

# The Quest for Speed: Muscles Built for High-Frequency Contractions

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***Vertebrate sound-producing muscles can contract at frequencies greater than 100 Hz, a feat impossible in locomotory muscles. This is not accomplished by unique proteins or structures but by qualitative shifts in isoforms and quantitative reapportionment of structures. Speed comes with costs and trade-offs, however, that restrict how a muscle can be used.***

The vast array of different motor tasks performed by animals, from making explosive movements over ~50 ms, to swimming hundreds of miles in ocean migrations, to producing sound at several hundred hertz for communication, requires very different outputs from the muscles. Although one might anticipate marked structural and physiological differences underlying muscles' abilities to perform these different tasks, all vertebrate skeletal twitch fibers share the same basic structure and operate by the same contractile mechanism (8). There are, however, a number of important qualitative modifications (myosin, troponin, and Ca<sup>2+</sup> pump isoforms with different kinetic rates) as well as quantitative modifications [densities of sarcoplasmic reticulum (SR) and mitochondria] within muscle fibers, which underlie these differences in performance. Shifts in protein isoforms and muscle structure result in trade-offs that may maximize performance of one function at the expense of another, and which, in extreme cases, can lead to different designs that are mutually exclusive (i.e., a muscle designed to operate at high frequencies cannot operate effectively at low frequencies and vice versa).

Perhaps the most extreme set of modifications is seen in those muscles that operate at very high frequencies. Our laboratories, both in collaboration with each other and separately (in collaboration with other groups), have been examining modifications and trade-offs in two remarkable examples of rapid muscles—the sound-producing swim bladder muscle of toadfish (which is the fastest known vertebrate

muscle) and the tail shaker muscle of rattlesnake (certainly the most feared and one distinguished by its endurance).

## What's needed for speed?

The male toadfish (*Opsanus tau*) produces a "boat whistle" mating call 10–12 times/min for many hours to attract females to its nest. A tone is generated by oscillatory contractions of the muscles encircling the fish's gas-filled swim bladder at 200 times/s. Such high-frequency stimulation of typical locomotory muscles (which relax relatively slowly) would produce a completely fused (i.e., constant force) tetanus because they would be unable to relax between stimuli. Because maintained tension would simply compress the bladder and prevent it from vibrating, sonic muscles must be specifically modified to turn on and off rapidly.

To better understand these modifications, we (9) examined the changes in physiological properties of different fiber types from toadfish because the muscle activation-relaxation rates vary by ~50-fold between locomotory and sonic fiber types. Toadfish red muscle (used for slow, steady swimming at ~2Hz) has a twitch half-width (time duration at the 50% force level) of ~500 ms, compared with ~200 ms for white muscle (used for burst swimming at ~5Hz) and ~10 ms for the swim bladder muscle (Fig. 1A, top).

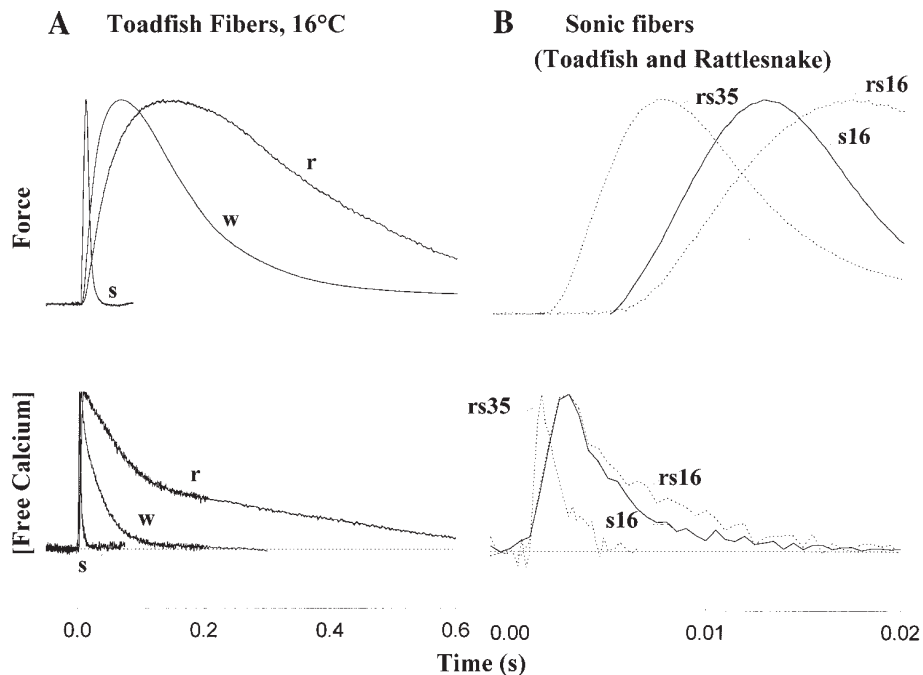
For any muscle to activate and relax rapidly, two conditions must be met. First, calcium, the trigger for muscle contraction, must enter the myoplasm rapidly and be removed rapidly (Fig. 2, steps 1 and 4). Second, myosin cross bridges must attach to actin and generate force soon after the calcium level rises and then detach and stop generating force soon after the calcium level falls (Fig. 2, steps 2, 3, 5, and 6).

