ORIGINAL PAPER

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Power and control muscles of cicada song: structural and contractile heterogeneity

Abstract Sound production in cicadas is powered by a pair of large muscles whose contractions cause buckling of cuticular tymbals and thereby create sound pulses. Sound is modulated by control muscles that alter the stiffness of the tymbals or change the shape of the abdominal resonance chamber. Muscle ultrastructure and contractile properties were characterized for the tymbal muscle and two control muscles, the ventral longitudinal muscle and the tymbal tensor, of the periodical cicada *Magicicada septendecim*. The tymbal muscle is a fast muscle that is innervated by a single motoraxon. The control muscles are an order of magnitude less massive than the tymbal muscles, but their innervation patterns were considerably more complex. The tensor muscle is innervated by two axons, each of which evokes rather slow twitches, and the ventral muscle is innervated by at least six axons, some of which produce fast and the others slow contractions. Muscle contraction kinetics correlated well with ultrastructure. Fibers of the tymbal muscle and the portions of the ventral muscle thought to be fast were richly supplied with transverse tubules (T-tubules) and sarcoplasmic reticulum (SR); slow portions of the ventral muscle and the tensor muscle had relatively little SR.

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Introduction

Singing in cicadas (Insecta: Homoptera: Cicadidae) is a male specific behavior that functions mainly to attract female consorts. Sound production involves the coordinated activities of diverse body parts—the structures that produce the sound, the body chambers that act as sound resonators and radiators, and the muscles that power and control the sound system. The basic sound-producing structures are a bilateral pair of dome-shaped tymbals and associated tymbal muscles. The tymbal is a dorso-lateral specialization of the cuticle of the first abdominal segment. The detailed structure of the tymbal varies from species to species (Bennet-Clark 1997; Fonseca and Hennig 1996; Hagiwara 1955; Moore and Sawyer 1966; Myers 1929; Pringle 1954a, 1957; Reid 1971; Simmons and Young 1978; Weber et al. 1987; Young 1972; Young and Bennet-Clark 1995; Young and Josephson 1983a), but in general it consists of a flexible membrane reinforced with highly sclerotized ribs. The tymbal muscles are the power muscles of singing. They attach to the inner surface of the tymbals. Contraction of a tymbal muscle buckles the tymbal inward, producing a sound pulse. The tymbal muscles on the two sides typically are activated out of phase with one another during the normal calling song, so the overall sound pulse frequency is twice the contraction frequency of an individual muscle (Hagiwara 1955; Young and Josephson 1983a). Large tracheal air sacs in both the thorax and abdomen serve as resonators of the sound, which is radiated outward largely through a pair of thin diaphragms, the tympana, situated in the ventro-lateral floor of the meta-thorax. Finally, in many species the tympanum on each side is covered by an operculum, a ventro-lateral extension of the thoracic cuticle that also covers the membranes of the metathoracic-abdominal junction. The gap between the posterior edge of the operculum and the abdomen can be widened or narrowed by a raising or lowering of the abdomen. The position of the

abdomen also alters the volume of the air sacs, and the sound pulses that radiate from them.

If the tymbals and tymbal muscles alone were responsible for sound output, the songs of cicadas would be expected to consist of a series of sound pulses of unvarying pitch and amplitude. But cicadas produce modulated songs in patterns that are species specific and context specific (e.g., Pringle 1954b; Hagiwara 1955; Young 1972; Simmons and Young 1978; Fonseca 1991, 1996; Fonseca and Bennet-Clark 1998; Fonseca and Hennig 1996; Fonseca and Popov 1994; Hennig et al. 1994). The periodical cicada, *Magicicada septendecim*, produces at least three different songs: the calling (=congregational), protest (=distress), and courtship songs (Alexander 1957; Alexander and Moore 1958; Weber et al. 1987; Young and Josephson (1983b). The calling song (=pharaoh call) of *M. septendecim*, which is familiar to most rural and urban inhabitants of the Eastern United States, consists of a sustained buzz lasting for about 1.5 s, ending with a decrease in amplitude and frequency. The drop in pitch at the end of the pharaoh call is associated with a relaxation and lowering of the abdomen (Weber et al. 1987). Control muscles that modulate sound output by changing the mechanical properties of the tymbal or the resonance characteristics of the thoracic and abdominal air sacs are largely responsible for cycle-to-cycle changes in sound output during singing, and for the breadth of the song repertoire of cicadas.

The anatomy and probable action of five muscles or sets of muscles thought to be control muscles are described by Pringle 1954b. Two of them, the tensor muscle and the ventral longitudinal muscle (VLM), along with the tymbal muscle, are considered here. The detensor tympani, dorsal muscle group, and the set of abdominal muscles, all proposed to be involved in sound modulation, were not included in this study.

One of the goals of this study was to extend observations on correlations between muscle contraction kinetics and ultrastructure in insect muscles. Previous studies (Josephson and Young 1987) have focused on structural and functional correlations derived from comparisons between different species. In this study we compare muscles with different properties from a single species.

Details of the muscles examined

Tymbal muscle

The paired tymbal muscles originate at the ventral midline of the metathoracic segment on a broad skeletal structure called the chitinous V (Myers 1928; Pringle 1954b). The muscle fibers are arranged in parallel and insert dorso-laterally on the chitinous tymbal. The muscle is attached to the tymbal by a thin apodeme that originates from the middle of the tymbal-plate. In some species of cicadas (e.g., *Platypleura*; Pringle 1954b) the

tymbal is an asynchronous muscle; however, in *M. septendecim,* the species considered in this paper, it is a synchronous muscle. Tymbal muscle action is antagonized by the elastic properties of the tymbal; during muscle relaxation, the tymbal returns passively to its rest state. Repetitive action of the tymbal muscle produces a series of in and out movements of the tymbal and associated auditory clicks. Each tymbal muscle is innervated by a branch of the auditory nerve. Most anatomical and physiological studies have concluded that there is but a single motoraxon to each tymbal muscle and that the muscle is effectively a single motor unit (Hagiwara 1955; Popov 1981; Pringle 1954a; Simmons 1977; Young and Josephson 1983a). However, detailed anatomical studies by Wohlers et al. (1979) and by Wohlers and Bacon (1980) report that in addition to the large efferent axon to the tymbal muscle in the auditory nerve—the motoraxon identified in other studies—there is also a set of much smaller efferent axons. There is no physiological evidence for the function of the smaller axons, which are possibly neurosecretory fibers.

