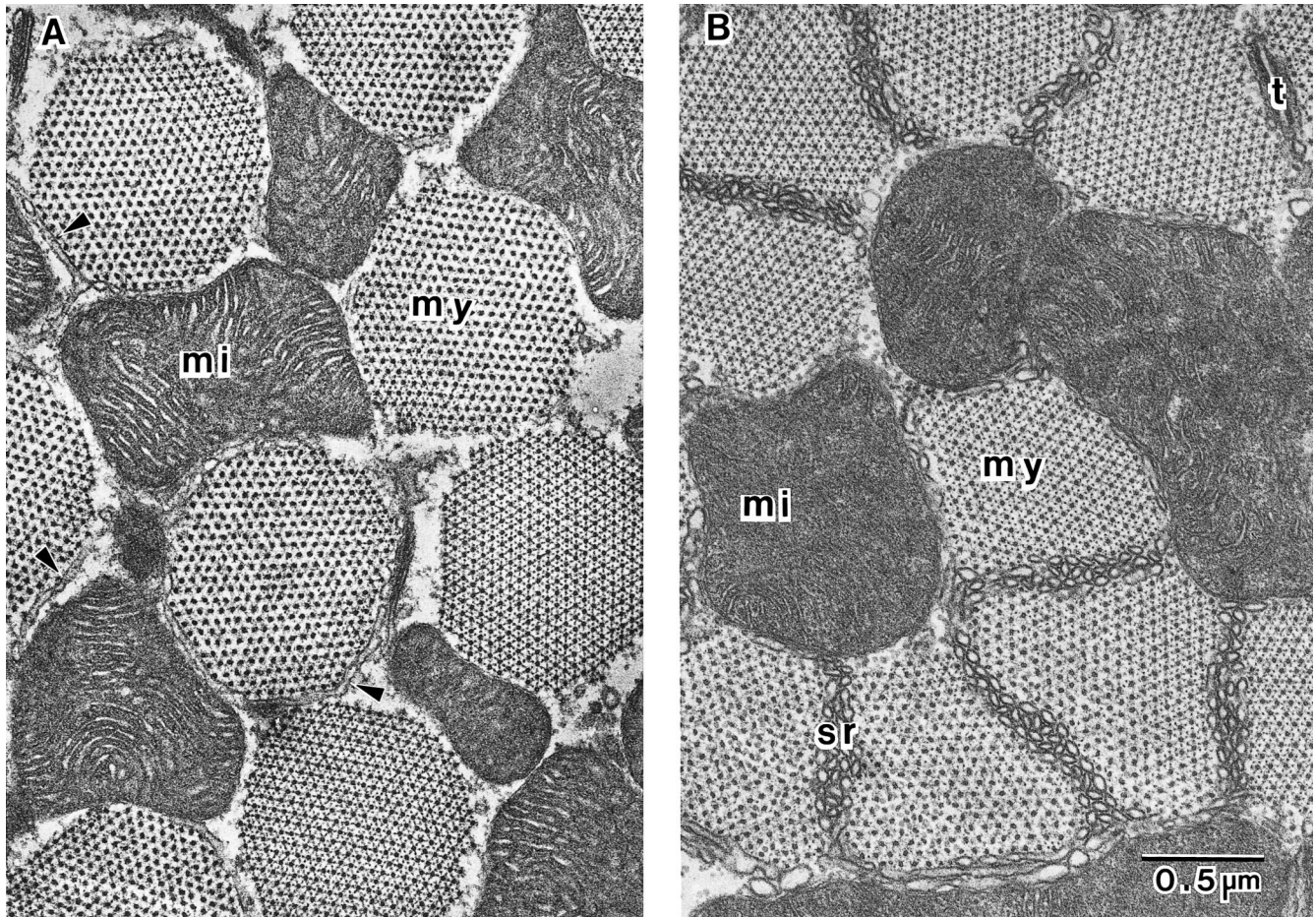


FIG. 4. Transverse electron micrographs of fibers from an asynchronous muscle (A) and a synchronous muscle (B). The asynchronous muscle is the tymbal muscle of the cicada *Platycleura capitata*; the synchronous muscle is the tymbal muscle of the cicada *Tamasa tristigma*. The contraction frequency of the tymbal muscle during song production is 390 Hz for *P. capitata* and 82 Hz for *T. tristigma* (Josephson and Young, 1981; Young and Josephson, 1983). The abbreviations are: mi-mitochondrion; my-myofibril; sr-sarcoplasmic reticulum; t-transverse tubule. Arrowheads indicate some elements of the sparse sarcoplasmic reticulum in *P. capitata*.

at all suitable for fine motor control while manipulating objects, nor for accurately maintaining body posture.

Asynchronous muscles almost certainly evolved from synchronous ones and they represent a design breakthrough. For high-frequency operation they are both more powerful and more efficient than their synchronous counterparts. It is presumably for these reasons that asynchronous control has been favored by evolution in several different insect lines. And it is presumably in part because of the presence of asynchronous muscles that insects have been so successful as terrestrial life forms.

ACKNOWLEDGMENTS

Supported by NSERC (DAS).

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