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*"...muscles must be specifically modified to turn on and off rapidly."*

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**FIGURE 1.** Twitch tension (*top*) and  $\text{Ca}^{2+}$  transients (*bottom*) of 3 fiber types from toadfish at 15°C (A). In each case, force and  $\text{Ca}^{2+}$  records have been normalized to their maximum value. The twitch and  $\text{Ca}^{2+}$  transient become briefer going from slow-twitch red fiber (r) to fast-twitch white fiber (w) to superfast-twitch swim bladder fiber (s). B: force and  $\text{Ca}^{2+}$  records for sonic fibers and swim bladder at 16°C (solid lines) and rattlesnake at 16°C and 35°C (rs; dotted lines). Note that time scale is expanded by  $\sim 30\times$  in B. Adapted from Ref. 9.

In collaboration with Steven Baylor's laboratory, we determined the time course of  $\text{Ca}^{2+}$  release and reuptake during contractions by injecting muscle cells with a fluorescent  $\text{Ca}^{2+}$ -sensitive dye (fura-2) and tracking fluorescence with time. We found that the  $\text{Ca}^{2+}$  transient in the sonic muscles is the fastest ever measured for any fiber type (a half-width of  $\sim 3.4$  ms at 16°C and 1.5 ms at 25°C). The importance of the  $\text{Ca}^{2+}$  transient duration in setting the twitch duration can be seen in Fig. 1A, which shows that both durations sped up in parallel by  $\sim 50$ -fold between the slow-twitch red fibers and the superfast-twitch swim bladder fibers.

The significance of a fast  $\text{Ca}^{2+}$  transient is most apparent during repetitive stimulation. During stimulation of slow red muscle at a modest 3.5 Hz (Fig. 3), the time course of  $\text{Ca}^{2+}$  uptake is so slow that  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]$ ) does not have time to return to baseline between stimuli. Even the lowest myoplasmic  $[\text{Ca}^{2+}]$  between stimuli was above the threshold required for force generation in this fiber type, thus resulting in a partially fused tetanus. By contrast, the swim bladder's  $\text{Ca}^{2+}$  transient is so rapid that even with 67-Hz stimulation, the  $[\text{Ca}^{2+}]$  return to baseline between stimuli is complete (Fig. 3; note the  $50\times$  faster time base). In addition,  $[\text{Ca}^{2+}]$  is below the threshold for force generation more than one-half of the time in all but the first stimulus. Hence, the  $\text{Ca}^{2+}$  transient is sufficiently rapid to permit the oscillation in force required for sound production.