Tensor muscle

The tensor and the much larger tymbal muscle are the only muscles of the sound system that are directly attached to the tymbal. The tensor is a fan-shaped muscle, originating on a cuticular protuberance on the posterior edge of the metathorax and inserting dorsolaterally on the anterior frame of the tymbal (Hennig et al. 1994; Pringle 1954a, 1954b). At least three neuron cell bodies have been identified in the metathoracic-abdominal ganglion complex that project axons through the tensor nerve to innervate the tensor muscle (Popov 1981; Wohlers et al. 1979). Contraction of the tensor muscle increases the stiffness of the tymbal (Hagiwara 1955; Pringle 1954b; Simmons and Young 1978) thereby increasing the force necessary for tymbal buckling to occur by action of the tymbal muscle (Fonseca and Hennig 1996). The tensor muscle is active during singing (Fonseca and Hennig 1996; Hagiwara 1955). Stimulation of the tensor muscle can increase or decrease the amplitude of sound pulses from a singing cicada, depending on the stimulation frequency and the cicada species considered.

Ventral longitudinal muscle

The VLM originates on the anterior part of the metathoracic sternum and inserts on the anterior extension of the base of the chitinous V. Contrary to the observations of Pringle (1954b) who reported that the VLM consists of two small muscle bands, we have observed three small muscle bands in *M. septendecim*. A central band of fibers originates on the sternum and is surrounded by two smaller bands of fibers that originate on the sternum ventral to the origin of the central band. Contraction of

the VLM depresses the abdomen (Pringle 1954b), thereby diminishing the gap between the metathoracic and abdominal segments and altering the volume of the resonating air sacs in the thorax and abdomen.

Materials and methods

Experimental animals

The 17-year periodical cicada, *Magicicada septendecim* (Linnaeus), was studied from three populations representing two geographically distinct broods. Animals from brood XIV (notation of Marlatt 1907) were collected near Falmouth, Massachusetts during successive emergences late in June and early July in 1974 and 1991. Additional animals from brood II were collected north of town center, Meriden, Connecticut, in June of 1996. Because of asynchrony in emergence, animals in any given year were available over a 3-week period. Many dead and dying animals were found in the field and most collected animals died within a few days of capture. Caging cicadas with freshly cut terminal oak branches or with living potted *Hydrangea* sp. improved survival. Only male animals that gave audible squawks when handled were used for experiments. All animals were used within 2 weeks of capture.

Gross measurements of whole muscle and muscle fibers

Dimensions of whole muscles were determined from animals fixed in 70% ethyl alcohol. The muscles were dissected free from the animals and stored separately for periods varying from 1 to several weeks. Both muscle length and muscle mass were measured following overnight rehydration in insect saline. Muscle length was measured to the nearest 0.1 mm using an ocular micrometer in a dissecting microscope. The tensor muscle, unlike the tymbal and VLM, is somewhat fan-shaped. For this muscle, the length was calculated as the mean of that of the longest fibers, which are those on the medial edge, and the shortest fibers, which form the lateral edge. Muscle masses were corrected for the expected weight loss associated with alcohol fixation and subsequent rehydration ($=11.2\%$) determined from a set of six tymbal muscles that were weighed before and after fixation and rehydration. Muscles were weighed on an electrobalance to the nearest 1 µg. Accuracy was improved by removing and storing the ventral muscle bands as bilateral pairs, and then weighing each pair together. The weight given is the average weight for the two.

Mechanical recordings

Muscle tension was recorded with a transducer made from two silicon semiconductor elements (Pixie 8101) that formed two arms of a bridge. A stainless steel hook, waxed to the transducer array, was used to hold one end of the muscle. The resonant frequency of the transducer and attached hook was greater than 500 Hz. The entire transducer assembly was mounted on a micromanipulator that was used to adjust the length of the muscle for optimal tension during stimulation. The temperature of the preparation was monitored with a thermocouple or thermistor probe (typical o.d. = 0.5 mm). For experiments with the tymbal or the VLM, the temperature probe was placed in the muscle contralateral to that from which mechanical recordings were made; with the tensor muscle the probe was placed in tissue adjacent to the origin of the muscle. The muscle temperature during mechanical recordings was maintained at 25 or 35°C by adjusting the intensity of a microscope lamp whose beam shone on the preparation. The muscles from which recordings were made were kept moist with the cicada saline described by Hagiwara and Watanabe (1954).

Tymbal muscle

The ventral nerve cord was transected between the 2nd and 3rd thoracic segments. Wings and legs were removed. One of the paired tymbal muscles was exposed by pinning one side of an animal to a dissecting dish and removing, from the upper side, the tymbal and pleuron of the last thoracic segments. The transducer was attached to the muscle by placing a hook around the apodeme that normally inserts on the tymbal. Muscle length was adjusted to the in vivo length by means of a micromanipulator. The muscle was stimulated by inserting a pair of wire electrodes, insulated to the tip, into the base of the muscle.

Tensor muscle

The ventral nerve cord was transected between the second and third thoracic segments and the wings and legs were removed. The right side of the animal, including the right tymbal, was embedded in a pool of quick-setting epoxy on a microscope slide. Mounting the animal in this way immobilizes the exoskeletal insertion of the tensor muscle on the right side. The tensor nerve to the muscle was exposed by dissecting away the first five abdominal segments on the left side of the animal. In so doing, the tymbal muscles were completely removed and the VLM transected. The tracheal sheathing overlying the tensor muscle was moved to one side to expose the nerve that was then cut at a length suitable for use with a suction electrode. The exoskeleton surrounding the muscle insertion was cut to fit onto a hook attached to the transducer on a micromanipulator and the length adjusted to the in vivo length. Stimulation of the muscle was through the suction electrode attached to the cut length of nerve.

Ventral longitudinal muscle

The initial preparation was similar to that for the tensor muscle, with the exception that the animal was fixed ventral side up to a glass microscope slide with epoxy. Abdominal segments 2–5 were then removed ventrally and laterally to expose the tymbal muscles in the 1st abdominal segment. The tymbal muscles were dissected cleanly from their ventral apodeme, detached dorsally and discarded. The VLM muscles are attached anteriorly to the 3rd thoracic segment and posteriorly to the 1st abdominal segment on the same apodeme as the tymbal muscles, but anterior to the tymbal muscle attachments. The nerves to the paired VLM muscles course between them at the ventral midline. Both nerves were exposed by a posterior to anterior midline cut through a tracheal sheathing surrounding both muscles. The tracheal sheath over one of the muscles was pinned to one side, but not removed. The nerve to the VLM was cut as far as possible from its midline entry into the muscle. The posterior apodeme with both VLM muscles attached was then dissected free from its connection to the first abdominal segment. Mechanical responses were recorded by placing an insect pin fixed to the transducer against an anterior groove at the midline of the apodeme. One of the VLM muscles was then activated by stimulating the cut VLM nerve held by a suction electrode filled with saline.

Preparation for electron microscopy

Animals chosen for electron microscopy were beheaded and surgically immobilized by severing the nerve cord between the 2nd and 3rd thoracic segments. All thoracic appendages were amputated and the abdomen severed between the 1st and 2nd abdominal segments. The preparation was pinned ventral aspect down in a small dissecting dish. This preparation was

immediately immersed in cold (4°C), 3% glutaraldehyde in 0.1 mol l^{-1} phosphate buffer containing 3% sucrose (pH 7.4). After 20 min the muscles were further exposed in the thoracic box and dissected free, often with a segment of the motor nerve attached. The muscles were removed to fresh glutaraldehyde. The total fixation time was for 1 h. Tissues were rinsed three times in 0.1 mol l^{-1} phosphate buffer and then postfixed in 1% OsO4 in 0.1 mol l^{-1} phosphate buffer (pH 7.4) for 40 min. Tissues were then rinsed in fresh buffer, dehydrated, and embedded in Spurr's plastic or a mixture of Epon/Araldite. Thin sections were stained with 50% alcoholic uranyl acetate followed by lead citrate (Reynolds 1963) and examined in either a Zeiss 9S or 10S electron microscope.

Stereology

The fraction of individual muscle fibers occupied by myofibrils, by mitochondria, and by sarcoplasmic reticulum (SR) and T-tubules combined was quantified by the stereometric techniques of Freere and Weibel (1967). A transparent acetate grid with 14×20 orthogonally intersecting lines was placed over electron micrographs and the structure underlying each intersection recorded. Points lying over tracheoles were excluded from the tabulations. The fractional area of each structure was calculated as the ratio of grid intersections overlying that structure to the total number of intersections overlying the muscle fiber in the entire grid field. Assuming no bias in the selection of fibers analyzed, and assuming that the muscle fibers are structurally homogeneous along their length, the fractional areas also represent the fractional volume for each structure. Mean volume density and standard error were determined for five fibers for each of six animals. Only those micrographs with a minimum of 200 structural intersections lying within fibers were included in the analysis.

Muscle fiber cross-sectional areas and sarcomere lengths were determined from muscles embedded for electron microscopy. Thick sections (1 μm) were stained with a 1% mixture of azure blue/methylene blue in a borate buffer (Richardson et al. 1960). Sections were examined in a light microscope. NIH image software was used to calculate cross-sectional areas of ten muscle fibers for each muscle from five animals; sarcomere lengths were measured as the average of ten consecutive sarcomeres.

Statistical treatment

Statistical significance was determined using the Bonferroni adjustment (Fry 1993). Paired comparisons of data were considered to be significantly different if $P < 0.05/n$, where n is the total number of comparisons made.

Results

Muscle and muscle fiber morphology

The tymbal muscle, the power muscle of singing, was an order of magnitude more massive than either the tensor muscle or the three bands of the ventral longitudinal muscle considered collectively (Table 1). Muscle fibers from the tymbal muscle were the largest of the muscles or muscle bands examined. The mean cross-sectional area of fibers from the tymbal muscle was 1.7 times greater than that of fibers from the VLM central band, though the difference between the two was not significant, and about ten times greater than fibers of the lateral and medial VLM and the tensor muscle (Table 1). Fibers of the central band were significantly larger in cross-sectional area than the fibers of the other two bands of the VLM and those of the tensor muscle. Sarcomere lengths were inversely related to cross-sectional areas (Table 1). Sarcomeres of the tensor muscle were the longest, and were about 2.7 times longer than those of the tymbal, which were the shortest. Sarcomere lengths of the lateral and medial band fibers of the VLM were not significantly different than the tensor muscle, and were 1.5 times longer than sarcomeres of central band fibers.

Ultrastructure

The set of fibers making up a muscle or muscle band were structurally homogeneous. However, there was considerable structural heterogeneity between fibers of each of the three muscles, and between fibers of the VLM bands (Figs. 1, 2). Fibers of the tymbal muscle and the central band of the VLM were organized into distinct circular or polygonal myofibrils, each delineated by a well-developed SR. Diadic structures of the transverse tubular system (TTS) often occurred with the cisternae of the SR. In transverse sections the mitochondria of the tymbal muscle fibers were often arranged side by side forming rows that alternate with a row of contiguous myofibrils. Mitochondria of the central band of the VLM were smaller than those of the tymbal muscle and

Table 1 Structural characteristics of tymbal and control muscles

|	Muscle length (mm)[1]	Muscle mass (mg)[1]	Fiber cross-sectional area (μm^2)[2]	Sarcomere length (μm)[3]
Tymbal	4.18 ± 0.10	10.50 ± 0.63	3072 ± 379[A]	2.7 ± 0.2[a, b, c, d]
Tensor	2.31 ± 0.05	0.70 ± 0.05	300 ± 16[a, b, c]	7.3 ± 0.5[A]
VLM-Medial	2.83 ± 0.03	0.14 ± 0.02	441 ± 41[a, b, C]	6.5 ± 0.2[C]
VLM-Central	3.31 ± 0.07	0.61 ± 0.04	1853 ± 281[B]	4.5 ± 0.4[a, c, D]
VLM-Lateral	2.74 ± 0.06	0.12 ± 0.02	279 ± 50[a, b]	6.6 ± 0.7[B]

Values are mean ± SE
Sample sizes: [1] $n = 6$–11 muscles, each from a different animal;
[2] $n = 5$ muscles, diameters of 10 fibers measured and averaged for each muscle except for those of the tensor for which 18 fibers were measured per muscle;

[3] $n = 5$ muscles, the length of 10 consecutive sarcomeres was measured for 5 fibers from each muscle
Statistically significant differences in fiber area and sarcomere length are coded as follows: A is significantly greater ($P < 0.05$) than a, B > b, C > c, D > d