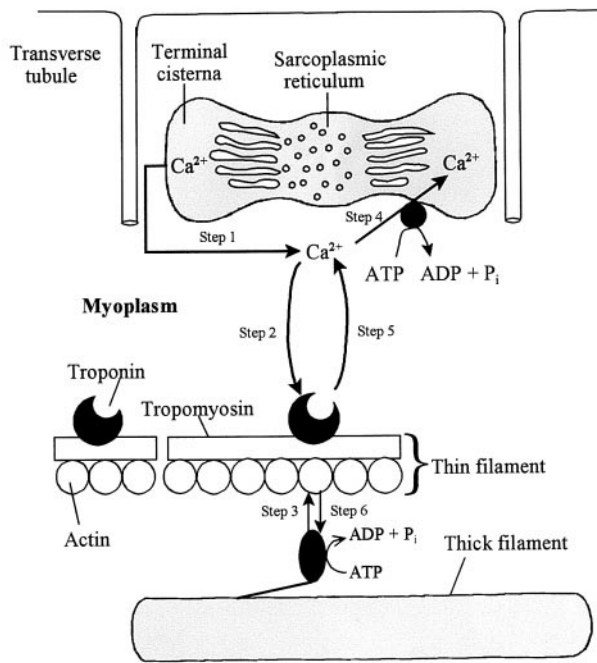
Even though  $[\text{Ca}^{2+}]$  returns rapidly to baseline, the swim bladder fiber could not relax quickly unless its troponin rapidly released bound  $\text{Ca}^{2+}$  (Fig. 2, *step 5*). Indeed, kinetic modeling (9) indicates that if the swim bladder troponin had the same off-rate for  $\text{Ca}^{2+}$  ( $k_{\text{off}}$ ) estimated for fast-twitch fibers of frog ( $115 \text{ s}^{-1}$ ), then occupancy of its troponin sites with  $\text{Ca}^{2+}$  would not decline sufficiently rapidly to permit the observed rapid fall in force. To assess possible modifications in the  $\text{Ca}^{2+}$ -troponin control system, the force-pCa relationship (force as a function of  $[\text{Ca}^{2+}]$ ) was measured in permeabilized ("skinned") muscle fibers. A right shift (decreased  $\text{Ca}^{2+}$  sensitivity) was found in the curves for the white fibers with respect to the red fibers, and an even lower  $\text{Ca}^{2+}$  sensitivity was found for the superfast fibers. On the basis of the threefold right shift of the force-pCa curve of swim bladder fibers with respect to frog fibers, these results suggest that  $k_{\text{off}}$  of swim bladder troponin is likewise three times faster than that of frog. With this higher  $k_{\text{off}}$ , the modeled rate of troponin deactivation no longer appears limiting. Thus the less-sensitive troponin with its more rapid  $k_{\text{off}}$  may be another modification for faster relaxation (9).

The final requirement for force to drop quickly after the dissociation of  $\text{Ca}^{2+}$  from troponin is a fast cross-bridge detachment rate (Fig. 2, *step 6*). Indeed, the maximum velocity of shortening ( $V_{\text{max}}$  which is thought to be affected by cross-bridge detachment rate) of swim bladder muscle

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*"The final requirement... is a fast cross-bridge detachment rate..."*

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**FIGURE 2.** Major kinetic steps in muscle activation and relaxation. Activation: *step 1*,  $\text{Ca}^{2+}$  is released from sarcoplasmic reticulum (SR) into myoplasm; *step 2*,  $\text{Ca}^{2+}$  binds to troponin, releasing inhibition of thin filament; *step 3*, cross bridges then attach and generate force. Relaxation: *step 4*,  $\text{Ca}^{2+}$  is resealed from myoplasm by  $\text{Ca}^{2+}$  pumps; *step 5*,  $\text{Ca}^{2+}$  comes off troponin, thereby preventing further cross-bridge attachment; *step 6*, cross bridges then detach. For a muscle to relax rapidly, *steps 4–6* must all be very fast.

[~12 muscle lengths (ML)/s] is exceptionally fast, 5- and 2.5-fold faster than toadfish red and white muscle. Recently, in collaboration with Yale Goldman's laboratory, we measured the cross-bridge detachment rate directly and found that it is exceptionally fast (~170  $\text{s}^{-1}$  or ~70 times faster than the toadfish red fibers and the well-studied rabbit fast fibers). This fast detachment rate would permit the rapid relaxation rate observed (5).

The toadfish experiments have thus identified three kinetic variables that change progressively as twitch speed increases from the slow twitch of red fibers to the superfast twitch of swim bladder fibers. 1) The duration of the  $\text{Ca}^{2+}$  transient must become shorter, which in turn requires more rapid  $\text{Ca}^{2+}$  release and reuptake. As discussed in more detail below, this is achieved principally by an increased density of SR  $\text{Ca}^{2+}$  pumps (1). 2) Troponin needs a faster off-rate for  $\text{Ca}^{2+}$ , which requires molecular modification of troponin to a lower affinity type. 3) Cross bridges must detach more rapidly, which involves molecular modification of myosin (5).