Fig. 1 Electron micrographs (*left panels*) of the tymbal muscle (**A**) and tensor muscle (**B**) cut in cross-section. *Insets* are enlarged profiles of the myofilament array for each muscle. The tymbal has 6 thin filaments surrounding each thick filament; the tensor 10–12 thin filaments surround each thick filament. Histograms in the *right panels* show the volume percent (mean ± 2SE) of myofibrillar structure (*M*), mitochondria (*Mc*), and sarcoplasmic reticulum/transverse tubules (*SR*) for each muscle. *d* dyad of the transverse tubular system. Scale bar = 0.5 μm and 0.2 μm (*inset*)
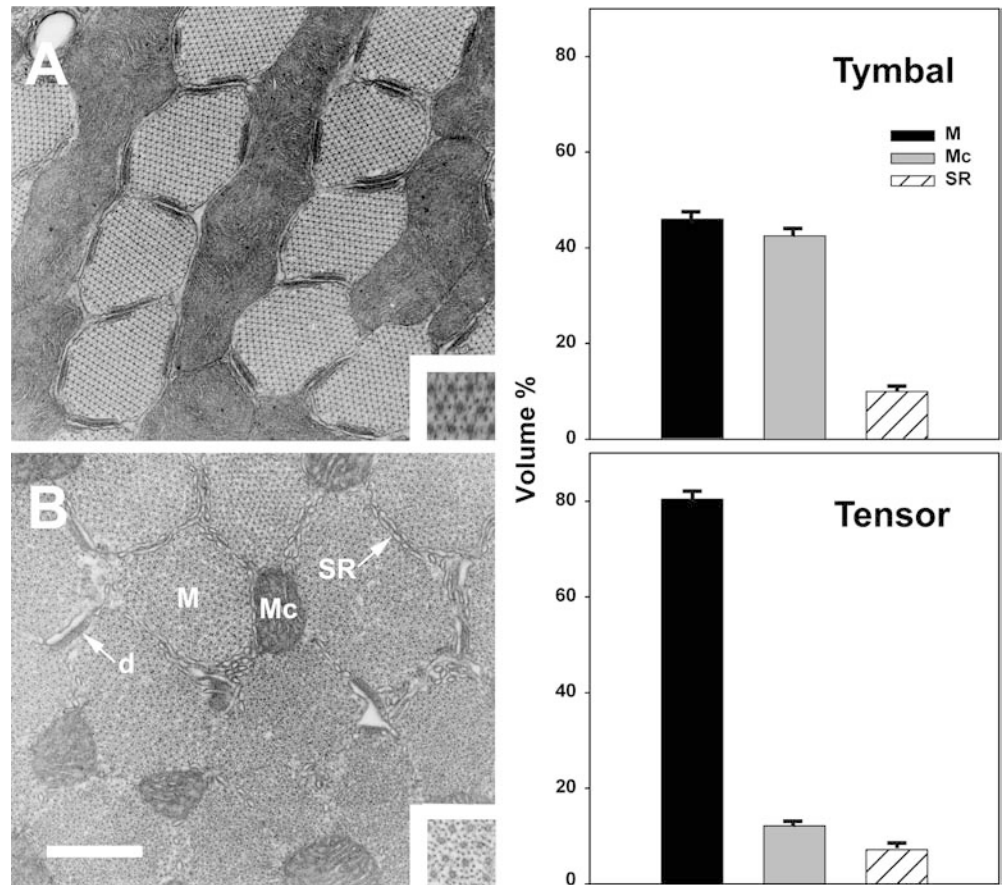
were scattered between myofibrils throughout the fiber. Tymbal muscle fibers and central band fibers shared two other features: (1) myofilaments arranged with six thin filaments surrounding a single thick filament (see insets in Figs. 1A, 2B), and (2) the presence of intrafiber terminal branches of the tracheal system.

Fibers of the tensor muscle and of the medial band fibers of the VLM were also arranged as parallel sets of circular or polygonal myofibrils with small, individual mitochondria scattered throughout; however, the SR was not as apparent as in tymbal and central band VLM fibers. In the lateral band of the VLM the thick and thin filaments within a fiber were not organized into discrete myofibrils. The SR in these fibers was scattered, as were the very small mitochondria. Tensor fibers and fibers of the lateral and medial VLM were similar in that in each there are ten thin filaments surrounding a single thick filament (see insets in Figs. 1B, 2A, C), and there were no intrafiber tracheoles. Muscle fibers that we interpret to be degenerating because they were enlarged, highly vacuolated and with disorganized myofibrillar structure were frequently observed in tensor muscles and occasionally in the VLM.

Stereology

Myofibrils made up much or most of the cellular volume in all the muscles examined (Figs. 1, 2). Fibers

of the tymbal muscle and of the central band of the VLM had the smallest fractional volume of myofibrils (46% and 54%, respectively). Tensor muscle fibers and fibers of the medial and lateral bands of the VLM were 1.5–1.8 times greater in myofibrillar volume density (about 80%). Mitochondrial volume densities, on the other hand, were highest for tymbal muscle fibers and VLM central band fibers (43% and 26%, respectively), and lowest for tensor fibers and fibers of the lateral and medial VLM (10–12%). The greatest investment in SR/TTS was in central band fibers of the VLM (19%), followed by tymbal muscle fibers (10%). Fibers of the tensor muscle and the lateral and medial VLM had the least investment in SR/TTS (6–7%).

Motor unit organization

The number of motor units for the tymbal, tensor and VLM were determined by recording muscle twitches from each muscle following stimulation of the muscle or its motor nerve with single current pulses of gradually increasing or of gradually decreasing intensity. Each force step in the sequential records of twitch amplitude signaled the recruitment of a new motor unit or the loss of a previously responding one as the stimulus intensity passed the threshold for activation

Fig. 2 Electron micrographs (*left panels with insets*) of the ventral longitudinal muscle: **A** lateral band; **B** central band; **C** medial band. *Line drawings* alongside each panel show the ventral longitudinal muscle (VLM) from the left side in dorsal view with the represented band for each panel highlighted. All three bands of the VLM originate anteriorly on the ventral side of the chitinous V (*V*). The tymbal muscle (not shown) originates dorsally on the chitinous V and would project upwards and laterally out of the plane of the drawing. Vertical scale bar = 1 mm. *Insets* are enlarged profiles of the myofilament array for each muscle band. The *central band* is similar to the tymbal muscle with 6 thin filaments surrounding each thick filament. The *lateral and medial bands* are similar to the tensor muscle with 10–12 thin filaments surrounding each thick filament. *Histograms* in the *right panels* show the volume percent (mean ± 2SE) of myofibrillar structure (*M*), mitochondria (*Mc*), and sarcoplasmic reticulum/transverse tubules (*SR*) for each muscle band. *t* tracheole. Micrograph scale bar = 0.5 μm and 0.2 μm (*inset*)
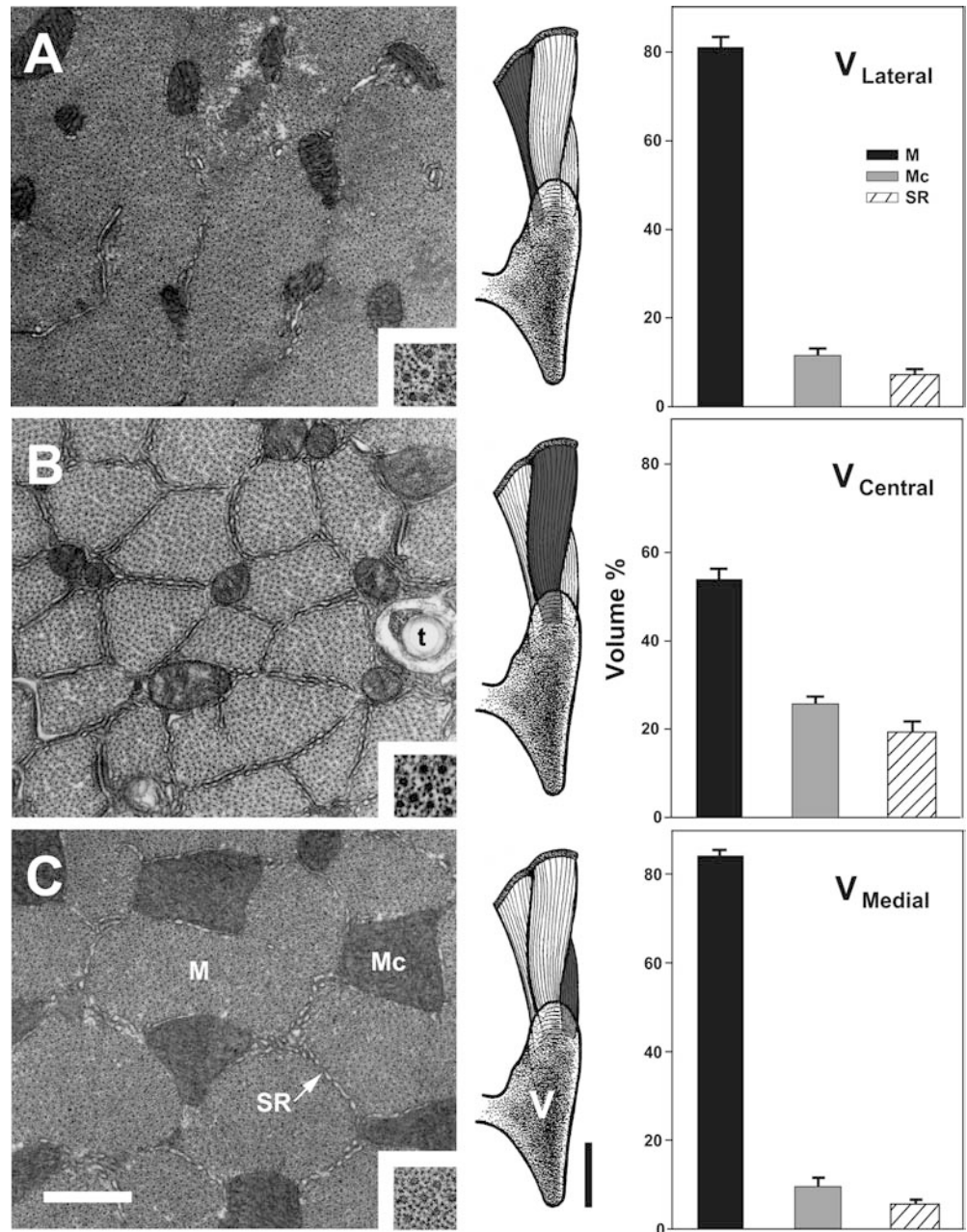
of a motoraxon to the muscle. The amplitude and time course of individual motorunit responses in the tensor, which contains at least two units, and the VLM, which has at least six, were obtained by digitizing and averaging three or more twitches collected at one stimulus intensity, and subtracting from this waveform the digitized and averaged response collected with a stimulus intensity that was sufficiently lower than the first that one motorunit has dropped out of the response (see Fig. 3). Representative mechanical responses along with the cross-sectional profile of the nerve supplying each muscle, obtained from muscle sections taken at or near the nerve entry to the muscle, are shown in Fig. 4.

Tymbal muscle

Stimulation of the tymbal muscle gave rise to a fast unitary twitch of constant amplitude at all suprathreshold intensities of stimulation (Fig. 4, upper panel). Thus, the tymbal muscle appears to be a single, fast, motor unit. Correspondingly, the nerve supplying the tymbal muscle has a single large axon profile (12–15 μm diameter) which presumably is the motoraxon supplying the fibers of the tymbal muscle. There are smaller axons ventrally positioned with respect to the large axon profile which may be axons that branch to innervate other muscles; also there are many small axon profiles in the nerve, presumably sensory fibers.
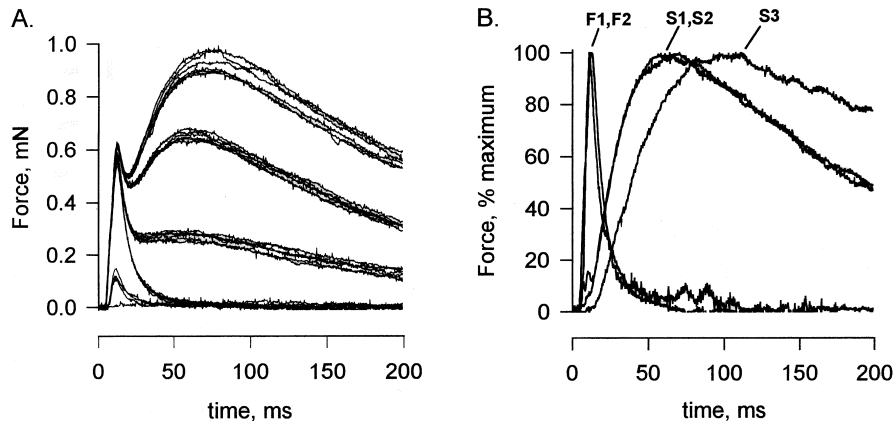
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Fig. 3A,B Ventral longitudinal muscle. **A** Superimposed twitches obtained during a series of twitch stimuli during which the stimulus intensity, which was initially greater than that required to get a maximum response, was progressively decreased (25°C). Although the stimulus intensity was decreased gradually, force decreased in discrete steps, each step corresponding to the dropping out of a motor unit. Five force levels were discernible in this preparation (plus a zero level when the stimulus was below threshold), indicating that the muscle contains at least 5 motorunits. Two of the motorunits produced quite fast twitches (*F1, F2*), the other three distinctly slower twitches (*S1, S2, S3*). **B** The traces within each of the 5 clusters in **A** were averaged to obtain a typical trace for that stimulus intensity range. The difference in force between adjacent averaged traces, which represents the force increment due to the recruitment of a new motor unit, was obtained by subtracting the smaller from the larger of two adjacent units. These contributions for the successive motorunits were normalized and superimposed to facilitate comparison of their time-course

The twitches from the tymbal muscle had durations, onset to 50% relaxation, of about 15 ms at 25°C and 10 ms at 35°C (Table 2). In this study, we were unable to evoke clean, well-fused tetanic contractions from the tymbal muscle. Force rose abruptly during tetanic stimulation but typically the force quickly peaked and then became irregular and fell. Typical tetanic responses have been obtained using similar techniques in other studies of cicada tymbal muscles (Josephson and Young 1981); why we were unsuccessful here is not known. The maximum force reached during tetanic stimulation averaged 1.38 times greater than that during a twitch (SD = 0.27, n = 5, 35°C) but, as indicated, the tetanic tension failed to approach a clear plateau, and it is likely that the ratio of tetanic force would have been greater than 1.38 had more fused tetanic contractions been obtained.