Changes in all of these parameters in concert enable swim bladder fibers to perform mechanical work at high operating frequencies. Sound production requires mechanical work to produce sound energy and to overcome frictional losses in the sound-producing system. Work-loop experiments show that swim bladder fibers can perform work at oscillation frequencies in excess of 200 Hz at 25°C, the highest frequency for work production recorded for any vertebrate muscle (9).

By contrast, locomotory muscles lack the combination of fast  $\text{Ca}^{2+}$  pumping and fast cross-bridge cycling necessary to generate work at these high frequencies. The highest frequency for vertebrate locomotory muscles is an order of magnitude lower, 25–30 Hz for mouse and lizard fast-twitch muscles at 35°C, whereas the red and white muscle of toadfish cannot generate work at >15 Hz.

### Rattling in snakes

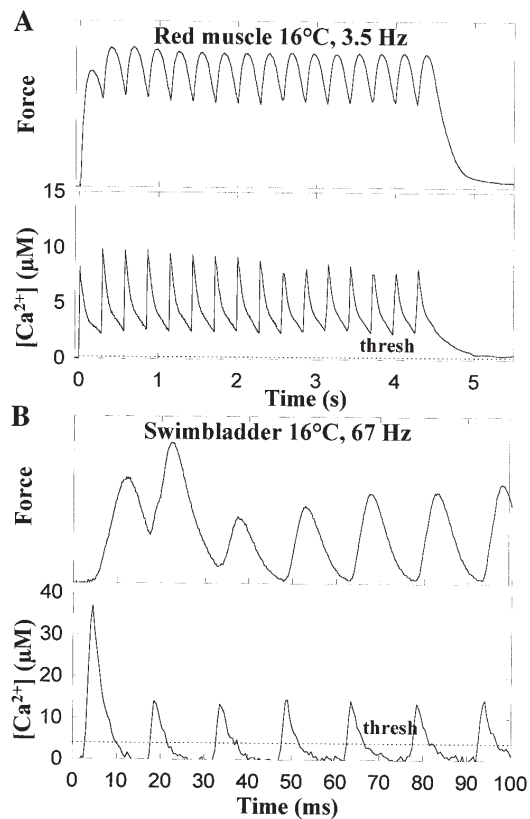
Rattlesnakes provide us with another example of a muscle design for sound production and with further evidence that all three of the above modifications are necessary for high-frequency contractions. Rattlesnakes in the genus *Crotalus* use sound produced by rattling their tails as a loud and effective warning device that makes these animals, like many venomous animals, very conspicuous. Sound is produced by the temperature-dependent contraction of the tail shaker muscles at frequencies up to 90 Hz.

At 16°C, the shaker fibers have a very rapid  $\text{Ca}^{2+}$  transient (half-width = 4–5 ms), which is only 1–2 ms slower than that of the swim bladder (Fig. 1B, top). However, the twitch duration of the shaker muscle twitch is far longer (half-width = 25 vs. 10 ms) than that of swim bladder (Fig. 1B, top). This slower twitch likely reflects the effects of a slower cross-bridge detachment (the shaker  $V_{\text{max}}$  of ~7 ML/s is only about one-half that of the swim bladder) and probably a slower

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*"Rattlesnakes... use sound produced by rattling their tails..."*

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**FIGURE 3.** Force production and  $\text{Ca}^{2+}$  transients during repetitive stimulation. *A*: slow-twitch red fiber stimulated at 3.5 Hz. Threshold  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]$ ) for force generation was derived by force-pCa experiment on skinned fibers and is shown with a dotted line. *B*: swim bladder fiber stimulated at 67 Hz.  $[\text{Ca}^{2+}]$  threshold for force production is much higher for swim bladder than for red fiber; note different scale for ordinate. Adapted from Ref. 9.

troponin  $k_{\text{off}}$ . This analysis suggests that in fact all three systems, not just the  $\text{Ca}^{2+}$  transient, must be very rapid to produce the fastest contractions. Thus, at 15°C, shaker fibers can be stimulated up to only ~20 Hz before force begins to summate. Accordingly, rattling frequency at 15°C is only ~30 Hz, although unlike toadfish, these snakes may never be active at temperatures this low.