Tensor muscle

We were able to demonstrate two motorunits in the tensor muscle, both of which produced rather small and slow twitches relative to that of the tymbal muscle (Fig. 4, middle panel). The nerve to the tensor muscle had two large axon profiles (10–12 μm diameter), presumably the motoraxons to the two demonstrable units, and a third much smaller (2–3 μm diameter) axon profile which may be that to yet another motorunit whose

twitches we were not able to detect. A motorunit which produced very small twitches and which had a higher threshold than the two identified units could easily have gone undetected in trials examining changes in twitch height associated with changes in stimulus intensity.

We will identify the unit in the tensor muscle that produced the lower tension and generally had the lower threshold as unit 1, the other as unit 2. Twitches from unit 1 were typically less than 10% as large as those from unit 2 [average ratio of twitch tension (unit1/unit2) = 6.7%, SE = 1.3%, n = 9, 25°C]. It was because unit 1 usually had the lower threshold that it was possible to record twitches from this unit alone. If it were not so, twitches of unit 1 would have been difficult to detect and quantify, given the much larger size and somewhat variable amplitude of twitches from unit 2. The contraction kinetics of the two units were similar but not identical. Unit 2 was somewhat faster than unit 1, with a shorter twitch rise time and duration (Table 3) and a more rapid rise of tetanic tension. The tetanic tension generated by unit 1 was less than that from unit 2 but not statistically significantly so (ratio of tetanic tension from unit 1 to that from unit 2 = 78%, SE = 32%, n = 6; 0.1 > P < 0.05). The maximum stress in the muscle during simultaneous tetanic activation of both units was 282 kN m^{-2} at 25°C (SE = 22, n = 8) and 270 kN m^{-2} at 35°C (SE = 18, n = 7). At 25°C the ratio of twitch to maximum tetanic tension was about 1% for unit 1 and 12% for unit 2 (Table 4). The twitch:tetanus ratio increased by about three times for both units 1 and 2 when the muscle was warmed from 25°C to 35°C. As expected, warming the muscle decreased both the rise time and the total twitch duration measured from onset to 50% relaxation. The average Q_{10} for twitch rise times and durations for the temperature range 25–35°C were 1.49 (SE = 0.22, n = 5) and 1.81 (SE = 0.38), respectively, for unit 1 and 1.30 (SE = 0.11, n = 5) and 1.69 (SE = 0.19) for unit 2.

VLM

The motor unit composition of the VLM is considerably more complex than that of the tymbal and tensor muscles; both for the number of motor units and their heterogeneity. It was possible to identify up to six motorunits in
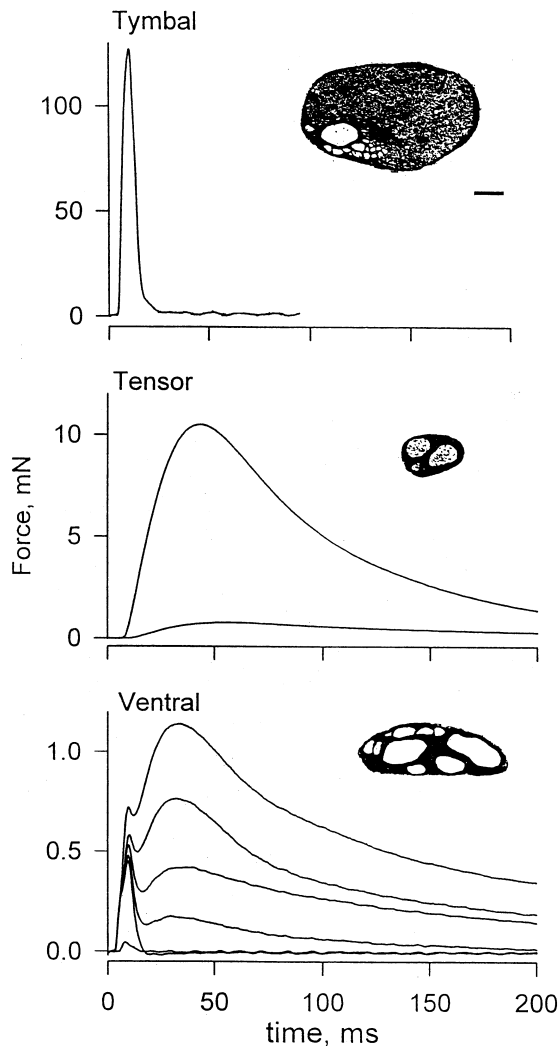
Fig. 4 Motorunit organization of the tymbal (*upper*) tensor (*middle*) and ventral longitudinal (*lower*) muscles. *Insets* show motor axon profiles in cross-sections of the motor nerve supplying each muscle. The force traces show the change in twitch height obtainable by gradual increases in stimulus intensity. Each force increment results from a new motorunit having been recruited. In order to reduce the noise, the traces shown for the tensor and the ventral longitudinal muscles are averages of several force records. The traces from the tensor muscle are the averages of three force records obtained at each of two stimulus intensities; one intensity sufficient to excite the motorunit with the lower threshold (here the low-force trace), the other adequate to excite both motorunits detectable in the muscle. Each of the traces from the ventral longitudinal muscle is the average of five force records at a given stimulus intensity (see Fig. 3) for additional information on the collection of these data. The temperature was 35°C for all recordings, chosen because the motorunit composition of the ventral longitudinal muscle selected was clearer at 35°C than at 25°C. Scale bar = 10 μm

different preparations by varying the intensity and polarity of the stimuli given the motor nerve. A preparation with five identifiable units is shown in Fig. 3; one with six units in Fig. 4. A motorunit that produced a vanishingly small twitch, or one whose stimulation threshold was very close to that of another unit could easily have been missed in evaluating the motorunit composition of a

Table 2 Characteristics of isometric twitches from the tymbal muscle

	Rise time (ms)	Onset to 50% relaxation (ms)	Peak to 90% relaxation (ms)	Stress (kNm^{-2})
25°C	9.2 ± 0.4	14.4 ± 0.7	10.5 ± 0.7	24.8 ± 5.4
35°C	6.1 ± 0.2	9.4 ± 0.4	6.4 ± 0.4	36.6 ± 12.4
Q_{10}	1.51 ± 0.03	1.52 ± 0.0	1.66 ± 0.08	

Values are mean ± SE, $n = 7$

muscle. Thus there are seemingly at least six and possibly more motor units in the VLM.

The distribution of these six motor units within the three bands of the VLM has not been elucidated clearly. In a preparation in which only slow unit activity was observed, there was no detectable movement of the central band following nerve stimulation. In yet another preparation fast unit activity was eliminated following cutting of the central band. It appears that the fast motor units are not found in the lateral and medial bands of the VLM. The nerve to the VLM may contain as many as ten moderately large axon profiles of varying diameter; two of them are noticeably larger (20–25 μm diameter) than the others and may be the axons to the fast units.