To provide faster contractions and rattling frequencies, these reaction rates must be sped up. This is accomplished by an increase in muscle temperature. At 35°C, at which snakes rattle at 90 Hz, the  $\text{Ca}^{2+}$  transient (Fig. 1) and  $V_{\text{max}}$  (18 ML/s) are even faster than those of the swim bladder muscle at 15°C. In addition,  $k_{\text{off}}$  is likely very rapid as well. The resulting briefer twitch (half-width = 8–9 ms; Fig. 1B) enables the shaker fibers to be stimulated at high frequencies without complete fusion of force and to perform the requisite mechanical work at 90 Hz. The similarity of the modifications in this sound-producing muscle to those in toadfish is suggestive of convergent evolution, supporting the contention that these modifications are necessary for high-frequency operation. Although somewhat slower than the swim bladder at a given

temperature, the shaker muscle can operate continuously for hours, which, as we will see, requires additional modifications.

### Speed costs—the trade-offs for speed

Building muscle to operate at high frequencies requires not only “qualitative” modifications in key muscle protein isoforms but also quantitative modifications in the amount of structure. These modifications are compounded when the muscle is used continuously. Collectively, these modifications result in important trade-offs both in energetic cost and in space. Because these trade-offs greatly restrict the force, frequency, and endurance range over which a given muscle fiber type can be effectively used, they can lead to mutually exclusive designs.

### The trade-off for energetic cost of contraction

The energetic cost of contractions is set by two components, the ATP used by  $\text{Ca}^{2+}$  pumps and the ATP used by the cross bridges (Fig. 2). Fibers used for high frequencies have extraordinarily high ATP utilization rates when active because the rate of ATP utilization by both of these components is extremely rapid. For instance, swim bladder fibers appear to pump  $\text{Ca}^{2+}$  back into the SR at ~50 times the rate of red muscle (9). Thus the swim bladder fiber  $\text{Ca}^{2+}$  pumps use ATP 50-fold faster than those of the red muscle. We have further found that the cross bridges of swim bladder fibers use ATP about six times faster (when expressed on a volume basis) than red muscle fibers (5). Thus, during rapid stimulation (where  $\text{Ca}^{2+}$  is being continuously pumped and cross bridges are continuously generating force and splitting ATP), the swim bladder will use ATP at a rate between 6- and 50-fold faster than the red muscle (because the relative contribution of  $\text{Ca}^{2+}$  pumps and cross bridges to the total ATP utilization is unknown, only a range can be given.)

### Trade-off for space—the zero-sum game

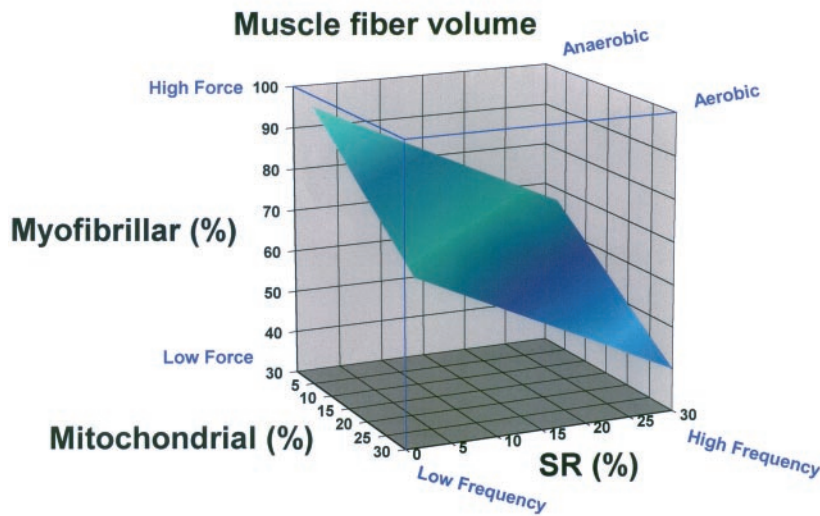
Although many of the adaptations for performance are qualitative (i.e., different isoforms of myosin and troponin with different kinetic rates), many of the modifications are quantitative; that is, they are dependent on the apportionment of different structures in the muscle cell. Different structures in muscle are necessary for different functions—thus competition for space can greatly impact muscle performance.

Only a very small volume of the muscle fiber is typically devoted to fuel storage. The combined volume of lipids and glycogen is usually

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“...these modifications result in important trade-offs...”