One or two of the motorunits of the VLM produced quite fast twitches with durations, measured from force onset to 50% relaxation, typically being between 10 and 20 ms at 25°C (mean duration = 14.2 ms, SD = 3.9, $n = 12$ units from nine preparations). One of the fast units, to be identified as F1 because it generally had the lower threshold, produced twitches that were much smaller than F2, the other fast unit. In several muscles the twitches from F1 were detectable but so small in comparison to the noise level that their time course could not be readily measured. The slower units in the ventral muscle produced twitches whose durations were typically 100–400 ms at 25°C (mean = 241 ms, SD = 101 ms, $n = 23$). It was clear that some slow units were slower than others, and that there was heterogeneity even among the slow units of a single muscle (Figs. 3, 5). Available data are insufficient to determine if there is a standard pattern in the distribution of contraction kinetics among the slow units of a muscle.

The tetanic force (stimulation frequency = 200 Hz) recorded from the VLM was quite variable, and ranged from 3.7 to 35.8 mN in different preparations (mean = 22.2 mN, SD = 13.9 mN, 25°C, $n = 10$). The average cross-sectional area of a VLM, calculated from the data in Table 1, is 2.8×10^{-3} cm^{-2}. Using this value for area gives a mean stress of 79.3 kN m^{-2} with a range of 13–127.9 kN m^{-2}. The twitches in all units were a small fraction of the maximum tetanic force of the muscle. F2, the second fast unit, generally produced the largest peak force among the muscle units. The size of the largest fast unit—the F2 response when both an F1 and F2 could be distinguished or that of the single fast unit when only one fast unit could be detected—averaged 2.3% of the tetanic force from the same muscle (SD = 2.4%).

Table 3 Features of twitch and tetanic contractions from two motor units of the tensor muscle

	Rise Time	Onset to 50% relaxation (ms)	Tetanic stress (kNm^{-2})	Twitch force/Tetanic force (%)
25°C				
Unit 1	70 ± 12 (*n* = 9)	229 ± 44 (*n* = 9)	74 ± 23 (*n* = 6)	0.96 ± 0.03 (*n* = 6)
Unit 2	49 ± 4 (*n* = 9)	141 ± 16 (*n* = 9)	168 ± 22 (*n* = 6)	12.1 ± 4.3 (*n* = 6)
35°C				
Unit 1	52 ± 6 (*n* = 5)	155 ± 19 (*n* = 5)	139 ± 21 (*n* = 4)	3.3 ± 1.4 (*n* = 4)
Unit 2	41 ± 4 (*n* = 5)	97 ± 11 (*n* = 5)	167 ± 28 (*n* = 4)	30.4 ± 13.4 (*n* = 4)

Values (mean ± SE) are based on three twitches from each preparation and temperature in which unit 1 alone was activated; the twitches in which unit 1 and 2 were excited simultaneously; and one tetanic contraction with unit 1 alone and one with both unit 1 and 2 activated. The twitches from each preparation were digitized and averaged. The amplitude and time-course of unit 2 alone was obtained by subtracting the averaged twitch of unit 1 from the averaged twitch of the two units combined. In some preparations it was not possible to obtain twitches from unit 1 alone at 25°C and 35°C, or to reliably activate unit 1 alone throughout a tetanic burst; therefore, the sample size, which is the number of different preparations represented, varies from cell to cell in the table

Table 4 Features of power and control muscles of *M. septendecim*

	Tymbal (power)	Tensor (control)	Ventral (control)
Mass (mg)	11	0.7	0.9
Fiber volume as mitochondria (%)	43	12	26/11[a]
Number of motorunits	1	2	≥6
Twitch duration, onset to 50% relaxation at 25°C (ms)	14	170	15/264[b]
Twitch tension/tetanic tension (%)	70(60[c], 56[d])	12	5

[a] Central band/mean of medial and lateral bands
[b] Fast units/mean of slow units
[c, d] Values for *Cyclochila australasiae* and *Psaltoda argentata* from Figs. 5 and 8 of Josephson and Young (1981)
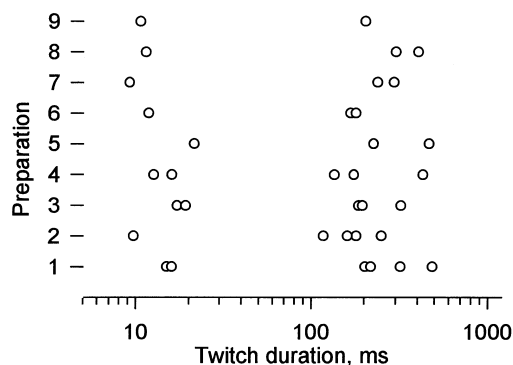

Fig. 5 Ventral muscle. Twitch duration (onset to 50% relaxation, 25°C) for identified motorunits in nine ventral muscles. Individual motorunits within a muscle were isolated as in Fig. 3. Muscles are placed, from top to bottom, in order of increasing number of identified motorunits. In one additional preparation the motorunits were too weak and the force levels too variable to allow characterization of individual units with any confidence

Discussion

Power muscles and control muscles

In its functional division into power muscles and control muscles, the musculature of the sound system of cicadas is rather like, albeit much simpler than, the flight systems of insects. In locusts, for example, there are about a dozen pairs of power muscles and one pair of control muscles, the pleuroaxillary muscles, in each of the two winged segments (Tiegs 1955). Flight in dipteran flies is powered by two pairs of "big and dumb" power muscles, and aerodynamic performance is modulated by 18 pairs of small, control muscles, most of which insert on skeletal elements associated with the wing hinge (Dickinson and Tu 1997). Some of the features of the tymbal, tensor and ventral muscles of *M. septendecim* that seem particularly important for their functioning as power or as control muscles are listed in Table 4 and enumerated as follows:

1. *The power muscle is large, control muscles are relatively small.* The power available from a muscle is proportional to the muscle mass. To produce loud calling songs, ones that can be heard and are attractive over long distances, requires large muscles. In contrast, selection for overall efficiency would tend to minimize control costs and allow control to be achieved with small muscles.

2. *The power muscle is richly supplied with mitochondria, control muscles less so.* High, sustained power output requires a high supply rate of ATP that, in aerobic muscles like insect flight and singing muscles, is

supplied by mitochondria. The abundant mitochondria provide the metabolic energy needed for high mechanical power output and with high-frequency, synchronous muscles such as the tymbal of *M. septendecim*, for the calcium cycling costs associated with rapidly turning the muscle on and off. Control muscles presumably operate with lower energy requirements than do power muscles, and require less investment in mitochondria.