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**FIGURE 4.** Skeletal muscle is composed of 3 components (myofibrils, SR, and mitochondria), which together comprise 100% (less a small volume devoted to lipid and glycogen fuel) of muscle fiber volume. Each of these elements is responsible for a different aspect of muscle function: myofibril volume sets force generation, SR volume sets frequency of operation, and mitochondrial volume sets aerobic ATP synthetic rate. In this 3-dimensional graph, all muscle fiber types must exist as 1 point on the depicted plane. According to this graph, continuous operation at high frequencies seems to preclude the high force (and thus power generation) necessary to fly. Adapted from Ref. 7.

under 3%. The remainder of the muscle fiber volume is occupied by three structures—myofibrils, SR, and mitochondria in various proportions. Because these structures collectively make up nearly 100% of the fiber, every muscle fiber type must exist as a point on a single plane of a three-dimensional graph depicting these muscle components (Fig. 4). Hence, any increase in one structure (and its concomitant function) must be at the expense of another.

In typical muscle fibers, myofilaments make up the vast majority of the cell—often up to 90% of the fiber volume. As the force per cross section generated by muscle is thought to be proportional to the number of cross bridges working in parallel, this quantitative feature sets force production. Although myofibrils form the basic structure of muscle, the ability to turn muscles on and off quickly or to produce prolonged activity requires that myofibril volume (and hence force) be reduced in favor of the two other main structures.

The SR is important for turning muscle on and off. The speed of relaxation, and more particularly, the speed at which  $\text{Ca}^{2+}$  is pumped back into the SR, is critical in setting the maximum frequency at which a muscle can operate. Although there are different  $\text{Ca}^{2+}$  pump isoforms (named SERCA1 and SERCA2; Ref. 12), in general the speed at which  $\text{Ca}^{2+}$  is pumped back into the SR is set by the number of  $\text{Ca}^{2+}$  pumps. Because the  $\text{Ca}^{2+}$  pump density on the SR membrane is often maximal in fast fibers, the number of  $\text{Ca}^{2+}$  pumps is tightly correlated to the volume taken up by the SR. Thus the swim bladder fibers' ability to sequester  $\text{Ca}^{2+}$  50 times faster than red fibers requires a high SR

volume (30%) (1). Similarly, the SR volume in shaker fibers of rattlesnakes is ~26% (10).

Finally, if the activity is to be sustained rather than intermittent, aerobic metabolism is required to replenish the ATP used during contraction. The amount of  $\text{O}_2$  consumed and ATP produced is again tightly coupled to the mitochondrial volume. In mammals, in which it has been studied most extensively, mitochondria on average consume 5 ml  $\text{O}_2$  per  $\text{cm}^3$  of mitochondria per minute. Assuming a P-to- $\text{O}_2$  ratio of 6, this is equivalent to an ATP production rate of 1.3 mM ATP· $\text{cm}^3$ · $\text{min}^{-1}$ . The requirement of continuous operation obviously requires increased mitochondria volume, which in turn comes at the cost of space for myofibrils. Toadfish call intermittently. Hence, they do not rely heavily on aerobic metabolism, and only 4% of their muscle volume is devoted to mitochondria (1). By contrast, rattlesnakes shake their tails continuously for hours at a time, requiring a very high mitochondrial density—26% (10).

In a sense, cellular structures are competing for space in muscle fibers. Increases in SR and mitochondrial volumes necessarily translate into reduced myofibrillar volume and hence, lower force. In muscle fibers used at low frequencies, however, the rate of  $\text{Ca}^{2+}$  uptake is sufficiently slow, so that relatively little SR is required, thus effectively removing the SR from the space competition. Furthermore, because the SR uses little ATP, few mitochondria are necessary to supply ATP to the  $\text{Ca}^{2+}$  pumps. Thus, even in highly aerobic muscle such as the slow-twitch red muscle of swimming fish, the myofilament volume is

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*“...small volume of the muscle fiber is... devoted to fuel storage.”*

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*“...high performance in certain attributes precludes...”*

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reduced only to ~70%—still allowing for significant force generation.

In contrast, the “zero-sum game” becomes a significant factor in muscles designed to operate at high frequencies, both because of the large volume that must be devoted to SR and because of the large number of mitochondria necessary to supply ATP to the  $\text{Ca}^{2+}$  pumps and the cross bridges. For instance, the aerobic, high-frequency shaker muscle of rattlesnake, because of the large volume devoted to SR and mitochondria, has only 31% myofilaments (this muscle also contains an extraordinary amount of glycogen, which makes up the bulk of the ~17% of the muscle volume not accounted for by myofibrils, SR, and mitochondria). The faster (500 Hz) aerobic sound-producing muscle of cicadas (6) has even fewer myofibrils (22%). The swim bladder muscle of toadfish has a comparatively high myofibrillar volume, 50%, because very few mitochondria are required for its intermittent use (note that the fibers have an unusual annular deposition of myofilaments—the center of which is devoid of myofilaments; Ref. 1). In all three cases, the reduced myofilament volume results in reduction in force.