3. *The innervation of the power muscle is quite simple, that of control muscles less so*. During singing the tymbal muscle is turned on and off rapidly, and in a nearly all-or-nothing manner. The innervation of the tymbal muscle by a single axon insures that all fibers are simultaneously activated. It is likely that the force in control muscles is graded, and that gradation is achieved in part by recruitment of different moto-runits within the muscles.

4. *Twitches from the power muscle are short, those from control muscles mostly long*. High frequency contraction, as with the tymbal muscle during singing, requires a rapid muscle activation and deactivation. The activity of control muscles in modulating the sound extends over many cycles, and is best achieved with muscles whose contraction time course is long compared with the sound cycle duration. It is interesting that the medial portion of the ventral muscle produces twitches as short as those from the tymbal muscle. Perhaps this part of the ventral muscle contracts phasically during singing at a frequency similar to that of the tymbal, but what the function of such contractions might be is unknown.

5. *The twitch/tetanus ratio is high in the power muscle, low in the control muscles*. During singing the tymbal muscle contracts in a series of repetitive twitches. For maximal power output the tymbal muscle needs to become fully activated or nearly so during the brief time-course of a twitch. On the other hand, the force in the control muscles can vary from a small fraction of tetanic force to full tetanic force depending on the frequency and number of incoming motorneuron action potentials. The potential for grading muscle force over a wide range increases the versatility of the control muscles in modulating sound patterns.

Ultrastructural and functional correlations

The quantitative investment of mitochondria, SR, and myofibrillar protein in muscle fibers is expected to correlate with muscle functional properties (Josephson 1975). Muscle fibers well endowed with mitochondria should have high endurance; those with a large investment in SR can quickly release and resequester large amounts of calcium and should have brief twitches; and those with a large investment in myofibrillar protein are expected to be capable of high force production and work. Heretofore correlations have been on muscle homologues from several different insect species (Joseph-

son and Young 1985, 1987) or from a single muscle of heterogeneous muscle fibers (Stokes et al. 1975). In this study, we extend these observations to functionally different muscles of the sound system of a single species, *M. septendecim*. The speed of isometric twitch development for the muscles of *M. septendecim* is inversely related to the volume density of SR/TTS. The muscles with the greatest investment in SR/TTS are the central band of the VLM (19.4%), followed by the tymbal muscle (10.0%). The central band is thought to contain the fast units of the VLM. It is somewhat surprising that this part of the muscle has more SR/TTS than does the tymbal muscle. However, the kinetics of the fast units of the VLM are indeed faster than the tymbal muscle for twitch rise time (7.39 ms versus 9.22 ms, respectively). Total twitch durations (onset to 50% relaxation), however, are very similar (14.92 versus 14.36 ms, respectively). Slow units of the VLM and the tensor muscle have twitch rise times that are six to eight times greater than the tymbal muscle and fast units of the VLM. The slow kinetics of these muscles correlate well with a much smaller investment in SR/TTS (volume percent ranges from 5.6% to 7.4%). Josephson and Young (1987) compared the relative development of the SR/TTS with twitch kinetics of synchronous tymbal muscles from eight species of Australian cicadas. They used the ratio of the volume of the muscle fiber as myofibril to that as SR/TTS as an index of the relative development of the tubular system within the fibers. Twitch rise time, relaxation time, and total duration at 30°C were all significantly correlated with the ratio of myofibril volume to SR/TTS (Fig. 2B in Josephson and Young 1987). An estimated least-squares regression line from their data for twitch duration (twitch onset to 50% relaxation) corrected for 25°C using an assumed Q_{10} of 1.57 is shown in Fig. 6. The tymbal muscle of *M. septendecim* is apparently faster for its SR volume, by a factor of about 2, than are the Australian cicada muscles (Fig. 6A). Interestingly, the fast part of the VLM lies right on the regression line. What is also obvious is that the two slower muscles, the tensor and slow parts of the VLM, are much slower than might be expected by extrapolating the regression line for tymbal muscles out far further than is statistically justifiable (Fig. 6B). What is also clear is that present knowledge and models are not adequate to accurately predict relationships between muscle ultrastructure and twitch time course.

Contraction kinetics are evolutionarily stable

The time-course of twitches recorded in the experiments of this paper are remarkably similar to those found for the tymbal muscle of *M. septendecim* in an earlier study of this species (Young and Josephson 1983b). For example, in the earlier study the twitch rise times at 25°C and 35°C were 9.2 and 6.4 ms, respectively, whereas in the muscles of the present study they were 9.2 and 6.1 ms. The earlier study was done with animals of
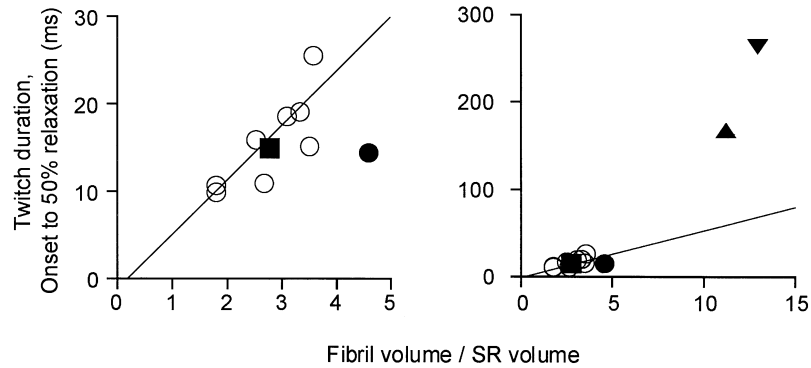
Fig. 6 Mean values for twitch duration and the relative development of the tubular systems in tymbal muscles from eight species of Australian cicadas (*open symbols*) and in muscles of the sound system of *M. septendecim* (*filled symbols*). Data are plotted twice (*left and right panels*), at different ranges for ordinate and abcissa. The data for the Australian cicadas are from the same experiments plotted in Fig. 2B of Josephson and Young (1987), but are for the twitch duration measured at 25°C rather than at 30°C as in that figure. For two species the lowest temperature at which twitch duration was measured was 30°C. For these the value for twitch duration measured at 30°C was adjusted to the expected value at 25°C using the average Q_{10} (1.57) obtained for the range 25–30°C for the other six species. The muscles for *M. septendecim* are: *filled circles* tymbal muscle; *filled squares* fast units of VLM, *filled triangles* tensor; *filled triangles* (*inverted*) slow units of VLM. The line in each plot is a least squares regression line for tymbal muscles of the Australian animals

brood IV collected in Kansas: those of the present study from animals of brood XIV collected in Massachusetts. Because the animals of the two studies are in separate broods, they have been totally isolated reproductively from one another since the brood pattern was established. Muscle contractile properties are apparently quite conservative in the species.

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