### Mutually exclusive designs

From the above discussion, one would anticipate that the price of operating at high frequencies is a far higher cost of force generation (ATP/force). This is caused by both the rapid rate of ATP utilization ( $\dot{A}TP$ ) and the low force produced. However, the force produced may be even lower than expected on the basis of the reduced volume of myofibrils because myosin designed to operate at very high speed may intrinsically generate low forces. In collaboration with Yale Goldman’s laboratory, we found that in toadfish swim bladder fibers the force generated per cross section of myofibrils is only about one-fourth that generated by locomotory fibers (5). As mentioned above, to relax quickly swim bladder fibers need a high myosin cross-bridge detachment rate constant ( $g$ ). In locomotory muscles, the attachment rate constant ( $f$ )  $> g$ , and 60–70% of the cross bridges are attached during isometric contraction, whereas in swim bladder fibers  $g \gg f$ , and thus only ~10% of the cross bridges are attached [the number of attached cross bridges is equal to  $f/(f + g)$ ]. Thus, although the force per attached cross bridge is similar for all fiber types, the force production in swim bladder fibers is very low because there are few attached cross bridges. Low force per cross section of myofibril also has been observed in synchronous sound-producing muscle of insects (6), suggesting that this finding may be a general phenomenon.

The fact that necessary trade-offs can lead to mutually exclusive designs is vividly illustrated in toadfish. Neither the red nor the white swimming muscle can physically produce high-frequency sounds because they cannot relax fast enough and thus produce a fused tetanus when stimulated at high frequencies (Fig. 3). By contrast, the combination of high energetic cost and low force production in swim bladder fibers makes their cost of generating force at least 30-fold higher than in red fibers. Hence, these fibers would be incapable of efficiently powering swimming movements that occur at low frequencies (2–5 Hz). Thus both the qualitative and quantitative modifications of muscle necessary for one activity likely make it unsuitable for another activity.

Furthermore, solutions to the competition for space within the fiber may exclude certain designs altogether. Because of the zero-sum game, high performance in certain attributes precludes high performance in other attributes; the underlying structures cannot physically occupy the same space (4, 7). For instance, as seen graphically in Fig. 4, muscle designed to generate the highest forces must necessarily be used at low frequencies and anaerobically (and thus be used intermittently). By contrast, muscles designed to operate continuously at high frequencies must necessarily generate low force. Thus, as shown in Fig. 4, high force (and power) output, continuous aerobic operation, and high-frequency operation are seemingly mutually exclusive. It would seem to be impossible for an animal’s muscle to have all three attributes—yet that is precisely what they must have to fly. How is it done?

### The design for continuous high power at high frequencies—beating the zero-sum game

The apparent trade-off for speed resulting in low force output is evidently adequate for sound-producing muscle where the force (and power) output per muscle weight is not crucial. This is not adequate, however, for the muscles of continuously flying animals, some of which operate at even higher frequencies. Because the mechanical power necessary for flight is so high, the flying muscles make up a substantial part of the animal’s mass and thus the power output per weight ratio becomes crucial. Because power output is the product of frequency, displacement, and force, and force in turn depends on myofilament volume and myosin isoform, a high power per weight ratio seems impossible for synchronous vertebrate muscle used continuously at high frequencies (Fig. 4).

Through evolutionary time, important strategies for circumventing the zero-sum game have

emerged. In collaboration with Kevin Conley's lab, we have been investigating three general ways that the "game has been beaten." The first two are found in vertebrates, whereas the third, and perhaps the most significant, evolved only in insects.

*Temperature increases rates for a fixed volume.* The rates of many biological reactions are very temperature sensitive. Thus any increase in operating temperature results in greater rates for the same amount of structure. This has great importance in two of the systems.

Because mitochondria have an apparent  $Q_{10}$  of 2.2, increasing body temperature from 30 to 40°C results in a 2.2-fold increase in ATP synthetic rates. Functionally, this is equivalent to doubling the effective volume density of mitochondria within the cell. Hence, one reason that many insects are unable to fly without warming up is that they are unable to produce ATP at an adequate rate to power flight (3). High temperature also permits  $Ca^{2+}$  pumps to pump faster, resulting in faster  $Ca^{2+}$  transients and faster relaxing muscles capable of operating at a higher frequency for a given SR volume (Fig. 1). This factor is no doubt important for high-wingbeat-frequency birds (i.e., hummingbirds, 40 Hz) and large insects using synchronous muscle contractions—but as we will see below, even more drastic measures are required for the smallest flying insects, which have frequencies approaching 1,000 Hz (4).

*Double-packed mitochondria.* In many flying animals, the sustained weight-specific power requirements of flight muscles are extreme. In hummingbirds, for instance, the flight muscles account for 25% of the animal's body mass and are composed of ~35% mitochondria. When one divides the total  $O_2$  consumption by the volume of mitochondria, they consume  $O_2$  at about twice the rate of mammals (7–10 vs. 4–5 ml  $O_2 \cdot cm^{-3} \cdot min^{-1}$ ) (11). If hummingbird mitochondria were identical to those of mammals, the flight muscle would have to contain ~70% mitochondria to produce the required ATP for flight, but this would not leave a sufficient volume of myofibrils to generate the required lift. Hummingbirds appear to have achieved the higher mitochondrial  $O_2$  consumption rate by having twice the packing of mitochondrial inner membranes (cristae) as is found in mammalian mitochondria. It remains a mystery why this "double packing" is not the norm for mitochondria in general. Importantly, all flying insects have densely packed mitochondria as well, allowing more space to be devoted to myofibrils (2).

*Asynchronous muscle.* Although the above two factors help to beat the zero-sum game, it is evidently still not adequate for animals to fly at the highest wingbeat frequencies. No animal can fly

at frequencies of >100 Hz with synchronous muscle (6). Perhaps the single greatest space- and energy-saving modification in skeletal muscle is the development of asynchronous muscle. In synchronous muscle, the muscle is activated by release of  $Ca^{2+}$  and deactivated by  $Ca^{2+}$  reuptake, that is, there must be one  $Ca^{2+}$  release and one  $Ca^{2+}$  reuptake for each cycle (Figs. 2,3). By contrast in asynchronous muscle, a single  $Ca^{2+}$  release can keep the muscle "turned on" for many activation-relaxation cycles. The muscle becomes activated by "stretch activation" and becomes deactivated by "shortening deactivation," all this in the presence of nearly constant myoplasmic  $[Ca^{2+}]$ .

As noted by Josephson and Young (6), insects possessing this type of muscle save space in two ways. The first is a direct saving of space that would otherwise be occupied by SR. For example, synchronous sonic muscles in cicadas, which operate at 500 Hz, have ~34% SR. Asynchronous muscles have <1/10th that volume of SR (6). The second savings is indirect. As noted above, a large amount of ATP is required to release and re-sequester  $Ca^{2+}$  at high frequencies, and this may rival the amount of ATP that cross bridges use. Thus, in continuously used synchronous muscles, a large volume of mitochondria must be present simply for supplying ATP to the SR for pumping  $Ca^{2+}$ . By slowing the  $Ca^{2+}$  pumping rate in asynchronous muscles by more than an order of magnitude, the space occupied by these mitochondria is freed up and can be replaced by both force-generating myofibrils and additional mitochondria whose sole function is supplying ATP for the cross bridges. In this regard it is interesting to note that flight muscles in all flying animals have at least 50% myofibrils (3).

Finally, reducing the amount of ATP used to power  $Ca^{2+}$  pumping not only saves space but also reduces energetic cost (6). This makes work generation during flight cheaper and thus more efficient. This, too, may be an important requirement from an ecological viewpoint.

To conclude, muscles are called on to power a vast array of motor activities. Muscles can be configured in an equally vast number of types by altering important protein isoforms, amounts of structure, and even modes of activation-relaxation (synchronous vs. asynchronous). Because of inherent design trade-offs, configuring a muscle to perform one activity necessarily reduces its ability to perform another activity. At the extremes, this leads to mutually exclusive designs. Thus no one muscle fiber type can perform all activities effectively—a fact that explains why muscle may show the greatest diversity of any tissue.

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*"... muscle becomes activated by 'stretch activation'..."*

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